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Gene Therapy for Sickle Cell Disease: Present Insights

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ABSTRACT
Sickle Cell Disease (SCD) is an inherited red blood cell disorder resulting from a single-point mutation. It results in complications such as acute and chronic pain, infections, stroke, kidney disease, and heart failure that reduce the quality of life not only for affected persons but also for their families. It can be treated with drugs and bone marrow transplants, but there is no known cure for it. One of the potential cures is gene therapy, which involves adding, editing or silencing and correcting genes by bioengineering techniques including CRISPR/Cas9 system; zinc finger nucleases (ZNFs); transcription activator-like effector nuclease (TALEN) technology; and base editors. The therapy procedure involves the retrieval of affected genes from patient hematopoietic stem cells, engineering them ex vivo and reintroduction into patients as therapeutic genes. Researchers have achieved significant progress in the use of CRISPR technology by, amongst others, repressing BCL11A gene enhancers and editing erroneous genes via base modifications as well as prime edits. This has proven effective in the reduction of adverse impacts that SCD have on patients. Nonetheless, therapeutic genes can cause insertional mutagenesis and cancer after integration into the patient. Finding a significant cure for SCD via gene therapy is in the clinical stage; methods to combat therapeutic cancer-inducing genes, as well as expertise and infrastructure provision, are highly required. Gene therapy for SCD has room for further research and development.

Keywords: *SCD, Gene Therapy, CRISPR/Cas9, Mutation, Transplants*

1 Introduction

1.1 Brief Overview of Sickle Cell Disease (SCD) and Its Impact on Affected Individuals

The first description of sickle cell disease (SCD) was given in 1910 by James Herrick, who termed the disease due to abnormally sickle-shaped red blood cells (Herrick, 1910). Sickle cell disease is an inherited blood disorder that affects the production of hemoglobin. The condition makes the red blood cells sickle-shaped, and this may result in blockage of normal flow, which may cause injury or atrophy to vital organs. SCD affects millions of the world's inhabitants, predominantly members of the African population group as well as people descending from Mediterranean and Middle Eastern countries together with Indians. It can lead to multiple complications, such as acute and chronic pain, infections, stroke, kidney disease, and heart failure, resulting in a shorter lifespan. SCD is diagnosed through a blood test and can be treated via drugs, transfusions, and stem cell replacement. Interestingly, no established treatment is available to cure SCD, and still, many patients are restricted or deprived of getting enough access to quality treatments or research facilities (Brandow & Liem, 2022; The Foundation for Sickle Cell Disease Research; Kato et al., 2016; Yawn et al., 2014).

Imagine people are drowning in water, and inflated boats are needed to rescue them, but some of the boats have broken valves or holes in them, and in the course of rescuing the people, the boats are deflated. The shape of the boats will change, and the people will not be saved. In the same manner, red blood cells are shaped or structured for carrying oxygen; if the subunits of the hemoglobin within them are affected by an abnormal amino acid, their shape will change and the body will lack oxygen. This will in turn weaken the organs of the body, which depend on oxygen to generate ATP via the electron transport chain.

It is important to note that SCD is derived from

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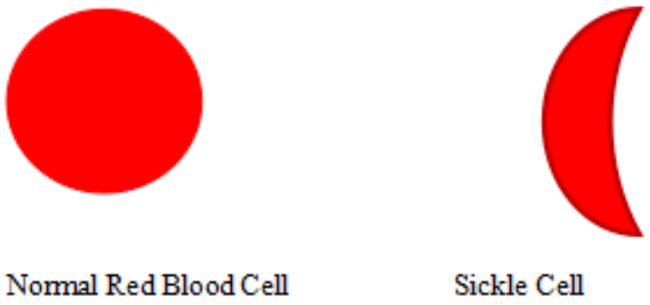

Figure 1: A Normal Red Blood Cell and a Sickle Cell

a one-point mutation. It is explicitly described as a monogenic disorder caused by a single base-pair point mutation in the β-globin gene, replacing valine for glutamic acid in the β-globin chain (Inusa et al., 2019). The unusual valine amino acid at position 6 in the beta-globin chain has a weak interaction with the adjacent sickle hemoglobin molecule (Harvard Medical School, 2013). The sickle shape of the red blood cells is due to valine and later results in SCD.

The presence of sickle cell disease results in the emotional drainage of the parents, and this affects interpersonal relationships between them and other family members (Burlew et al., 1989). A critical review of 116 articles emanating from the search of four different databases focused on the impact of sickle cell disease in Nigeria, and this country was identified as having the highest global burden of sickle cell disease (Adigwe et al., 2023). A cross-sectional survey was used to look at 2,145 SCD patients from seventeen different countries throughout the world. In their statements, the patients testified that the disease imposed a significant load on them regarding their quality of life while they were suffering from it and led to an impairment of their emotional state (Osunkwo et al., 2021). The presence of a sickle cell trait has stopped lovers from having weddings because they are either sickle cell carriers or both partners carry the disease. If a cure for SCD is discovered, the quality of life for patients and carriers will significantly improve. Even though there is no known treatment for sickle cell disease, gene therapy can offer a revolutionary solution to this condition. This review aims to harness information on emerging cures for sickle cell disease via gene therapy.

