# Review Article

# Sickle Cell Disease in Sub-Saharan Africa: Is CRISPR-Cas9 the Breakthrough We've Been Waiting For?


## ABSTRACT

Sickle cell disease, which results from a single nucleotide substitution in the beta-globin gene (HBB) is recognized as a significant global health concern. Approximately 7.74 million people worldwide were living with sickle cell disease in 2021. Sub-Saharan Africa carries the highest disease burden, with mortality rates ranging from 50 to 90% among affected children within the first five years of life. Current FDA-approved therapies (hydroxyurea and glutamine) offer symptomatic relief but are not sufficient to fully prevent the disease from progressing into a chronic condition. Allogeneic hematopoietic stem cell transplantation is the only curative treatment but is limited by donor availability and immunological complications. Advances in gene-editing technologies, particularly CRISPR-Cas9, present promising solutions by enabling precise genetic modifications. CRISPR-Cas9 is employed to treat sickle cell disease either through direct correction of the causative mutation in the *HBB* gene or by inducing fetal haemoglobin production. The FDA's recent approval of CASGEVY™ marks a historic milestone as the first CRISPR-based therapy for sickle cell disease. CASGEVY™, which induces fetal haemoglobin production, showed 93.5% efficacy in preventing severe vaso-occlusive crises in sickle cell disease patients, with no graft failures or rejections reported.Despite its promise, challenges remain, including technical barriers such as delivery strategies, off-target effects, and unintended genetic alterations, as well as ethical, societal, and regulatory concerns. In Sub-Saharan Africa, inadequate healthcare infrastructure, high treatment costs, and limited public awareness further hinder widespread adoption. To harness CRISPR's potential, Africa must invest in advanced genomic laboratories, interdisciplinary training for healthcare professionals, and robust educational programs in molecular biology and biotechnology. Regional and international collaborations are essential to overcome these barriers, streamline regulatory processes, and foster public acceptance as CRISPR-Cas9 holds transformative potential for addressing sickle cell disease in Africa, offering a pathway toward reducing mortality and improving quality of life for affected populations.



*Keywords: Sickle cell disease, CRISPR-Cas9, Sub-Saharan Africa, Genome editing*


## 1. INTRODUCTION

The World Health Organization (WHO) recognizes sickle cell disease (SCD) as a global health concern affecting millions of people globally [1]. Although comprehensive global estimates of the SCD burden are scarce, according to the Global Burden of Disease Study 2021, approximately 7.74 million people worldwide were living with SCD in 2021, marking a 41.4% increase from 5.46 million in 2000 [2]. Sub-Saharan Africa bears the highest burden

of this disease [3, 4], with around 75% of more than 300,000 children born annually with SCD occurring in this region [5, 6]. Nigeria alone accounts for approximately 150,000 infants born with SCD each year[7]. The mortality is also disproportionately high in sub-Saharan Africa, as it contributes to 5-16% of under-five mortality[2,5]. The significant mortality rate, particularly among children in this region, is driven by insufficient public health interventions for SCD [5], and limited access to adequate healthcare services [1]. In medium- to well-resourced countries, nearly all affected infants now have a high chance of surviving into adulthood, though their overall life expectancy remains 20–30 years shorter than that of individuals without SCD [8-10].

Effective management of SCD involves early detection through neonate screening programmes, followed by comprehensive preventive care [11]. In most hospitals, treatment goals for SCD primarily focus on managing acute complications caused by vaso-occlusive crises [12]. This involves pain management [13]; adequate hydration which is essential to maintain proper blood flow and prevent the sickling of red blood cells [14]; and blood transfusions to enhance oxygen delivery and lower the risk of complications [15]. Hydroxyurea and glutamine remain the sole US Food and Drug Administration (FDA) approved medications for treating SCD. However, these therapies are not sufficient to completely prevent the progression of the disease into a chronic condition [16].

Allogeneic haematopoietic stem cell transplantation (HSCT) remains the only curative treatment for SCD [17]. Clinical studies have demonstrated its effectiveness [18]; however, its widespread use is limited by the availability of suitable donors and by complications and mortality rates, which increase with age [19]. When performed before the age of two, allogeneic HSCT can fully restore bone marrow function in patients with SCD. Nonetheless, immunological complications, such as graft-versus-host disease (GVHD), restrict the use of unrelated matched donors. This issue could potentially be addressed through gene editing and autologous HSCT [17].

Genetically engineered autologous cells eliminate the need for a matching HSCT donor, making this treatment accessible to all patients. Since the cells are derived from the patient's own stem cells, there is no requirement for immunosuppression, thereby reducing the risks of GVHD and immune-mediated graft rejection [20, 21]. With the advancement of clustered, regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein 9 (Cas9) technology, which is a gene editing tool that makes it possible to correct errors in the genome, autologous transplant of gene-edited haematopoietic stem cells could possibly provide a cure for most patients with SCD[22]. The discovery of CRISPR-Cas9 systems has transformed gene therapy by enabling precise gene targeting [23]. It has already been demonstrated that it can be used to repair defective DNA in mice curing them of genetic disorders[24].

In treating SCD, CRISPR-Cas9 is primarily used in two approaches: directly repairing the gene responsible for haemoglobin S (HbS) or boosting the production of fetalheamoglobin[22]. This revolutionary approach has already shown promising results in early clinical trials[25], raising expectations for its broader application in regions like sub-Saharan Africa, where SCD prevalence is the highest globally. This transformative technology has the potential to lessen the long-term healthcare burden of SCD in sub-Saharan Africa, in addition to enhancing the quality of life for those with SCD. This review explores the developments of CRISPR-Cas9 genome editing, its potential to treat SCD, and the opportunities and challenges of implementing these therapies in sub-Saharan Africa.

## 2. Sickle Cell Disease Overview

Sickle cell disease is an autosomal recessive genetic disorder affecting red blood cells, inherited from parents who are carriers of the sickle cell trait (AS) [26]. It belongs to a group of diseases caused by inherited disorders of haemoglobin. These disorders are generally referred to as haemoglobinathies and SCD is the most severe and common haemoglobinopathy [27]. It primarily results from a mutation in the beta-globin gene (HBB) on the short arm of chromosome 11 [28, 29]. This mutation leads to the formation of HbS, which differs structurally from normal adult haemoglobin (HbA). HbS alters the shape of red blood cells, reducing their deformability and changing their membrane adhesive properties. These changes lead to cell deformation and blood vessel blockage (vaso-occlusion) under conditions such as deoxygenation and acidosis [30, 31].

