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The CRISPR-Cas9 induced *CCR5* Δ32 mutation as a potent gene therapy methodology for resistance to HIV-1 variant: a review

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Abstract. – Human Immunodeficiency Virus (HIV) has continuously been the greatest epidemic for humanity over a period spanning almost five decades. With no specific cure or treatment available to date despite extensive research, the C-C Chemokine Receptor 5, Delta 32 (*CCR5* Δ32) allele genetic point mutation plays an imperative role in the prevention of acquired immunodeficiency syndrome (AIDS). This comprehensive study aims to review the induction of the homozygous recessive deletion genotype using the Clustered Regularly Interspaced Short Palindromic Repeats, Cas 9 Enzyme (CRISPR-Cas9), and hematopoietic stem cell transplantation under positive selection pressure for active immunity in seropositive patients' populations as the phenotype. A methodology is proposed to trigger a significant increase in the expression of Delta 32 beneficial mutant alleles within controlled modern healthcare facilities utilizing totipotent stem cells through somatic gene therapy. It acts upon two dysfunctional *CCR5* genes, translating mutant G protein-coupled co-receptors, whose primary function is similar to that of C-X-C Motif Chemokine receptor 4 (*CXCR4*), by blocking the entry of viral RNA into the CD4+ T helper lymphocytes, halting infection and seizing viral life cycle. This modification is endemic in Northern Europe, where it naturally pertains to the Caucasian descent population samples in the form of polymorphism, *p* (X=0.01), where X is the probability of frequency of complete immunity against HIV-1 in population samples. The epigenetics of the single nucleotide polymorphism (SNP) are analyzed as they play a significant role in immunity distribution. Furthermore, a comparative analysis within the ethical boundaries of CRISPR-Cas9 is conducted to discuss the practical aspects and challenges of the presented methodologies and treatment alternatives. Additionally, the study assembles all available data and summarizes preexisting research while providing a promising solution to this ethical dilemma. Finally, a methodology is devised to answer the question of whether the variant-specific epidemic of AIDS caused by HIV-1 can be cured via artificially inducing immunity by CRISPR-Cas9.

Key Words:
CCR5 gene/receptor, T helper cells, Beta-Chemokine *CXCR4*, CRISPR-Cas9, Hematopoietic stem cells.

Introduction

Acquired Immunodeficiency Syndrome (AIDS) is caused by the Human Immunodeficiency Virus (HIV), which is a pathogen instigating adverse effects on the immune response, leading to lowered potency of the immune system in fighting against infections. This health condition is called the acquired immunodeficiency syndrome and is more commonly referred to as AIDS. Studies[1] have reported the origin of the virus within the Democratic Republic of Congo and the inter-species transmission to humans that takes place from feeding on chimpanzees, which dates back to the 1920s. The very first detection of the virus occurred in 1959 in a native individual from Kinshasa[2]. However, the first official human infection of the virus was documented in 1981 in the United States when patients suffering from mild but extremely rare cases of *Pneumocystis carinii* pneumonia (PCP) died uncharacteristically due to a weakened and suppressed immune system after sexual transmission of HIV[3]. Since then, HIV has proven to be a pressing health calamity of modern times, with multiple variants infecting up to 76 million people. These include deaths exceeding 33 million and 38 million active infections worldwide by the end of 2020 and the highest density in the Sub-African continent[4].

Nonetheless, a modern method to potentially tackle the epidemic of HIV-1 and develop resilience in the general population against the common M

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tropic HIV-1 is made possible by the introduction of mass application of gene therapy. CRISPR-Cas9 is used on the totipotent stem cells that originate from the seropositive patient to induce a mutation known as *CCR5* Delta 32[5]. Gene therapy involves alterations made in the genome for the treatment of hereditary diseases and other disorders influenced by translational products of various gene variants. This is followed by the reintroduction of certain genes into an individual's cells along with their donor tissues in order to treat the disease with the help of CRISPR-Cas9[6]. In this case, a genetic point mutation affects the gene coding for the *CCR5* receptor that is targeted for specific chemokines on the white blood cells in the body. This technique can be executed in different ways, such as replacing the gene that is causing the disease with the healthy gene instead or inactivating the faulty gene that is not functioning in the way it is supposed to[7]. This paper analyses the latter methodology to inactivate the *CCR5* gene for immunity against HIV-1 infection.

The two main types of gene therapies are the somatic cell therapy and the germline cell therapy. In somatic gene therapy, the RNA is induced inside the somatic diploid cell type in bodily tissue in a way that alters the pattern of the cells' gene expression. This results in a therapeutic effect on the targeted tissue[8]. In sharp contrast, germ-line cell therapy is performed in which the DNA is inserted into the fetal specialized cells, which are responsible for producing gametes. This method enables the fixation of the gene that is causing the disease to be prevented from passing down from one generation to another. In this way, the disorder is permanently tackled at any given point during early disease progression[9]. With all aspects considered, this study reviews the model focused on somatic cell therapy in fighting the HIV-1 infection by introducing homozygous mutant copies of the *CCR5* genes called Delta 32 in totipotent somatic stem cells. The cells under discussion are extracted only from the seropositive patient with an active HIV infection. Instead of altering the genome of each individual with germ-line therapy, the selective mass application of somatic gene therapy towards infected patients with health and age-related prioritized medical care will provide an efficient and practical method to tackle the epidemic[10]. In this way, the reintroduction of the cell will ensure the natural uptake of the gene during cell differentiation, pertaining to positive selection pressure for survival and immunity against HIV.

Additionally, individuals can develop autoimmune and systemic diseases over time after prolonged exposure to HIV due to immune dysregulation as a result of chronic infection[11]. Consequently, expressing significantly fewer chronic symptoms than normal infections. This provides control over the immune system by halting infection spread with the natural defense mechanism when operational *CCR5*-specific antibodies are manufactured for HIV control[12]. Therefore, the body gains natural autoimmunity in predominantly healthy individuals with superior functioning immune response to disintegrate the morphology of *CCR5* co-receptors from binding to GP120 spike proteins on the viral bodies, making HIV dormant[13].

The paper further delves into the subject by providing a detailed elaboration on the *CCR5* gene itself to establish the molecular basis for the genetic point mutation. The evolutionary pathway for the mutation and its origins are then traced back, inferring the history of the mutation to better understand the succession of each variant in the C-C motif chemokine receptor family. This enables adaptive interaction towards all strains of HIV and influenza virus from a geographical point of view. The endemism of Delta 32 is addressed, and the distribution in various populations is discussed to devise epigenetic relationships with beneficial allele expression frequencies for immunity. The study also discusses Gaussian statistical modeling as a way of devising such a relationship. An insight into the fusion-co-receptors[14] that interact with the pathogen upon binding and their role in the infection is then outlined. The paper also covers a detailed overview of the mechanism involved during the entire infection cycle and the stages that constitute the successful reproduction of HIV-1 viral bodies that compromise the immune cells against other standard pathogenic encounters[15]. Furthermore, all the ligand-receptor interactions and chemical aspects of viral RNA replication inside the host cell to synthesize pathogenic proteins are also covered. The infection types are then briefly introduced for a much better understanding of the genomic engineering using CRISPR-Cas9 for the *CCR5* gene. The possible side effects of using CRISPR-Cas9 are also mentioned in order to weigh the advantages and drawbacks of the presented methodology. Finally, all the aspects mentioned are collectively considered for a conclusive comparative discussion. This comprises the efficacies of both the CRISPR-Cas9 technology towards mass application on HIV-1 specific seropositive patient treatment, and each

type of C-C motif chemokine receptor family member is analyzed for possible genetic alteration to halt infection[16]. Figure 1 below describes the layout of the prevention of HIV-1 in a step-wise manner using hematopoietic stem cell (HSC) transplantation after CRISPR-Cas9 action. Lastly, a final proposal for outweighing the ethical issues of modern genome editing techniques with healthcare innovations for the treatment of the most enduring epidemic of HIV-1[17] is made. A glimpse of what the future holds for humanity and the treatment of other disorders, such as the autoimmune disease that has a coupled molecular basis with that of HIV-1 infection, is presented.

CCR5 Gene

The cytogenic location of the *CCR5* gene is the 3rd chromosome from the normal human set of diploid-2n 46 chromosomes. It is a member of the 10 C-C motif chemokine receptors family (CCR1-CCR10) that specifically binds to ligands encoded by genes on the 17th chromosome[18]. This receptors family comprises seven members that are transmembrane proteins at the surface of the host cells. *CCR5* is a G protein-coupled receptor (GPCR) that is responsible for controlling the effector capacities of T-lymphocytes as well as managing the trafficking of immature dendritic cells and macrophages. It is the main co-receptor for the binding and reception of R5 strains of HIV-1 and HIV-2[19]. The cytogenetic band location of the

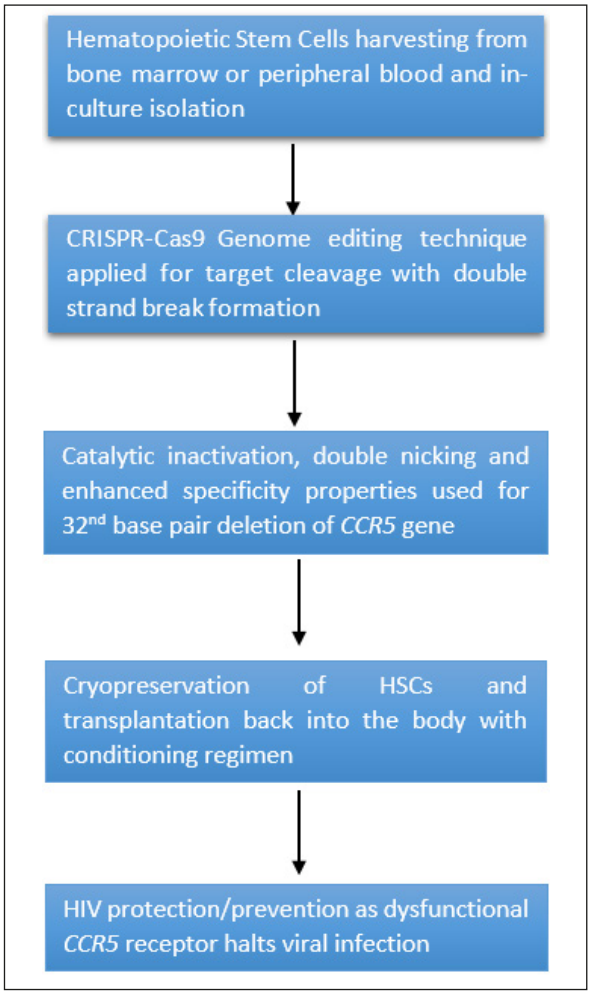

Figure 1. The action plan for inducing active immunity against HIV-1 using HSCs for gene therapy.

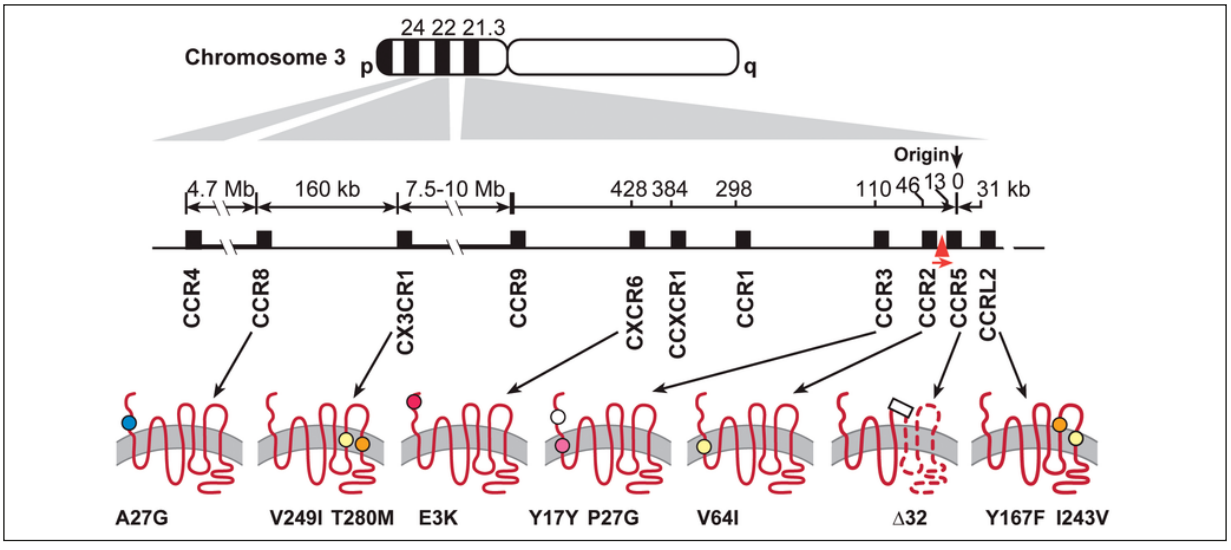

Figure 2. The seven possible polymorphisms in the chemokine receptor family and the location of the *CCR5* with Delta 32 deletion, leading to dysfunctional receptor morphology[24].

CCR5 gene is expressed as 3p21.31[20]. This locus nomenclature translates into the corresponding region 2, band 1 and sub-band 31 on the shorter P arm of the 3rd chromosome. However, it is simply referred to as the 21.31 band on the 3p chromosome with the *CCR5* gene comprising 6,065 nitrogenous bases[21].

The CCR5 protein is one of the members of the 10 GPCRs. Accordingly, it constitutes one of the beta morphology chemokine receptors. These receptors are categorized under the morphology of integral proteins that are found especially for the *CCR5* protein in the membrane of white blood cells. Another receptor that supports the CCR5 during HIV-1 binding and antigen formation is the *CXCR4* co-receptor. It does this by facilitating the lock-key configuration model of the spike protein on the viral body towards the *CCR5* receptor on the lymphocyte host cell[22]. By an artificially altered genetic expression, the *CCR5* protein becomes structurally dysfunctional. Specifically, in the case of the Delta 32 mutation, the 32nd base pair in the gene base sequence is deleted. This prevents the infiltration of the white blood cells by the invading and active pathogenic HIV[23].

