How the CRISPR-Cas9 Gene Editing Complex Can Be Used to Treat Human Immunodeficiency Virus 1 and Sickle Cell Disease with Immune Cell Therapies and Genetic Engineering with Insight Into its Societal Impacts

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HIV affects approximately 38 million people in the world today, according to hiv.gov, and Sickle Cell Disease affects an estimated 1 to 3 million people worldwide (U.S. Department of Health & Human Services, 2021). Today, modern drugs used to treat HIV-1 make living with it more manageable, however, this is not the case with Sickle Cell Disease. No effective treatments are available to provide a cure, but CRISPR has provided new research opportunities that have been proven effective in treating these diseases. This paper highlights the use and effectiveness of both the CRISPR gene editing system and the processes of experimentation in multiple fields. More specifically, researchers focus on the effectiveness and potential for future treatments for gene editing in inherited diseases like Sickle Cell Disease, and contagious diseases like Human Immunodeficiency Virus 1 (HIV-1). The potential of CRISPR-Cas9 is paramount in discovering gene-editing methods within these diseases, by allowing excision of the RNA and genomic DNA to delete, replace, and discover new vulnerabilities to guide effective treatment. This paper seeks to explain how the biochemical applications of the CRISPR-Cas9 protein complex can be used in genetic engineering to treat HIV-1 and Sickle cell disease (SCD), using immune cell therapies and other treatments. It can be supported and discussed within the context of previous gene-editing technologies and where we stand with future technological development. This can provide insights into social development if given the resources to alter the attributes of humans and the natural world.

Keywords: CRISPR, Cas9, HIV-1, SCD, Hematopoietic Stem cells, Germline, Somatic, HDR, NHEJ, mutation, PAM, Reverse Transcription.

Mechanisms of CRISPR/Cas9-directed cleavage

The Cas9 protein forms a complex with a sgRNA, which guides the nuclease to a specific genomic address for cleavage. Cas9 catalyzed DNA cleavage is guided by a 17-20 nucleotide sequence within the sgRNA. Cas9 "scans" genomic DNA for regions of homology with the guide sequence, where it unwinds the DNA and its nuclease domain direct site-specific cleavage. This results in deletions generated by non-homologous end joining (NHEJ) at the binding site, or in homologous-dependent repair (HDR). Homology-directed repair (HDR) is one method that could be used for hemoglobin gene replacement, which is where the DNA essentially is being fixed via the mechanism available in cells to repair the double-stranded DNA lesions. In CRISPR, this naturally occurring nucleic acid repair system can be used to help the homologous DNA join together and modify genomes in many organisms. A key part of HDR is that you add in a small DNA segment containing the gene you want to be inserted into the genome.

Introduction to CRISPR

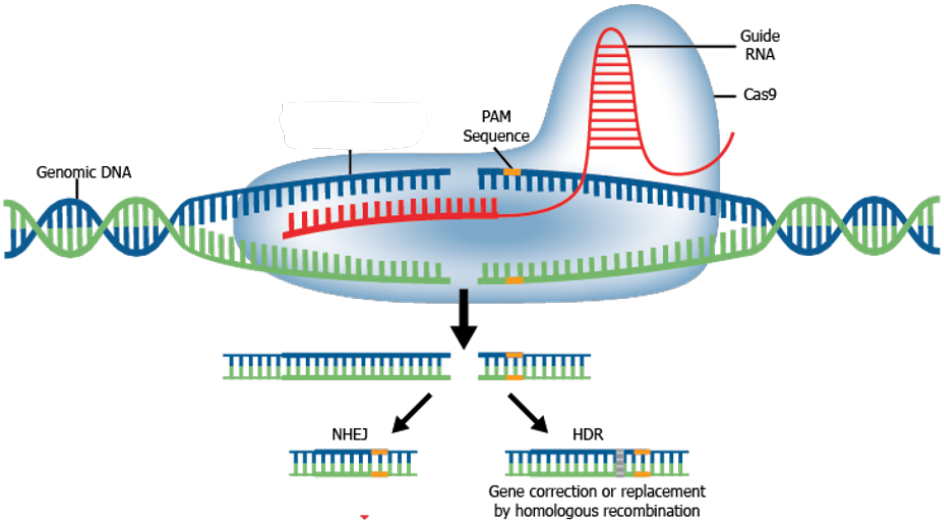

FIGURE 1. CRISPR/Cas9 gene-editing complex diagram depicting the functional placement of the Cas9 protein, target genomic DNA, and gRNA. Two outcomes of a double-stranded DNA cut are described: non-homologous end joining (NHEJ) and homology-directed repair (HDR) which can lead to gene disruption and replacement, respectively (Rockland Immunochemicals, 2016).

