JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

CRISPR CAS 9 AS A POTENTIAL THERAPY AGAINST SICKLE CELL ANEMIA

Arundhati rej, Madhurima Tiwari, & Neerja Masih*

Department of Biotechnology, Isabella Thoburn College, Lucknow, India.

ABSTRACT

Sickle cell Anemia is a severe inherited blood disorder that develops due to a mutation in the beta chain protein of hemoglobin, a crucial protein in red blood cells responsible for carrying oxygen from the lungs to all parts of the body. This genetic alteration causes the red blood cells to assume an abnormal sickle or crescent shape, which can lead to blockages in the blood vessels. The blocked vessels can limit the amount of oxygen reaching the body tissues, causing intense pain and other complications such as organ damage, infections, and stroke. The current treatment options for sickle cell disease are limited and pose significant risks. However, gene therapy, specifically CRISPR-Cas9 technology, has emerged as a promising new treatment avenue for this condition. This innovative approach involves editing the DNA to correct the mutation that causes sickle cell anemia. This correction can potentially lead to healthy red blood cells that can carry oxygen efficiently and reduce the risk of complications. CRISPR-Cas9 is a revolutionary gene-editing technology that can accurately target and modify specific base pairs in DNA. Researchers use a guide RNA molecule to direct the Cas9 protein to the specific location in the DNA sequence where the mutation causing sickle cell disease occurs. The Cas9 protein then cuts the DNA, and the cell's repair mechanisms replace the mutated DNA with the correct version, fixing the genetic defect. Moreover, CRISPR-Cas9 technology demonstrates the potential to treat various other genetic disorders beyond sickle cell disease. The technology is still undergoing research and development, and more investigations are necessary to determine its safety and efficacy for treating sickle cell disease and other genetic conditions. In this review, we will delve into the details of treating sickle cell anemia through the application of CRISPR-Cas9.

KEYWORD: Gene therapy; Hematopoietic stem cell transplantation; Sickle cell Diseases; Gene delivery**;**

CRISPR CAS 9; Sickle cell anemia .

1. INTRODUCTION

About 100,000 people in the USA and over 3 million people worldwide suffer from sickle cell anemia, a type of hereditary genetic blood condition caused by abnormalities affecting the βglobin chain of hemoglobin. The condition is defined by a defect in the haemoglobin protein, which is a component of red blood cells and is in charge of distributing oxygen throughout the body. Instead of producing hemoglobin A as is normal, people with this condition create hemoglobin S, a faulty form of the protein [1].

The tetrameric protein known as hemoglobin (Hb) is made up of various combinations of globin subunits; each globin subunit is linked to the cofactor haem, which has the ability to carry an oxygen molecule. Both reticulocytes, or immature red blood cells, and erythrocytes, or mature red blood cells, express hemoglobin (Hb). distinct types of globin proteins are encoded by multiple genes, and these globin protein combinations result in distinct forms of Hb. These types of Hb are generally expressed at different stages of life, including embryonic, fetal, and adult. With two α-globin subunits (encoded by the duplicated HBA1 and HBA2 genes) and two β-globin subunits, Hb A (HbA) is the most common (>90%) form of adult Hb.

A solitary nucleotide alteration in HBB yields the sickle Hb (HbS) allele βS. The resulting mutant protein, the sickle β-globin component, undergoes an amino acid substitution [2] .

When Hb is not linked to oxygen, or in a state known as deoxygenation, two of these mutant sickle β-globin subunits, or HbS, can form Hb tetramers. These tetramers can polymerize and give the erythrocytes the sickled or crescent shape that gives rise to the disease. Though not as effectively as HbS, Hb tetramers with a single sickle β-globin subunit can also polymerize. Recurrent vaso-occlusive episodes, which are the hallmark of SCA, can be brought on by sickle erythrocytes. Instead of being soluble, the mutant hemoglobin precipitates in red blood cells, giving rise to crescent (sickle)-shaped red blood cells as opposed to normal spherical ones.

Monogenic sickle cell anemia is an autosomal recessive disorder. Recessive implies that an individual only gets the disease if the mutation is inherited from both parents, autosomal means that the gene is not on a sex chromosome, and monogenic means that it is caused by a mutation in a single gene [3] .Sickle cell trait, which typically does not result in symptoms but puts carriers of the mutation at risk of passing it on to their progeny, is caused by inheritance of only one copy. Because of how it is inherited, sickle cell anemia is also occasionally referred to as a Mendelian disease, and as such, it is frequently used to explain genetics.