Figure 2 showcases the complete set of receptors and their morphology coded by the genes on the given cytogenetic locations with an elaboration of the morphology of the receptor with the Delta 32 deletion in *CCR5*. The interaction and expression levels of the *CCR5* gene upon inheritance with the sub-receptor *CXCR4* can be determined by gene mapping. Genetic mappinginvolves the use of a genetic map, which is another type of chromosome mapping that enables the identification of the correspondinggenes along with other key characteristics. The most intriguing feature of the map is that it is made on the idea of linkage, meaning that two genes that are close together on the chromosome have a far greater chance of being inherited together[25]. Subsequently, knowing the expression rate of *CCR5*, the same application can be based on the *CXCR4* receptors for further prevention of viral genome entry in the host immune cells. However, the receptor must also be able to participate in further natural mechanisms, which would be compromised given the variety of ligands to bind with *CXCR4*[26]. This ultimately leads to a natural resistance for individuals towards M tropic HIV-1 virus based purely upon *CCR5* morphology change. The *CCR5* gene mutation is a point-nonsense mutation as it leads to the premature induction of a stop codon in the gene translation. After code-specific deletion of the 32nd base in the *CCR5* protein-encoding gene, the protein product that is synthesized becomes truncated and non-functional throughout the life of the individual with the mutation. The *CCR5* gene characteristically determines the CD4 structural morphology and functionality of receptors, which interact with and bind to the protein spike receptors on the HIV bodies[27]. Therefore, *CCR5* itself acts as the HIV-1 fusion co-receptor that is a product of the protein synthesis derived from the gene expression of the gene. *CCR5* encodes the 5th member of the beta chemokines, and it is abundantly present in the T helper cells and macrophages. These are also referred to as phagocytes, which carry out phagocytosis by engulfing the target pathogenic foreign bodies and cells during infection and digesting them within through special digestive enzymes. Thus preventing infection in the body. However, viral particles such as M tropic HIV-1 take advantage of this property using the *CCR5* co-receptor as a transduction pathway, allowing the virus itself to penetrate and continue its life cycle inside the host cells.

Subsequently, HIV is able to significantly compromise the immune system and reduce natural immunity over time. Since there are not sufficient phagocytes working effectively and efficiently for the elimination of pathogens, even mild infections can lead to fatal consequences in the body.

In this manner, multiple phagocytes, which are usually paramount in an effective immune response, are compromised as they are no longer able to engulf the pathogenic viral bodies of M tropic HIV-1. Neither can they digest them inside by their digestive enzymes. Following this, a significant proportion of the leukocytes comprising macrophages, as well as T helpers, are destroyed. This can largely lower efficiency and compromise immunity, suppressing the immune response[28]. Consequently, even mild and regular infections can test the immune system to its limits[29].

Therefore, the genetic point mutation halts the genetic expression of *CCR5* from taking place and halts the synthesis of the protein that characteristically determines the structural integrity and functionality of the *CCR5* co-receptor. Hence, HIV will not be able to interact with and bind to the co-receptor and is unable to penetrate and enter into the host immune cell. Therefore, the cell does not undergo destruction caused by HIV. Neither does it perform apoptosis for self-elimination and damage control. Ultimately, HIV

is prevented from infecting the phagocytes. The number of white blood cells remains constant, halting infectious spread.

History of the CCR5 Delta 32 Mutation

This mutation arose naturally in humans several centuries ago, but today, it carries the potential to save millions of lives from the greatest modern calamity brought about by HIV[30]. The initiation of the mutation dates back to almost 2500 years ago amongst individuals of Northern European descent. Every 1 person in a sample size of 20,000 people[31] who had their genomes genetically screened possessed an active infection. As of today, one in every 10 Europeans has the *CCR5* Delta 32 genetic mutation and complete immunity[32]. This massive increase in the frequency of mutated genes proves that it is a beneficial allele. Over time, the frequency of the beneficial *CCR5* Delta 32 mutation allele increased due to natural selection across generations. The selection pressure for resistance against HIV forced the passing on of the allele. Thus, it became more common amongst individuals in the form of an evolutionary adaptation for greater chances of survival by resisting HIV. However, to actually benefit from the *CCR5* Delta 32 mutation, an individual must be homozygous recessive for the *CCR5* mutated gene. Only being a homozygote proves complete resistance against an HIV infection by disabling the C-C chemokine co-receptors.

Figure 3 displays the impact of molecular morphological variance on the immunity against HIV-1 strains. In Figure 3A, it is clear that the *CCR5* receptors protruding from the cell surface provide an entry pathway for viral injection into the host. By comparison, Figure 3B indicates the absence of the receptors that disable the virus from injecting the viral RNA into the immune blood cell, and this inhibits infection[33]. Replication of the viral proteins is halted in such cases in Figure 3B, and therefore, stem cell transplant is to be carried out in individuals to prevent infection[34].

Thus, the HIV entrance into the immune cells via the receptor is halted completely. Nonetheless, despite only individuals with two copies of the CCR5 Delta 32 allele on their 3rd chromosome showcasing complete resistance, individuals with only one mutated gene copy are still able to exhibit some minimal resistance in terms of the degree and frequency of AIDS-infected individuals[35].

The CCR5 Delta 32 mutation holds an extremely intriguing and localized epidemiology. It is not typically found amongst individuals of African, Southeast Asian, Asian, native American, native Tasmanian, or Amerindian but only in Eu-

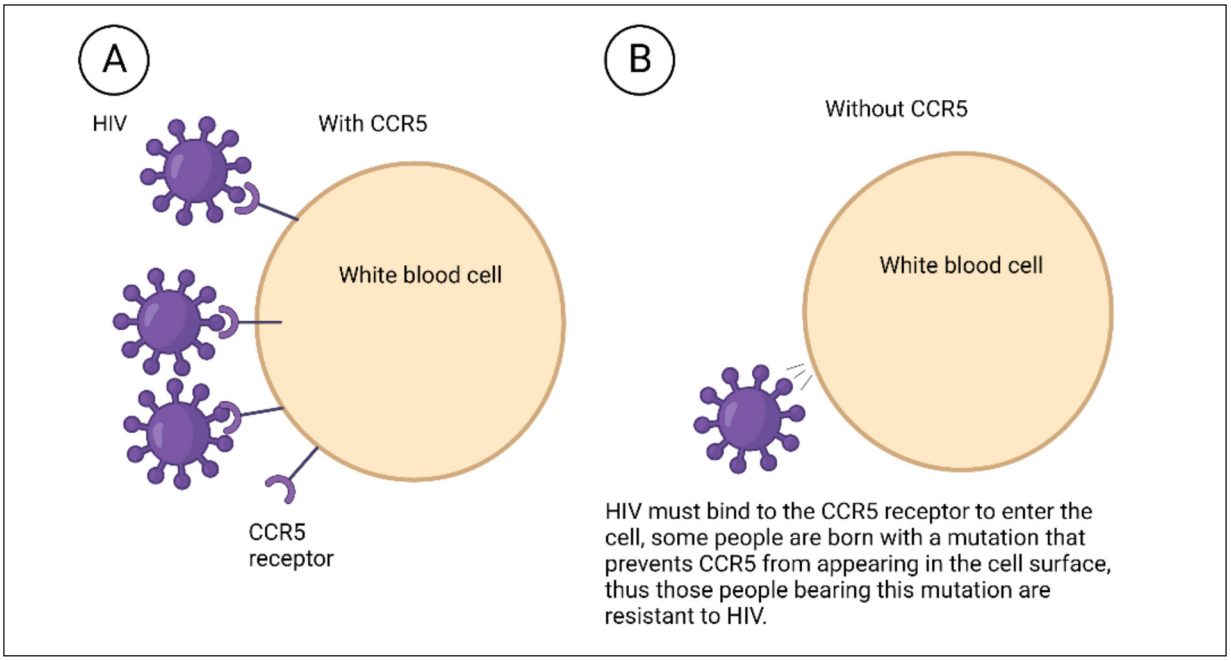

Figure 3. The general overview of the importance of *CCR5* Δ32 mutation in protecting people from AIDS on a molecular level by preventing HIV-1 binding with host immune white blood cells. **A**, Entry pathway for injection of the viral particle by *CCR5* receptors. **B**, Inhibition of infection from the absence of *CCR5* receptors.

ropean descent[36]. This is because the CCR5 Delta 32 mutations can be found mainly in the Eurasia region. According to one theory[37], the mutation had originated from Vikings and then spread to Europe through invasions and raids. Also, through raids, Vikings brought these alleles to many other countries, such as Scandinavia, Iceland, and Russia[38]. Based on multiple previous research[39] upon the evolutionary pathway of homozygous *CCR5* Δ32 expressions in the population, these mutated alleles were traced back predominantly in the nation of Scandinavia around one thousand years ago. It is certain that this mutation was beneficial since it kept on passing from one generation to another, allowing natural selection to occur with the resistance to HIV. Hence acting as a beneficial selective pressure. It was an advantageous mutation for the people from 700 years ago since that was the time when the Black Plague Death began[40]. Moreover, this mutation increased the survivability of the people of that era and triggered the passing down of mutated alleles in generations over time, resulting in further expression of it in the population[41]. The localization of the mutation within the Caucasian ethnicity refers to epigenetic signatures that exist between the genome of European ancestry and the *CCR5* Delta variant research. It is believed[42] that the mutation came to protect humans from smallpox disease, which has been ongoing for centuries. Moreover, both HIV and smallpox have shared receptors of *CCR5* that are used to penetrate into other cells. So, it is possible that the mutation survived in order to provide protection against HIV after being prevalent and staying in the population due to smallpox infections. Additionally, it is known[43] that this mutation can be found primarily in Northern Europe and Central Europe mainland only in individuals having the 32nd base pair mutation. They are resistant to HIV-1 disease for homozygous carriers since this specific mutation blocks the expressional function of the gene that codes the receptor. Without this receptor, HIV-1 is not able to enter CD4+ T cells.

Delta 32 variant comprises a deletion in the genetic code that halts HIV spread by inhibiting receptor binding. Although this variant was not around for too long, it has reached large numbers in Europe, quantifying 1% of the Caucasian Northern-European population. This infers that it is a beneficial mutation as it has been under positive selection for a long time. However, partially due to the limited spread of the virus in the human population, the *CCR5*-Delta 32 allele does not directly correlate with the present number of cases of AIDS and mutation expression rate in the global human population. This ignites a debate about whether selective pressure is responsible for the increase in the number of alleles for this *CCR5* gene and whether only the 32nd base pair deletion would individually qualify as HIV-1 resistant. Figure 4 below illustrates the spread of the variation in the general population where next-generation sequencing (NGS) was performed at

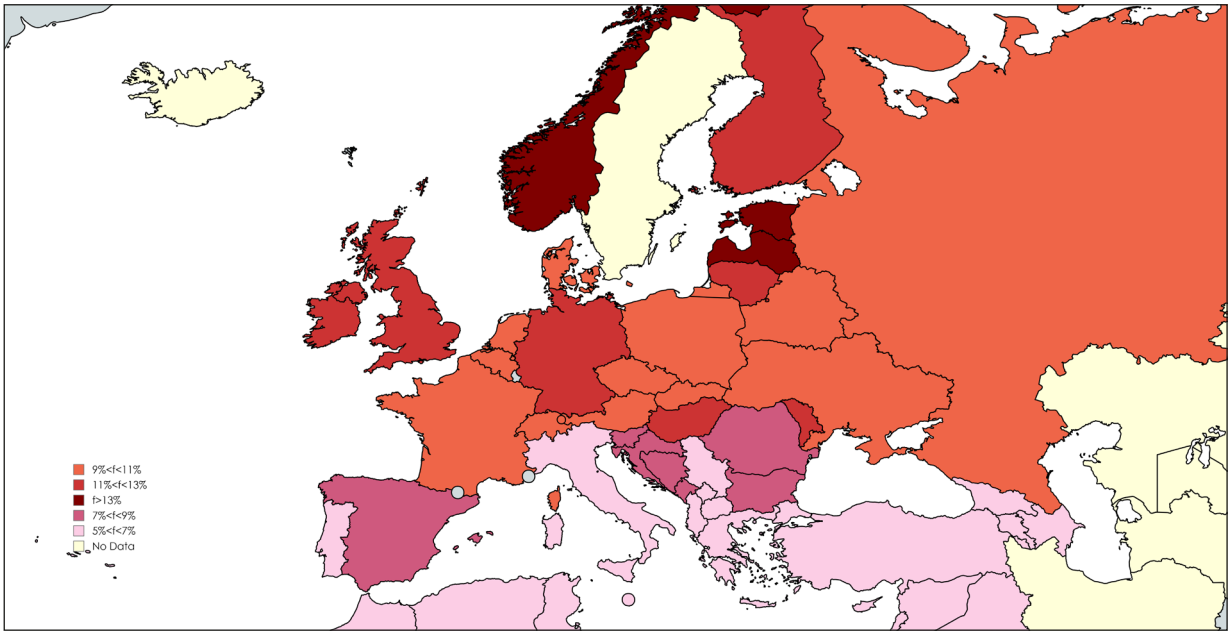

Figure 4. The spread of the *CCR5* Δ32 within European descents identified from Next Generation Sequencing of 1.3 million individual samples in three national DKMS donor centers[44].

Deutsche Knochenmarkspenderdatei (DKMS) donor centers, demonstrating percentages of up to 16.4% in the European populations. The debate continues about the historical selective pressures and epigenetics acting up on the *CCR5*-Delta. However, an argument can be formulated by the geographic placement of the variant as the single nucleotide polymorphism becomes critical in successful association with the environmental factors and types and level of expressions of the gene. This, in turn, dictates the overall prevalence and occurrence in the population[45].

In addition, a study conducted by Stephens et al[46] depicted the haplotype analysis of 192 Caucasian chromosomes, which revealed that there is a strong connection with the correlation between *CCR5* and two microsatellite loci. Furthermore, through the usage of coalescence theory along with haplotype genealogy, it can be approximated that the mutation originates from a haplotype found to be 700 years old. Predictions suggest a possible range that lies between 275 and 1875 years[47]; the geographical representation of the *CCR5*-Delta 32 numbers and its recent presence are both compatible and demonstrate a historically strong selective event. This justifies a strong correlation of the increase in mutation among Caucasian populations[48].

