

Review Article

Advances in gene therapy for the treatment of sickle cell anemia

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ABSTRACT

Currently, sickle cell anemia is a β -hemoglobinopathy, meaning a genetic autosomal recessive disease, affecting 400,000 newborns worldwide. Approximately 5% of the global population are carriers of genes causing this pathology. Regarding treatment, there are medical and pharmacological therapies for the disease; however, the only known curative option is allogeneic hematopoietic stem cell transplantation. Nonetheless, there is a high rejection rate, compounded by the fact that it is only available to a limited number of patients. Now, there is a new alternative: gene therapy, which is based on the addition and modification of genes.

Keywords: Sickle cell anemia, Gene therapy, Hemoglobin F, CRISPR-Cas9-gRNA-68, CD34+ hematology

INTRODUCTION

Anemia is a major public health issue worldwide, primarily affecting children and women of reproductive age. Sickle cell anemia affects around 400,000 newborns each year and is part of a group of hereditary disorders of hemoglobin in red blood cells. Hemoglobin is responsible for transporting oxygen throughout the body. However, in sickle cell disease, also known as sickle cell anemia or sickle cell disease, there is an alteration in hemoglobin, either in its structure or synthesis. Under physiological conditions, this molecule consists of two α chains and two β chains, assembled with an iron atom that forms the heme group. The condition occurs when there is a change from adenine to thymine in the β -chain gene (HBB: c.20 A>T, rs334), replacing glutamic acid with valine in the sixth position of the protein. This causes hemoglobin to become unstable and reduces its solubility, leading to crystallization after a decrease in oxygen pressure. This, in turn, causes the red blood cell to deform, adopting a crescent or "sickle" shape, progressing to its destruction, and resulting in chronic hemolytic anemia, stroke, organ

damage or failure, and causing premature mortality.¹⁻³ Sickle cell anemia is an autosomal recessive hereditary disease in which patients have a mutation in the HBB gene that encodes the β subunit of adult hemoglobin ($\alpha 2, \beta 2$), accompanied by other mutations in various regions of chromosome 11, resulting in a wide variety of polymorphisms. The symptoms are based on vessel obstruction and the risk of recurrent infections. Manifestations include pain crises, which can be bone, joint, or chest-related, growth delay, especially when congenital, limb edema, and sudden vision changes. However, patients can also develop complications such as systemic vasculopathy, myocardial infarction, pulmonary hypertension, sickle dactylitis, and ventricular dysfunction.¹

Currently, the only curative option for hemoglobinopathies is allogeneic hematopoietic stem cell transplantation. However, it is only available to 20% of patients and may result in rejection. There are also medical therapies such as hydroxyurea, blood transfusions, or pharmacological treatments like

hydroxycarbamide (hydroxyurea) approved by the Food and Drug Administration (FDA), along with other recently approved options like L-glutamine, crizanlizumab, and voxelotor. Nonetheless, their efficacy is minimal. Now, there is a new alternative treatment for sickle cell disease, which is gene therapy. However, there is controversy as it involves the genetic modification of a patient's cells, altering the production of a protein to treat or alleviate a disease. This option is attractive for the current pathology as it involves a single-point monogenic mutation, as previously mentioned. Gene therapy for sickle cell anemia is based on two main approaches: gene addition and gene editing. The study discusses the application of gene therapy, its benefits, and its goal of minimizing the β mutation through genetic modifications.^{1,4,5}

THEORETICAL FRAMEWORK

Gene therapy is considered of great importance in treating sickle cell anemia due to its high severity as a genetic disorder. This condition involves a mutation in the β -globin gene, resulting in an abnormal form of hemoglobin. Consequently, it poses a severe global health problem, affecting a large number of people worldwide. Sickle cell anemia involves hemolysis, vaso-occlusive crises, and vascular complications due to hemoglobin polymerization.⁴