This pathological process contributes to intravascular inflammation and the obstruction of small blood vessels which is the hallmark of the disease and is the most common cause of frequent hospital visits for affected individuals [31, 32]. It also leads to a wide range of complications including retinopathy, nephropathy, acute chest syndrome (ACS), stroke, venous thromboembolism and chronic pain [33]. The long-term complications of SCD arise from a combination of persistent haemolytic anemia and the functional damage to organs caused by vaso-occlusive crises [34].

### 2.1 Impact of Sickle Cell Disease in Sub-Saharan Africa

SCD is recognized as a significant public health concern, particularly prevalent among some of the most socioeconomically disadvantaged groups with limited access to healthcare services [35]. In high-income industrialized countries, over 94% of individuals born with SCD now survive into adulthood, with a current life expectancy ranging between 40 and 60 years [36]. This stands in stark contrast to sub-Saharan Africa, where 50% to 90% of affected children may die within the first five years of life [37]. This high mortality is largely attributed to the fact that many children with SCD in Africa remain undiagnosed beyond their second year of life [38, 39]. When these children die without a confirmed diagnosis, their deaths are frequently attributed to other causes, rendering SCD an invisible killer of children [40].

Sadly, most of the African countries with high burden of SCD have no budgetary allocation for the prevention and control of this disease [41]. Also, the healthcare infrastructure in many Sub-Saharan African countries is often inadequate to meet the needs of SCD patients. There is a lack of specialized care, limited access to diagnostic tools, and insufficient availability of essential medications [42]. For example, in Uganda, there are only a few specialized centers for SCD care, and many patients must travel long distances to access these services [43].

Also, in many African countries, health insurance systems are either non-existent or inadequate, leaving families affected by SCD struggling to afford essential care [44]. These families often face high out-of-pocket expenses for medical treatments which places significant financial strain on both households and the broader healthcare system [45, 46]. Hospitalization represents a major driver of SCD-related healthcare costs, while the lifelong nature of the disease further worsens the financial burden. Patients typically require continuous prophylactic treatments, including penicillin and folate supplements, and in some cases, additional therapies such as hydroxyurea [47].In Nigeria, SCD patients often experience catastrophic healthcare expenditures due to frequent hospitalization for managing complications, which are frequently aggravated by delayed presentation often linked to poverty [35]. The loss of productivity due to illness and caregiving responsibilities

leads to further economic hardships. In Ghana, it is estimated that families spend up to 25% of their annual income on SCD-related healthcare costs [48].

SCD significantly impacts the quality of life (QoL) by affecting physical and mental health, social interactions, work productivity, and academic performance [35, 49, 50]. Children with SCD often miss school due to recurrent illness, resulting in educational setbacks [49]. When assessing the quality of life of individuals with SCD, it is essential to consider the social, emotional, and psychological dimensions of the disease [51]. A study conducted by Tunde et al. [52] in Ilorin University in Nigeria revealed that social impairment, limitations in physical and social activities, reduced academic achievement, and feelings of depression are prevalent among individuals with SCD. Also, pain and other complications associated with SCD adversely affect patients' physical, social, emotional, psychological, and spiritual well-being [53]. These challenges also undermine patients' self-efficacy and ability to achieve self-sufficiency [54]. Adults with SCD may also struggle to maintain consistent employment due to recurrent health issues, limiting their economic opportunities and contributing to poverty cycles [55]. In Kenya, studies have shown that individuals with SCD have a 30% lower employment rate compared to the general population [56].

## 2.2 Genetic Basis for Sickle Cell Disease

Sickle cell disease is characterized by the production of abnormal haemoglobin, called HbS[31]. Haemoglobin molecules consist of four globin subunits; each globin subunit is associated with the cofactor haem, which can carry a molecule of oxygen. Hb is expressed by both mature and immature red blood cells [57]. Several genes encode different types of globin proteins, and their various tetrameric combinations produce multiple types of Hb, which are expressed at different stages of life - embryonic, fetal, and adult. Fetalheamoglobin (HbF), composed of two α-globin and two **γ**-globin molecules is normally expressed during the development of the fetus and starts to decline just before birth, when it is replaced by HbA [58]. HbA is the most abundant form of adult haemoglobin (over 90%) and consists of two α-globin subunits (encoded by the duplicated HBA1 and HBA2 genes on chromosome 16) and two β-globin subunits (encoded by the HBB genes on chromosome 11) [57].

A single nucleotide substitution in the HBB gene results in the sickle Hb (HbS) allele βS, where GTG replaces GAG in the sixth codon of the β-globin gene [57]. This substitution changes a hydrophilic glutamic acid residue (Glu) to a hydrophobic valine residue (Val) at the sixth position in the β-globin chain, leading to the formation of the mutated Hb tetramer HbS ($\alpha 2\beta^{S}2$) in the erythrocytes of individuals with sickle cell anemia [59]. Homozygous inheritance of the $\beta^{S}$ mutation (HbSS) or coinheritance of $\beta^{S}$ with other mutations such as $\beta^{C}$ (HbSC), $\beta^{D}$ (HbSD), $\beta^{O}$ (HbSO/Arab), $\beta^{E}$ (HbSE), or a β-thalassemia allele (HbS/β-thal$^{0}$ or HbS/β-thal$^{+}$) leads to other forms of SCD through multiple interlinked molecular and cellular mechanisms [59].

During deoxygenation, healthy Hb rearranges itself into a different conformation which enables binding with carbon dioxide molecules, and reverts to normal when released, however, Hb tetramers containing two mutant sickle β-globin subunits (HbS) can polymerize, causing erythrocytes to take on a crescent or sickled shape, which gives the disease its name [57, 60]. Haemoglobin tetramers with one sickle β-globin subunit can also polymerize, though less efficiently than HbS. These sickle-shaped erythrocytes can lead to recurrent vaso-occlusive episodes, which are the hallmark of SCD [57].

During fetal and early postnatal life, the lack of expression of the HbSS phenotype is explained by the production of HbF, which is sufficient to limit, by dilution, the effects of

sickling. As the red cells that emerge from the bone marrow carry increasing amounts of HbS and smaller amounts of HbF, the results of sickling gradually appear. Therefore, newborns begin to manifest the disease from the sixth month of life, when the amount of HbF begins to approach adult levels [61].