Several studies[49] suggest that this allele could be found to be prevalent in the gene pool within Scandinavia from about one thousand to two thousand years ago. There are theories[50] suggesting that the Vikings were the first ones to introduce these alleles to Europe, Russia, and Iceland. However, due to the lack of experimental studies and scientific research to support this theory, it is not suitable for explaining the spread of the mutation. Subsequently, another theory[51] is that the allele rose and spread across central Europe due to a geographical gradient in selection intensity, causing a higher frequency of expression in the northern part of Europe. Also, the Vikings alone would not be able to spread the mutations as they played only a small role in the spreading of this allele. Moreover, from the data and statistics gathered over the years, it can be conceived that there would only be up to two biological factors that determine selection[52]. The primary selective advantage that the mutation may have conferred in the northern regions is due to its association with the epigenetics of that area and environmental factors such as climate and temperature gradients, which interact with gene variants and their genetic expression[53]. This includes various examples, such as smallpox being much more severe in the northern parts of Europe. The second one is the selective cost of the mutation that might have been higher in the southern region, resulting in the selection intensity of the allele being lower in the southern regions of Europe and higher in the northern parts[54].

The selective cost theory[55] has a higher chance of being true than the rest of the theories mentioned before since chemo-kinesis is a paramount part of the inflammatory response to the infection.

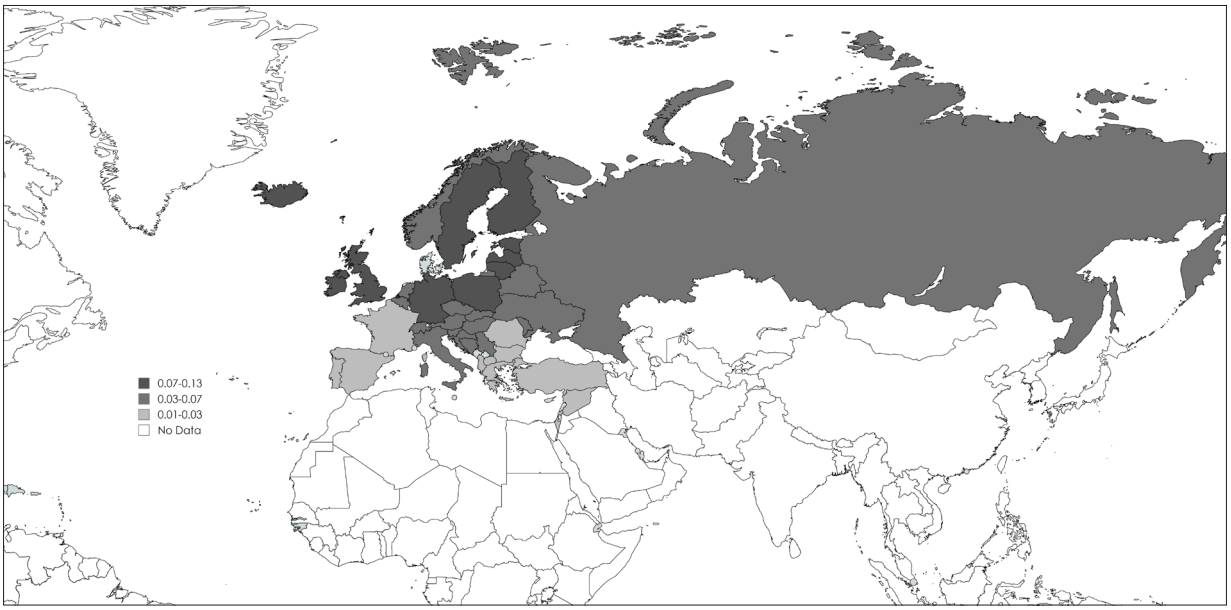

Figure 5. A global overview of the distribution of the mutation in the population. Figure obtained from official PALFIR Genetics resource on *CCR5* Δ32, 2022.

Additionally, it has been observed[56] with mice that *CCR5* genetic knockout responses towards pathogenic infections result in the failure of effective immune responses. Therefore, these results suggest that certain pathogens have an advantage when it comes to infecting Delta 32 carriers. Their immune response has been damaged due to functional *CCR5* chemokine receptors not being present in the case of pathogens. They are endemic in general or just thrive much better and frequently throughout their life cycles and across generations in the temperate climates of southern Europe[57]. These conditions would cause a rise in the selection gradient for the mutation to prevail up north, which would ultimately justify its numbers and rate of expression in both the hemispheres of the European continent amongst individuals of Caucasian ethnicity and pure European ancestry[58].

In other cases, it is also possible that the Delta 32 mutations might be harmful in certain areas, and this, in return, is going to exceed the protective effects of the mutation by the harmful effects on the immune system[59]. Overall, multiple factors have played a role in spreading the allele. However, geographic isolation and spatially influenced variable selection are different from the Viking theory. In the meantime, it stands as the only explanation as to why Delta 32 is geographically localized and endemic to Northern Europe and, to some extent, the west of Asia.

Gaussian Statistical Modelling and Sampling Technique

In this statistical-based scientific sampling, the data points were used to devise trend lines and showcase the frequency distribution using the Gaussian statistical modeling of different geographical distinctions. The isolated locations across the European continent are composed of samples of equal proportion of males to females, making up a sample size of at least greater than 30 samples in total per data entry. Moreover, the individuals who depicted resistance were genetically screened to correlate the presence of homozygous *CCR5*-Δ32 with HIV-1 immunity[60]. These individuals were of the same age range and weight and presented other epigenetic altering factors as all non-smokers. However, they have varied genes pertaining to differences in ancestry and other pure European endemic genetic expressions and epi-genomes with different methylation marks. While a simple distribution is a collection of data and frequency of a variable, a Gaussian distribution, also known as a normal distribution or bell curve, is a probability distribution that follows the bell curve distribution. The symmetric distribution curve indicates that the data analyzed is more likely to occur near the mean than near the extremes. Any growing dataset with independent feature probabilities and a finite variable can be displayed in a Gaussian distribution model. The symmetric distribution curve indicates that the data analyzed is more likely to occur near the mean than near the extremes. Any growing dataset with independent feature probabilities and a finite variable can be displayed in a Gaussian distribution model, making it the most preferable and commonly used statistical model in scientific research compared to others, such as the binomial and the Poisson distribution models. Although the receptors for smallpox have yet to be discovered, it is still one of the prime examples of selective pressure for the fixation of the *CCR5* Delta HIV-1 resistance allele in Caucasians today.

Figure 5 shows the map indicating the expression of the mutation with the individuals showcasing homozygous *CCR5* Δ32 allele genotype and HIV-1 resistant phenotype across the globe. The main concentration can be seen to be around the European continent, with Northern Europe and Western Asia showcasing the maximum prevalence of 0.13% for homozygous Δ32. Lastly, there is almost no phenotypic existence across the North/South American and Australasian continents.

Background of CCR5 and Fusin Co-Receptors

During early stages, it was believed that HIV functioned and gained entry by infecting and destroying specific cells, known as CD4+ T-cells, that manage immune responses[61]. Hence, it shuts off and disables the immune system once those cells are depleted, making the human body vulnerable to any minor pathogenic infection. These CD4+ T-cells coordinate upon infection through a network and transduction cascades of chemical interactions to activate other cells in order to fight off the invading microorganisms[62]. However, upon the discovery of the proteins *CCR5* and *CXCR4* in the immune cells succeeding several experiments in test tubes, which were linked to a doorway used by HIV to gain access to the inner compartments of the cell, it became evident that CD4 was solely

not enough for viral microorganisms to enter into the cells[63]. Scientists now believe that depending on the type of HIV infection, HIV targets cells that either have a *CCR5* or a *CXCR4* co-receptor. The *CCR5* co-receptor appears to be targeted by the non-syncytium-inducing (NSI) strain, while the *CXCR4* receptor is mainly targeted by the SI strain of the virus. Furthermore, the viral body needs to bind to the CD4+ receptor for full entry into the host via viral genome injection.

During the early onset of infection, the CD4+ receptors become critical in allowing the T-tropic HIV-1 strains to enter the host, which is a process that involves the functioning of *CXCR4*. In the case of T lymphocytes, the *CXCR4* becomes vital in the expression and regulation operations of T cell migration alongside CXCL12[64]. However, when it comes to *CCR5*, the expression is solely activated by T-cells that direct their migration along with CCL3, CCL4, and CCL5 gradients.

Moreover, certain proteins such as CD4 and *CXCR4* that operate from the cell surface on T lymphocytes function as co-receptors in the event that a virus gets into the body[65]. CXCR4 can be expressed by the majority of the stem cells of various classes, such as hematopoietic, endothelial cells, and neurons. Furthermore, CD4 has the tendency to bind to the nonpolymorphic region of MHC II presenting antigens to T cells. Also, CD4 functions as the co-receptor in the simian immunodeficiency virus, i.e., SIV[66].

CD4 cells, also known as T cells, are leukocytes that mainly halt infection by fighting viruses and increasing the strength of the immune response. The CD4 quantitative measurement/count can be inferred for qualitatively judging the immune system for positive infection of HIV. Subsequently, HIV first attacks dendrites to destroy CD4 cells. Moreover, a certain ligand called CD40L attaches itself to the CD40 receptor. The CD40 molecules are present for immune Beta cells operating from their surface. These beta cells are important for the synthesis of proteins known as antibodies or immunoglobulins that provide aid to protect the body against infections and other viruses[67].

Figure 6 illustrates the various sites of the *CCR5* and the positioning of the Delta 32 deletion next to the 2D7 binding site for the HIV molecules. Mutational variant surrounding the coding region site initiates the premature induction of the stop codon, and this, in turn, inhibits the synthesis of the set of proteins. Thereafter, and consequently, this leads to the 2D7 site used for binding to be lost with *CCR5* protein. Clustered regularly spaced short palindromic repeats (CRISPR), more commonly referred to as Cas-9, is a modern experimental scientific technique for carrying out selective genetic manipulation to tackle human health concerns and hereditary disorders such as cystic fibrosis, which are determined by a predominantly genetic fallback. This method of treatment involves the usage of a novel protein, which is the RNA complex, that is

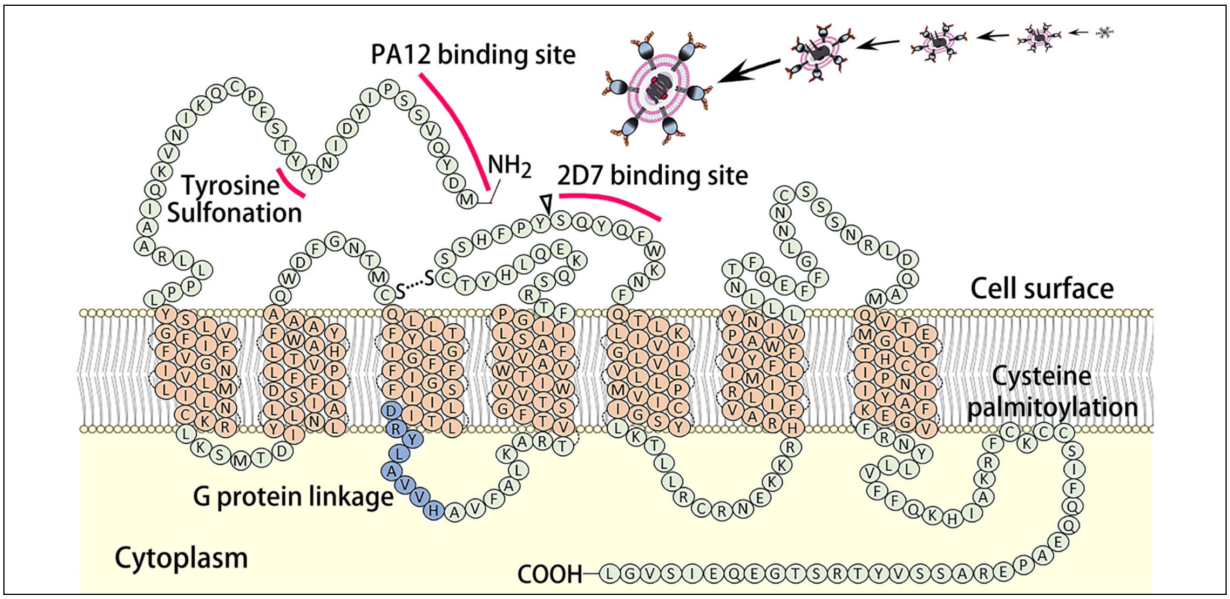

Figure 6. The morphological structure of the membrane-integrated *CCR5* showcases the domains, and the triangle represents the position of Δ32 next to the 2D7 binding site, which will inhibit the synthesis of the whole *CCR5*. Domains such as the Tyrosine sulfonation and the PA12 remain unchanged[68].

used for the fixation of genetic mutations that are harmful to humans by editing and correcting the genetic makeup of the patient. For illustration, the respiratory tract is infected alongside the lungs in the autosomal recessive inheritance mode disease called cystic fibrosis. This disease may also partially affect the digestive system and is caused primarily by a mutation of the CFTR[69].

CRISPR could be used to edit the mutation on the gene called CFTR, which can cure cystic fibrosis. For example, recently, scientists[70] in the Netherlands were able to use base editing to fix CFTR mutations *via in vitro* CRISPR-Cas9 application on cystic fibrosis cells without causing any harm to the patient's genetic code.

Figure 7 below depicts the coordinated action of both receptors for viral entry. Figure 7A showcases the combinatorial binding effect of the CD4 receptors with *CCR5* co-receptors. Figure 7B also includes the post-induction mechanism with the conformational change of the envelope in detail. The R-5 tropic HIV-1 particle comprises envelope trimers that are complementary in their interactions with the receptors. This mechanism precisely facilitates the fusion of the virus with the host cell membrane.

As a result, the HIV capsid becomes localized within the host cell, facilitating the establishment of infection following entry. Similarly, CRISPR could be used to cure AIDS, which is the root cause of a major health crisis around the globe. In such cases, CRISPR is able to edit out DNA from the pathogen, that is, HIV, from the host genetic material. This enables an attack on the virus while it is still inactive, thus making it possible to get rid of it before it activates. Consequently, it makes humans more resilient to HIV infections. In some cases, few individuals are born bearing a natural resistance to HIV pertaining to a mutation that takes place on the *CCR5* gene. This gene encodes receptor proteins on the surface of immune cells, and HIV uses them as a means of entry for infecting the cells[71]. Accordingly, with CRISPR, it is possible to change the structure of the protein to prevent the virus from binding to it. Furthermore, scientists[72] in China have recently used this unorthodox practice of genetic manipulation on human embryos, gaining resilience to HIV. In the modern science world, this experiment has been heavily looked down upon in the scientific community, barring a lack of scientific ethicalities.