The RNA molecule guiding Cas9 is the gRNA or guide RNA. The guide RNA is a chimeric molecule made up of two distinct RNA molecules. The PAM site is a functional domain that is vital in the use of CRISPR/Cas9 gene editing. The protospacer adjacent motif (PAM) is a

short sequence of nucleotides in DNA around 2-6 base pairs in length, following the target DNA sequence for the CRISPR complex (Uddin et al., 2020). The PAM site is required for the Cas9 nuclease to cut the DNA at the target site. The crRNA or CRISPR RNA is the spacer RNA, which binds to the target DNA cut site with natural binding affinities of the nucleotides through complementary base pairing. There is also the tracrRNA, which serves as the scaffold, which acts to bind the RNA oligonucleotide to the Cas9 protein, and the spacer sequence (Medline Plus, 2020). The spacer RNA is complementary to the target DNA site, which the CRISPR-Cas9 complex is going to cut, whereas the scaffold RNA sequence holds onto the Cas9 protein to form a functional complex. We can extract the Cas9 protein from bacterial cultures for use in new promising genetic research. CRISPR interest is best invested in the chimeric molecules of gRNA. A chimeric molecule is a molecule that combines parts of various molecules. In the case of gRNA, it is the combination of crRNA and tracrRNA together used to cut the target gene. By synthesizing an oligonucleotide sequence to act as the gRNA, Cas9 can be used to cut a DNA site-specifically and efficiently, taking only about 15 minutes. This creates a new horizon for specific splicing of DNA to dissociate and remove the HIV-1 genome from human cells.

Earlier gene-editing methods

There are many predecessors to CRISPR/Cas9 in the field of genetic engineering, all of which have been used in the development of commonly used drugs and medicines. These methods have played a foundational role in biotechnology and the technological revolution, as medicine has been developed to extend quality of life and life expectancy. One major milestone in biochemistry was the discovery of restriction enzymes, also called a restriction endonuclease. Restriction enzymes are proteins coming in different forms produced by bacteria to specifically cleave DNA at target sites. Different forms of enzymes have different sites of digestion, which gave a wide area of application of their use. In bacteria, restriction enzymes act as a defense to invading species, by cleaving foreign DNA, eliminating infections from the cell.

These small proteins, usable in almost any DNA sequence, have been used to pioneer some of the world's most effective medicines and innovations. One example includes insulin. In the past, insulin was harvested from porcine and bovine pancreases. Today, insulin is produced using multiple cloning site gene insertion in bacteria using restriction enzymes. The restriction enzyme is used to cleave the desired expression vector at a target insert site, which is what controls the gene's production, and the gene is inserted to allow production in bacteria. Thus, removing the need for expensive breeding and harvesting of cattle, and making insulin production more effective.

The use of restriction enzymes has been reliable and efficient for the most part, allowing an easily produced, quickly sourced protein with a simple procedure. However, the restriction enzymes lacked one thing that CRISPR/Cas9 gene editing could do better, and that was target specificity. Throughout any given genome, four nucleotides can only create so many patterns in the six-nucleotide space that restriction enzymes target. Because of this, restriction enzymes can often end up cleaving DNA off-site, limiting the ability of genetic engineering beyond things like gene insertion in small vectors. This is why the development of CRISPR was such a monumental feat in science, as it had allowed scientists to explore the genome in ways they couldn't before. This included experimentation with gene function, including using CRISPR to knock out genes to determine their function. However, it is arguable that the lack of specificity in restriction enzymes that limit the capabilities of gene editing had a positive influence. This prevents the use of genetic engineering from being used in controversial ways.