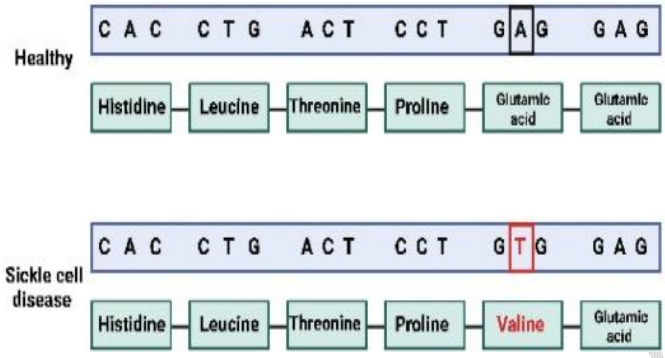


**Fig. 1.A GAG to GTG point mutation in the 6th codon of the HBB gene results in the substitution of glutamine to valine and is responsible for causing SCD [62].**

## 3. CRISPR-Cas9 Genome Editing

While the genetics of human diseases are often complex, many are characterized by alterations in gene expression *in vivo*, particularly genetic disorders caused by single-gene mutations [63, 64]. Genome editing has emerged as a revolutionary field, offering the potential to address diseases at their genetic level [64]. This technology enables precise modifications of the genome, facilitating targeted insertions, deletions, or base substitutions [65]. Over time, gene-editing technology has evolved through three key generations. The first generation utilized zinc-finger nucleases (ZFNs), followed by the second generation with transcription activator-like effector nucleases (TALENs). The most widely used third generation gene-editing technology is the CRISPR-Cas9 system [66].

CRISPR refers to the unique organization of short, partially repeated DNA sequences found widely in the genome of bacteria and archaea (prokaryotes) [67, 68]. CRISPR-Cas9 exploits a natural DNA-snipping enzyme in bacteria, called Cas9 to target and edit particular genes [69]. This technology has transformed genome editing by offering highly accurate and efficient methods for modifying genetic material [70-72]. Unlike ZFNs and TALENs, which rely on protein-DNA interactions for targeting, CRISPR technology employs a guide RNA sequence to direct Cas proteins to specific genome locations. This innovation significantly enhances editing accuracy and broadens the technology's applicability across diverse fields [73]. The applications of CRISPR-Cas9 are vast, spanning medical research, human gene therapy, plant science, and crop improvement [74]. In biomedical research, CRISPR has advanced precise investigations into gene functions and disease mechanisms. It has enabled researchers to create targeted gene knockouts, develop accurate disease models, and explore innovative therapeutic approaches [63].

### 3.1 From Bacterial Immunity to Genome Editing

CRISPR which emerged in 1987, has been hailed as the greatest genetic tool of the century due to its outstanding advantages, including low cost, simplicity, high efficiency, and speed [75]. The CRISPR system is essentially a natural tool bacteria uses to protect themselves by remembering parts of invading viral DNA and then targeting it if it enters the bacteria a second time [76]. The CRISPR defense mechanism protects bacteria from repeated viral

attacks through three basic stages: adaptation (spacer acquisition), crRNA synthesis (expression), and target interference. During the adaptation process, bacterial cells become immunized by the insertion of short fragments of viral DNA (spacers) into a genomic region called the CRISPR array, serving as a genetic memory of previous viral infections [77]. Secondly, the CRISPR array is transcribed into a long precursor CRISPR-RNA (pre-crRNA) that is further processed into mature guide crRNAs containing the memorized sequences of invaders [78]. In the last stage of immunity, Cas protein recognizes the target with the help of mature crRNAs which are used as guides to specifically interfere with the invading nucleic acids [79].

The discovery of CRISPR began when Japanese scientist Ishino and his team accidentally found unusual repetitive palindromic DNA sequences interrupted by spacers in *Escherichia coli* while analyzing a gene for alkaline phosphatase [80]. However, they did not ascertain its biological function at that time. It was not until 2007 that CRISPR was experimentally confirmed as a key element in the adaptive immune system of prokaryotes against viruses [77]. The use of CRISPR-Cas9 to edit genes was thrust into the spotlight in 2012 when George Church, Jennifer Doudna, Emmanuelle Charpentier, and Feng Zhang harnessed it as a tool to modify targeted regions of genomes. They discovered that by designing guide RNA to target a specific region in the genome, the CRISPR-Cas9 system can be instructed to cleave DNA at the target site to modify genomes [81]. Since after its discovery, it has been adapted and repurposed as a ground-breaking technique that allows scientists to edit regions of the genome by deleting, inserting, or modifying DNA sequences[82].

### 3.2 Mechanism of CRISPR-Cas9 Genome Editing

CRISPR-Cas9 is a simple two-component system consisting of a single guide RNA (sgRNA) and a Cas9 protein [83, 84]. The sgRNA is made up of two parts: crRNA, which includes a 20-nucleotide protospacer sequence targeting the DNA, andtrans-activating CRISPR RNA(tracrRNA), which binds to both the crRNA and Cas9[82, 84, 85]. In gene editing, the crRNA and tracrRNA are combined to form a synthetic sgRNA, which can target almost any gene sequence for editing [83]. The Cas9 protein is a DNA endonuclease responsible for cleaving the target DNA and creating a double-stranded break (DSB)[83]. Cas9 has two lobes: the recognition (REC) lobe which consists of the REC1 and REC2 domains is responsible for binding to the guide RNA, and the nuclease (NUC) lobe which contains the RuvC, HNH, and Protospacer Adjacent Motif (PAM) interacting domains. The RuvC and HNH domains cut each strand of the DNA, while the PAM-interacting domain ensures binding to the correct target sequence by recognizing a Protospacer Adjacent Motif[86, 87].

The CRISPR-Cas9 genome editing mechanism can be divided into three steps: recognition, cleavage, and repair[88]. Firstly, The CRISPR-Cas9 components are introduced into the target cells, commonly via viral vectors or direct injection[89, 90]. Inside the cells, the Cas9 protein and sgRNA form a complex that identifies the target DNA sequence[89]. The sgRNA directs Cas9 to the chromosomal location of interest through its 5' crRNA complementary base pair component. Cas9 remains inactive without the sgRNA. Once activated, the Cas9 nuclease searches the target sequence by binding with a sequence that matches the PAM sequence (5′-NGG-3′) and makes double-stranded breaks (DSBs) at a site 3 bp upstream of the PAM sequence using its HNH and RuvC domains[91]. The HNH domain cleaves the DNA strand that is complementary to the 20-nucleotide sequence of the sgRNA (target strand) and the RuvC domain cleaves theopposite strand (non-target DNA strand), resulting in resulting in blunt-ended DSBs. Finally, the DSB is repaired by the host-mediated DNA repair mechanisms[83, 87].There are two primary mechanisms for repairing DSBs created by Cas9: non-homologous end joining (NHEJ) and homology-directed repair (HDR)[92]. In the absence of a repair template, the NHEJ pathway is activated, causing random insertions

and deletions (indels) or substitutions at the DSB site[87]. NHEJ is the predominant and most efficient cellular repair mechanism, but it is error-prone, potentially resulting in small indels that generate frameshift mutations or premature stop codons[93].