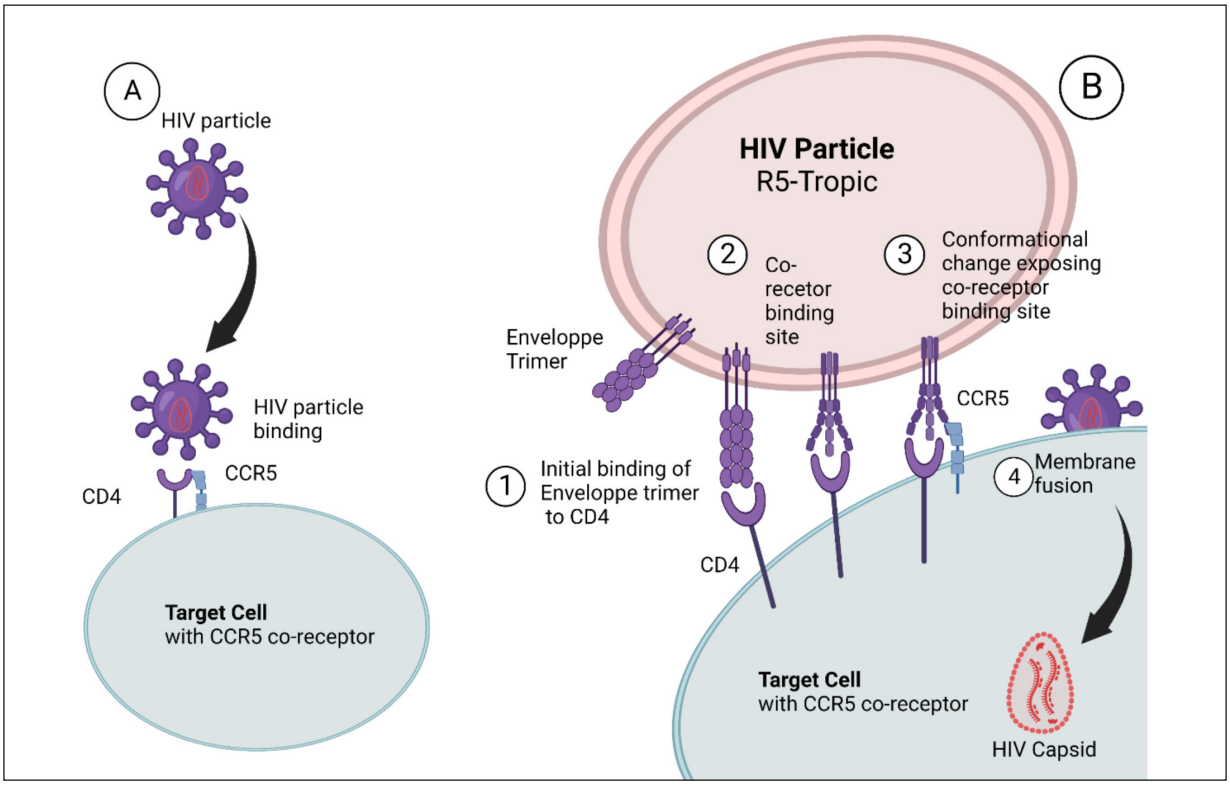

Figure 7. The entry pathway for HIV-1 towards the host is done by utilizing the *CCR5* and the *CD4* receptors in coordinated action and specificity towards the envelope trimer and cell surface markers. **A**, Combinational binding of the virus towards the *CCR5* and the *CD4* co-receptors. **B**, Conformational change induced upon binding to co-receptors and fusion.

The ideology carried forward by the scientists initially was to prevent possible reduced life span of human life being developed from the embryo. By altering the *CCR5* gene in such a manner, the contraction of HIV-1 later on in life would be harmless to the immune system. The virus could be easily terminated with phagocytosis and antibody response.

Furthermore, in 2008, the allogeneic transplantation of stem cells carrying a homozygous mutation demonstrated resistance to viral infection. This resistance resulted in the natural immune system's elimination of the virus, as it was unable to integrate its RNA into the donor cells, thereby preventing the synthesis of viral DNA[73]. This infers that there is no translation and synthesis of viral proteins, hence, no replication of pathogenic HIV bodies. Pertaining to the common characteristic of viruses, if they fail to replicate in large numbers, the virus, such as HIV, cannot establish a potent infection and is easily eliminated by the human immune response. Since this implementation of HIV-related genetic manipulation in human embryos, there have been only two cellular ways to achieve this efficient response[74]. A particular cellular approach involves utilizing hematopoietic stem cells to replicate the initial findings. The other method is gene therapy to prevent the expression of *CCR5*.

Furthermore, five more new methods have been discovered and are being tested clinically in the present scientific community. The application of zinc finger nucleases (ZFN) and the CRISPR/Cas9 system are possible methods. Other methods include the transcription activator-like effectors nuclease (TALEN), ribozyme, and short hairpin RNA (shRNA)[75]. In a subsequent investigation[76], dual-function gene therapy was employed, integrating a conditional suicide gene to trigger apoptosis, along with a *CCR5* knockout strategy. This combination aimed to alleviate the limitations associated with the *CCR5* knockout that previously proved insurmountable. To achieve these results, a two-vector system and the addition of the integrating lentivirus vector (LV) were used. This vector expresses the GFP reporter gene, as well as the mutant variant of the SR39 version of the Tat-dependent kinase. Following this, another possible non-integrating lentivirus vector (NIL) can be applied to induce the expression of Tat protein, as well as the *CCR5* system coupled with gRNA-CRISPR/Cas9. The integration and transduction of cells alongside the NIL vector allows the insertion of the suicide gene that induces apoptosis[77]. Furthermore, the KO (knockout) of the *CCR5* gene and transient expression of GFP are used to improve the modified cells. This method is used to modify TZM cells and to manufacture a cell line that is resilient against various *CCR5* tropic viruses. While allowing for the infection of *CXCR4*, upon binding of viral spike proteins with the cell surface co-receptors, the tropic viruses continue to operate, which can be controlled by the Ganciclovir treatment[78].

Furthermore, besides the discovery of both *CCR5* and *CXCR4* co-receptors, multiple new natural anti-HIV immune chemicals known as beta-chemokines have been found. These chemicals bind to either *CCR5* or *CXCR4* and assist in blocking any further binding to the HIV and, hence, preventing any HIV infection[79]. Gallo et al[80] diagnosed these chemokines, produced by CD8+ cells, and identified them as MIP-1-alpha, MIP-1-beta, and Rantes[81]. He also concluded that the production of large quantities of these chemicals fills the *CCR5* or *CXCR4* co-receptors binding sites and blocks the entry doorway of HIV, thus suppressing the viral infection of the CD4+ cells[82]. Conversely, the absence or lack of production of these chemokines in the human body facilitates the CD4+ cells infection by HIV. Moreover, similar studies by Morvan et al[83] also documented the presence of another CD8+- derived antiviral factor (CAF) that plays a role in inhibiting HIV replication. Nevertheless, to date, the origin of this CAF is still unidentified[84]. However, these studies[85,86], alongside all other discoveries in regard to the chemokine's structural function, do not have an immediate impact on HIV patients. They have shed important light on the HIV infection mechanism and the reason why each person's immune response differs from one to another.

These discoveries have opened new aspects of research for the stem cell transplantation of defective *CCR5* genes. This transplantation consists mainly of the removal of a stem cell from an individual acquiring the *CCR5* defective gene and reinfusing it in an HIV-infected patient. If the process has been successfully completed, these transplanted stem cells will produce lymphocytes and macrophages that are naturally immune to NSI strains of HIV[87]. Hence, they could eliminate the M-Tropic HIV in the patient. In addition, the findings can be applied to enhance animal models for HIV research. The limitation of HIV infection in certain animals has not made much room for viral infection studies. Therefore, there was a stagnation in the advancement of prospective HIV

treatments. Though in the cases in which HIV infects animals, it rarely imitates the same conditions of decline in immune operations and disease symptoms as in humans. Therefore, engineering animal cells with *CCR5* and *CXCR4* co-receptor genes could potentially provide better research conditions and test additional efficient therapies.

In the past couple of years, however, new information surfaced about the complexity of HIV infection mechanisms that urged further research in order to fully understand the virus' operating system and its interaction with human immunology. The recently presented information involved the American scientific group's findings[88] on CD4+ cells' resistance to HIV infection despite having the *CCR5* gene. Moreover, some scientists have backed these findings by identifying a resistant stem cell against HIV infection bearing both the CD4+ protein and the two co-receptors on its surface[89].

An Italian research collective[90] pinpointed 52 additional factors contributing to long-term non-progression (LTNP) status, supporting the argument that inheriting a single mutated *CCR5* gene from one parent is not the sole determinant for LTNP in HIV infections. This research was done by examining HIV-positive patients who were infected for seven years or more and maintained their CD4+ cell count at 500 or above. However, they never experienced any HIV symptoms nor took any anti-HIV drugs. The findings of this study[91] suggest that LTNP is a complex process that does not solely depend on inheriting or acquiring a single copy of the defective *CCR5* gene. This highlights the need for further research to gain a better understanding of the mechanisms involved in HIV infection. More interestingly, some studies[92] showed that people diagnosed with both hemophilia and HIV demonstrated greater survival rates due to a gene deletion of another newly identified co-receptor known as CCR2. These back-to-back findings raised questions on the possibility of the existence of more HIV-related co-receptors but also helped in expanding the HIV research field. Although it might lead to short-term confusion as conflicted reports are coming out lately due to the rising extensive work on comprehending HIV, it still provides promising opportunities for a rapid finding of an HIV treatment.

Besides, a New York research team[93] has discovered that the use of GM-CSF (Leucine) in their laboratory setting decreases the cell's gene expression of *CCR5* and *CXCR4*, thus leading to enhanced immune defenses against HIV[94]. This GM-CSF technique shows its capability of releasing anti-HIV chemicals that make other cells immune to HIV infection. These promising results led to getting approved for clinical trials in the laboratory and are now considered a potential and efficient treatment for HIV infection prevention. As all these recent discoveries[95] have shown prominent results, further and thorough research is necessary to assist and support these findings as well as develop potent treatments to interfere with the cellular proteins of HIV[96].

Figure 8 illustrates the chemokine action during viral entry. While Figure 8A elaborates on the normal combinational binding scenario for active infection, Figure 8B demonstrates the sliding action role of chemokines for defense against infection. Chemokines effectively block the binding of the GP120 spike glycoproteins with the *CCR5* on macrophages and *CXCR4* co-receptors on T helper cells in the immune system. Chemokine action

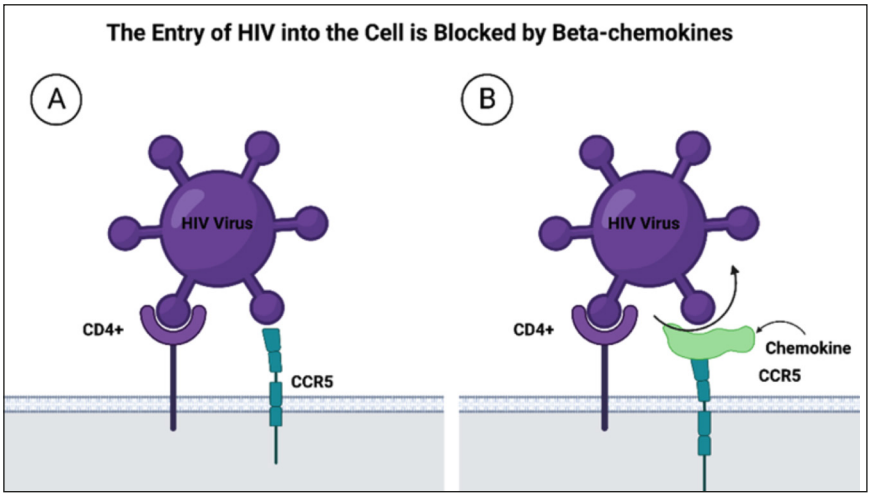

Figure 8. The chemokine barrier between the *CCR5* and *CXCR4* co-receptors and the envelope glycoprotein - GP120 on the HIV bodies preventing viral RNA injection. **A**, Stable combinational binding leading to infection. **B**, Blockage by sliding action of chemokines between spike glycoproteins and *CCR5*.

on the *CCR5* inhibits HIV-1 from the injection of viral RNA, and thus, infection in this way also becomes impossible to establish.

Mechanism of the *CCR5* Δ32 Mutation

The deletion of the 32nd base pair in the genetic sequence for the *CCR5* coding sequence located on the (petite) p-arm of the 3rd chromosome in humans results in the mutated allele known as (Delta) Δ32, corresponding to the occurrence of the point mutation. The Δ32 comprises a frameshift mutation altering the reading sequence surrounding the 185th amino acid being coded for by the gene, which comprises a combination of amino acids that have a total molecular weight of 40.6 kilo Daltons for 352 units[97].

Multiple variations in mutation types of the *CCR5* gene have been discovered. The mutations in the coding regions of the *CCR5* gene have a direct impact on the potency of a resistive mutation. However, alterations to the noncoding sequence could also possibly affect the regulation of the *CCR5* gene expression indirectly through passive genomic regulation. Coding sequence mutations include both synonymous and nonsynonymous mutations. The synonymous mutations end up encoding the same amino acid, given that the genetic code is redundant and degenerated. Accordingly, the mutations have no potency to bring about resistance, and this is referred to as a silent mutation. However, nonsynonymous mutations direct nonsynonymous codons, which lead to missense mutations as the change in the genetic sequence alters the amino acid[98]. Furthermore, in the case of the Δ32 alleles, most of the missense mutations prove to be conservative. Hence, they do not hugely impact the structure and functionality of the C-C chemokine receptors. Thus, no resistance against M-tropic strains of the HIV-1 variant is gained.

Nonetheless, the most common consequences of alterations in the genetic sequence are nonsense mutations from nonsynonymous codons as they produce truncated proteins due to the early induction of the stop codon. This results in truncated RNA and proteins, making them dysfunctional and the C-C chemokine receptors smaller in size. So, they do not hang out through the cellular membrane, making them undetectable on the cell surface. In return, the co-receptors are not functional anymore and leave no binding sites for the HIV-1 variants to establish infection within the host body. Ultimately, the human body is able to gain resistance against the virus[99].