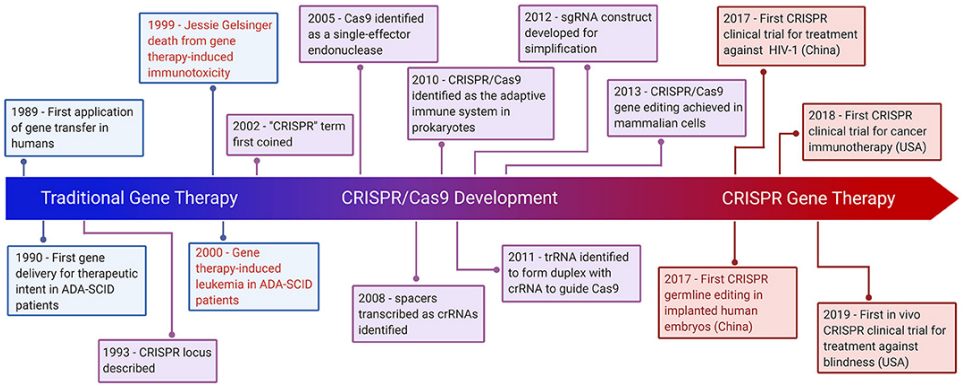

FIGURE 2. This chart shows the development of CRISPR/Cas9 gene-editing from the use of traditional gene therapies to modern gene editing using CRISPR (Uddin et al., 2020).

Introduction to HIV-1

Human Immunodeficiency Virus 1 (HIV-1) is a virus that infects different cell types through multiple receptor binding sites. It is a target for CRISPR/Cas9 gene-editing technology, because it is a retrovirus, meaning that it inserts its DNA converted RNA into the human genome. As a result, it uses human cell machinery to transcribe and translate the HIV genes to produce more viral proteins and assemble more of the virus. Sickle Cell Disease is also a result of abnormalities in the human genome. In this case, it is a mutated human gene causing a malformation in a human hemoglobin protein. CRISPR can create treatments for this disease by removing and replacing the harmful mutations and DNA sequences in several ways.

Academic Context

HIV-1 (Human Immunodeficiency virus 1) is an immunodeficiency virus and the most common among its counterpart HIV-2, however, both viruses are known to cause the often fatal or terminal AIDS disease (acquired immunodeficiency syndrome). HIV-1 is also well known as a sexually transmitted disease. HIV-1 retroviral infection is achieved when a virus inserts its genome into a target cell, which is most frequently immune cells. After the viral entry into the target cell, the viral genome is then incorporated into the cell genome, and this has proved difficult to remove safely from a human cell. However, with new research in CRISPR gene editing, new treatments for HIV-1 have been explored. These treatments, however, have not been researched to determine effectiveness, and are not yet available for all patients.