When a donor template with the desired sequence and matching homology arms is available, the error-free HDR pathway can be initiated. HDR creates desired mutations through homologous recombination, allowing precise gene modification, such as gene knock-in, deletion, correction, or mutagenesis[87]. HDR is most active in the late S and G2 phases of the cell cycle. In CRISPR gene editing, HDR requires a large amount of donor DNA templates containing the sequence of interest. This pathway executes precise gene insertion or replacement by adding a donor DNA template with sequence homology at the predicted DSB site [93, 94].

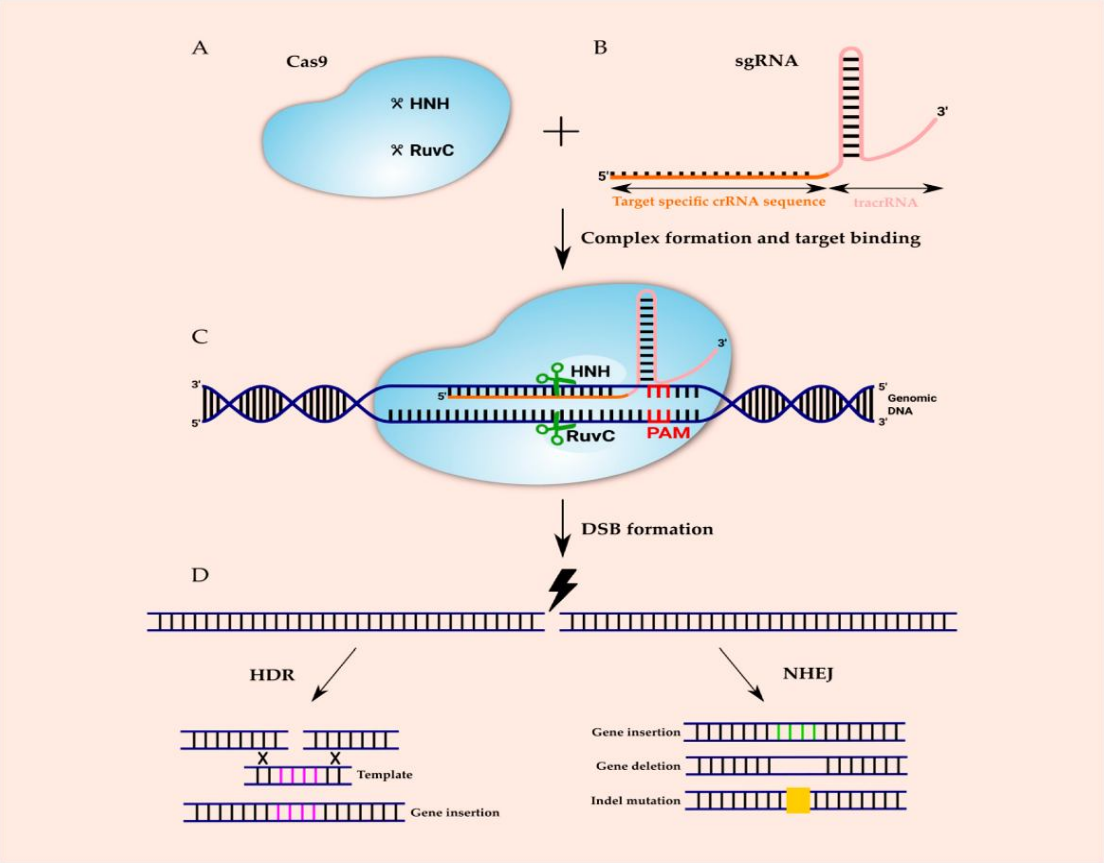


**Fig. 2. The Cas9-sgRNA complex binds to and unwinds the double-stranded DNA, and the complementary sequence in sgRNA anneals to one of DNA strands. The HNH domain targets and cleaves the DNA strand that pairs with the sgRNA, while the RuvC domain cuts the opposite strand three bases upstream of the Protospacer Adjacent Motif (PAM). The resulting double-strand break is repaired by the cell through either non-homologous end joining (NHEJ), which introduces small insertions or deletions, or homology-directed repair (HDR), allowing precise edits if a donor template is present [95].**

Efficient HDR editing in HSPCs is particularly crucial for achieving successful therapeutic outcomes and enabling effective clinical applications [96].Li and Mandal[95] noted

thatachieving high levels of HDR in primary cells is notably more challenging than performing gene deletions. When compared to the non-template correction mechanisms like NHEJ, the overall efficiency of HDR gene editing remains low [96].Notably, there are several factors that may influence HDR efficiency.

One major limitation of CRISPR-Cas9-mediated HDR is that the NHEJ pathway which is the predominant mechanism for repairing DSBs has a major impact on HDR [97]. The competition between HDR and NHEJ can reduce HDR efficiency [98]. Various strategies have been developed to suppress NHEJ activityto enhance HDR. These include inhibiting or depleting key NHEJ factors using small-molecule compounds, ubiquitination of target proteins, and gene knockdown or silencing. Common NHEJ targets include the Ku complex, DNA ligase IV, DNA-PKcs, and 53BP1 [99, 100].Although these approaches effectively inhibit NHEJ at specific sites, they also disrupt the natural DSB repair process. Since NHEJ plays a crucial role in maintaining genome stability, its widespread suppression could lead to significant adverse effects on genome integrity [97].

HDR occurs predominantly during the S/G2 phase of the cell cycle, whereas NHEJ functions throughout the entire cycle [101, 102]. The phase-specific nature of HDR further limits its effectiveness, particularly in non-dividing cells [96]. One approach to enhance HDR is by increasing the proportion of cells in the S and G2 phases. Restricting gene editing to these phases using a Cas9-geminin fusion enables transient Cas9 activity, thereby improving the HDR/NHEJ ratio [96].