There are 16 other identified mutations primarily found in the coding region of the *CCR5* gene, which can potentially provide resistance to HIV tropic-1 strain[100]. This comprises 3 non-synonymous codons, which further comprise 11 variants of their own that are codon-altering and thus are counted as missense mutations. There is 1 nonsense mutation resulting in chain termination, 1 specific trinucleotide deletion, and lastly, there are 3 synonymous mutations in the coding region, which act as silent mutations. In return, over several years and with the accumulation of selective pressures, natural selection coupled with epigenetic signatures have played a part in ensuring the prevalence of resistance in the Caucasian population. Numerous prior research studies[101] indicate that individuals possessing a homozygous recessive genotype, characterized by the deletion of the 32nd base pair in both copies of the *CCR5* gene, showcase an asymptomatic phenotype. This genetic configuration, resulting in the *CCR5* Δ32 alleles, confers absolute resistance. A study[102] involved the sampling of African-American and Caucasian populations to understand the relationship between the variance of mutations pertaining to the *CCR5* genes. The research managed to replicate this accomplishment synthetically through human intervention in individuals who either lacked the homozygous mutation entirely or were heterozygous for the Delta 32 allele. It is necessary to induce the mutation in such cases using the most convenient and modern method available, which is CRISPR technology. The mutation introduced at the 32nd base pair of the *CCR5* gene, coupled with modifications to the 185th amino acid, leads to the creation of non-functional co-receptors for the transmembrane *CCR5* receptor protein, thereby impeding the entry of HIV-1 variants. Thus blocking its entry into the human immune system. With natural T helper cells, the immune system can continue producing effective immune responses by phagocytosis and antibody production against other mild infections[103].

Figure 9A exemplifies the detailed amino acid sequence and the three-dimensional structure (Figure 9B) of the *CCR5* protein integrated at the membrane junction. It comprises seven domains folded up where only one mutation will lead to alteration in conformation. The Delta 32 deletion will inhibit viral HIV-1 from binding and the synthesis of the *CCR5* starting from the 2D7 following the induction of a premature stop codon.

The beta chemokine receptors are comprised of novel receptors, *CCR5* and *CXCR4*. They bind

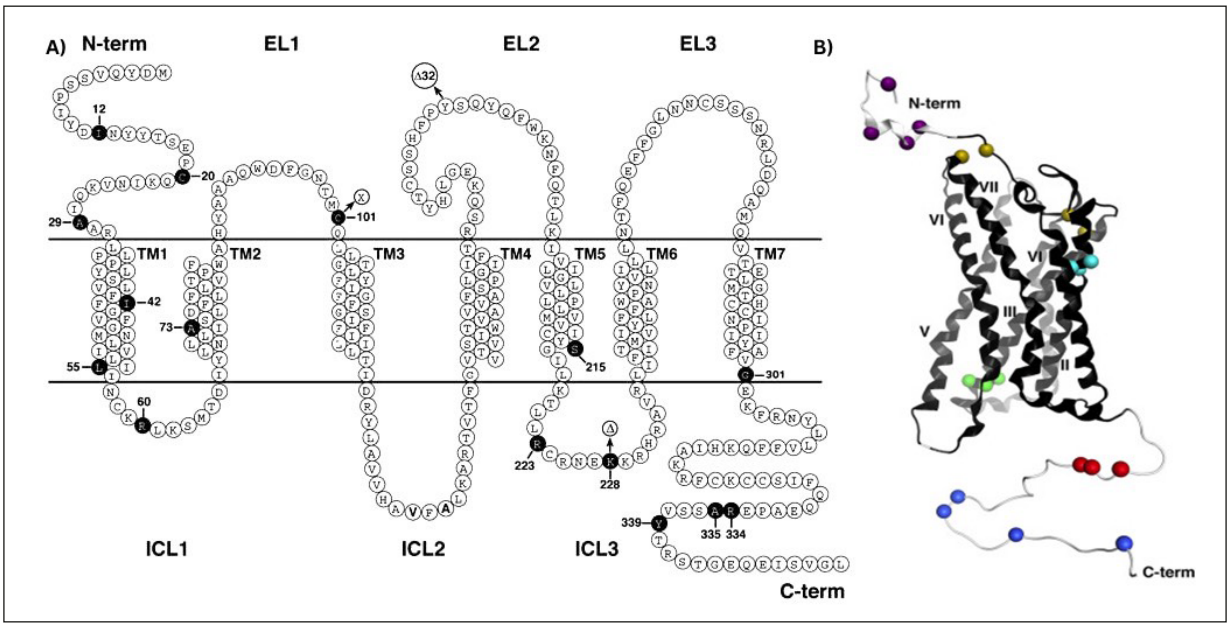

Figure 9. The multiple domains of the *CCR5* protein with each amino acid are displayed, and the expanded three-dimensional structure spans from the N-term to the C-terminus[104]. **A**, The structure arising from the linkage between the sequence of constituent amino acids. **B**, The three-dimensional conformation of the domains for the *CCR5* integrated into the membrane.

to beta chemokines, which are immune chemicals capable of acting as a barrier between the envelope glycoproteins 120 on the viral particles and the receptors on the cell surface of the host immune macrophages and T helper cells in the body for HIV resistance[105]. The HIV-1 strain binds to the cell surface of CD4 receptors on the immune cells. They fuse with the plasma membrane with the help of the *CCR5* and *CXCR4* co-receptors.

This allows the injection of the viral RNA of HIV into the cytoplasm of the host cells, where it is actively converted into viral HIV DNA by the reverse transcriptase enzyme. A mirror image of the original RNA template is produced. Integrase enzyme permits the integration of the viral DNA into the host genome for natural transcription in T helper cells to produce more viral proteins. The formation of new HIV particles in the bloodstream and the emergence of various active viral strains facilitate their invasion into the immune system. This occurrence leads to a diminished or absent immune response to other standard infections, resulting from an impaired and suppressed immune system.The mutation is able to alter the extracellular, intracellular, and transmembrane domains of the chemokine receptor molecule coded for by the mutated *CCR5* gene with highly dense alterations near the N-terminus of glycoproteins. This makes up the co-receptor configuration on the mucosal surface. In this manner, it alters the ligand binding ability of the HIV-1 variant to the *CCR5* receptor. Moreover, naturally occurring antibodies specific to the *CCR5* receptor have been discovered in both seropositive people who display long-term control over infection and in people who are largely exposed to the virus but remain uninfected. Therefore, it can be concluded that natural autoimmunity to HIV is more prevalent in the population than previously expected, and thus, it can play an imperative role in attaining widespread HIV control in the human body[106].

The *CCR5* receptor works in complementary action with the *CXCR4* receptor. The expression of these genes is interdependent, allowing them to function as chemokines, which are a subset of cytokines. The cytokines are a family of small proteins that are actively secreted by cells to cause the induction of neighboring cells through chemotaxis upon a chemokine gradient[107]. The *CCR5* receptor belongs to the CC chemokine family and is able to perform coupled action with the *CXCR4* receptor. Thus, the receptor is a member of the CXC chemokine family of protein co-receptors. They both collectively act as co-receptors for the CD4 antigen molecules found on the surface of the immune cells, which secrete cytokines by leukocytes, such as the macrophages, lymphocytes, granulocytes, mast cells, T helper cells, fibroblasts, and endothelial cells. Therefore, the deletion of the 32nd

base in both the *CCR5* alleles pertains to a fully dysfunctional morphology of the *CCR5* co-receptor, which does not allow the HIV binding to the T helper lymphocytes, and proper fusion with the plasma membrane does not occur[96]. Ultimately, this prevents the injection of the viral RNA into the lymphocytes. Therefore, no integration with the host cell genome takes place, and the proteins for HIV construction are not synthesized, limiting the reproduction of the virus in the human body.

No reproduction of HIV in the human body translates into complete ineffectiveness of the virus against human immunology. Thus, resistance occurs in individuals who are homozygous recessive with mutated copies on both the p-arms of the *CCR5* on the 3rd pair of chromosomes in humans. The HIV section locks within the viral envelope of the glycoprotein complex, a receptor of chemokine origin around the *CXCR4* and *CCR5*, particularly towards the ending stages of infection. The envelope of the infection consists of the GP120 and GP41 proteins, which mediate the virus's attachment to the cell[108].

The outside protein called GP120, as well as the transmembrane gp41 subunits, are formed via the proteolytic cleavage of the GP160 and the structural morphology of trimeric spikes for the envelope complexes[109]. In spite of this fact, the morphology and three-dimensional formation of the complex comprising the envelope protein and receptor molecule is still subject to further immunological and spectrometric examination based on hereditary and biochemical parameters. These techniques produce data that proves and justifies the protein spaces participating in this process. HIV exhibits an affinity for binding to the extracellular segments of *CCR5*, specifically targeting the domain-specific N-terminal, which shows a propensity for binding with usual ligands. The ligands include RANTES for CCL5 and MIP-1 beta for CCL4. These ligand ties are distinct for each receptor and uniquely fight for the limited number of binding sites as well as other pathogenic bodies for infection.

Only a limited number of monoclonal antibodies that have the capacity to advance receptor signaling have been discovered. Furthermore, specific conformational alteration in association with oligomerization allows for internalization. *CCR5* receptors with a C-truncated structure, which are unable to internalize, may facilitate infection. Consequently, this structural feature seems to impede effective cellular infection, rendering the cells less proficient in disease propagation. Drugs such as Maraviroc are able to focus entirely on the biochemical backdrop and immunological aftereffects of drug application towards *CCR5*[110].

Infection Types

HIV infection can be classified into two types of infections depending on the strain of the virus: a syncytium-inducing (SI) or a non-syncytium-inducing (NSI) virus infection[111]. The SI strain of HIV is a more aggressive version that causes rapid disease escalation as no anti-HIV drugs have proven effective as a treatment. This strain results in an accelerated CD4+ decrease rate compared to the NSI strain since it specifically targets the CD4+ T-cells. On the other hand, the NSI strain of HIV is less aggressive than the SI strain but is more commonly sexually transmitted as it infects, specifically, macrophages found in skin and mucous membranes and is hence called macrophage-tropic or M-tropic. However, in some cases, the sexually transmitted HIV recognized as M-tropic at first tends to develop later on into T-cell tropic virus (SI strain that targets T-cells) and becomes more prevalent during the late stages of the disease. Although the reason for this conversion is still unclear and yet to be determined, people with the aggressive strain tend to have a 3- to 5-fold increase in regard to the rate of disease progression[112].

CRISPR-Cas9 as Treatment Methodology Proposal and Side Effects

The clustered regularly interspaced short palindromic repeats technology, commonly referred to as CRISPR or Cas9, can be used as a genome editing tool to induce the two Delta 32 copies of the *CCR5* gene *via* progenitor and hematopoietic stem cells in the body. The *CCR5* null T-killer lymphocyte blood cells can be transplanted into the human body to allow natural selection due to selective pressure for immunity against HIV[113]. Upon artificial infection, the uptake of the *CCR5* Delta 32 mutation is promoted in the immune cells in the body with CD4+ as the main receptors. The induction of *CCR5* co-receptors with ablation and Delta 32 mutations on the cell surface of the majority of the cells makes the HIV incompetent to reproduce and, thus, becomes completely ineffective[103]. Additionally, further research studies should be carried out to enhance the rate and level of effectiveness achieved in the uptake

of mutation in blood cells upon transplantation of cells into the human body comprising of defective variant *CCR5* co-receptor or *CXCR4* proteins on their cell surface.

Figure 10 above depicts the layout of the homozygous naturally occurring *CCR5* Delta 32 mutations as well as the CRISPR-Cas9 induced deletion and the respective advantages/drawbacks.

Thus, artificial induction of immunity is converted into complete autoimmunity for the body against AIDS in this anti-HIV strategy, with the help of CRISPR to delete the 32nd base from both *CCR5* genes on the 3rd chromosome pair in genome[114].

This permits natural selection to occur for greater chances of survival of immune cells and suppression of HIV. In this way, no reproduction is possible, and insufficient numbers of HIV particles that are capable of carrying out pathogenic activity become incompetent due to no viral RNA replication. As time progresses, internalization of the HIV protein particles in the bloodstream occurs by phagocytosis where the phagosome then merges with the lysosome. Inside the phagolysosome, the digestive enzymes like nucleases such as RNases and different proteases break down and disintegrate the HIV bodies into extinguishable components, clearing the body free of the pathogen[115]. This whole cycle of termination of the human immunodeficiency virus is repeated in the same manner upon possible reinfection from the environment. However, the individual maintains an HIV-negative status due to the acquisition of natural immunity over time, a consequence of evolutionary natural selection. Moreover, there is no prevalence of the virus in the blood, and the body is completely resistant to the HIV-1 variant, which uses the *CCR5* co-receptor as an entrance component into the T-killer lymphocytes with the CD4+ surface receptors[116].

The T helper lymphocytes are specified as CD4+ T helper cells due to the main CD4 receptor on their cell surface. This receptor is capable of binding to GP120 protein on the HIV-1 surface and thereby acts as one of the two subunits complementary to the second main GP160 subunit on the spikes of the viral HIV body[117]. Moreover, the *CXCR4* co-receptor is also known as the Fusin protein receptor and can selectively become dysfunctional. Rather, the *CCR5* delta 32 mutation obstruct the annihilation of CD4+ T helper cells following infection by the HIV-1 strain, owing to the impediment of binding. The Nef proteins found on the HIV-1 strain are not able to hijack and take control of the membrane traffic for the fusion of the virus with the host cell and transport of viral RNA injected inside the cell towards the Golgi apparatus. In this case, the Nef proteins are unavailable and not able to initiate and control binding.

The Fusin co-receptor is only selective for particular strains of HIV, which act as a doorway for viral infection primarily in CD4+ cells only. In comparison to this, the *CCR5* receptor is more widely available for viral entry into host immune cells. Thus, selective targeting of *CCR5* receptors to become ineffective for reception will provide greater protection against HIV. Moreover, the *CCR5* receptors are able to effectively bind with the common NSI (non-syncytium inducing) strains of HIV during early disease spread[100].