2 Gene Therapy and Its Potential as a Treatment for SCD

Gene therapy refers to a genetic engineering process through mutated gene alteration, correction, or specific target modification aimed at treatment (Paulo, 2017). Several gene therapies differ significantly and include the removal of the damaged gene and its replacement with a healthy one, the blocking or suppression of mutated genes, and the introduction of new or modified genes into the organism (FDA, 2022). Gene therapy is used in the management of some inherited diseases like hemophilia and sickle cell disease; however, it can be applied to treat acquired disorders like leukemia (NHGRI, 2022). As a future curative approach, gene therapy is questionable for its effectiveness, making it necessary to conduct sufficient research work, clinical trials, and regulate gene treatment before applying it to patients (MedlinePlus, 2022). Currently, there is no permanent solution to SCD; however, some procedures that can be offered are medicine alongside an allogeneic hematopoietic stem cell transplant from a healthy donor. SCD is either treated by using drugs or through transplantation; at non-serious levels, patients treat their illnesses with medication, but those with severe cases need a transplant. Nevertheless, some problems may occur because of poor matching and a shortage of blood donors (Dever et al., 2016). As a consequence, it is crucial to have a substantial cure for each type of SCD whether mild or severe. Gene therapy is a possible emerging cure for SCD. It is constituted by hematopoietic (blood-forming) stem cells (HSCs) harvesting, ex vivo modification with a vector gene carrying the normal or antisickling globin gene, and

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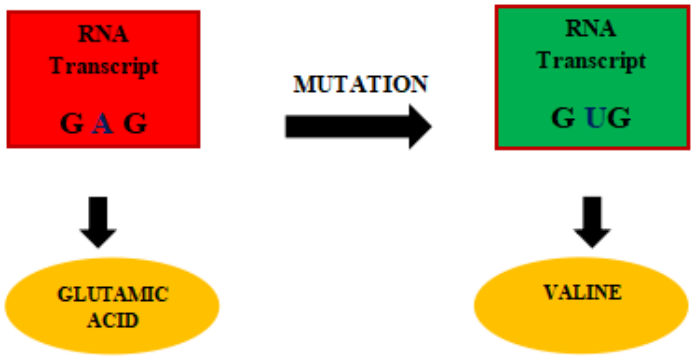

Figure 2: Single Point Mutation Leading to the Production of Valine

reinfusion after chemotherapy to allow engraftment of the corrected cell (Abraham & Tisdale, 2021).

Allogeneic blood or marrow transplantation is often limited by donor availability and graft-versus-host disease; gene therapy can circumvent these barriers (Kanter & Falcon, 2021). On the other hand, gene therapy has some risks, including insertional mutagenesis, which may result in leukemia or other malignancies (Jones et al., 2021). Advancements in the latest gene editing technologies like CRISPR have made up for decreasing its risks and enhancing the efficiency of gene therapy via exact, long-lasting modifications to the genome. The first CRISPR-edited gene therapy for SCD was approved by the FDA in 2023 (FDA, 2023). Gene therapy remains an experimental and expensive treatment that requires specialized centers and skilled personnel. People with SCD with extreme severity tend to show a high level of risk for gene therapies, but they still need information and counseling to be sufficiently informed about making decisions (ASH, 2023).

2.1 Types of Gene Therapy Currently in Use in SCD and Their Potential Risks and Benefits

There are four main types of gene therapy for SCD: gene addition, gene editing, inactivation or silencing of genes, and gene correction. The strengths and weaknesses of each group depend on safety, effectiveness, and feasibility. Gene therapy for SCD is still at a clinical trial level, and some researchers have demonstrated success in fetal hemoglobin (HbF) induction with lasting effects associated with relief from transfusions, increased antisickling Hb levels, and prevention of vaso-occlusive crisis (VOC) episodes (Abraham & Tisdale, 2021). On the other hand, gene therapy also introduces various barriers and challenges that need to be addressed before it can become a viable option for SCD patients, such as cost, infrastructure, side effects, and social and ethical issues. Gene therapy is not an all-in-one solution, but a targeted and sophisticated intervention that must be guided by personal considerations, inclinations, and hazardous prospects when applied to patients' health (Kanter & Falcon, 2021).