Another key factor influencing HDR is the availability and design of donor templates. The HDR pathway requires donor templates, which can be either endogenous or exogenous [103]. Endogenous templates primarily consist of sister chromatids, while exogenous templates include plasmids, viral and bacterial vectors, or synthetic single-stranded oligodeoxynucleotides (ssODNs) [97]. Several factors including donor template concentration, structure, type, and length affect HDR efficiency [97]. To maximize HDR success, donor templates must be carefully designed, considering homologous arm length, polarity, PAM shielding, and backbone modifications [104].The delivery method of Cas9/sgRNA reagentsalso influences HDR efficiency. Ensuring the safe and effective delivery of the CRISPR-Cas9 genome-editing system to target cells or tissues is essential for successful genome modification [105].

Due to the wide range of possible outcomes and the ability to make significant alterations to the genome offered by the HDR gene-editing technique, there is increasing interest in developing methods to enhance HDR efficiency, with numerous studies actively exploring ways to improve this process [97, 98].

### 3.3 Mechanism of CRISPR-Cas9 Genome Editing in Treating Sickle Cell Disease

Over the past decade, numerous genome editing approaches have been explored to correct the mutation responsible for SCD [106]. The introduction of genome editing technologies utilizing designer nucleases has enabled the development of novel and safer strategies for the treatment of SCD [107]. CRISPR-Cas9 presents a potentially effective therapeutic approach. By permitting the synthesis of normal haemoglobin and halting the development of sickled red blood cells, this genetic adjustment has the potential to treat the underlying cause of SCD[108]. Although there are still issues to be resolved regarding delivery strategies, side effects, and ethical concerns, the use of CRISPR-Cas9 in gene therapy for SCD is a revolutionary step in the direction of creating a treatment that can cure the genetic condition [109]. In 2019, CRISPR editing was trialled as a treatment for patients suffering

from SCD [25]. Several gene editing strategies for curing SCD have shown promise in recent preclinical studies [110-112].

CRISPR-Cas9 technology is being employed to treat SCD through two primary approaches. The first involves directly repairing the haemoglobin S gene either by addition of an anti-sickling variant or by correcting the causative point mutation in the *HBB* gene [114, 115]. The second approach focuses on boosting fetal γ-globin levels, either by disrupting γ-globin (*HBG*) repressors to induce HbF production [111, 115] or by introducing beneficial hereditary persistence of fetal haemoglobin (HPFH) mutations in the β-globin locus [112, 114, 116].

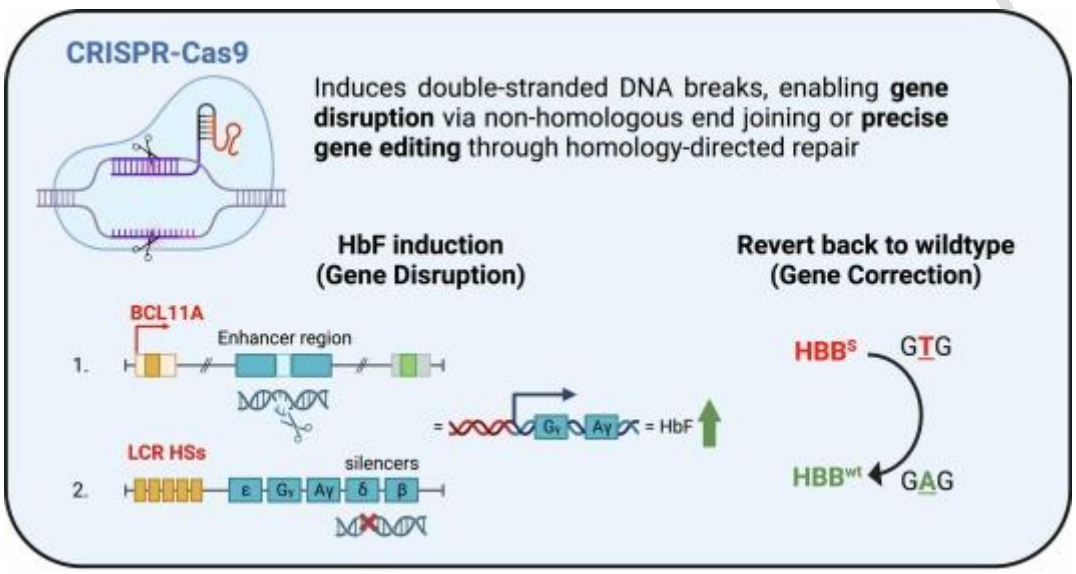


**Fig. 3. CRISPR-Cas9 can be employed either to correct the mutation and restore the wild-type sequence or to enhance fetal haemoglobin production[62].**

### 3.3.1 Using CRISPR-Cas9 to Correct Haemoglobin S

Correction of the disease-causing sickle mutation using gene-editing represents the most direct therapeutic strategies for SCD [117]. CRISPR-Cas9 genome editing offers a promising approach for efficient correction of the A-to-T base mutation of the HBB gene in SCD patients[118]. By specifically targeting and repairing the mutated HBB gene in HSCs, CRISPR-Cas9 eliminates the expression of pathologic HbS from the cell [28].Several Cas9-based gene correction approaches for HBB and other genes have now been validated in human haematopoietic stem and progenitor cells (HSPCs) and are rapidly progressing toward clinical trials [28, 110, 118].Recent studies suggest that optimizing the process can enable therapeutically significant correction of the *HBB* gene [28, 114]. However, the repopulation function of gene-corrected human HSPCs modified with Cas9 has only been assessed using xenograft transplantation into immunodeficient mice and no studies have evaluated this approach in the context of autologous transplantation for SCD [119].While most recent *HBB* gene correction techniques have employed CRISPR/Cas9, a GMP-compatible TALEN-based gene editing method, utilizing either viral or non-viral donor templates, has been shown to successfully correct the *HBB* gene. This method achieved over 50% normal globin expression in red blood cells without triggering a β-thalassemic phenotype [121].

Clinical translation of SCD mutation correction using the corrective donor template is currently hindered by the low efficacy of homology-directed repair pathways in long-term reconstituting HSCs [28, 110, 118]. Also, the possibility of inducing β-thalassemia major, intermediate or minor due to Cas9 cutting of *HBB* has not been carefully evaluated. In addition, the *in vivo* effects of Cas9 cleavage of HBB and reduction in functional β-globin levels in a patient with SCD remain unclear and will need to be addressed in a clinical trial [117].