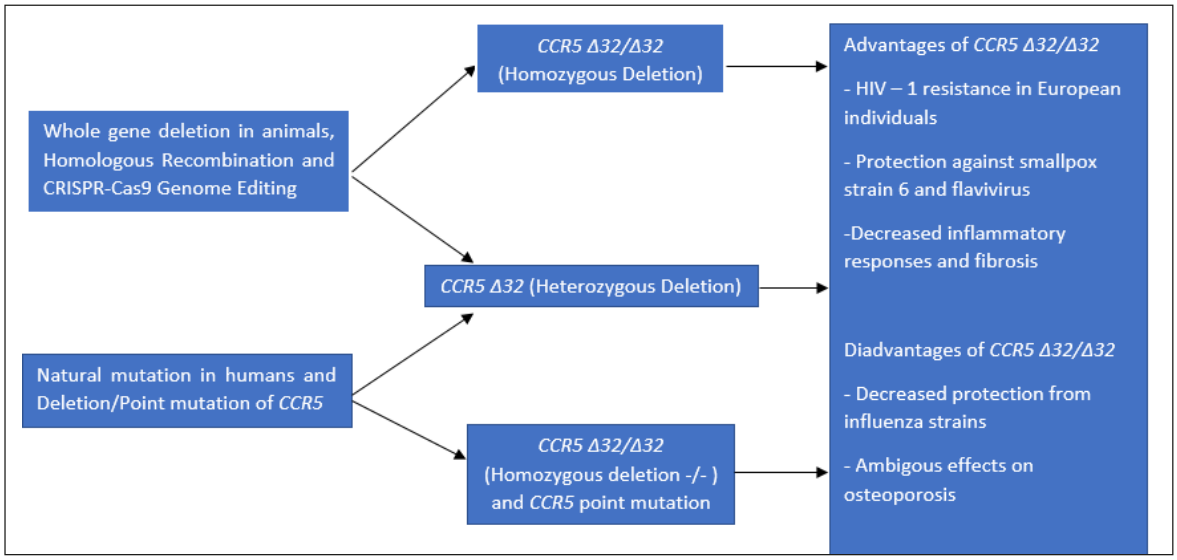

Figure 10. A detailed overview of the advantages and drawbacks of *CCR5* Δ32/Δ32 genotype and CRISPR-Cas9 action.

Furthermore, these cells are characterized as Mtropic (macrophagetropic) rather than syncytium-inducing (SI) strains. Upon infection, the predominantly M-tropic viruses mature into Ttropic viruses with the conversion of NSI strains into SI strains during the later stages of infection. Nonetheless, more than half of the deaths pertaining to AIDS are due to the NSI strains of HIV. The SI strain is in positive correlation with the rate and level of progression of AIDS. Additionally, SI strain depicts rapid suppression of the immune system, actively destroying CD4+ cells with great intensity of disease progression[118].

On a larger scale, the mutation can be successfully introduced in non-Caucasian ethnicities, which predominantly do not possess the mutation. Eventually, natural selection will lead to complete HIV resistance in the population. This will prevent any cases of AIDS from causing harm. This demonstrates a successful methodology to prevent the spread of HIV as well as treat AIDS across generations who will pass on the mutated copies of the *CCR5* alleles in generations over time. Increasing the frequency of the beneficial allele in the population, as well as its expression, will ensure active immunity against the virus. Additionally, this occurs within a broader genetic pool that includes the Delta 32 mutation, known for providing resistance, and exhibits a higher frequency of allelic expression within the population.

The CRISPR-Cas9 genome editing tool can be used to selectively and precisely crop out the 32nd base pair in the *CCR5* gene on the 3rd chromosome in humans and allows homologous recombination as a natural DNA repair process to take place[119]. The Cas9 molecule is a 160-kilo-Dalton protein showing an imperative role in the defense of bacteria against bacteriophages by allowing alteration of the genome. This is done by cutting the integrated part of the component of viral DNA in the cell genome itself and cutting it out in order to prevent further duplication of more viral proteins upon translation of the viral genes. Cas9 is an enzyme able to cut the DNA double helix at two different points precisely by acting as molecular scissors for editing the DNA. Furthermore, it comprises the guide RNA (gRNA) composed of a scaffold RNA around 100 bases long RNA sequence, which is predetermined and prepared in the laboratory. It is placed inside the scaffold and is complementary to the target base sequence for complementary base pairing to ensure proper cutting with CRISPR-Cas9. This ensures selective targeting of the genomic region complementary to the 20 base pairs towards the 5′ end of the guide RNA (gRNA)[120]. The binding of the DNA to the scaffold allows the pre-designed sequence to be read, giving direction to the Cas9 enzyme for correctly cropping the identified part of the genome. Lastly, the cut made by Cas9 is now considered a mutation.

Moreover, the CRISPR-Cas9 system provides other opportunities for the overall editing of the DNA, such as deletion, insertion, and substitution, with great precision. Unless the bases on the guide RNA are present repeatedly on more than one or multiple possible target RNAs, the CRISPR system does not go off target throughout the genome being applied with large pieces of mutations[121]. Additionally, this genome editing system with stem cell technology provides gene function that is controllable, and the expression is dictated by artificial mutation induction. This includes modifications to the promoter base sequences located external to the primary gene, such as the *CCR5* gene and its promoters. These alterations aim to regulate the expression level and synthesis of receptors, thereby influencing the number of available binding sites for the spike protein GP120 of HIV-1. Lastly, genetic modification also provides the possibility of treating other diseases by focusing on mutating genes outside of the *CCR5* gene. These include the cystic fibrosis transmembrane receptor (CFTR) gene for individuals who suffer from cystic fibrosis and other diseases caused by point mutations. From a virology point of view, only a dysfunctional *CCR5* receptor translates into immunity from the reception of viruses like smallpox variants and flavivirus[122]. Moreover, in the context of evaluating anticancer pharmaceuticals such as Gecko, among other drug screening initiatives, these form integral parts of the drug development and testing process. This process is designed to ensure safe human application, encompassing three distinct phases of clinical trials.

Figure 11 depicts the usage of CRISPR Cas9 for host factor discovery, validation with multiple genes, and assembly of screen hits and libraries. In this case, thousands of genes are inserted into CRISPR-edited cells for re-infection for therapeutics upon validating host factors with CRISPR from the original CD4+ T cells. In addition to examining the regulation of genes related to the cell cycle, which govern DNA damage checkpoints and cyclins, the interplay and expression of various other genes within the body is also a factor to take into consideration. This includes an analysis of how these genes influence the expression

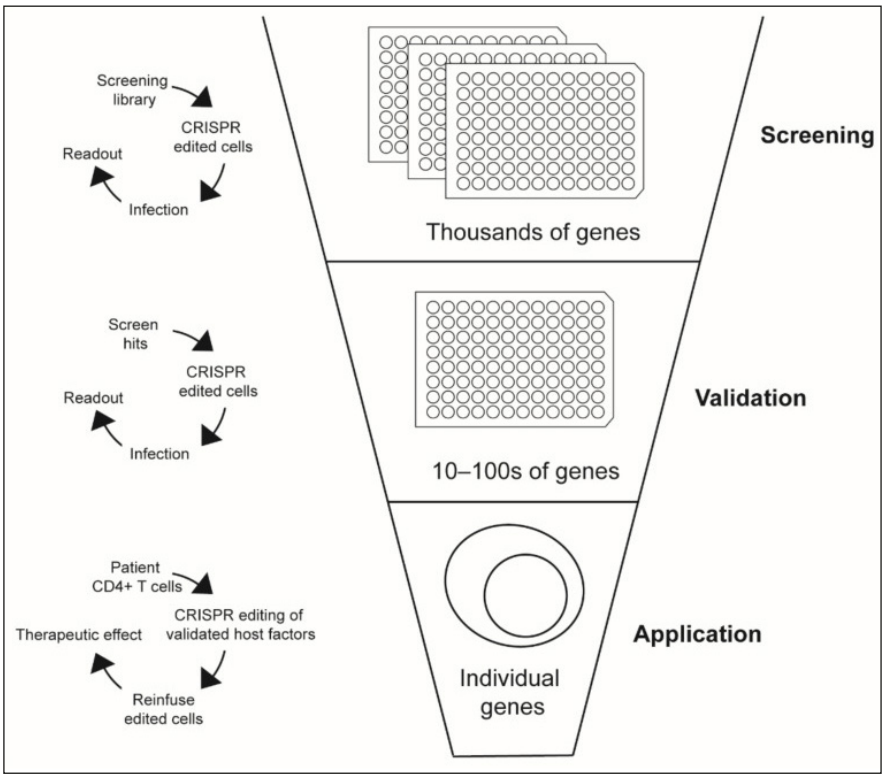

Figure 11. CRISPR Cas9 enables high throughput genetic screens of up to a thousand host factors in primary cell lines for validation, screening, and therapeutic applications[123].

of tumor suppressor genes and proto-oncogenes. The most important one of these is the p53-guardian of the human genome, which acts as a tumor suppressor and arrests the cell cycle in case of uncontrollable cell division. In this way, it is possible to treat diseases such as Alzheimer's disease by using CRISPR technology and the Cas9 protein to edit the human genome for better functioning and control over homeostatic conditions[124].

Table I summarizes a collection of recent studies on the application of the CRISPR-Cas9 system against HIV. Host sources include B cells and the *CCR5* and *CXCR4* co-receptors. The target of genetic modification can also be the viral molecules themselves. This includes the Env, Pol, and Nef proteins. Single crRNA and double crRNA molecules are supplied by lentiviral or adeno-associated vectors. The studies[142] showcase findings of successful inhibition of viral synthesis and elimination of HIV. The Cas9 system is the most effective method with widespread application for the *CXCR4* and *CCR5* receptors in the host, as well as the Env and Pol targets on the virus bodies. After this, Cas12a targets the Nef and Tat proteins in the virus. Cas13a and Cas13d are only effective for Pol protein modification on the virus, which will lead to inhibition of viral synthesis. The studies[143] carried out demonstrate CRISPR-Cas9 as the best option to fight against HIV infection by targeting the receptors in the host. This will require adeno-associated vectors and lentiviral vectors along with single and dual copies of crRNA. This results in the alteration of the genetic code, synthesizing the receptors on host cells and inhibiting viral replication.

Efficacy of CRISPR-Cas9

The efficacy of CRISPR-Cas9 varies depending on several important factors. CRISPR-Cas9 system acts as a site-specific gene editing tool. The *in-vivo* application of this technology is dependent on the selection site to be targeted on the DNA and the design of the single guide RNA (sgRNA)[144]. Moreover, nucleases that are programmable, such as Zinc Finger Nucleases (ZFN), can be used to enhance the precision of genome editing with paramount transcription regulation and protein interactions. Other factors include the frequency of occurrence of homology-directed repair (HDR), as well as the second mechanism known as non-homologous end joining (NHEJ) and its interference with Cas9 enzyme during operation. Furthermore, the efficiency of Cas9 action and its delivery method

Table I. Comparative analysis summary of studies carried out on CRISPR-Cas9 action to counter HIV infection with delivery vectors [i.e., Lentiviral (LV) or adeno-associated vectors (AAVs)].

Target	CRISPR System	Delivery Vector	Single/Dual crRNA	Study Conclusion	References
Host – *CCR5*	Cas9	AAV	Single and Dual	Genetic editing of *CCR5* receptor for HIV resistance by inhibiting viral replication.	125-127
Host – *CXCR4*	Cas9	LV	Dual	Genetic editing of *CXCR4* receptor for HIV resistance by inhibiting viral replication.	128
Virus - *Env*	Cas9	LV	Dual	Inhibition of viral synthesis and elimination.	129
Virus - *Pol*	Cas9, Cas13a and Cas13d	AAV and LV	Single and Dual	Inhibition of viral synthesis.	130-134
Virus - *Nef*	Cas12a	LV	Single	Inhibition of viral synthesis.	135
Virus - *Tat*	Cas9, Cas12a	LV	Single and Dual	Inhibition of viral synthesis, elimination of HIV.	136-140
Host - B cells	Cas9	AAV	Single	Antibody neutralization by induction of synthetic anti-HIV	141

for *in vivo* application are crucial for the precise and effective modification of the *CCR5* gene. This aspect must be considered within the broader context of legal implications and regulatory frameworks. These methods of delivery include transfection and viral entry via lentivirus and lentivirus adeno-associated virus (AAV)[145]. The efficiencies pertaining to the Cas9 system type vary with either *Staphylococcus aureus* or *S. pyogenes* derived cas9 protein. Other factors are the exact target sequence of the gene at the specific locus and gene region differentiated by the cell types. These combinations give different options for cutting the DNA at the targeted location with the Cas9 enzyme at different rates, giving specific efficiency of gene code alteration while providing disruption of the *CCR5* and *CXCR4* receptors. The small guide RNA (gRNA) is yet able to cause additional unwanted off-target effects by complementary base pairing with repeated sequences, leading to errors in the binding of the Cas9 enzyme, producing single nicks called off-cuts.

Cas9's ability to selectively target specific gene sequences, known as PAM codes, positions it at the forefront of treatment and prevention strategies. This attribute enhances its application in contemporary healthcare practices and programs through the targeted action of the Cas9 enzyme. For Cas9 to operate, the nuclear localization signal directs the Cas9 into the nucleus of the eukaryotic organism. The Cas9 enzyme activities can be derived from multiple different species, such as *S. thermophilus, Staphylococcus aureus,* and *Neisseria meningitidis*. Moreover, inhibitors such as Scr7 are able to target DNA ligase IV components involved in non-homologous end joining, showcasing greater efficiency in HDR to edit genes faster[146]. Finally, potential human genome editing could be conducted *in vitro*, involving the extraction of totipotent stem cells and the application of the CRISPR-Cas9 system. This procedure aims to induce a deletion at the 32nd base pair, steering the DNA repair mechanisms toward producing a mutated *CCR5* gene. This system requires electroporation inside an *in-vitro* laboratory setup to degrade the cellular integrity by the formation of pores in the cell membrane for the system to actively act upon the *CCR5* gene and reintroduce the cells for the uptake of modified genes in the DNA-producing mutated chemoreceptors and eventually providing resistance.