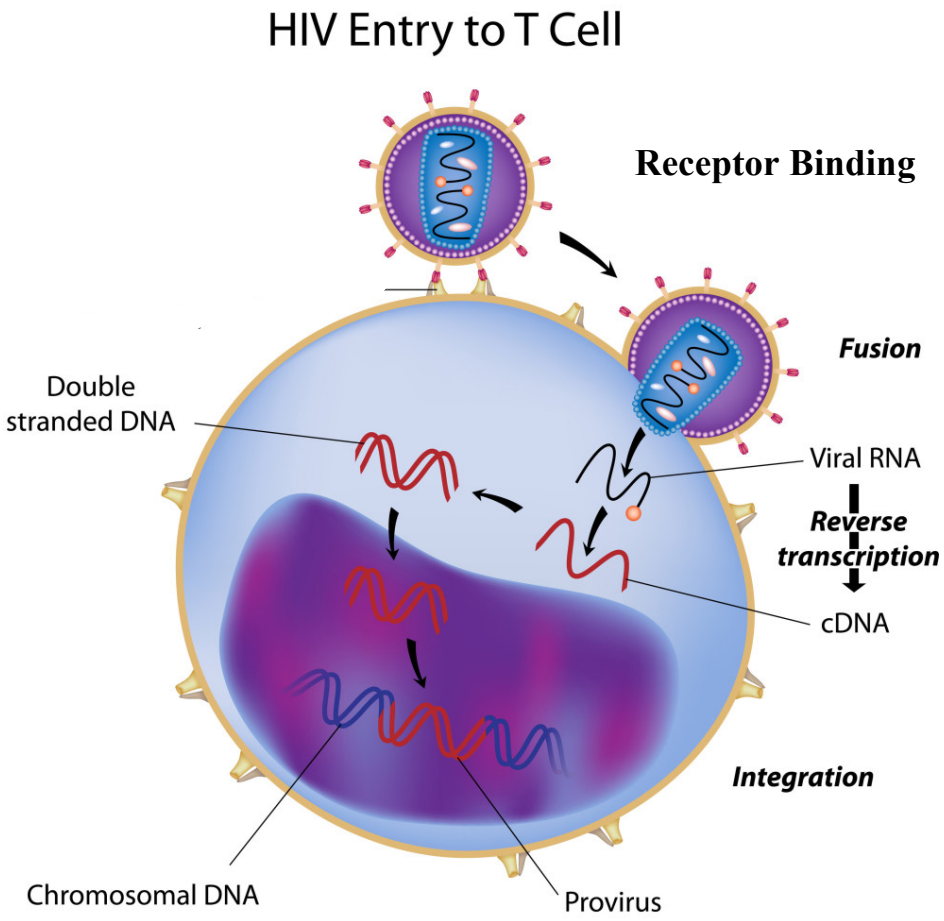

FIGURE 3. Outline of HIV-1 viral infection in human cells. Sequential stages of infection are shown as the infection progresses through receptor binding and DNA integration (Interactive Biology, n.d.).

This diagram illustrates HIV-1 cell infection. The virus first binds to the target receptor, such as the CCR5 receptor present on many helper T cells. This then allows the virus to fuse its membrane with the target cell and insert its viral RNA and contents such as proteins into the cell. The reverse transcriptase converts the viral RNA to cDNA (complementary DNA) as it is transported to the nucleus. This process is very important since, without the conversion into DNA, an RNA sequence will not be able to be integrated into the human genome composed of DNA. After the viral DNA is integrated into the cell DNA, it is officially in its proviral state and can use the cell machinery to transcribe and produce viral components to reproduce and infect more cells.

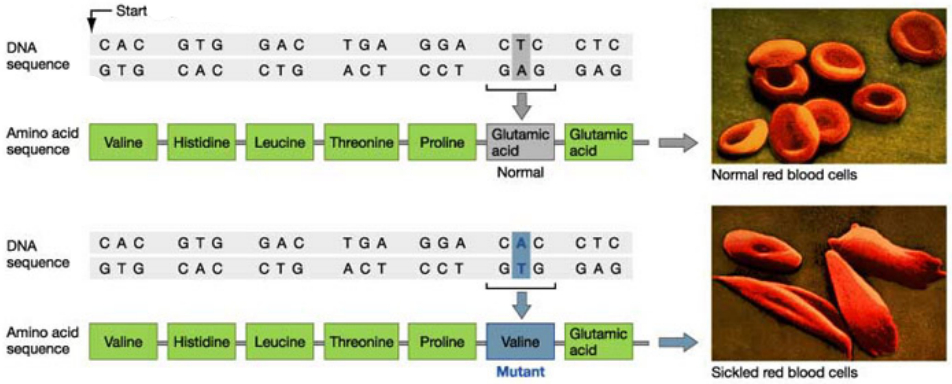

FIGURE 4. The causative gene mutations of Sickle Cell Disease. The grey rows indicate the nucleotide sequences of the corresponding hemoglobin gene in each person. The first row shows a healthy gene, while the second shows a mutated gene causing SCD. The green rows indicate which groups of nucleotides encode which amino acids in their protein sequence. In the first green row, the non-mutated gene encodes a glutamic acid amino acid in its chain whereas the second mutated row encodes a valine amino acid (McKinney et al., 2021).

As Figure 2 shows, the mutation in the adult beta hemoglobin gene results in the nucleotide sequence encoding valine instead of glutamic acid, which induces the hemoglobin amino acid chain to form a sickle-shaped protein (McKinney et al, 2021). Specifically, a healthy hemoglobin gene would have a nucleotide set of CTC-GAG, encoding the glutamic acid. However, the mutation results in a switch of the nucleotides adenine and thymine. This results in the sequence CAC-CTG, encoding the amino acid valine. Due to the mutation of a gene in the human genome, the resulting Sickle Cell Disease can be difficult to treat as it affects all cells in the body. However, most importantly, it affects those producing hemoglobin in red blood cells. These cells, called hemopoietic stem cells, are part of the bone marrow cells that produce a diverse array of cells in

the blood. Since the disease is caused by a genetic mutation, a practical treatment method is the CRISPR/Cas9 gene-editing complex. There are multiple applications of this method of treatment, and many have been effective in clinical trials (Frangoul et al., 2021) which gives hope to SCD patients everywhere.

Earlier treatments to sickle cell disease include methods like blood transfusions, which is when a healthy donor gives blood to a patient of a matching blood type with sickle cell disease via transfusion. This requires lifelong visits to the hospital and can even result in dangerous and potentially deadly effects like iron overload. AS a result, this traditional treatment method is less reliable than more modern methods like genetic engineering and stem cell treatments. One reason for this is because CRISPR provides more specificity for stem cell treatments, reducing the risk of rejection, and complications associated with blood transfusions like iron overload can be eliminated.

