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CRISPR-CAS9: A STEP TOWARD A CURE FOR SICKLE CELL DISEASE

Moore-Igwe, B. W.* and Chukwu, Priya Homa
Department of Haematology and Blood Transfusion Science, Faculty of Medical Laboratory Science, Rivers State University, Nkpolu-Oroworukwo, P.M.B. 5080, Port-Harcourt, Rivers State, Nigeria
***Corresponding Author:** B. W. Moore-Igwe; E-mail: beatrice.moore-igwe1@ust.edu.ng
Tel: +2348030983200
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ABSTRACT
Background: Sickle cell disease (SCD) is an inherited blood disorder caused by a GAG to GTG mutation in the 6th codon of the Hbβ gene, resulting in a glutamine to valine substitution in the β-chain of hemoglobin. This leads to the production of abnormal hemoglobin molecules. Currently, there are four Food and Drug Administration (FDA) approved drugs to manage acute complications, and blood transfusions are used in some cases. Hematopoietic stem cell transplantation (HSCT) from a matched related donor is the only curative therapy available. Gene therapy, specifically using CRISPR-Cas9, is being studied as another curative option. CRISPR-Cas9, a powerful genome editing system that emerged in 1987, acts as molecular scissors that can precisely cut Deoxyribonucleic Acid (DNA) strands to add or remove specific sequences.
Aim: This review examines how CRISPR-Cas9 technology can be used to treat sickle cell disease, emphasizing its mechanism, current developments in clinical practice, and potential as a gene-editing cure.
Methodology: The review utilized about 30 primary studies sourced from various electronic databases, including Semantic Scholar, PubMed and Google Scholar. The selection criteria focused on research related to CRISPR-Cas9 technology used in the treatment of sickle cell disease. Boolean operators were utilized to refine the search and ensure relevant studies were obtained. It will examine how CRISPR-Cas9 technology can be used to treat sickle cell disease, emphasizing its mechanism, current developments in clinical practice, and potential as a gene-editing cure. The review focuses on the use of CRISPR-Cas9 technology to treat SCD by either correcting the HbS mutation or promoting fetal hemoglobin (HbF) production. The most common clinical approach emphases on boosting HbF by downregulating the BCL11A transcription factor, a validated repressor of HbF. Disrupting a BCL11A erythroid-specific enhancer with CRISPR-Cas9 followed by autologous HSCT has shown to elevate HbF levels and reduce SCD symptoms. On December 8, 2023, the FDA approved CASGEVY™, the first cell-based CRISPR-Cas9 gene therapy for treating SCD in patients aged 12 and older with recurrent vaso-occlusive crises.
Result: CASGEVY™ inactivates BCL11A to convert abnormal HbS in blood stem cells into HbF. It offers a more sustainable and comprehensive solution by targeting the genetic basis of SCD. Its use's limitations and ethical issues are also covered in the review.
Conclusion: A novel method of treating sickle cell disease (SCD) is provided by CRISPR-Cas9, which either directly corrects the HbS mutation or stimulates the synthesis of fetal haemoglobin (HbF).
Keywords: CRISPR-Cas9, Fetal hemoglobin, gene therapy, genome editing, Sickle cell disease.

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Sickle cell disease (SCD) is an inherited blood disorder caused by a mutation in the beta-globin gene (Hbβ), leading to the formation of hemoglobin S (HbS) that alters red blood cell shape, causing vaso-occlusion and pain. Thein, 2013; Barabino *et al*., 2010; Nwabuko *et al*., 2016; Piel *et al*., 2017). SCD's severity varies, with infants often asymptomatic, but complications worsen with age, including recurring vaso-occlusive crises (Marouf *et al*., 2003; Tantawy *et al*., 2020). It affects individuals of African, Mediterranean, Middle Eastern, and South Asian descent, with Sub-Saharan Africa accounting for 75% of cases and 70% of global births, and many children dying before age 5 (Rees *et al*., 2017; Nwogoh *et al*., 2012; Stephen *et al*., 2018; Adigwe *et al*., 2023). SCD imposes economic and quality-of-life burdens (Barrangou *et al*., 2017; Ogamba *et al*., 2020). CRISPR/Cas9 gene therapy offers a potential cure by repairing the Hbβ gene or boosting fetal γ-globin production, with advances reducing off-target effects (Hsu *et al*., 2014; Yin *et al*., 2014; Redman *et al*., 2016; Demirci *et al*., 2019; Park and Bao *et al*., 2021 Youssry and Ayad, 2023).