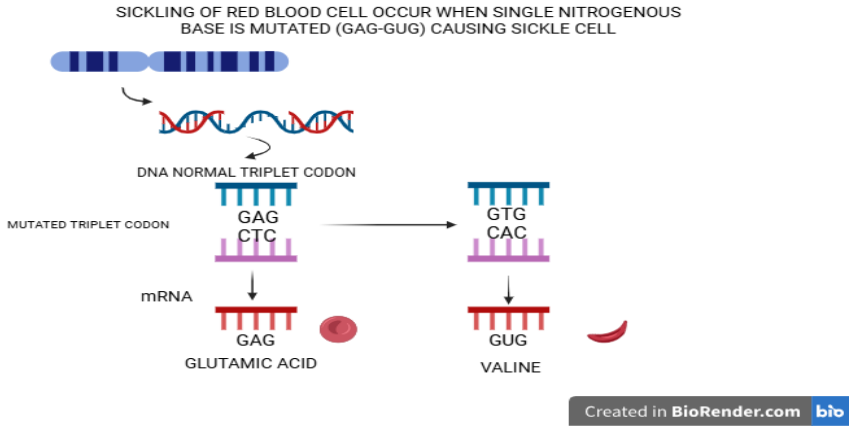
Early diagnosis, preventing complications, and managing end-organ damage are all necessary components of treatment. In this review, we go over recent developments in diagnostics made possible by gene therapy. Recently, a revolutionary new gene treatment method has been created that doesn't have any negative side effects. CRISPR-Cas9, which stands for CRISPR-associated protein 9, is incorporated into this version, which is less expensive and simpler to employ than the others. CRISPR-Cas9 is a cluster of regularly spaced short palindromic repeats. This review's objective is to objectively evaluate CRISPR-Cas9 as a potentially effective therapeutic tool for treating sickle cell anemia by analyzing its safety, and effectiveness, and present clinical application problems.

2. Understanding Sickle Cell Anemia

Encoded by HBB, normal hemoglobin A (HbA) is composed of two α globin subunits and two β globin subunits. In the mature β globin chain, at position 6, the sickle Hb (HbS) allele, βS, is an HBB allele where valine replaces glutamic acid due to an adenine-to-thymine substitution. When both of the HBB alleles are mutated, and at least one of them is the βS allele, sickle cell disease (SCD) results. HbS that is not linked to oxygen can polymerize and stiffen erythrocytes when they do so [4] . Those with sickle cell anemia (SCA), the most prevalent SCD genotype, have two βS alleles (βS/βS). Those with one βS allele have the sickle cell trait (HbAS) but not SCD.

There are potential genotypes for SCD that are not as common. The HbSC genotype is characterized by the presence of one βS allele and one HBB allele (HBB Glu6Lys, or βC allele) with a distinct nucleotide substitution, which results in the production of the HbC structural variant. The βC allele is primarily seen in people of West African heritage or in those who are from that region [5]. Compared to SCA, HbSC disease often causes less severe hemolytic anemia and fewer acute and long-term consequences, while it is frequently associated with retinopathy and osteonecrosis—also referred to as bone infarction, which is the loss of bone tissue due to a disruption in blood flow.

With the exception of microcytosis, which causes erythrocytes to be abnormally tiny, HbSβ0 thalassaemia is a clinical disease that is identical to SCA. It is caused by the combination of the βS allele and a null HBB allele (Hbβ0), which results in no protein translation. The βS allele in combination with a hypomorphic HBB allele (Hbβ+; a reduced quantity of normal β globin protein) causes HbSβ+-thalassaemia, a clinical disease that is typically less severe than SCA because normal HbA is not expressed at a high level. While mild variants of HbSβ thalassaemia are frequent in populations of African descent, severe and moderate types are more common in the eastern Mediterranean region and parts of India. Compound heterozygous SCD genotypes, such as HbS paired with HbD, HbE, HbOArab, or Hb Lepore, are uncommon [6] .