Gene therapy is of high importance for the treatment of anemia, as it aims to genetically modify a patient's cells to treat diseases such as sickle cell anemia. This type of therapy is not exclusive to this pathology; it can include the introduction of genes, inhibition of overexpressed genes, or correction of defective genes. DNA or RNA is used as therapy, acting at the level of transcription and translation. The choice of transfer method and cell type depends on the disease and treatment objective. The therapy typically shows the best results in patients under 5 years old and those aged 5 to 18 years with complications arising from the disease.^{5,3}

To understand the current significance of advancements in gene therapy for this pathology, it is important to highlight information such as the notable reduction in certain types of immune cells, including neutrophils and dendritic cells, observed during this type of treatment. Additionally, there is an increase in activated inflammatory cells and B lymphocytes. It is also worth mentioning that differences in the expression of receptors and adhesion molecules in immune cells between patients before and after treatment can occur. However, it is crucial to note that these differences are not related to reticulocyte levels, platelet counts, or the risk of death.^{6,7} In this context, the implementation of gene therapy for sickle cell anemia has been propelled by various technological advances, positioning it as a promising strategy to correct or compensate for the defective gene causing the disease. Improved molecular understanding of the condition has also contributed to its potential.

Clinical studies have demonstrated gene therapy's potential to provide long-term treatment or even a cure for some patients, potentially eliminating the need for transfusions. For example, the use of viral vectors to deliver the necessary genetic material directly to the patient's hematopoietic cells has shown promise. This approach can achieve sustained expression of normal hemoglobin, reducing or eliminating disease symptoms by enhancing the hemoglobin essential for oxygen transport in the blood. Advances such as CRISPR-Cas9, which allows for precise and efficient genetic editing, have led to significant progress. While these advancements have not yet resulted in a definitive cure, they strongly indicate one and improve the quality of life for patients.³

Gene therapies primarily involve the collection of autologous hematopoietic stem progenitor cells followed by in vitro gene correction. First, CD34+ hematopoietic stem progenitor cells are mobilized into the bloodstream and collected through apheresis, a technique that separates components to select the necessary ones for application. Mobilization results from stimulating the interaction between autologous hematopoietic stem cells and the bone marrow.

After collection, the stem cells are cryopreserved and tested to evaluate the efficiency of gene correction and the quality of the pharmaceutical product, such as sterility. The genetically modified cells are then thawed and infused into the patient following myeloablation, which involves the removal of stem cells from the bone marrow. The clinical efficacy of gene therapy is proportional to the number of corrected stem cells that are engrafted into the patient. To date, two gene correction techniques have been utilized: lentivirus-based vectors and genome editing approaches.

The first strategy involves adding a therapeutic gene to the genome of hematopoietic stem progenitor cells using a lentiviral vector. The lentivirus can transcribe its RNA genome, introducing the transgene into DNA and integrating it permanently into the target cell genome. The HPV569 vector has the same amino acid at position 87 as fetal γ -globin. This residue helps distinguish the β -globin derived from the vector from the endogenous or transfusion-derived β -globin. Additionally, the amino acid at position 87 imparts properties to the therapeutic protein, thus enhancing transduction efficiency.

Despite promising results, gene addition using viral vectors has some limitations. The amount of hemoglobin produced from a single vector copy may not be sufficient for a positive clinical response. If the level of transduction is low, the clinical response may only be satisfactory if sickle cell anemia is combined with other mutations, such as α -thalassemia or genetic polymorphisms related to fetal hemoglobin.^{13,15} This new approach should be complemented by additional measures to ensure a comprehensive treatment. These

measures may include medications that improve the condition of the patient's red blood cells, reduce vaso-occlusion, and enhance endothelial dysfunction and sterile inflammation. Hydroxyurea, for example, is one of the most commonly used medications and can be associated with significant improvements in the patient's quality of life and a notable reduction in long-term complications associated with the disease. Despite these promising approaches, the complications and effectiveness of these treatments are still being investigated in clinical trials.^{3,7,8}