Overview of Sickle Cell Anaemia

Sickle cell Anaemia (SCA) is a common and severe hemoglobinopathy, affecting 5-7% of the global population (Modell *et al*., 2008; Cappelli Yin *et al*., 2024). It is an autosomal recessive disorder, inherited with a 25% risk if both parents are carriers (AS) (Mwaiswelo *et al*., 2020; Tshilolo and Gonzalez, 2024). If one parent has sickle cell anemia (SS) and the other has the trait (AS), there is a 50% chance of the child inheriting SCD (SS) or the trait (AS) (Grosse *et al*., 2011). SCD results from a mutation in the β-globin gene, substituting valine for glutamic acid, causing hemoglobin polymerization and sickling of red blood cells (Grosse *et al*., 2011, Cappelli *et al*., 2024).

Epidemiology of Sickle Cell Disease

Sickle cell disease (SCD) has significant global prevalence, especially in malaria-endemic regions, with the βS allele distribution influenced by malaria and population movements, such as the slave trade. About 50 million people live with SCD, with 75% of 300,000 annual births occurring in sub-Saharan Africa (Aygun and Odame, 2012). SCD prevalence is highest in sub-Saharan Africa, with under-5 mortality rates reaching 90% and accounting for 7.3% of under-5 mortality (Grosse *et al*., 2011; Wastnedge *et al*., 2018). Nigeria has a prevalence of 1–3%, with 4–6 million people affected and 100,000–150,000 newborns annually (WHO, 2006, Charlton, 2015; Nwabuko *et al*., 2022). SCD contributes to high pediatric and maternal mortality, particularly in sub-Saharan Africa, where 50–90% of affected children die before age 5 (Makani *et al*., 2011; Ugboma *et al*., 2015, Wastnedge *et al*., 2018).

Genetic Basis for Sickle Cell Disease

Sickle cell anemia is inherited, with hemoglobin type determined by genes (Grosse *et al*., 2011). Hemoglobin (Hb) is a tetramer of globin subunits and is expressed in reticulocytes and erythrocytes (WHO, 2006). Different globin genes express various hemoglobin types during life stages. Adult hemoglobin (HbA) consists of two α-globin and two β-globin subunits (WHO, 2006). A mutation in the gene (GAG to GTG) causes sickle hemoglobin (HbS), replacing glutamic acid with valine, forming HbS (α2βS2) (Rees *et al*., 2017; Cappelli *et al*., 2024). Homozygous HbSS or coinheritance with other mutations causes SCD (Sundd *et al*., 2019). Under deoxygenated conditions, HbS polymerizes, deforming erythrocytes and causing vaso-occlusion (Makani *et al*., 2011; Sundd *et al*., 2019). High HbF levels in fetal life prevent sickling, but symptoms appear when HbF decreases after birth (Sundd *et al*., 2019).

Pathophysiology and Symptoms

HbS red blood cells polymerize in deoxygenated conditions, becoming rigid and prone to hemolysis, causing vaso-occlusion, ischemia, and infarction (Sundd *et al*., 2019). Hemolysis disrupts nitric oxide and endothelial function, leading to complications like stroke and ulcers (Hebbel, 2014). Sickled cells survive 10–20 days, unlike normal cells (120 days) (Hebbel, 2014). HbS forms rigid strands, causing sickling, hemolysis, free hemoglobin release, calcium pump impairment, and nitric oxide inhibition, which promotes adhesion to endothelium (Frenette and Atweh, 2007, Gardner, 2018). This worsens vasoconstriction, ischemia, and inflammation, exacerbating vaso-occlusion (Sundd *et al*., 2019).