The Vaso-occlusive pain crises is the iconic complication linked to SCA. HbS polymerization is the primary pathophysiological event in SCA, despite the fact that vaso-occlusion is a complicated issue [4] [5]. The physical characteristics and shape of erythrocytes are altered by HbS polymerization, which can harm any organ by causing hemolytic anemia and blood flow obstruction, especially in tiny and some big vessels. Reticulocytes, which make up around 20% of red blood cells in people with sickle cell anemia, are another cell type that can undergo HbS polymerization. The course and complications of sickle cell disease (SCD) can be altered by both direct and indirect effects of hemolysis. HbS polymers also cause additional cellular anomalies that add to the overall pathophysiological mechanism of sickle cell disease (SCD). This section describes the common pathophysiology of the several alternative genotypes of SCD (double heterozygous states or SCA with modifying genes). The variations offer subtle phenotypic variations or milder forms [7] .

2. The Role of CRISPR-Cas9 Technology

Starting in the mid-1990s, gene therapy was thought to be a promising treatment for sickle cell disease. γ-globin or modified β-globin genes that have been designed to minimize sickling have been inserted into hematopoietic stem cells using lentiviral vectors; these vectors are currently undergoing clinical trials[8] and have produced encouraging preliminary results. Three major generations of advancement have occurred in gene editing technologies throughout the years. Zinc-finger nucleases (ZFNs) were employed by the first generation to modify the genome. The DNA sequence was altered by the second generation using transcription activator-like effector nucleases (TALENs). Notwithstanding, the third generation of technology, which is based on CRISPR/CRISPR-associated protein 9 (Cas), is the most sophisticated and extensively employed. In contrast to the first two generations, CRISPR technology modifies the base sequence of a brief guide RNA segment to target Cas proteins to a particular region in the genome. This broadens the application of gene-editing technologies and greatly increases the efficiency of gene editing. Gene editing presents an unprecedented opportunity to improve healthcare and pave the way for the creation of novel treatments for a wide range of hereditary disorders.

Design and production of guide RNA (gRNA):A scaffold sequence that attaches to the Cas9 enzyme and a specially created spacer sequence that is complementary to the target DNA sequence make up the brief synthetic RNA sequence known as gRNA.By attaching to the DNA sequence that corresponds to the spacer region of the gRNA, this gRNA guides the Cas9 to the appropriate area in the genome. Protein binding of Cas9:Cas9 is a DNA-cutting nuclease, an enzyme. After binding to the gRNA, it creates a complex. After that, Cas9 searches the DNA for a sequence that coincides with the gRNA spacer sequence. Target identification and PAM order:Only when a particular PAM sequence (Protospacer Adjacent Motif) is present close to the target DNA may Cas9 cut the DNA. The PAM is often a short DNA sequence, such 5'-NGG-3', where N can be any nucleotide.The Cas9-gRNA complex hooks onto the DNA when it locates the corresponding DNA sequence close to a PAM.Cleavage of DNA:

At the precise target spot, Cas9 breaks the DNA strand twice. This break happens at a specific spot that the gRNA has identified.A few nucleotides upstream of the PAM sequence is often where the cut occurs.

DNA restoration:The cell uses its own natural repair processes to mend the damaged DNA. Repairing the break can be done in two ways:NHEJ, or non-homologous end joining, This is a prone to mistake repair process that can result in haphazard insertions or deletions (indels) at the severed location, frequently causing gene disruption.Repair directed by homology (HDR): The cell can repair a cut with high precision if it is given a repair template, which is a segment of DNA containing sequences similar to the regions surrounding the cut site. This allows for exact modifications, including the insertion of new sequences or the correction of mutations.

3.Discovery And Development Of CRISPR Technology In Sickel Cell Diseases

In 2012, researchers found that by combining the tracrRNA/crRNA complex into a single RNA, the CRISPR-Cas system could be utilized to cut a particular stretch of DNA. The new RNA molecule may then be engineered to be complementary to almost any target DNA sequence for alteration by joining the crRNA to the tracrRNA. Later on, this modified RNA molecule came to be known as guide RNA (sgRNA or gRNA). This method first showed that CRISPR-Cas9 may be used to activate or deactivate the transcription of particular bacterial genes and cause targeted changes in bacterial genomes. After years of research and development, it was shown that CRISPR-Cas9 could also be used to change the genes of yeast, certain plants, animals, and human cells [10].