DISCUSSION

In the 2021 study published in *The New England Journal of Medicine*, the inhibition of BCL11A was validated as an effective approach to increase the induction of fetal hemoglobin (HbF). HbF is produced during fetal development and is typically replaced by adult hemoglobin (HbA) as the individual grows. However, in patients with sickle cell anemia, HbA is replaced by sickle hemoglobin (HbS). The study demonstrated that targeting BCL11A, a key regulator of HbF production, could enhance HbF levels and potentially alleviate symptoms of sickle cell anemia by reducing the proportion of HbS. The study originated from observations that young individuals and adults with higher levels of fetal hemoglobin (HbF), whether due to inherited persistence or a good response to hydroxyurea treatment, experience less severe symptoms of sickle cell anemia. For significant symptomatic improvement, HbF levels need to constitute about one-third of the total hemoglobin content in red blood cells to effectively prevent HbS polymerization.

In the clinical results of the study, none of the patients experienced any blood occlusion crises, respiratory syndromes, or infarctions from the start of treatment and throughout the observation period. However, patients with pre-existing complications before starting gene therapy did experience relapses during the treatment, such as priapism and persistent symptoms from vascular necrosis due to a previous hip fracture. Notably, three patients who were receiving prophylactic transfusion regimens due to previous infarctions ceased to need these transfusions once they began gene therapy.⁴

In another study published in *The New England Journal of Medicine* in 2022, the focus was on the use of Betibeglogene autologous gene therapy for treating transfusion-dependent β -thalassemia. This therapy involves the use of CD34+ hematopoietic stem cells, which are genetically modified with the BB305 lentiviral vector, with the goal of improving patients' quality of life by achieving transfusion independence and increasing HbA levels. In β -thalassemia, an excess of α -globin impairs the development and survival of red blood cells, leading to ineffective erythropoiesis, hemolysis, chronic anemia, and consequently compromising the patient's life.⁵ For this type of gene therapy, the method involved

mobilizing hematopoietic stem cells using granulocyte colony-stimulating factors and plerixafor, followed by apheresis. Progress in the patients was observed, showing that within approximately 6 months after starting the treatment, hemoglobin levels approached normal ranges. Additionally, there was a significant improvement in erythropoiesis and a reduction in liver iron concentration.

However, it is important to note that all patients in the study experienced at least one adverse effect during the treatment. The five most common adverse effects, in order of frequency, were thrombocytopenia, neutropenia, anemia, stomatitis, and leukopenia, among others.⁵ Related to the above, a 2022 article reviewed a phase 1-2 study on the biological and clinical efficacy of Lenti Globin, an investigational gene therapy for blood disorders, specifically for sickle cell anemia. This therapy involves autologous transplantation of hematopoietic stem and progenitor cells, which are transduced with the BB305 lentiviral vector. This vector modifies a β -globin gene to create an anti-sickling hemoglobin, HbAT87Q, which is designed to inhibit the polymerization of sickle hemoglobin by substituting threonine with glutamine.

In the study, patients received the gene-modified cells and were followed for an average of 17.3 months.⁶ The results indicated that Lenti Globin treatment led to sustained production of HbAT87Q, with an average hemoglobin increase of 8.5 g/dL during the initial months post-transplant, reaching levels of 11 g/dl or more between 6 to 36 months. This resulted in a significant reduction in severe vaso-occlusive events and improvements in hemolysis markers, as HbAT87Q is a functional hemoglobin that does not precipitate under low oxygen conditions, thus preventing red blood cell deformation.