Subsequently, taking these aspects into consideration, it is clear that modern CRISPR systems can be introduced in hospitals for *in vitro* manipulation, which is already a common therapy worldwide in reproductive biology. A comprehensive method involves modifying stem cells extracted from patients and then reintroducing them with consideration for antigen-based hematological blood group compatibility. This approach can effectively confer resistance to HIV-1 strains in patients. This is feasible in practice, as it leverages the globally established infrastructure of *in-vitro* facilities in IVF laboratories, with CRISPR-Cas9 serving as an advanced extension to this existing setup. The last step of the patient's *CCR5* genetic alteration would be simply the reinjection for uptake of the modified gene-carrying stem cells, which will be replicated over time carrying receptor types that are non-binding to HIV-1, giving immunity[147]. Thus, CRISPR-Cas9 proves to be a cost-effective and reliable system for immunity against HIV-1 by genetic alteration of the *CCR5* gene.

Relative HIV-1 Resistance Efficacy of Mutated C-C Motif Chemokine Receptor Family

The C-C motif chemokine receptor family comprises a total of 10 members. These vary from *CCR1* to *CCR10*. As described before, the *CCR5* is the most significant route for the pathogenic activity of HIV-1, but the rest of the chemokine receptor subtypes still play an imperative role in blocking the viral entry of certain influenza to some varying extents and, additionally, by mediating and regulating diverse immune responses emanating from leukocytes. All these receptor subtypes are coded by the genes on the 3p21 genotypic location[148]. For example, the *CCR7* receptor is homeostatic and is responsible for the trafficking and transport of mature T helper lymphocytes and dendritic cells towards other secondary lymphoid organs in comparison to the *CCR9* and *CCR10* receptors, which perform immune action in cells of the intestinal lining, gut, and skin tissues. Moreover, the *CCR1, CCR2, CCR3, CCR4, CCR6,* and *CCR8* receptors are involved in the trafficking of effector lymphocytes toward the sites of the inflammation[149]. However, by tracing the evolutionary biology of human chemokine receptors, their epigenetic signatures, and variation in genetic expression over time in a population, it can be deduced that the *CCL2* gene is selectively coding for the reception of viral GP proteins on the spikes of HIV-1, allowing the infection of host immune cells. Additionally, *CCR7* is greatly overregulated during the maturation of dendritic cells, making it the prime option for infection and ligand binding for internal inflammations. Accordingly, *CCR2*, *CCR3*, *CCR4,* and *CCR8* hold the potency to work as co-receptors on HIV-1 surfaces under *in vitro* conditions. It is mainly the *CCR2* and *CCR5* receptors expressed in monocytes and T-killer lymphocytes as their specialization allows for effective binding with pathogens[150]. Furthermore, *CCR5* acts as the first method of entry for HIV-1 tropic strain due to its independent and specialized effectiveness upon binding with strains of different influenzas. Meanwhile, *CCR2* plays a secondary yet significant role in antigen processing during cell maturation and participates in various allergic reactions, leading to a substantial reduction in its availability. Despite this, mutations in *CCR2* indicate a degree of partial resistance to influenza infection. It binds to the chemokine signaling hydrophilic ligand 2 to initiate a signal transduction pathway with a cascade of events constructing a cellular response. The *CCL2* gene is located on the long q-arm of the 17th chromosome at band loci 11.2 until reaching the sub-band designated as 21.1, which pertains to a specific configuration of area and region on the same chromosomal arm. The complementary chemokine of the *CCR2* receptor is the *CCL2* protein. The genetic expression of *CCL2* comprises a total of 1,927 bases configuring polypeptide chains composed of up to 76 amino acids[151]. This constitutes a molecular weight of approximately 11.025 kilo-daltons pertaining to glycosylation levels. Most HIV-1 infected subjects showcase significant proportions of *CCR2* receptors in association with Chemokine ligand 2 upon viral replication and pathogenesis. This *CCL2*/*CCR2* combination is responsible for playing a part in the progression of AIDS, but it is not the main determining factor, like the independent effect of *CCR5* and *CXCR4* reception pathways upon Delta 32 mutation. Nonetheless, *CCR2* acts as an alternative for HIV-1 viral GP spike proteins to attach and inject viral RNA into the host immune cell. The *CCL2* provides a signaling framework for the transmission of the pathogen to occur; therefore, a homozygous mutant genotype can hinder partial HIV-1 infection and delay the progression to acute AIDS, in contrast to the standard *CCR5* gene, which provides resistance when it is dysfunctional[152].

The R5 strains of the virus are the most prominent as they are most compatible with the co-receptors. Additionally, cellular enactment occurs upon successful binding with the receptor and ligand, and this process may use the flagging pathways with G protein. Identical to several G-protein coupled receptors (GPCRs), *CCR5* is controlled by agonist-dependent forms, which include G protein-coupled receptor kinase (GRK)-dependent phosphorylation, beta arrestin-mediated desensitization, and internalization. *CCR5* regulation is subject to phosphorylation from kinases that are dependent on agonists as well as beta arrest for internalization. Furthermore, an illustration of the three-dimensional structure and work of *CCR5* demonstrates the structural configuration that, in turn, regulates the expression of *CCR5*. In sharp comparison, *CCR2* can carry out communication on basophils[142]. Other receptors in the family, such as *CCR6* and *CCR5*, are able to establish communication with the dendritic cells

for maturation of the cell and to be able to handle the antigen binding activity. However, the receptors such as *CCR1* and *CCR2* cannot participate in strong binding interactions towards the neutrophils. The receptors serve as biomarkers and targets for several conditions, such as atherosclerosis and rheumatoid joint pain, while for HIV-1, both the association of co-receptors that are *CXCR4* and *CCR5* will ensure binding[153]. This only leaves the homozygous carriers of the mutation to resist the infection successfully due to failure of binding. *CCR8* is one of the members of the chemokine receptors, and it is useful for *in vitro* application as a substitute co-receptor to bind with HIV. Other infections, such as herpes, also use the same set of receptors[154].

CCL4 and *CCL3* serve as cognate ligands to the main *CCR5*. *CCR5* interacts with the structure of the *CCL5* receptor protein. Furthermore, *CCR4* operates towards the thymus-dependent ligands with high affinity, particularly in the case of *CCL17*[155]. Additionally, the *CCR3* functions as a binding site for inflammation-inducing ligands, which include an extensive number of receptors like CCL15 and *CCL2*6. For instance, *CCL2*8 is known for its unique role in mucosal tissues, affecting immune cells like killer cells and B cells. Neurodegenerative damage linked to Multiple Sclerosis has been predominantly connected to the interaction between *CCR2* and its specific ligands. From this, it can be noted and observed that *CCR3* possesses the widest variety of ligands, making it the most diverse in this context.

The CC-chemokine receptor 7 ligands are CCL19 and *CCL2*1, which are conveyed by different subsets of resistant cells. *CCR7* and its ligands are basically included in the same category amongst different populations of killer lymphocytes. The *CCR3* and *CCR5* genes are unique for accumulating and activating inflammatory cells in the respiratory tract and airways. Moreover, since the expression does not occur in various populations, it is essential to conserve and isolate these genes due to their rare frequency of expression.

All versions of the mutant chemokine receptors provide varying protection against influenza strains due to the incompatible binding with the altered morphology of receptors and antigens on the cell surface proteins and spikes of viral bodies. In return, this stimulates antigenic shifts and drifts. For this reason, a heterozygous genotype is least effective in providing immunity for HIV-1 for a viral entry method that is already less favored.

Ethicalities of CRISPR-Cas9 for HIV-1 Treatment

The breakthrough discovery of the CRISPR Cas9 system has sparked considerable interest in the scientific community. It displays a quick and efficient solution that, in theory, will advance in some unresolved and yet-to-process diseases. Scientists Jennifer Doudna and Emmanuelle Charpentier, who discovered this tool and made it applicable for use in 2012, have only been awarded in 2020 with the revolutionary scientific contribution award of the Nobel Prize[156]. However, the CRISPR Cas9 system has also been a source of apprehension as the prospect of deliberately modifying the DNA raises some serious international concerns if applied to humans. On the other hand, the CRISPR technique has already started to benefit the global economy as it was used in the agriculture industry to boost yields and enhance crops, making them further genetically variant and resistant to pests and insecticides. It is an alternative to the zinc finger nucleases and transcription activator-like effector nuclease techniques due to CRISPR-Cas9's efficiency, precision, and lower cost. Since 2012, many human clinical trials have been performed with the aim of treating somatic cell diseases such as those of cystic fibrosis and AIDS.

In contrast, no clinical trials have been allowed in regard to germline genome editing. Whether in germ cells or embryos, this approach raises ethical and safety issues, particularly because it suggests that such modifications would be passed on to offspring and become hereditary[157]. Nonetheless, the debate on the ban of CRISPR-Cas9 continues, whether it is applied to germline or somatic cells. Nevertheless, it has mostly shifted from somatic cells to germline cells due to the extra ethical worry it carries. Somatic cell editing is much closer to clinical implementation. These ethical issues derive mainly from the concern that germline modification might lead to the creation of 'designer babies' whose DNA has been precisely selected to enhance multiple human features. These features could be intelligence and other traits that are not vital for human survival. This misuse could lead to the resurgence of discriminatory practices akin to eugenics, perpetuating unequal distribution of enhancements worldwide and resulting in a societal divide between those who are enhanced and those who are not, achieved through reproductive and genetic control[158]. Yet, with all these issues, they still divert attention and point of focus away from more pressing

matters related to clinical applications of somatic cells' genome modification.

The debate is ongoing on whether this modern genome editing technique creates more novel ethical considerations. Ethical reasoning is even greater than the ones already existing with previous genome editing tools, such as identifying and determining with certainty which cases are serious enough to warrant genetic changes. Patients will have the option to choose between a one-time cure for a disease or lifelong immunity to it rather than relying solely on other medical alternatives, especially since these genome editing toolkits' potential adverse effects have not yet been completely acknowledged and understood. Moreover, there have been some controversial incidents in the scientific community. For example, a criminal investigation commission was assembled against Chinese biophysicist He Jiankui in 2018[159]. He made an announcement by confessing to having genetically modified human embryos that were successfully implanted in an illegal effort to make the resulting offspring completely resistant to HIV infection.

This action, which was widely condemned by scientists worldwide pertaining to the illegal and unauthorized editing of embryos, did bring out to the world that genetic manipulation can save lives if performed legally.

With all that being stated, on the other hand, we cannot ignore that this discovery opened a portal into a new era in science with enormous perspective. Thus, the regulatory laws are to be developed to support wider clinical uptake of such technology for future use in healthcare. It offers considerable treatment for several challenging conditions. This does not mean it will happen anytime soon, but until the prospects and mechanism of this tool and its secondary effects are fully understood, its use should only be permitted in a narrow set of circumstances due to its immense scale of potent application.

Medication History for HIV-1 and Side Effects

The medicines that have been used to treat HIV are called antiretroviral drugs (ART)[160]. There are numerous types, and each fights the virus in the body in a unique and different way. Researchers[161] suggested that taking a combination of drugs is an efficient way to lower the chance of the virus becoming resistant to the treatments. It is recommended to take three-in-one medical pills one time per day, and every type of medication shall be taken depending on what strain the virus is, if it is resistant to drugs, what medications have been consumed, and how strong the immune system is.

Using nucleotide reverse transcriptase inhibitors (NRTI) will prevent the HIV virus from being equipped for further replication as the viral RNA will not be transcribed into DNA. So, the cells that have been infected by HIV will not be able to reproduce more HIV proteins for spreading infection. The NRTIs include examples like Abacavir, Didanosine, and Zidovudine, which will help in decreasing the amount of HIV produced in the body, as mentioned in Table II.

Another treatment methodology using medication involves Non-Nucleotide Reverse Transcriptase inhibitors (NNRTI), as mentioned in Table III. The NNRTI will bind to a selective protein that will disable the virus from copying itself, for example, Drotaverine, Rilpivirine, and Efavirenz. Furthermore, Protease inhibitors (PIs) types of drugs block the protein that the infected cells require to incorporate the new HIV-1 virus strain. Therefore, using this drug will disable it from doing so. Examples of these drugs are Atazanavir, Indinavir, and Ritonavir, as mentioned in Table IV. Thus, using antiretroviral therapy (ART) will help in the prevention of HIV from copying, multiplying, and increasing in number, subsequently giving the immune system the ability to produce more CD4 cells[165].

Plausible Alternate Treatment Methodologies

Latency-Reversing Agents (LRAs)

The most imperative and efficient paths towards HIV-1 treatment are shock and kill methods that utilize different latency-reversing agents in order to significantly increase the quantity of viral infection and induce their gene expression in the host organism[166]. The amount of viral load in the host increases with the help of latency-reversing agents to such an extent that it is detectable to the host's defensive system. Thereby, it is exposed to various immune or cytotoxic mechanisms. There are numerous new latencies reversing agents that produce gene expression related to HIV-1 through changing the structure of integrated provirus at the chromatin level as well as initiating various transcription factors, including NF-κB, as mentioned in Table V. The latency-reversing

Table II. The NRTIS as medication for HIV-1 tolerance and the associated side-effects[162].

Nucleotide Reverse Transcriptase inhibitors (NRTIs)	Common side effects	Precautions
Abacavir	Hypersensitivity reaction, high level of cholesterol, high risk of cardiovascular diseases	Genetic testing must be done
Didanosine	Nausea, vomiting, fat loss in arms or face, abdominal pain	Used rarely
lamivudine	Skin rash	None
Emtricitabine	Skin darkening in palms and soles	None
Stavudine	Peripheral neuropathy, lactic acidosis, and fat loss	Should not be used
Tenofovir	Bone, kidney damage and weakening	Shall not be used if there is a kidney disease
Zidovudine	Anemia, nausea, fatty liver and increase in cholesterol	None

Table III. The NNRTIS as medication for HIV-1 resistance and the associated side-effects[163].

Non-nucleotide Reverse Transcriptase inhibitors (NRTIs)	Common side effects	Precautions
Rilpivirine	Tiredness, headaches, trouble in sleeping	People with liver or kidney problems must not use it, might cause problems in the rhythm of the heart
Efavirenz	Anxiety, depression, liver damage, skin rash	Should not be used by people who have depression or psychological problems
Doravirine	Weight gain and skin rash	none
Nevirapine	Liver damage, skin rash, difficulty in sleeping, depression	Must not be used with people who have liver problems, and women who have CD4+>250 and in men CD4+>400, drug must be taken after food consumption For people with <200 CD4+ or HIV viral load >100,000

Table IV. The Protease Inhibitors (PIs) as medication for HIV-1 resistance and the associated side-effects[164].