The idea of using CRISPR-Cas9 as a novel gene therapy tool was raised by research demonstrating that it could be used to edit the genome of eukaryotic cells, including human cells. Scientists reasoned that it would be easier and more effective than the methods currently in use to utilize site-specific gene editing to rectify genetic abnormalities in human genes [14]. Furthermore, compared to conventional gene therapy using viral vectors, this method would be less likely to trigger an immune response because the modified gRNA is put into the cells without the presence of viral proteins.

In a patient with lung cancer, researchers successfully employed CRISPR in 2016 to disable the gene that generates PD-1, an immune checkpoint inhibitor that is implicated in the genesis of cancer.In this investigation, immune cells called lymphocytes were extracted from the lung cancer patient, and CRISPR-Cas9 was injected along with a gRNA that targeted the PD-1 gene [87].In order to boost the body's ability to combat tumor cells, the genetically altered lymphocytes were subsequently reintroduced into the patient, and the PD-1 gene was effectively silenced in the patient's cells. Subsequently, researchers looked at whether this technique may be used to treat inherited genetic abnormalities that cause disease in addition to just deactivating genes.The same year, a point mutation in the SERPINA1 gene that results in alpha-1 antitrypsin deficiency, a disorder with symptoms primarily affecting the liver and lungs, was effectively corrected using CRISPR-Cas9 in laboratory-cultured cells [86]. Since then, cells with a variety of malignancies, including thyroid, breast, and lung tumors, have been effectively treated using CRISPR technology. Promising outcomes have been shown when it is utilized to treat cells with genetic disorders such beta-thalassemia, Huntington disease, muscular dystrophy, and Alzheimer's [11].

One of the illnesses that researchers intended to try treating with CRISPR-Cas9 is sickle cell anemia. The original goal of research was to try to alter the beta-globin gene mutation that causes red blood cells to sickle back to the normal sequence. An individual with sickle cell disease would have their red blood cell producing cells removed, and the DNA would be altered to reverse the mutation. After being reinjected into the patient's blood, the altered cells would be allowed to continue producing red blood cells. These people may manufacture regular hemoglobin and, as a result, have normal, healthy red blood cells since the modified cells would now have the normal beta-globin gene sequence [12].

In 2017, the first effort was made to modify human cells using this method. Sickle cell disease patients' bone marrow was used to identify and culture CD34+ hematopoietic pluripotent stem and progenitor cells, which are cells that produce red blood cells, in a lab. The CRISPR-Cas9 genome editing technology was used to try and fix the mutation in the mutant hemoglobin S gene (HbS). Following editing, examination of the CD34+ cells showed a rise in the synthesis of normal hemoglobin A [13].The prospect that CRISPR-Cas9 could be used clinically to treat sickle cell anemia was raised by the fact that the cells could be effectively modified in a lab to produce greater levels of normal hemoglobin A. However, there were still worries about potential negative repercussions from this method. The possibility of gene editing to off-target sequences, or the possibility that Cas9 would cut the beta-globin gene at a different site than the intended mutation and result in a variety of unforeseen modifications, was one of the main issues. These modifications could be dangerous or even lethal.

But by 2017, researchers were optimistic that this issue may be resolved thanks to a better grasp of the principles behind CRISPR-Cas9 and the quickly developing technology that was being created. Because of the effectiveness of the study utilizing CD34+ cells and the ongoing advancements in our understanding of the CRISPR-Cas9 gene editing technology, scientists were prepared to employ the technology in clinical trials by 2018. Since then, numerous studies to treat sickle cell anemia have been started utilizing CRISPR-Cas9. According to reports, Graphite Bio intends to begin a CRISPR clinical trial in 2021 [14].The goal of

the experiment is to directly alter the sickle cell disease-causing beta-globin gene mutation. However, other clinical trials have used CRISPR technology to treat the condition in different ways.