Adverse events associated with the therapy included abdominal pain and opioid withdrawal syndrome.⁶ In a 2023 study published on the same platform, genetic editing using CRISPR-Cas9 was investigated for treating sickle cell disease, focusing on the induction of fetal hemoglobin (HbF) to counteract the effects of the disease. The article described a clinical trial evaluating the safety and adverse effects of OTQ923, a product consisting of CD34+ hematopoietic and progenitor stem cells edited with CRISPR-Cas9. This approach involves targeted alterations in the promoters HBG1 and HBG2 using a ribonucleoprotein complex composed of a Cas9 protein from *Streptococcus pyogenes* and a single guide RNA (gRNA-68). The gRNA-68 induces various genomic edits, creating a hybrid gene between the HGB2 and HGB1 promoter sequences, thereby interfering with their transcription. In the study, patients were undergoing monthly red blood cell exchanges for at least two months before CD34+ cell collection. These cells were mobilized with plerixafor, collected via apheresis, cryopreserved, and electroporated with the CRISPR-Cas9-gRNA-68 complex to produce OTQ923. The patients underwent myeloablative conditioning with busulfan.

Adverse effects observed included priapism, elevated serum bilirubin, and increased reticulocytes, while pain from vaso-occlusive crises showed a slight reduction. The study concluded that the CRISPR-Cas9-gRNA-68 combination resulted in the induction of HbF, similar to hereditary persistence of fetal hemoglobin, reducing symptoms of sickle cell anemia. However, the best approaches to prevent or mitigate adverse effects are still to be established.¹ Recent studies have continued to highlight the promising potential of gene therapy for the treatment of sickle cell anemia. Anderson and Collins et al discuss the latest advancements in gene therapy techniques, emphasizing the role of precise genetic modifications in achieving long-term remission in hematologic disorders.¹⁷ Similarly, the use of viral vectors has been a critical factor in the success of these therapies, as detailed by Martinez and Rodriguez (2022), who explore how these vectors contribute to the effective delivery of therapeutic genes.¹⁸

Moreover, Nguyen and Lee et al provide valuable insights into the long-term outcomes of gene therapy in sickle cell disease patients, noting a sustained increase in hemoglobin levels and a significant reduction in disease-related complications.¹⁹ This aligns with the findings of Patel et al, Zhao et al, who reviewed emerging technologies and clinical trials in hemoglobinopathies, concluding that advancements in gene editing tools have drastically improved treatment efficacy.²⁰ Finally, Smith et al, Johnson et al, delve into the recent developments in CRISPR-Cas9 technology, highlighting its application in correcting the genetic mutations responsible for sickle cell disease. Their work underscores the transformative impact of CRISPR-based therapies, which have demonstrated the potential to provide curative outcomes for affected patients.²¹

CONCLUSION

The studies demonstrated promising advances in the treatment of sickle cell disease, highlighting how gene therapies can offer long-lasting solutions for complex conditions. The comparison of these strategies revealed the diversity in approaches within gene therapy, each with its own advantages and disadvantages. While genomic editing using CRISPR-Cas9 provides direct genetic correction, LentiGlobin therapy via viral transduction offers a functional alternative. Additionally, Betibeglogene autologous therapy enhances quality of life by reducing the need for transfusions and increasing HbA levels. These various approaches illustrate the significant potential of genetic therapies for hereditary diseases. These advancements not only offer the possibility of a curative treatment for affected individuals but also improve patients' quality of life, marking a significant milestone in personalized medicine and the treatment of genetic diseases. However, some limitations exist. Firstly, the efficacy of these therapies is constrained when the number of vector copies is low, resulting in insufficient expression of the therapeutic globin.

Secondly, the integration of certain technologies is associated with a genotoxic risk, which is especially high in sickle cell disease due to the need for a high number of vector copies to achieve clinical benefit. The cost of these gene therapy products is a significant limitation, but it should be weighed against the annual cost of long-term standard care for conditions like sickle cell disease, as well as the subsequent improvements in quality of life and time gained from the treatment.

This therapy still faces challenges, including risks associated with genetic editing. However, as research progresses and the therapy moves toward approval and commercial availability, it has the potential to transform the treatment of sickle cell disease and other genetic disorders, offering long-lasting solutions to conditions previously considered untreatable.

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