Gene addition refers to the incorporation of a healthy or bioengineered gene into a patient's hematopoietic stem cells using an internal vector, like lentivirus or adeno-associated virus. The goal of gene addition is to increase the expression of normal or antisickling globin genes such as gamma-globin or beta-globin variants (HbA2 or HbG16A). These expressions can impede the effect of sickle globin (HbS) gene expression. Among the different SCD gene therapy techniques, gene addition is the most advanced type that has been scientifically proven to be safe in several clinical trials for reducing vaso-occlusive crises, transfusion needs, and complications (Abraham & Tisdale, 2021). Gene addition therapy relies on the process of lentiviral transduction and applies autologous HSC-targeted gene therapy that binds to proper genes (Dever et al., 2016). Evidence highlights that lentiviral transduction is successful in terms of sustainability and long-term gene expression (Pirona et al., 2020).

Gene editing uses molecular tools to make modifications in the patient's genome. These changes have been beneficial using either zinc finger nucleases or transcription activator-like effector nucleases, among others. Another area of gene editing is using transcription factors to disrupt gene

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Figure 3: Steps in HSC Gene Therapy

enhancers such as BCL11A or LRF/ZBTB7A, which normally suppress the production of HbF. Gene editing intends to rebalance HbF and HbS or eliminate sickle hemoglobin (HbS) in place of normal beta-globin (HbA). Gene editing is a recent type of gene therapy that still needs several clinical trials and long-term observations to assess its safety and efficiency (Abraham & Tisdale, 2021). CRISPR/Cas9 can be used to modify the HbS gene directly. The process involves using a patient's bone marrow cells and editing them in vitro. Genome editing can also reverse the sickling of red blood cells by promoting fetal hemoglobin production (Parc & Bao, 2021).

Gene silencing inhibits the expression of specific genes, including alpha-globin or erythropoietin receptors, associated with SCD pathophysiology. Mediators for this gene silencing include siRNAs and shRNAs. HbS knockdown may revert to normal hemoglobin, abolish or reduce HbS, or modulate the erythroid response in hypoxic and inflammatory conditions. Despite many positive findings, researchers are still at the pre-clinical stage regarding gene silencing for SCD because siRNAs or shRNAs fail to get delivered to target cells and prevent off-target effects (Abraham & Tisdale, 2021). The mechanism involved in gene silencing also includes regulating the expression of a particular gene to keep its synthesis and consequent production of proteins restrained. This therapy is injected into the body, similar to gene editing, which suppresses BCL11A and leads to an increase in HbF while reducing HbS production (Kanter & Falcon, 2021). Gene silencing relies on transfer vectors, similar to gene addition, but targets messenger RNA to suppress gene products rather than cutting the specific sequence as done in gene editing.

Gene correction uses homologous recombination or base editing to repair the sickle mutation in the beta-globin gene without introducing any foreign DNA. Gene correction can restore normal beta-globin expression and function in the patient's red blood cells. Gene correction is also in preclinical development for SCD, with technical difficulties in achieving high efficiency and specificity of gene repair (Abraham & Tisdale, 2021).

3 Advances in Gene Therapy for SCD

Frangoul et al. (2021) developed a method to treat two extreme genetic abnormalities, namely -thalassemia and sickle cell disease. The scientists utilized an intervention approach by altering specific genes using a gene editing technique based on CRISPR-Cas9 applied to cells from healthy donors. By targeting the BCL11A gene, which reduces fetal hemoglobin production, they managed to alleviate the symptoms of these diseases. After genetic correction, the cells were transplanted back into two separate patients with -thalassemia and sickle cell disease. One year later, both patients showed remarkable improvement with high levels of edited genes in the bone marrow and blood, increased fetal hemoglobin throughout their cell types, no longer needing blood transfusions, and a reduction in painful episodes of sickle cell disease. This implies that the gene editing method using CRISPR-Cas9, targeting the BCL11A gene enhancer, proved effective in treating these genetic disorders, thus offering an alternative avenue for therapeutic intervention.

Zarghamian et al. (2023) focused on using modern DNA technology platforms, including CRISPR-Cas nucleases and base editors, to correct the defective gene resulting in SCD. These tools are designed to restore the production or functionality of fetal hemoglobin (HbF) that can compensate for the mutated hemoglobin (HbS). They discussed various approaches, either by rectifying specific genes responsible for inhibiting HbF formation or by correcting the mutant directly in the hemoglobin gene

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packaging. They noted that in animal model studies and early-phase human clinical trials, these genetic editing techniques have shown preliminary promise for efficacy and safety.