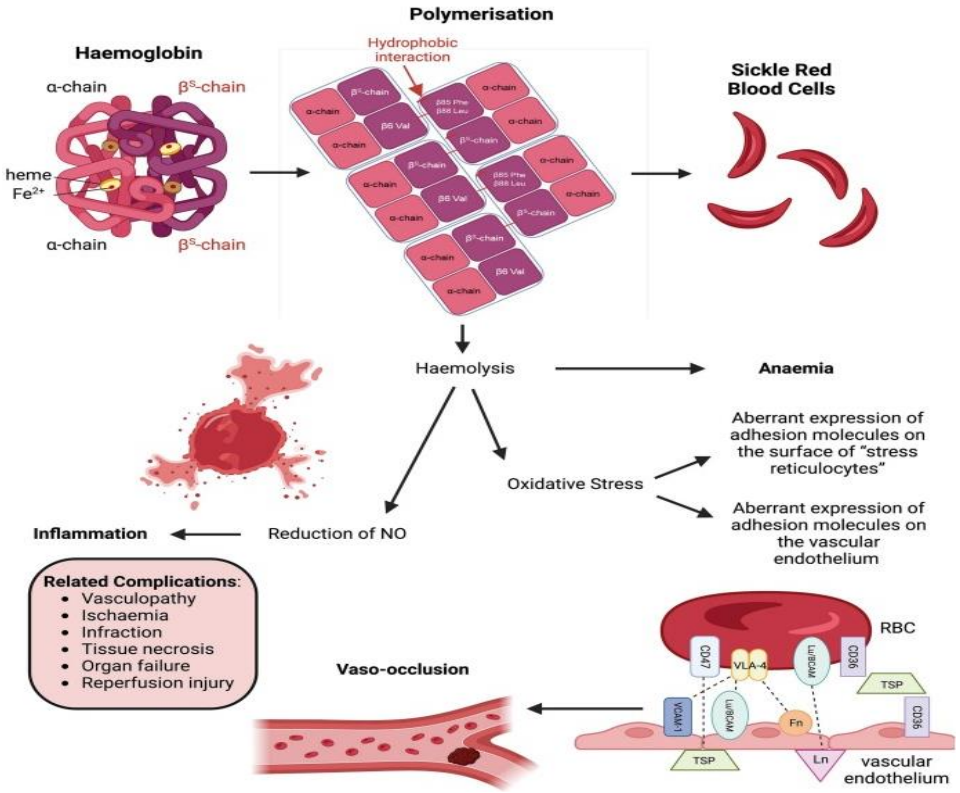

Figure 1: Pathophysiology of sickle cell disease. Sickle hemoglobin polymerization leads to hemolysis (causing anemia) and oxidative stress. This stress triggers adhesion molecule expression on "stress reticulocytes" and endothelial cells, leading to vaso-occlusion. Hemolysis also reduces nitric oxide (NO), causing inflammation (Kato *et al*., 2017).

Impact of Sickle Cell Disease

Sickle cell disease (SCD) imposes a significant economic burden and negatively impacts quality of life (QoL), affecting physical, mental, social, and educational well-being (Hsu *et al*., 2014; Yin *et al*., 2014). In Nigeria, common issues include social impairments, restricted activities, and depression (Grosse *et al*., 2011; Gardner, 2018). Pain and complications reduce self-efficacy and independence (Sebastiani *et al*., 2007, Hebbel, 2014). With improved survival rates, over 94% of SCD patients in high-income countries live into adulthood, compared to 50-90% childhood mortality in sub-Saharan Africa due to limited healthcare (Mwaiswelo *et al*., 2020) Life expectancy in high-income countries is 40-60 years (WHO, 2006). SCD remains a public health issue in impoverished populations with limited healthcare access.

Current Treatment Options and Limitations

Treatment goals focus on managing acute vaso-occlusive crises. Wealthy countries offer curative therapies, while less-wealthy countries often provide only symptom management (Crossley *et al*., 2022).

Blood Transfusion

Blood transfusion is key in SCD treatment, aiming to increase oxygen carrying capacity and reduce HbS to prevent complications. Simple transfusion raises Hb but risks hyper viscosity, targeting 10 g/dL for HbSS patients (Crossley *et al*., 2022). Exchange transfusion increases oxygen carrying capacity and reduces HbS%, minimizing vaso-occlusive crisis risk. Long-term transfusion, while maintaining low HbS%, requires iron chelation due to iron overload (Ziemba *et al*., 2021, Crossley *et al*., 2022).

Drug Therapy

Despite advances in SCD pathophysiology, few drug treatments exist. Hydroxyurea (HU) remains the primary therapy, increasing HbF and reducing SCD severity (Mwaiswelo *et al*., 2020). New drugs approved include L-glutamine (2017), Crizanlizumab, and Voxelotor (2019), which reduce oxidative stress, endothelial adhesion, and HbS polymerization, respectively (Sebastiani *et al*., 2007, Charlton, 2017). Research for new treatments is ongoing.

Haemotopoietic Stem Cell Transplant

Hematopoietic stem cell transplant (HSCT) is the only cure for SCD, with an 87% cure rate in severe cases (Hsieh *et al*., 2014), and over 90% long-term survival in clinical trials. However, it's mainly available to pediatric patients, with limited use in adults due to high cost, severe side effects, and the need for a matched donor (Sebastiani *et al*., 2007). Advances in immunosuppressive therapy and graft manipulation are improving outcomes, but fewer than 15% of patients have a matched sibling donor (Charlton, 2017). Alternative donors like haploidentical or cord blood are being explored. GvHD remains a major risk (WHO, 2006).