### 3.3.2 Using CRISPR to Promote Fetal Haemoglobin Production

While correcting HBB mutations directly addresses the root cause of SCD, promoting HbF production offers a different approach thathas been linked to reduced morbidity and mortality [121]. Different studies have shown a correlation between elevated HbF levels in adults and reduced SCD severity [122]. This is because it can mitigate the manifestations of SCD by reducing sickle haemoglobin polymerization and erythrocyte sickling [123, 124]. In recent years, research has focused on achieving elevated levels of HbF in SCD patients by either modulating transcriptional repressors or introducing mutations associated with HPFH[62]. CRISPR-Cas9 has proven to be a highly effective and widely available strategy to achieve these therapeutic HbF levels [125].

The regulation of HbF expression and repression is a complex process influenced by numerous genes and can operate through multiple distinct pathways[114]. HbF is a minor component of normal adult haemoglobin but has significant clinical implications for SCD. The γ-globin chain of HbF is encoded by two nearly identical genes, HBG1 (Aγ) and HBG2 (Gγ), located in a developmentally regulated gene cluster on chromosome 11p15 [124].Around the time of birth, the expression of HBG1 and HBG2 is repressed, and the HBB gene, responsible for β-globin production, is activated. This leads to the switch to HbA production, which is expressed throughout adult life [126]. The switch from γ-globin to β-globin is an important model of developmental gene regulation and has clinical significance because β-hemoglobinopathies can be treated byinhibiting this switch [127]. The perinatal switch is regulated by the BCL11A gene which encodes the transcriptional repressor protein B cell CLL/lymphoma 11A (BCL11A) and leukemia/lymphoma related factor (LRF) which is also known asZBTB7A or FBI-1 [128]. This BCL11A proteinbind to cis-regulatory elements in the HBG1 and HBG2 promoters [114, 129]. Strategies to downregulate *BCL11A* includetargeting and disrupting the erythroid-specific enhancer that regulates the *BCL11A* gene or disrupting the binding sites for *BCL11A* in the γ-globin gene promoters [130].

The erythroid-specific enhancer region that regulates the *BCL11A* gene contains a binding site for the transcriptional activator GATA1. Disruption of the GATA1 binding site by Cas9 nuclease in the *BCL11A* erythroid-specific enhancer decreases its expression specifically in the erythroid lineage. This, in turn, allows for increased production of HbF [130, 131].Frangou*et al*. [25] demonstrated that using the CRISPR-Cas9 system to target and disrupt a BCL11A erythroid-specific enhancer followed by autologous HSCT has resulted in elevated HbF levels and reduced SCD symptoms[25]. Several other studies have also demonstrated the promising efficiency and safety of increasing γ-globin levels by disrupting the erythroid enhancer controlling the *BCL11A*gene using CRISPR-Cas9 [112, 115, 130]. Notably, the initial patients treated with this approach have shown no adverse effects even after more than three years [132-134].The second approach involves disrupting the promoter regions of the *HBG1* and *HBG2* genes, which act as binding sites for the *BCL11A* repressor [135]. This disruption significantly impairs the ability of *BCL11A* to bind in adult red-cell precursors, thereby preventing the γ- to β-globin switch and enhancing HbF expression [112, 136].This strategy has shown to effectively reactivate γ-globin expression in both cellular and animal models [130, 135,137-139].

Another CRISPR-cas9 nuclease strategy being explored to increase HbF in treating SCD involves introducing indels into the HBGpromoters to mimic the effects of HPFH. These mutations either create new binding sites for erythroid activators or eliminate the binding sites for repressors, resulting in increased HbF expression [138, 139]. This approach is exploredbecause patients who co-inherit large deletions, indels, or point mutations within the *HBG* gene cluster, which result in persistently elevated HbF expression, are typically asymptomatic [125]. HPFH deletions vary in size from 12.9 to 84.9 kb, covering the HBG1, HBBP1, HBD, and HBB genes within the β-globin cluster, and lead to uniform (pancellular) HbF production[140]. Introducing HPFH deletions into adult haematopoietic stem and progenitor cells activates γ-globin expression, which subsequently alleviates the SCD phenotype [123, 141]. According to Steinberg [142], deletional HPFH mutations produce higher levels of HbF when compared to the various genetic variants that induce HbF expression.

Multiple studies have demonstrated proof-of-concept for CRISPR-Cas9-mediated gene editing to replicate large deletional HPFH mutations within the β-globin gene cluster, presenting a promising therapeutic approach for treating SCD [116, 142].However, CRISPR-Cas9 editing produces a variety of indel combinations at target sites, making it difficult to pinpoint the specific mutations responsible for gene regulation [139].A study by Antoniani et al.[123] showed that CRISPR-Cas9-mediated deletion of a 13.6-kb region, analogous to the naturally occurring 12.9-kb HPFH-5 deletion, encompassing the δ- and β-globin genes and the δ-γ intergenic region, successfully derepressedHbF expression in erythroblasts and reduced RBC sickling. Similarly, Ye et al. [141] demonstrated that using RNA-guided CRISPR-Cas9 genome-editing technology to delete a 13-kb segment of the β-globin locus in normal HSPCs effectively mimicked the naturally occurring Sicilian HPFH mutation. Erythroid colonies derived from CRISPR-Cas9-edited HSPCs exhibited significantly higher γ-globin gene expression compared to colonies without the deletion [141]. Also, several HPFH mutations located in the −115 and −200 regions of the *HBG* promoters disrupt the binding sites of the potent HbF repressors *BCL11A* and *LRF*, respectively [128]. The study by Ravi et al. [139] demonstrated that base editing at the −123 and −124 positions of the *HBG* promoter resulted in higher HbF levels compared to the disruption of the well-known *BCL11A* binding site in erythroblasts derived from human CD34+ hematopoietic stem and progenitor cells.Cas9 nuclease effectively introduces indels in these regions, leading to HbF reactivation and correction of the SCD phenotype [112, 131].

Lamsfus-Calle et al. [143]compared various CRISPR-Cas9 strategies for inducing HbF expression and found that targeting genes such as *KLF1* and *BCL11A* was a more clinically relevant approach than disrupting transcription factor binding sites like *HBG1* and *HBG2*. Despite all strategies achieving therapeutic levels of HbF expression, gene knockdown approaches showed greater potential for clinical application.

### 3.4 Current Progress in Utilizing CRISPR-Cas9 for Sickle Cell Disease

The FDA on December 8, 2023, approved CASGEVY™, marking the first-ever cell-based CRISPR-Cas9 gene therapy for SCD in patients aged 12 and older experiencing recurrent vaso-occlusive crises[144, 145]. This milestone followed the approval of CASGEVY™ by the United Kingdom's Medicines and Healthcare products Regulatory Agency (MHRA) on November 16, 2023, for the treatment of both SCD and transfusion-dependent β-thalassemia[146].