Methods

In this paper, I have compiled research based on keywords like CRISPR, Cas9, Sickle Cell Disease, and HIV-1. I used multiple search engines to find reputable sources regarding the target topic and found multiple articles to explain these diseases and their treatments. I then used keywords in researched treatment methods like hematopoietic stem cells to find further research and clinical trials on the topics. Sources I used included NCBI and NIH.gov, HIV.gov, and science magazines like ScienceDaily and nature.com. Sequences of genes and proteins were derived from national databases like Genebase, NCBI, genome.gov, and Embase.

Another paper was published in early 2021 by researchers at the University of Illinois and Chicago (Frangoul et al., 2021). This paper contained the research from Dr. Frangoul et. al's lab. It described how CRISPR technology had been used to cure Sickle cell disease in a patient's blood cells. This was done by deleting the gene responsible for suppressing fetal hemoglobin, called BCL11A, and the mutation in the beta hemoglobin gene responsible for SCD. Fetal hemoglobin is only produced by the fetus in vitro and is suppressed after birth. Then the adult hemoglobin begins to be produced. The mutation for SCD is in the adult hemoglobin, which means that removing the suppression of fetal hemoglobin will allow a healthy production of red blood cells (Frangoul et al., 2021).

Sickle Cell Disease is a genetic disorder caused by a mutation in the beta hemoglobin gene. This mutation causes the red blood cells in the body to develop into a crescent moon shape or a sickle shape. This disease affects approximately 100,000 Americans with a disproportionate number of cases occurring in African Americans and Hispanic Americans. This connection has significant impacts on societal aspects, as disadvantaged minorities statistically have less access to healthcare and make up a large

population of lower-class Americans. This means that those who are already disadvantaged face more of an affinity for diseases like SCD, which further perpetuates the cycle of inequity and poverty associated with race. The beta hemoglobin cannot be simply removed, because then the body would have nothing to circulate its oxygen. Since Sickle Cell Disease (SCD) is a genetic disorder, and research has been done into many treatments, some of which with CRISPR. To remove, repress, or replace the mutated hemoglobin gene, a gene-editing complex functional in human cells needs to be engineered. CRISPR has proven to be a worthy candidate as it is much simpler than other gene-editing techniques used, and is faster, more efficient, and more affordable.

Abstract on CRISPR and HIV/SCD treatments

One paper; "B cells Engineered to Express Pathogen-Specific Antibodies Protect Against Infection" was published in 2019 by immunology researchers at Fred Hutchinson Research center on this topic using CRISPR-Cas9 gene editing complex to engineer naïve B-cells. Naïve B-cells are a type of immune cell that is not developed enough to function, in other words, it is still "learning" what signals should trigger an immune response. Since B cells function by receiving immune signals from other cells and then producing antibodies according to the antigens, engineering them can create a new means of HIV-1 treatments (Moffett et al., 2019). In this paper written by Dr. Howell F. Moffett et al., they use CRISPR-edited vectors to transport genes encoding antibodies to prevent HIV-1 antigen binding. These vectors are then inserted into naïve B cells to "teach" them how to respond to HIV-1 antigen detection (Moffett et al. 2019).

In the figure below, the representation of engineered B cells works for Respiratory Syncytial Virus (RSV) immunity, however, similar principles and methods apply to HIV-1 treatment and immunology. Researchers proposed using genetic engineering to treat viruses by inserting artificial antibodies into immune cells that would fight off disease. This was successfully used in the case of RSV (Moffett et al., 2019).

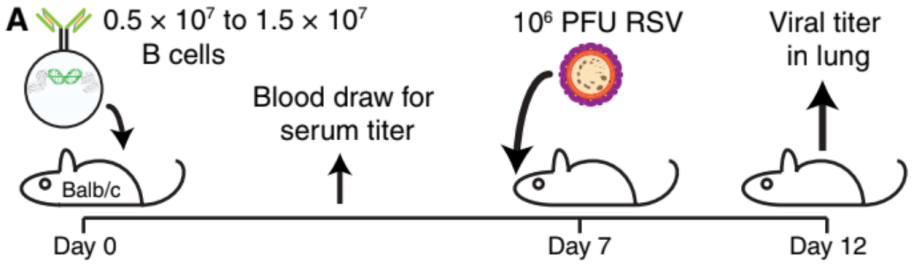

FIGURE 5. Adapted from the article "B cells Engineered to Express Pathogen-Specific Antibodies Protect Against Infection" (Moffett et al., 2019).