Targeting genes other than the defective betaglobin gene directly has been the goal of some trials attempting to treat sickle cell disease. 2018 saw the start of one such clinical trial conducted by Vertex Pharmaceuticals Incorporated and CRISPR Therapeutics. Similar to the previously discussed study on cultured cells, CRISPR-Cas9 was employed in this experiment to modify CD34+ hematopoietic stem and progenitor cells. Nevertheless, this strategy sought to create a distinct, nonmutated hemoglobin gene that is present in the genome but not expressed in adulthood, as opposed to trying to correct the defective beta-globin gene [16].Additionally, because it is less intrusive and uncomfortable for the patient, CD34+ hematopoietic stem and progenitor cells were obtained from the patient's peripheral blood in this research as opposed to their bone marrow.

The BCL11A gene is the beta-globin gene that is the focus of this investigation. This gene is activated before birth and creates the proteins that end the creation of fetal hemoglobin and start the production of adult hemoglobin. The main difference between fetal hemoglobin (abbreviated HbF) and normal adult hemoglobin (abbreviated HbA) is that the former has two gamma chains while the latter has two beta chains. Since supplying oxygen to a developing fetus is HbF's principal role, its structure enables it to bind oxygen more strongly than HbA, making it easier for HbF to take up oxygen from the mother's bloodstream and transfer it to the fetus through the placenta. Production of HbF stops around the time of birth, and production of HbA starts. The BCL11A gene is important in this process because it produces a protein that combines with other proteins to mute the gamma-globin genes. This prevents the production of gamma-globin and ultimately fetal hemoglobin. Moreover, it directly interacts with the beta-globin gene to generate beta-globin, which is a part of adult hemoglobin [17].

It has previously been demonstrated that when sections of the BCL11A gene are deleted, the generation of the protein it encodes is blocked and red blood cells produce more fetal hemoglobin. Compared to cells carrying adult hemoglobin with beta chains produced by the mutant beta-globin gene when the BCL11A gene is present, cells containing this type of hemoglobin are less likely to get sickled because the gamma chains in it transmit oxygen through the bloodstream more efficiently. Red blood cells would produce more fetal hemoglobin than adult hemoglobin and fewer sickled cells if the BCL11A gene were silenced, according to scientific reasoning [18]. Researchers are trying to fix the beta-globin gene defect that causes sickled red blood cells by utilizing CRISPR-Cas9 to cure sickle cell anemia. Red blood cells will be produced by the altered cells when they are reinjected into the patient's blood. It is hoped that people would be able to make normal hemoglobin and, as a result, have healthy, normal red blood cells [16].

In 2017, CRISPR-Cas9 was used for the first time on human cells to fix a mutation in the mutant HbS gene that resulted in an increase in the production of normal hemoglobin A. The possibility of Cas9 cleaving the beta-globin gene at a different position than the intended mutation, which could result in a variety of unforeseen modifications, is one danger associated with gene editing to off-target regions. In 2018, researchers were prepared to use the technique in sickle cell anemia clinical trials. Since then, numerous studies to treat sickle cell anemia with CRISPR-Cas9 have been started. Beginning in 2021, Graphite Bio will conduct a clinical trial utilizing CRISPR technology in an effort to directly modify the sickle cell anemia-causing beta-globin gene abnormality. Several clinical trials have used CRISPR technology to treat the condition in different ways.
Rather than trying to fix the mutated beta-globin gene, one such clinical trial that was started in 2018 used CRISPR-Cas9 to edit CD34+ hematopoietic stem and progenitor cells in an effort to produce a different hemoglobin gene that is present in the genome but not expressed in adults. The patient's peripheral blood was used in this trial to harvest CD34+ hematopoietic stem and progenitor cells, which is less invasive and painful for them [19]. The BCL11A gene, which is activated before birth and generates proteins that halt fetal hemoglobin production and start adult hemoglobin production, is the beta-globin gene that is the focus of this study. The main difference between fetal hemoglobin (abbreviated HbF) and normal adult hemoglobin (abbreviated HbA) is that the former has two gamma chains while the latter has two beta chains. Since supplying oxygen to a developing fetus is HbF's principal role, its structure enables it to bind oxygen more firmly than HbA, making it easier for HbF to take up oxygen from the mother's bloodstream and transfer it to the fetus via the placenta [18].