CASGEVY™ functions by converting HbS in haemaotopoietic stem cells to HbF. It achieves this through the inactivation of *BCL11A* by targeting its erythroid-specific enhancer[25, 133]. Using CRISPR-Cas9, the patient’s hematopoietic stem cells are genetically edited and then

reintroduced into the patient through a one-time, single-dose infusion, with the goal of enabling them to engraft in the bone marrow [25,144]. Prior to this infusion, patients must undergo myeloablative conditioning, a high-dose chemotherapy regimen designed to eliminate affected cells from the bone marrow and create space for the modified stem cells[144]. With a successful engraftment, the CASGEVY™-modified stem cells are expected to enhance fetalHbF production. This increase in circulating HbF levels aims to prevent the sickling of red blood cells, addressing the root cause of SCD[144].

The FDA assessed the safety and efficacy of CASGEVY™ in adult and adolescent patients with SCD who had experienced at least two severe vaso-occlusive crises (VOCs) annually over the two years preceding screening[144]. Impressively, 93.5% of participants (29 out of 31) reported no severe VOC episodes for at least 12 consecutive months during the 24-month follow-up period. Furthermore, all patients treated with CASGEVY™ achieved successful stem cell engraftment, with no cases of graft failure or rejection observed[144]. The most reported side effects included thrombocytopenia, mouth sores, nausea, musculoskeletal pain, abdominal pain, vomiting, febrile neutropenia, headache, and itching [144]. These significant advancements signal the anticipated integration of CRISPR-Cas9-mediated gene editing into modern therapeutic strategies for SCD. Furthermore, they provide substantial hope for SCD patients who have limited treatment options [147].

### 3.5 Challenges and Limitations of CRISPR-Cas9 Therapies in Sickle Cell Disease

Although CRISPR-Cas9 holds great promise in SCD treatment, it faces several challenges which includes off-targeting, polymorphism, delivery method, and ethical concerns [148]. Precise editing of the *HBB* gene in HSPCs is essential to correct the underlying mutation [28]. However, off-target effects in these cells may disrupt essential genes for hematopoiesis or other crucial functions, which may compromise the viability and functionality of cells that have been altered [148]. Off-target effects occur when the Cas9 nuclease cleaves DNA sequences like the target sequence but located elsewhere in the genome. These off-target mutations can disrupt vital genes or regulatory regions, potentially leading to unintended consequences such as genotoxicity or activation of oncogenes, raising significant safety concerns for clinical applications [149].Several techniques have been developed to reduce the potential off-target effect of CRISPR/Cas-9, including sgRNA optimization, Cas-9 nuclease modification, the use of anti-CRISPR proteins, and other Cas-variants [150]. High-fidelity Cas9 variants, including eSpCas9, SpCas9-HF1, HypaCas9, evoCas9, xCas9, Sniper-Cas9, and HiFi, have also been developed [151].

An essential first step in minimizing the off-target effect is choosing and creating a suitable sgRNA for the intended DNA sequence [152]. When designing sgRNA, strategies such as GC content, sgRNA length, and chemical modifications of sgRNA must be considered [148]. Research has shown that the best ways to increase the genome editing efficiency of CRISPR/Cas-9 are to have a GC content of 40% to 60%, truncate (short length of sgRNA), and incorporate 2'-O-methyl-3'-phosphonoacetate in the sgRNA ribose-phosphate backbone [152, 153]. Another strategy of reducing off-target effects is by modifying the Cas-9 protein to maximize its nuclease selectivity. For instance, mutating either one of the catalytic residues of Cas-9 nuclease (HNH and RuvC) will transform the Cas-9 into nickase that could only cause a single-stranded break instead of a blunt cleavage [154] Utilizing sgRNA in conjunction with the inactivated RuvC domain of Cas-9 has been shown to decrease the off-target effect by 100–1500 times [155]. According to Paul and Montoya [156], the nuclease Cas-12a, formerly known as Cpf1, is a type V CRISPR/Cas system that has excellent genome editing efficiency. In contrast to the CRISPR/Cas-9 system, CRISPR/Cas-12a can reduce the size of plasmid constructs by processing pre-crRNA into mature crRNA without

tracrRNA [154]. More recently, multicomponent Class I CRISPR proteins like CRISPR/Cas-3 and CRISPR/Cas-10 have been used to edit genomes more efficiently than Cas-9 [157]. A significant portion of DNA can be removed from the target region by the ATP-dependent nuclease/helicase Cas-3 with few off-target effects. Small proteins called anti-CRISPR (Acr) proteins have also been produced from phages that inhibit the CRISPR/Cas system. They are a newly developed technique that reduces CRISPR/Cas-9's off-target effects [158].

Choosing a safe and efficient delivery strategy for the CRISPR-Cas9 system also poses as a significant challenge in the treatment of SCD [159]. To ensure precise editing of the defective HBB gene within the nucleus, the CRISPR system must be delivered precisely to the HSPCs in vivo or ex vivo in SCD. Achieving focused distribution, preventing unforeseen effects on off-target sites, and guaranteeing effective packing of the CRISPR components are the primary challenges in choosing a suitable delivery mechanism [159, 160].

Another significant challenge in CRISPR/Cas9 gene therapy for SCD is immunogenicity [22]. Due to their preexisting conditions, many SCD patients receiving treatment may be more vulnerable to immunological reactions. Pre-existing Cas9 antibodies in certain individuals can intensify immune responses, which could result in a swift elimination of altered cells and reduce the efficacy of the therapy [161, 162]. If Cas9 proteins or components remain after reinfusion, altered cells may cause immunological reactions for ex vivo approaches [162, 163]. To overcome these obstacles and guarantee the safety and effectiveness of CRISPR-based treatments for SCD, it is necessary to optimize delivery techniques to minimize exposure to Cas9 proteins, create hypoimmunogenic Cas9 variations, and put strict off-target evaluation procedures into place [164].

Polymorphism in the HBB gene and its neighboring loci, can make editing more difficult[165]. The effectiveness and accuracy of CRISPR-Cas9 targeting may be impacted by patient genetic variations, which could result in off-target effects or less than ideal editing outcomes [166]. Also, after editing, gene expression may be impacted by polymorphisms in non-coding areas like enhancers or regulatory elements. To address these challenges, highly specific guide RNAs that are tailored to each patient's unique genetic profile must be created, guaranteeing accurate editing while lowering the possibility of off-target effects [167, 168].