This figure was adapted from the article "B cells Engineered to Express Pathogen-Specific Antibodies Protect Against Infection" (Moffett et al., 2021) and represents engineered monoclonal antibody treatment (emAb treatment) in mice for a Respiratory Syntactical Virus infection (RSV). Engineered monoclonal antibodies are made to bind to specific antigen sites on a protein, which allows for inhibition of viral and bacterial infection if natural antibodies are not able to, or not effective enough to bind to the antigen sites (Moffett et al., 2019). The emAbs are inserted into the mice B cells to fight the RSV infection in different groups. The B cells are the cells that produce antibodies, so engineering them to make the RSV-specific sequence, will help the body fight the infection more independently after treatment. The emAb treatment was successful in finding that it was efficient in inhibiting and protecting the mice from RSV in mice.

Testing the genetically engineered antibodies on different immune systems is effective because HIV-1 has a prominent affinity for infecting T cells. In treating different forms of HIV, HIV infection of T cells can be inhibited via an HIV infection. By ensuring treatments in immunocompromised patients' effectiveness, we can also ensure that it will function on those who have a higher level of infection. This also works on those with preexisting immunocompromised systems before becoming infected with HIV-1, therefore allowing a larger population for treatment in the sense that the emAb sequence will not induce an adverse immune response.

Genetic engineering provides new hope for patients with SCD and scientists, as multiple methods to treat the disease effectively and permanently are being developed. These treatments include using CRISPR to cut the dangerous mutated hemoglobin gene from the human genome and promoting what would be a suppressed hemoglobin gene called fetal hemoglobin. The fetal hemoglobin gene is only expressed in vivo of a fetus, and once born, the body makes the shift to the adult hemoglobin genes. If adult hemoglobin is mutated to cause SCD, then fetal hemoglobin provides a healthy alternative for the person's cells and can be reused with a matter of selective activation, which can be done using CRISPR-based treatment. This can provide healthy red blood cells for those affected with SCD (Frangoul et al., 2021).

Traditional treatments for SCD include blood transfusions from a healthy donor, however, there are multiple issues. One challenge is that it is a very temporary treatment, and it has to be done multiple times a month for it to be effective enough to improve quality of life. There are also risks if the donor was not a perfect match, and the patient could develop an iron overload. These treatments require frequent hospital visits and there is currently no cure.

Allogenic stem cells are harvested from a healthy donor. The allogeneic stem cell transplant is used to achieve a permanent cure for Sickle Cell Disease by replacing diseased cells with healthy ones that

persist over long periods. This is preferable in comparison to traditional treatments like blood transfusions as it is more permanent, less risky, and won't require patients to have lifelong regular hospital visits [9]. Initially, stem cell transplants for SCD included bone marrow grafts from healthy viable donors, which allowed the patients to make their normal hemoglobin in their bodies, providing a more permanent solution. One of the major limitations of this treatment was the risk of rejection. If the body does not accept the cells grafted into the body, it could cause an immune response and be very dangerous for the patient. CRISPR however, provides a solution to this limitation by reducing the risk of rejection to almost zero by engineering stem cells from the patient themselves to remove, replace, or suppress the mutate hemoglobin gene so they can produce healthy blood cells on their own. This is one promising possibility in the treatment of SCD. Stem cells will begin to produce healthy hemoglobin when correctly grafted into the bone marrow.

Stem cell treatments are a fairly new treatment. The introduction of CRISPR had inspired an even more innovative alternative treatment, which is the genetic modification of stem cells. This allowed patient specificity and reduced treatment risks, both in comparison to traditional treatments and normal stem cell transplants. This treatment was made possible by CRISPR/Cas9 gene editing, as previous gene-editing techniques would have resulted in off-site DNA cleavage, resulting in treatment failure.

Another paper was published in early 2021 by researchers at the University of Illinois at Chicago. This paper describes how CRISPR technology had been used to cure Sickle Cell Disease in patients' blood cells. This was done by deleting the gene responsible for suppressing fetal hemoglobin, called BCL11A, and the mutation in the beta hemoglobin gene responsible for SCD using CRISPR. Fetal hemoglobin is produced in utero. This gene is suppressed after birth, and the uterus encourages the adult hemoglobin to begin to be produced. The mutation for SCD is in the adult hemoglobin, which means that removing the suppression of fetal hemoglobin will allow a healthy production of red blood cells. Future research into this topic includes how HIV-1 researchers have invested interest in the endocytic nuclear targeting system. This system is used by the virus to enter the cell and the nucleus to incorporate its genome into human DNA without being harmed. This mechanism can be used to treat SCD, and the knowledge taken from HIV-1 research inspires future treatments for SCD.