Production of HbF stops around the time of birth, and production of HbA starts. The BCL11A gene is important in this process because it produces a protein that combines with other proteins to mute the gamma-globin genes. This prevents the production of gamma-globin and ultimately fetal hemoglobin. Moreover, it directly interacts with the beta-globin gene to generate beta-globin, which is a part of adult hemoglobin. Researchers have discovered that when sections of the BCL11A gene are deleted, the generation of the protein it encodes is blocked and red blood cells produce more fetal hemoglobin. Red blood cells that lack sickled cells and produce more fetal hemoglobin than adult hemoglobin if the BCL11A gene were silenced could have potential for treatment for SCD.
Victoria Gray is a lady who was among the first patients treated for sickle cell anemia with plate number 1. After removing her stem cells from her blood, the BCL11A gene was modified by CRISPR to increase the amount of fetal hemoglobin. The cells were edited and then reinfused into her blood. Her hemoglobin level rose from 7 g/dl to 11 g/dl in June 2020, a year following therapy, which is closer to the typical range for women's hemoglobin levels (12 to 15.5 g/dl).Gray's sickle cell anemia required seven hospital stays annually on average before therapy. But she didn't need to be hospitalized in the first year following the treatment. Scientists are planning later stages of clinical trials (phases II or III) that will involve more patients receiving this medication as a result of these encouraging outcomes [20].

4.Challenges and Future Prospects

There are several difficulties in implementing a SCD therapy plan in the clinic, including as the requirement for high editing efficiency and minimal off-target effects. For safe clinical applications, a quantitative understanding of the genotypic and phenotypic effects of a wide range of mutations in the CRISPR/Cas9 altered SCD CD34+ cells is necessary. It is still difficult to create editing techniques that produce significant quantities of long-term repopulating HSCs with a polyclonal and a high percentage of gene-edited cells upon engraftment.

There are certain drawbacks to the ex vivo gene-editing methods used today. Typically, HSCs make only a very minor portion of CD34+ cells obtained from SCD patients. It is invasive to remove HSCs from the bone marrow. Infections and low blood counts are among the negative effects of chemotherapy that patients receiving myeloablative chemotherapy encounter. HSC pluripotency and engraftment potential are lost when HSCs are cultured in vitro and have their genes altered. Furthermore, the high expense of ex vivo gene editing, which is driven by the requirement for highly specialized facilities and the technical skills necessary, may make it unaffordable for patients to receive a cure. However, there are significant obstacles to overcome before in vivo gene editing can be used as a therapeutic strategy. These obstacles include obtaining high editing and in vivo delivery efficiency.

Although CRISPR cell and gene therapies for sickle cell anemia have advanced, researchers are being cautious because it is still too soon to determine the long-term safety and effectiveness of this technique. Before CRISPR—Cas9—based medicines are widely used, there is still a lot of ground to be covered. Public perception is a huge obstacle; although brave individuals like Gray come forward, most people are still reluctant to receive CRISPR-based treatments.

The public may need some time to be persuaded of CRISPR-Cas9's value in treating sickle cell disease despite its safety and efficacy. A survey and focus group are presently being conducted to find out more about people's understanding, attitudes, and beliefs regarding CRISPR-based therapeutic approaches for sickle cell disease. This will help us better understand and address patient concerns. Compared to conventional CRISPR-Cas9 editing, the more recent CRISPR adaptations of base, prime, and epigenome editors may offer safer therapy options for sickle cell anemia and related illnesses. Whichever strategy is used, it is obvious that CRISPR editing offers great promise for treating sickle cell anemia. The gene-editing community will be closely following this development as the goal of curing this crippling illness gets closer to reality.

5. CONCLUSION

As CRISPR-Cas9 targets and corrects the genetic defect causing sickle cell anemia, it holds great promise as a potential therapeutic. With hematopoietic stem cells, CRISPR offers the possibility of a single, curative treatment by modifying the defective HBB gene. Red blood cell sickling has been successfully reduced by reactivating fetal hemoglobin synthesis, according to early clinical trials. However, before extensive clinical application becomes viable, issues like ethical considerations, long-term safety, and delivery

efficiency must be resolved. All things considered, CRISPR-Cas9 offers a revolutionary chance to treat sickle cell anemia.

6. ACKNOWLEDGMENT

The authors are highly indebted to President ,Principle and the Department of Biotechnology , Isabella Thoburn College , Lucknow India , for their great support and encouragement to complete this manuscript.