Gene Therapy

Over 6000 genetic disorders exist, but most lack effective treatments (Ocheni *et al*., 2007). From 1998 to 2019, 22 gene therapies, including CRISPR/Cas-9, were approved for human diseases. Gene therapy, involving gene replacement or editing, is a developing treatment for SCD, focusing on β- or γ-globin gene addition and reactivating HbF expression, offering results similar to hydroxyurea (Ocheni *et al*., 2007).

CRISPR-Cas9

CRISPR-Cas9 is a genome-editing method using the Cas9 enzyme to target and edit specific genes (Ball, 2024). CRISPR (clustered regularly interspaced short palindromic repeats) is a bacterial defense mechanism that 'remembers' viral DNA and targets it if reintroduced (Hille and Charpentier, 2016). The system works in three stages: adaptation (inserting viral DNA fragments), crRNA synthesis (creating guide RNAs), and target interference. CRISPR-Cas9 is now used in gene editing in eukaryotes, enabling mutations, gene knockouts, and expression changes. It belongs to Class 2 systems, with the CRISPR-Cas9 system (type IIA) being the most commonly used (Bhaya *et al.,* 2011).

Discovery and Development of CRISPR-Cas9

CRISPR, discovered in 1987, is a powerful genome-editing tool known for its low cost, simplicity, efficiency, and speed (Bhaya *et al.,* 2011).

It was initially identified in *E. coli* by Ishino in 1987 and later found in other bacteria and archaea (WHO, 2006). Researchers discovered its role in adaptive immunity when they linked spacer sequences to viral resistance in bacteria.

In 2007, CRISPR's role in immunity was confirmed, and in 2012, it was repurposed for gene editing by Doudna, Charpentier, and Zhang. By 2013, CRISPR/Cas9 achieved genome editing in mammalian cells, and in 2020, it earned Doudna and Charpentier a Nobel Prize (Barrangou and Doudna, 2016).

Mechanism of CRISPR-Cas9

CRISPR/Cas9 is a genome-editing system comprising a guide RNA (sgRNA) and the Cas9 protein (Deltcheva *et al.,* 2011). The sgRNA targets a specific DNA sequence, and the Cas9 protein, a 160 kDa DNA endonuclease, creates a double-stranded break (DSB) at the target site. The editing process involves recognition, cleavage, and repair (Gostimskaya, 2022). Cas9 binds to the target DNA via sgRNA, cleaving the DNA at a Protospacer Adjacent Motifm (PAM) sequence. DSBs are repaired by two mechanisms: non-homologous end joining (NHEJ), which causes random mutations, and homology-directed repair (HDR), which allows precise gene editing using a donor template (Yang *et al.,* 2020).

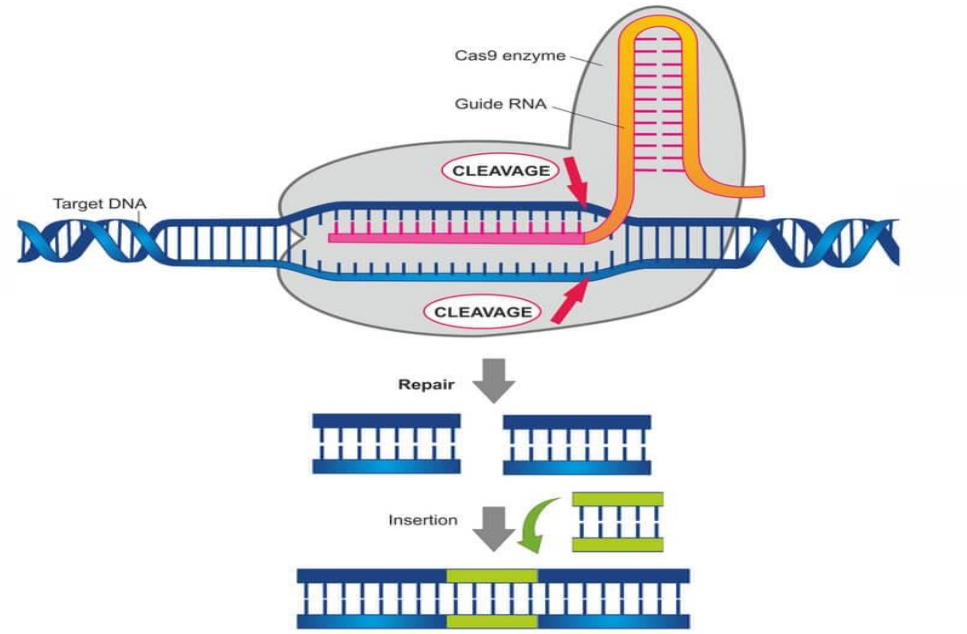

Figure 2: CRISPR/Cas9 uses a guide RNA and Cas9 protein to cut DNA at a specific site, enabling the addition or removal of DNA. After cutting, repair occurs via two pathways: non-homologous end joining (leading to random insertions/deletions) or homology-directed repair (allowing precise genome editing using a repair template) (Cong *et al*., 2013).