New HIV infection can be blocked by directly targeting pre-integrated proviral dsDNA and disrupting early-stage host dependency factors such as the co-receptor CCR5. C-C chemokine receptor type 5 (CCR5) is a protein on the surface of white blood cells that are involved in the immune system as it acts as a receptor for chemokines. CCR5 plays a vital role in HIV infection due to its major involvement in viral entry, which could lead to a new means of treatment. In a 2009 research article

published by the Indian Journal of Sexually Transmitted Diseases and AIDS, a therapeutic HIV infection strategy has included CCR5 inhibitors. CCR5 inhibitors are a new class of antiretroviral drugs used in the treatment of human immunodeficiency virus (HIV). They are designed to prevent HIV infection of CD4 T-cells by blocking the CCR5 receptor. The connection to CRISPR is between a human receptor gene mutation resulting in natural HIV resistance and the induction of this genetic modification to benefit a patient's viral resistance. The mutation occurs in the gene encoding the CCR5 protein so that it is no longer recognized by HIV which prevents virus binding of the viral antigens, thus preventing infection. This is one method that can be used to create an HIV resistance; however, limitations include that ubiquitous genetic modification amongst many different cell types has not been done in vivo and can be invasive to extract the cells for modification (Rao, 2009).

The comparison between previous gene-editing techniques, like restriction enzyme employment, and modern methods, like the CRISPR/Cas9 complex, gives an important perspective on progress and development in medicine and technology. Older methods are still very applicable to our lives today, as things like restriction enzymes play a large role in the development and synthesis of medicines, which some people cannot live without. New technologies like CRISPR can play a significant and foundational role in future medicine and technology. Researchers are using CRISPR to develop new treatments for diseases like cancer and Alzheimer's; this can play a pivotal role in the health of future generations. There are still many arguments being made however to whether or not the use of genetic engineering as effective as CRISPR can be applied ethically. It is ethically questionable to use CRISPR germline cell editing in future research.

Ethical issues

CRISPR is a very efficient, affordable, and specific way to accomplish gene editing. This poses a new possibility for innovation; however, it also poses some ethical issues. One highly controversial example is human germline genome editing. In 2018, Chinese professor He Jiankui had produced the first genetically altered babies. According to ScienceMag, He and two collaborators forged ethical review documents and misled doctors into unknowingly implanting gene-edited embryos into two women (Normile, 2019). One mother gave birth to twin girls in November 2018; it has not been made clear when the third baby was born. He's research was centered around genetically modifying an embryo from a couple with an HIV-positive father, and attempting to create HIV resistance. The court ruled that the three defendants had deliberately violated national regulations on biomedical research and medical ethics, and rashly applied gene-editing technology to human reproductive medicine. After pleading guilty, He was sentenced to 3 years in jail. This is a radical example of those in the scientific community experimenting

with genetic engineering. However, the issue is with how not only the governmental regulations on the ethical use of genetic engineering are participating in the international conversation, but how the individuals invested in gene editing participate.

In the case of Professor He Jiankui, the ethical considerations of medical practices are regulated by the government and were made clear to the public. The ethical implications of Jiankui however, cast a different light on his research, where he justified his actions for the sake of sparing children of HIV/AIDS infection in the future. The variation of ethical perspectives between the different parties creates regulatory problems, in terms of what research is permitted. This brings to light how the future of medicine can be affected if CRISPR is applied. It is important to balance both positive and negative impacts.

Many who have expressed strong opinions about the ethical implications of gene editing often focus on the alteration of the human genome. Most of the conversation involves how to restrict the genetic "enhancement" of future children. If nothing else, this shows the egocentric nature of the human mind, collectively, and individually. While it is true that humans are egocentric, and subconsciously collect their thoughts around their fears, could part of this conversation be the selfish instinct of humans compelling us to evade our inevitable fear of obsolescence, age, and mortality?