Protease inhibitors (PIs)	Common side effects
Atazanavir **Indinavir** **Saquinavir** **Ritonavir** **Tipranavir** **Lopinavir** **Nelfinavir**	**All have similar side effects, such as:** Fat redistribution, dizziness, diarrhea, tongue sensation and taste alteration, insulin resistance and mismanagement, nausea, vomiting, random rashes, liver failure, possible jaundice aggregation, High cholesterol and excess triglyceride levels.

agents (LRAs) are classified according to their respective mechanisms into four distinct groups such as modulators of non-histone chromatin, extracellular stimulators, histone modulators of post-translational alteration and stimulators in NF-κB pathway. The *CCR5* or protein kinase C (PKC) agonists stimulate inactive HIV-1 with the help of the activated NF-κB.

Moreover, the ability of diverse latency-reversing agents to activate gene expression in HIV-1 is primarily dependent on subdivisions of CD4+T cells. This is due to the several mechanisms of

Table V. The epigenetic-modification factors which affect the action of the latency-reversing agents[167].

Latency Reversing Agents	
Epigenetic Modifiers	Methylation inhibitors Methyl-transferase inhibitors Histone deacetylase inhibitors (HDACs) Bromodomain inhibitors P-TEFb activators

LRAs that initiate viral latency among these subdivisions of CD4+T cells. The latently infected cells help in the reactivation of HIV-1 and thereby are efficiently killed through cytotoxic T lymphocyte (CTL) arbitrated immune response or viral cytopathic effect[168].

Epigenetic Factors Affecting LRAs

There are several epigenetic modifiers that act on the same pathway of operation for latency-reversing agents. They increase the rate of detection by the natural immune system itself upon the alteration of the morphology of the viral spike proteins on the cell surface. The increased susceptibility of the pathogenic bodies ensures active immunity against HIV-1[169]. Therefore, epigenetics plays a vital role in determining the impact of environmental influence on the relative genetic expression of the coding regions via LRAs. These include alterations in methylation and acetylation patterns of the genome, leaving epigenetic signatures. Accordingly, the epigenetic factors include methylation inhibitors, which are drugs that are utilized to consider the section of DNA methylation binding to CpG islands. These sections bind to totally different tissues and showcase models in light of the numerous instances of Cytosine-Guanine (CG) dinucleotides on the entire genome, resulting in switched-off genes. This methylation can also target histone tails, conforming the chromatin structure into a tighter structure and reducing genetic expression by blocking accessibility for transcriptional factors. This methylation of the genome will ensure inactivation pertaining to switching off of genes. However, this natural action can be artificially prevented with methylation inhibitors and methyl transferase inhibitors to inhibit methylation completely[170]. The methyltransferase inhibitors are cytosine analogs that join to DNA and cause quality inactivation. Therefore, by temporarily blocking the methyl transferase protein, none of them undergo the transfer of methyl groups.

In addition, epigenetic modifiers better promote the activity of LRAs by reducing methylation for overexpression, giving variability to improved detection of viral bodies for T helpers cells. There are modifications that can be induced with increased acetylation to loosen the chromatin structure. This can be achieved with the Histone deacetylase inhibitors (HDACs)[171]. The remodeling of the chromatin during methylation can be further reduced with bromo-domain inhibitors.

After taking control of the accessibility for transcriptional factors, a cyclin-dependent kinase such as positive transcription elongation factor (P-TEFB) can be used as an epigenetic influence. P-TEFB refers to the positive transcription elongation factor B. Therefore, P-TEFB activators will allow the control of the regulation of the transcriptional factors in such a selective manner that only the required expressions for specific genes are switched on, which would result in diverse three-dimensional viral body structures for the latency-reversing agents to detect infection and trigger immune response[172]. Subsequently, the compounds that are actively transcribed peak to a conceivable restorative alternative for disarrangement that includes modified DNA methylation.

Furthermore, the most prominent hindrance in this type of HIV treatment is the inability of latency-reversing agents (LRAs) to cause an efficient and significant reversal of latently infected cells. The mRNA expression of HIV-1 activated by various LRAs is sometimes insufficient to elicit a significant number of infectious virions along with viral proteins. The inadequate number of virions or viral proteins in the host causes the inefficient action of cytotoxic T lymphocytes (CTL) through crucial histocompatibility complexes. The treatment of HIV-1 with the assistance of LRAs particularly inhibits the complete removal of virally infected cells because of impaired LRA as well as CTL function. Thereby, it produces the state of anti-apoptosis in the host despite the large production of viral infection. Moreover, the latency-reversing agents (LRAs) primarily lead to the reactivation of the global immune system, and this generates profound side effects along with increased pro-inflammatory cytokines[173].

Transcriptional Gene Silencing (TGS)

The recent novel treatment against HIV-1 primarily consolidates the viral latency in the host instead of the reversal of latently infected cells. This inhibits the reoccurrence of the virus upon the discontinuance of antiretroviral therapy (ART). The category of viral infection treatment, such as that with HIV, is known as the block and lock technique, which makes use of small interfering RNAs in order to generate transcriptional gene silencing. The production of TGS is largely generated through the disruption of the optimal structure of chromatin.

Hence, the latency of HIV-1 is maintained by conserving the various epigenetic mechanisms. The block and lock method utilizes diverse latency-inducing agents (LIAs) in order to prevent

(block) virions within the host from transcription at their promotor region along with freezing (lock) the embedded viral genetic makeup in an extremely permanent inactive state. The most profound agents of the block and lock technique are several small inhibitor molecules, RNA, and lastly, trans-dominant protein[174].

In addition, the block and lock method mimics the inactive natural form of HIV-1 by producing extreme latency in the host with the help of various LIAs. The traditional epigenetic silencing pathway involving epigenetically silenced human endogenous retroviruses (HERVs) is employed in the block and lock technique to permanently enforce the silenced phase on HIV-1[175]. The HERV largely supports the probable longevity and easy feasibility of the block and lock method since it comprises roughly 8% of the total human genetic makeup. The most imperative advantage of this HIV-1 treatment is that it is arbitrated by therapeutics involving RNA and, hence, is extremely specific in targeting the required genetic sequence. On the other hand, the most prominent obstacle in this specific type of HIV-1 treatment is the proper development of an efficient delivery system inside the host in order to particularly target inactive reserves of infected cells. This type of functional cure is still mainly in its preliminary developmental stages and thereby requires many more trials on humans since there might be some concealed negative effects of this HIV-1 treatment[176].

Highly Active Antiretroviral Therapy (HAART)

Highly active antiretroviral therapy (HAART) is a collection of various anti-HIV drugs that mainly help in limiting the mortality and morbidity connected with the infection of HIV-1 and AIDS. The collection of numerous antiretroviral therapies significantly reduces the replication of the virus inside the host and suppresses the plasma viral load (vLoad) of HIV-1 in order to enhance the effectiveness of the host defensive system[177]. Subsequently, the viral load of HIV-1 is drastically decreased in this particular treatment category through the consumption of several drugs and thereby is undetected by numerous highly sensitive assays. In addition, the combination therapy utilizes at least three potent antiretroviral drugs against a minimum of two primary molecular objects since it is the fundamental basis for anticipating resistance against the consumed drugs. The most plausible and potent drugs for the treatment of HIV -1 through HAART are produced with the help of a trial-and-error process.

On the other hand, the ineffectiveness of HAART for the treatment of HIV-1 causes about 25% of the patients using the therapy to terminate their medication since it is unable to prevent viral replication of HIV-1[178]. The majority of such patients usually discontinue their treatment in the early stages as the combination of antiretroviral therapy is unable to decrease the overall viral load in these patients. Additionally, there are various severe negative side effects on the human body, which are primarily caused by the combination of antiretroviral therapies. The preliminary side effects of various antiretroviral therapies are mostly mild. They comprise gastrointestinal-related problems, including diarrhea, bloating, and nausea. The primary negative impacts of therapeutic drugs such as zidovudine (AZT) along with efavirenz (EFV) are nightmares, headache, and fatigue[179]. Subsequently, there are numerous other severe adverse impacts caused by the combination of antiretroviral therapies, including peripheral neuropathy related to stavudine (d4T) drug, retinoid toxicity caused by some protease inhibitors (PIs), zidovudine (AZT) related anemia and lastly, the occurrence of hypersensitivity reactions due to non-nucleoside reverse transcriptase inhibitors (NNRTIs).

Novel Cure Strategies

There are some new strategies that are generating a permanent reduction of viral load (vLoad) in HIV-1 patients after successful treatment. The most recent method for treating HIV-1 uses a novel discovery that is primarily based on the expression of respective immune checkpoint markers or IC markers through potentially powerful CD4+T cells, and this includes CTLA4 and PD1 markers[180]. Subsequently, the immune checkpoint markers, also known as inhibitory receptors, help treat chronic viral infections such as HIV-1 by limiting their effector functions and reducing tissue damage within the host caused by prolonged activation of the immune system. The cells with IC markers consist of inactive provirus inside the host, which are subjected to apoptosis through various antibodies such as CTLA4, PD-L1, and PD1, or they are targeted through the delivery of particular drugs[181]. Therefore, this specific strategy in treating HIV-1 has proven to be extremely potent in recent research[182].

Moreover, there has also been a considerable amount of success in treating HIV-1 through the cells consisting of CAR-T or chimeric antigen

receptor T. This approach has already proven[183] to be effective in treating various types of cancer. The T cells, which have been genetically modified and retrieved from the same host's bloodstream, stimulate the production of more effective antibodies specific to the viral infection in question[184]. These T lymphocytes are reinjected into the bloodstream of the same host since they are interlinked with the receptors of intracellular T lymphocytes. These T cells regulate cytotoxic response towards cells containing epitopes of viral infection within the host. With this strategy, the HIV-1 infected cells are cleared through a CTL-mediated immune response. Subsequently, this type of treatment strategy helps in controlling the viral infection in the absence of any therapeutic treatment, and the multi-functional CAR-T cells with anti-HIV-1 characteristics demonstrate the ability to clear the virus inside the human host. Therefore, the CAR-T cells can also be used cooperatively with latency-reversing agents of the shock and kill method. This increases the efficiency of HIV type-1 treatment[185].

Is There a Link Between Autoimmune Diseases and Treatment with HIV-1-CRISPR/Cas9 Methodology?

The CRISPR/Cas9 technology has allowed new advancements in the field of gene editing. The CRISPR strategy for treatment against viral infection has more precision, easier usage, and wide versatility. It is more readily accepted around the world. In addition, the CRISPR/Cas9 technology can also be utilized for the treatment of a few immunological diseases as this specific type of technology has enhanced efficacy in treating various diverse autoimmune diseases[186]. The autoimmune diseases are a collection of different diseases that are arbitrated through the immune system. They possess complex mechanisms leading to their disease pathogenesis. The most common examples of autoimmune diseases include rheumatoid arthritis, psoriasis, diabetes mellitus type-1, and lastly, coeliac disease. Subsequently, the overall most potent treatment of autoimmune diseases is achieved through the suppression of the immune system of the host with the help of various immunosuppressant drugs[187]. These immunosuppressant drugs inhibit the defensive immune response of the host in order to control the spread of various autoimmune diseases.

Furthermore, the CRISPR/Cas9 technology has the ability to induce mutation upstream of the gene variant at the region with the scientific notation 'rs6927172'. This region of the genome is involved in inducing alpha-factored tumor necrosis, specifically pertaining to the third variant of the protein commonly referred to as TNFAIP3. In addition, previous scientific research[188] devises a relation between TNF alpha-induced protein 3 (TNFAIP3) and its contribution to bringing an autoimmune-positive phenotype. Therefore, this particular strategy of treating diverse autoimmune diseases is very similar to HIV-1 treatment since they both involve inducing mutation to specific regions of the genome in order to cure diseases. However, in autoimmune diseases, there are countless possibilities of the body's organ systems getting infected. Therefore, in cases where the aggregation of the disease can be predicted pertaining to specific genome methylation factors, it is best to selectively use CRISPR-Cas9 in the same potency methodology that it can be used for HIV-1[189].

Conclusions

The effect of *CCR5*-Δ32 with CRISPR-Cas9 on mortality appears to be negligible; however, it has the potential to significantly progress global healthcare in combating HIV. With all aspects considered, this study reviews the model focused on somatic cell therapy in fighting the HIV-1 infection by introducing homozygous mutant copies of the *CCR5* genes called Delta 32 in totipotent somatic stem cells. The cells under discussion are extracted only from the seropositive patient body with an active HIV infection. Instead of altering the genome of each individual with germline therapy, the selective mass application of somatic gene therapy towards infected patients with health- and age-related prioritized medical treatment will provide an efficient and practical method to tackle the epidemic. As stated, the *CCR5* ordinarily codes for a receptor on the surface of white blood cells, and it plays an indispensable part in ordinary resistant reactions. This is due to the fact that HIV co-opts *CCR5* as a way to urge into white blood cells. So, blocking HIV also means eliminating a small part of the normal immune system. After HIV researchers brought attention to the *CCR5* Δ32 mutation, scientists in other fields became interested as well. Influenza researchers studying the mutation discovered that it predisposes individuals to severe outcomes when infected with certain strains of influenza. Additionally, West Nile infection analysts found

the same with that infection. Neurobiologists have discovered[190] that *CCR5*-Δ32 aggregates and upgrades recuperation from stroke, leading to a faster recovery. The CRISPR technology showcases significant potential in the widespread application of genetic induction of Δ32 alleles, providing reliable resistance for HIV-1. In a simple, high-throughput method, selective mutation of both copies of the *CCR5* gene using CRISPR-Cas9 will induce two homozygous Δ32 copies in totipotent stem cells. These cells will be reintroduced in the patient's body for uptake of the mutant allele to undergo replication, increasing the frequency of expression in immune cells under positive selection pressure for survival. This will potentially prevent and treat the variant-specific epidemic of AIDS caused by HIV-1 via artificially induced immunity. This prepares humanity for high expectations from the health sector in understanding the total scope of *CCR5* to ensure it is not merely seen as an obsession among researchers. Therefore, the pressure for treatment will eventually lead to selective flexibility in the application of the CRISPR-Cas9 system and HSC transplantation. Amidst the various implications that may halt the application of CRISPR, the sustenance of the introduced mutation in the human genome will provide active immunity against HIV-1 and diminish the epidemic over time.

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Not applicable.

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