7. REFERENCES

[1]. Gregory J. Kato1, Frédéric B. Piel2, Clarice D. Reid3, Marilyn H. Gaston4, Kwaku Ohene- Frempong5, Lakshmanan Krishnamurti6, Wally R. Smith7, Julie A. Panepinto8, David J. Weatherall9, Fernando F. Costa10 and Elliott P. Vichinsky11Article number: 18010 doi:10.1038/nrdp.2018.10 Published online 15 Mar 2018

[2]. Serjeant GR, Serjeant BE, editors. *Sickle cell disease.* 3rd ed. Oxford: Oxford Univ Press; 2001.

[3]. Steinberg, M. H. Pathophysiology of sickle cell disease. Baillieres Clin. Haematol. 11, 163–184

[4]. Vekilov, P. G. Sickle-cell haemoglobin polymerization: is it the primary pathogenic event of sickle-cell anaemia? Br. J. Haematol. 139, 173–184 (2007).

[5]. Seakins, M., Gibbs, W. N., Milner, P. F. & Bertles, J. F. Erythrocyte Hb-S concentration. An important factor in the low oxygen affinity of blood in sickle cell anemia J. Clin. Invest. 52, 422–432 (1973)

[6]. Rogers, S. C.et al. Sickle hemoglobin disturbs normal coupling among erythrocyte O2 content, glycolysis, and antioxidant capacity. Blood 121, 1651–1662(2013).

[7]. Davila, J.et al. A novel inflammatory role for platelets in sickle cell disease. Platelets 26, 726–729 (2015)

[8]. Hoban, M. D., Orkin, S. H. & Bauer, D. E. Genetic treatment of a molecular disorder: gene therapy approaches to sickle cell disease. Blood 127,839–848 (2016)

[9] . Ribeil, J. A. et al. Gene therapy in a patient with sickle cell disease. N. Engl. J. Med. 376, 848–855 (2017) .

[10]. H. Frangoul, et al., "CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β-Thalassemia," New England Journal of Medicine, vol. 384, pp. 252–260, Jan. 2021

[11]. S. Demerci, N. Uchida, and J. F. Tisdale, "Gene Therapy for Sickle Cell Disease: An Update," Cytotherapy, vol. 20, no. 7,(2018)

[12]. W. Jianguo, T. Wenjing, H. Suyang, and Youli, Z, "Cellular function reinstitution of offspring red blood cells cloned from the sickle cell disease patient blood post CRISPR genome editing," J Hematol Oncol. vol. 10, no. 1, p. 119, June (2017)

[13]. Hsieh, M. M. et al. Nonmyeloablative HLA-matched sibling allogeneic hematopoietic stem cell transplantation for severe sickle cell phenotype.JAMA 312, 48–56 (2014).

[14]. Ribeil, J. A. et al. Gene therapy in a patient with sickle cell disease. N. Engl. J. Med. 376, 848–855 (2017) .

[15] "CRISPR Therapeutics and Vertex Announce Progress in Clinical Development Programs for the Investigational CRISPR/Cas9 Gene-Editing Therapy CTX001." Sec.Gov. https://www.sec.gov/Archives/edgar/data/1674416/000119312519049058/d712065dex991.htm. (accessed Nov. 2020).

[16].W. Jianguo, T. Wenjing, H. Suyang, and Youli, Z, "Cellular function reinstitution of offspring red blood cells cloned from the sickle cell disease patient blood post CRISPR genome editing," J Hematol Oncol. vol. 10, no. 1, p. 119, June (2017) .

[17]. V. G. Sankaran and S. H. Orkin, "The Switch from Fetal to Adult Hemoglobin," Cold Spring Harb Perspect Med., vol. 3, no. 1, p. a011643, Jan (2013) .

[18]. Karis Weisgerber, Howard Community College Mentored by: Kathryn S. Jones, Ph.D. Using CRISPR-Cas9 To Treat Sickle Cell Disease Journal of Research in Progress

[19]. R. Cross. "CRISPR start-up Graphite Bio launches with $45 million for targeted DNA integration." launches-45/98/web/2020/09. c&en.acs.org. (accessed Dec. 2020).

[20]. R. Plater. "First Person Treated for Sickle Cell Disease with CRISPR Is Doing Well." Healthline.com.cell-disease-with-crispr-is-doing-well#The-next-steps. (accessed Nov. 2020).