How CRISPR-Cas9 Works for Sickle Cell Disease: A Molecular Overview

CRISPR-Cas9 offers a potential cure for sickle cell disease (SCD) by synthesizing normal hemoglobin and preventing sickled red blood cells (Young *et al.,* 2011). Despite challenges in delivery, side effects, and ethics, CRISPR-Cas9 is a promising gene therapy for SCD. Trials in 2019 focused on repairing the hemoglobin S gene or boosting fetal γ-globin (Fernández *et al.,* 2018).

Using CRISPR-Cas9 to Correct Haemoglobin S

A mutation in the Hbβ gene causes sickle cell disease (SCD). CRISPR-Cas9 can target the mutated gene in hematopoietic stem cells, correcting the mutation or boosting fetal hemoglobin production. CRISPR can efficiently correct the A-to-T mutation in codon 6 of Hbβ responsible for the HbS allele and the E6V substitution.

Several Cas9-based approaches are advancing toward clinical trials, but gene-corrected human stem cells have only been tested in xenograft mice models. Clinical use is limited by low HDR to NHEJ ratios and potential risks like β-thalassemia (Young *et al.*, 2011).

Using CRISPR to Promote Fetal Haemoglobin Production

The most common CRISPR-Cas9 approach for SCD gene therapy focuses on boosting fetal hemoglobin (HbF), which can alleviate SCD symptoms. HbF, composed of α2γ2, is produced in infants and reduces sickle hemoglobin (HbS) polymerization. The Gγ-globin genes (HBG1) and The Aγ-globin genes (HBG2) are repressed post-birth, and β-globin (Hbβ) is activated. Inhibiting repressors like B-cell lymphoma/leukemia 11A (BCL11A) reactivates HbF. CRISPR-Cas9 can disrupt BCL11A to elevate HbF levels, reducing SCD symptoms. Elevated HbF levels correlate with reduced SCD severity, and hereditary persistence of fetal hemoglobin (HPFH) naturally reduces SCD symptoms (Abdel-Hadi *et al.*, 2023).

What has been done so far?

On December 8, 2023, the FDA approved CASGEVY™, the first CRISPR-Cas9 gene therapy for sickle cell disease (SCD) in patients aged 12 and older (FDA, 2024). It works by converting HbS to HbF by inactivating BCL11A, enhancing γ-globin expression. Patients undergo myeloablative conditioning before receiving the edited stem cells. CASGEVY™ was shown to reduce severe vaso-occlusive crises (VOCs) in 93.5% of patients. A clinical trial with CRISPR-edited HSPCs (CTX001) showed promising results, curing patients of transfusion dependence, though some adverse effects were observed.

Challenges and Ethical Considerations

CRISPR-Cas9 gene therapy faces challenges like immunogenicity, delivery, off-target effects, and ethical concerns (Young *et al.*, 2023). Delivery methods include physical (electroporation, microinjection, hydrodynamic injection), chemical (lipid and polymer nanoparticles), and viral vectors (Adeno-Associated Viruses (AAVs)) and Lentiviruses (LVs), with AAVs preferred for their low immunogenicity (Yip, 2020). Immunogenicity remains a concern, as the immune system may respond to Cas9 proteins (Zhang *et al.*, 2020). Off-target effects risk unintended genome edits, with Cas9 enzyme concentration affecting specificity. Ethical issues include germline editing, social inequality, and the potential for designer babies (Young *et al.*, 2023). The high cost of CRISPR therapies may limit accessibility.

CONCLUSION

Sickle cell disease (SCD) is a common monogenic blood disorder with limited treatments, including FDA-approved drugs (hydroxyurea, L-glutamine, crizanlizumab, voxelotor) and blood transfusions. The only cure is hematopoietic stem cell transplantation. CRISPR-Cas9 gene-editing offers a promising therapy by correcting the sickle mutation, with preclinical and clinical evidence supporting its effectiveness. Unlike symptomatic treatments, CRISPR targets the genetic cause, offering a more sustainable solution. Ensuring precise genome editing and minimizing off-target effects are key to its success as the technology improves.

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