Levesque and Bauer (2023) commented on a new treatment for sickle cell disease (SCD) and other blood conditions through the prime editing procedure. CRISPR, and particularly prime editing, is a viable treatment option for the genetic mutation in hematopoietic stem cells (HSCs). Prime editing is a more advanced technique that allows for high-accuracy targeting without causing breaks in the double-stranded structure of the DNA, considered much safer. The conventional prime editing machinery contains a molecular entity called PE2, which is made of Cas9 nickase (an enzyme producing cuts on the DNA) and reverse transcriptase fused. This tool is aided by special RNA molecules targeting the site of interest in the HBB gene. Specific changes are made by the prime editor without requiring a DNA template. To enhance prime editing effectiveness, they incorporated another RNA component (sgRNA) to generate a nuclease-induced DNA break in a single-stranded form. This helps the cell's repair machinery incorporate the desired genetic correction more effectively, although it introduces small genetic changes (indels) at the target site. The advantages of prime editing over other methods, such as base editing, include its ability to target non-dividing stem cells more efficiently and its flexibility in making precise changes without some of the limitations seen in other gene-editing techniques.

Casgevy and Lyfgenia are groundbreaking gene therapies recommended by the FDA for adults with SCD aged 12 and older. Casgevy works by CRISPR/Cas9 genome editing, while Lyfgenia uses lentiviral vectors and modifies the blood stem cells of patients with sickle cell disease to produce healthier hemoglobin that minimizes complications caused by the condition. These are one-time treatments that require pre-transplant chemotherapy and have been proven efficient in clinical trials. Both therapies obtained multiple FDA designations for fast-track development, marking a significant advancement in gene therapy for SCD (FDA, 2023).

4 Challenges and Considerations of Gene Therapy for SCD

Developmental gene therapy, with its goal of curing SCDs, is a complex and expensive application requiring experts. It involves appropriate infrastructure and rigorous clinical trials before it can be safely implemented to enhance human health (MedlinePlus, 2022). Gene therapy also has side or off-target effects, such as insertional mutagenesis, which can cause cancerous growth, potentially causing more harm than good in improving the health condition of a patient with SCD.

To mitigate the potential for hematologic malignancies (blood cancer) in Casgevy and Lyfgenia gene therapies, a black box warning is included in the labels, particularly for Lyfgenia, and patients will undergo lifelong monitoring. Long-term studies will assess the safety and effectiveness of both treatments (FDA, 2023). Baum et al. (2004) identified several cases of leukemia that were supposedly developed from an attempt to use retroviral transfer as part of clinical gene therapy. This method involved using retroviruses to insert therapeutic genes into blood cells. The issue of blood cancer appeared after the therapeutic genes accidentally activated a regular cell's proto-oncogen, which is an oncogene capable of producing tumors. Hackett et al. (2023) studied the use of the Sleeping Beauty (SB) transposon/transposase system in clinics. They observed that this bioengineering method assists in inserting Chimeric Antigen Receptors (CAR) into T cells. CARs act as navigation guides for T cells to target specific threats, such as cancer. Researchers may adopt this system to test the activity of therapeutic genes against cancer after the introduction of these genes into the patient.

Another possible way to reduce the effect of insertional mutagenesis is to change the type of vector used to insert the therapeutic gene(s). Lentiviral vectors can affect both stem and permanent cells and provide lasting stable gene expressions, but their transfer is not site-specific, which may lead to insertional mutations. The use of adeno-associated viruses is site-specific and safe, making it a preferable option for gene therapy (Zheng et al., 2018). The major drawbacks to developing and employing various types of gene therapies for SCD are not technical but financial. The costs

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are high for reagents like clinical-grade lentiviral and adeno-associated vectors, gene editing reagents, cell processing materials, personnel costs, and drug research and development costs. If the costs for transfer vectors production, cell processing, and other financially demanding materials improve, the cost of administering gene therapy to patients will decrease (Kohn et al., 2023).

Other difficult questions stemming from the treatment of SCD through gene therapy include the safety and efficacy of transgenic technologies, the consent process involving informed decisions for patients on this specific level of medical care provision, access and affordability of gene therapy by different socioeconomic groups in society, enhancement or modification of non-disease state traits, and the implications of germline gene editing for future generations. These concerns must be addressed with due deliberation and public participation to ensure that biotechnology, which involves gene therapy, is used appropriately and in a manner that respects both individuals and communities affected by SCD (Kanter & Falcon, 2021; Giacca, 2010; Spink & Geddes, 2004).

5 Conclusion

Gene therapy for sickle cell disease is still in its clinical stage. It has the potential to provide a lasting cure for SCD. It also has the advantage of overcoming the limitations of allogeneic blood transplantation, donor availability, and mismatching challenges. However, it comes with some off-target effects, such as insertional mutations, which may eventually develop into cancer. To mitigate the negative effects of this technique, current gene editing techniques such as CRISPR should be employed. There is also a need for expertise and adequate infrastructure to carry out more research and development in this area.

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