Conclusion

Human Immunodeficiency Virus affects a large population of people and has been proven difficult to treat as a retrovirus. Sickle Cell Disease affects a smaller population of people, but it is a lifelong debilitating illness. Even though both afflictions are very different in scope, they both are based on the human genome, and working to make them treatable without harming the healthy DNA has been given potential with CRISPR/Cas9 gene editing. It also presents a monumental innovation in gene editing, medicine, and science in comparison to previous methods. Potential CRISPR treatments for HIV-1 include engineering naïve B cells, CRISPR-based strategies using CCR5 receptors, inhibiting proteins like Tat, Rev, and LTRs, or eliminating latent pools of the virus using shock and kill, cutting proviral DNA out of the genome, and emAb treatments. More research was done into using CRISPR/Cas9 to successfully excise HIV-1 RNA from the human genome, however, further investigation must be done to apply this to an in vivo model like an HIV-1 positive patient. In the context of Jiankui's work of human experimentation, future efforts for medical science should be centered around editing somatic cells as opposed to germline cells in the prevention of ethical violations.

CRISPR has also been used to treat Sickle Cell Disease. CRISPR/Cas9 treatments for SCD include hemopoietic stem cell engineering and bone marrow grafts from viable donors. Stem cell engineering has included multiple methods for inactivating and/or

replacing the mutated adult hemoglobin gene. One method was to fully excise the mutated adult hemoglobin and insert a healthy hemoglobin gene from a short, synthesized DNA molecule. Another method included suppressing the SCD causing gene and using promoters to re-activate the fetal hemoglobin gene, which is only active in utero and becomes dormant after birth. These Sickle Cell Disease treatments have been proven to be a potential cure for patients and reduce the risk of rejection as opposed to donor grafts and are less risky and more permanent than blood transfusions.

For both illnesses, the biggest limitation of CRISPR/Cas9 treatments in vivo continues to be that CRISPR/Cas9 complexes cannot be used blatantly in human systems to prevent toxicity issues. While SCD can be treated by genetically editing stem cells outside the body, HIV is usually localized to T-cells. HIV-1 is more difficult to treat as it can spread from a single cell.

In the quickly advancing field of medical science, there are many ethical arguments to consider in research. He Jiankui's research is a case to study as it is quite controversial. Some support Jiankui and argue that it is beneficial to the human race to develop HIV resistance, and others argue that it is unjustifiable since there is no way for a child to consent to the procedure and accept the possible risks. In this case, the use of CRISPR leads to "designer babies". In this sense, genetic modification and germline editing becomes less regulated and is means for an arms race between families in an already competitive human nature. This can lead to regressing of social evolution by reducing forced adaption. Counterarguments include advancing science by measuring the implications of different research ventures individually, allowing the advantages of specified gene editing to intersect with the lives of human beings. The extensive reach of gene editing research can provide the improvement of both arts and sciences, to give diverse groups affected by things like HIV-1 and SCD the ability to make an impact in their fields.

The ethical controversies create enormous challenges for this new biotechnology. CRISPR is innovative and allows researchers and scientists to solve complex medical problems in a new, exciting way. The potential benefits to our society are huge. We could potentially control or eradicate some terrible diseases. The financial burden of treating these illnesses can be decreased which allows our society to use precious resources differently. However, there needs to be a careful balance between what we can do and what we should do. Medical ethicists need to be involved in all conversations about genome editing. No one is interested in creating designer babies, or picking and choosing genetic pools. We could treat pervasive illnesses in third-world countries that drain financial resources and instead divert these resources to feeding or creating infrastructure.

In healthcare, gene editing, both somatic and germline, can pose ethical issues beyond embryo alteration. This can include religious appositions to gene editing, as well as personal philosophies opposing the

treatments. If gene editing treatments are implemented in healthcare, it could spark controversy among ethical groups and private groups. Even when restricting gene editing's use in healthcare, it could be misused as examples like He Jiankui's research was for medical research. This means that the use of things like somatic cell gene editing can open the door for other treatment methods, including germline editing.

CRISPR/Cas9 gene editing has its limitations. This can be exemplified In its ability to be delivered in vivo within current treatments. People who contain genetic disorders, malformations, and/or mutations that exist within the body are present in cell populations that cannot be removed non-invasively. This means that the application of ubiquitous and specific genetic engineering within an in vivo model like the human body is highly sought after, and currently, not possible in CRISPR technology as the Cas9 enzymes can cause toxicity issues in human cells. Some perspectives, however, can argue that this is beneficial. This is because many gene-editing technologies can lead to the questionable applications of future research, and natural limitations can prevent humans from altering their current nature. Many ethical arguments for germline cell engineering are made in the prevention of starting an arms race between families, however, the use of genetic modification in vivo can create an arms race between people as well.

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