## 4. Challenges of Implementing CRISPR-Cas9 Therapies in Africa

The development of CRISPR-Cas9 gene-editing technology has ushered in promising therapeutic avenues for treating genetic disorders such as sickle cell disease (SCD). However, availability of advanced healthcare infrastructure in sub-Saharan Africa, high cost of gene therapy, long-term safety, ethical concerns, and potential risks associated with CRISPR-Cas9 therapies for SCD remains a primary concern [148, 169, 170].

In sub-Saharan Africa, the lack of advanced healthcare infrastructure and capacity capable of performing and supporting complex genetic modifications presents a major challenge. Complex laboratory sets up, stringent quality control measures, and highly trained personnel are necessary for CRISPR-Cas9 therapies, but these resources are frequently lacking in many African nations [169, 171].

Additionally, the cost of CRISPR-based therapies, is extremely expensive for most individuals and healthcare systems across Africa, where healthcare expenditure per capita is relatively low [172, 173]. This raises concerns regarding equity and accessibility, particularly for groups that are disproportionately impacted by SCD. Access to potentially curative therapies for underprivileged communities may be hindered by this financial barrier, which could worsen already-existing health disparities [174, 175].

The ethical, societal, and regulatory aspects associated with introducing CRISPR therapy also provides another significant challenge. The absence of thorough legal frameworks in many African nations to regulate genetic editing technology may raise issues regarding abuse or unforeseen repercussions [176, 177]. Additionally, there is a lack of public awareness and understanding of CRISPR technology, which may lead to resistance because of cultural or religious beliefs [177]. For diseases like SCD, where the technology must be applied at the germline or somatic level, the ethical implications are profound, particularly regarding equity in access and the potential for stigmatization of individuals receiving gene-editing treatments[178].

Safety, equality, and the possibility of unforeseen effects that could affect future generations are some of the many ethical concerns surrounding CRISPR-Cas9 technology [170]. Using CRISPR-Cas9 for germline editing raises serious concerns because it could result in heritable genetic alterations. The potential for CRISPR-Cas9 to promote "guerrilla eugenics," in which gene-editing technology is employed for objectives other than therapeutic ones, such as improving human traits or producing so-called "designer babies," is another source of concern [170, 179, 180]. This prospect raises issues regarding consent as future generations will be susceptible to genetic modifications done without their knowledge or involvement and leads to discussions about individual rights against collective genetic interventions [179]. The use of CRISPR-Cas9 also raises questions of accessibility and justice[181]. Although the potential of this technology to cure hereditary disorders like cystic fibrosis has been widely celebrated, socioeconomic constraints may limit access to such therapies [182, 183]. This discrepancy raises questions regarding social justice and equality in healthcare since it may exacerbate already-existing health disparities and create an ethical split where only specific communities benefit from genetic therapies[181]. The ethical implications of informed consent are paramount; ensuring that patients fully understand the benefits, risks, and uncertainties associated with gene-editing therapies is crucial for ethical clinical practice [184]. Also, off-target effects and unintended genetic alterations present health risks to patients, raising ethical questions regarding the safety and effectiveness of CRISPR-based treatments[185].

## 5. Current Regulatory Frameworks Governing Gene Therapies in African Countries

The regulatory environment around gene therapies in African nations is evolving, with several countries implementing important measures to establish frameworks that govern both the development and utilization of these cutting-edge medical interventions [186, 187]. Nigeria was the first African nation to publish genome editing guidelines, thus becoming a pioneer in this area [188]. Following suit, Kenya's National Biosafety Authority (NBA) published Genome Editing Guidelines in March 2022 to make it clear which genome-edited products and species are classified as conventional kinds and which are covered by the Biosafety Act. These guidelines place a strong emphasis on early consultation to identify the best regulatory pathway for genome editing initiatives [189]. To provide a favorable biosafety regulatory environment, Malawi has also achieved progress with the approval of its Genome Editing Guidelines in August 2022. These guidelines provide a step-by-step procedure for regulating genome editing and clarify which products are exempt from being regulated as genetically modified organisms (GMOs) [190]. In South Africa, the National Health Act of 2003 and the South African Health Products Regulatory Authority (SAHPRA) oversee regulating cellular therapies, including gene therapies. The nation mandates that unproven cellular therapies be investigated in clinical studies that are approved by SAHPRA and assessed by ethics boards [191]. Despite these advancements, complete gene therapy regulatory frameworks are still lacking in many African countries. The development of such

regulations is crucial to ensure the safe and ethical application of gene therapies across the continent.

## 6. Public Awareness and Acceptance of CRISPR-Cas9 Technology in Africa

In Africa, CRISPR-Cas9 technology is progressively becoming more widely known and accepted, especially in the agricultural sector [192, 188]. Nations like Kenya, Nigeria, and Eswatini have made significant progress in establishing guidelines to regulate gene editing and gene drive technologies, which reflects a growing institutional recognition of the potential benefits of CRISPR technology. Kenya's National Biosafety Authority, for example, has begun establishing guidelines for gene-edited items to offer a clear roadmap for their development and use [193]. Despite these advancements, public understanding of CRISPR technology remains limited across much of the continent [177, 192]. This gap is a result of the intricacy of gene-editing science as well as a dearth of extensive educational resources [192, 194]. Additionally, cultural and ethical concerns may influence public perception and acceptance [170, 184]. Comprehensive public engagement efforts that incorporate education and open communication of the advantages and dangers of CRISPR are required to solve these issues. These initiatives are important for building trust and supporting African populations in making well-informed decisions about the use of gene-editing technology.

## 7. Addressing Misconceptions and Promoting Education about Gene Therapies in Africa

To promote public awareness, acceptance, and appropriate use of gene therapies, it is important to eliminate myths and promote understanding of these technologies. Gene therapy misinformation frequently results from ignorance or misunderstanding of scientific principles, which can give rise to concerns about "playing God," moral dilemmas, or inflated dangers [195, 196]. To eliminate this, communities must be actively engaged by stakeholders, such as governments, scientists, and medical professionals, through easily available and culturally appropriate teaching initiatives. Making simpler complex scientific ideas into relatable terms, leveraging multimedia platforms, and involving trusted community leaders can help eliminate myths and clarify the purpose and safety of gene therapies [197, 198].

Education programs should highlight the potential advantages of gene therapies, including its ability to treat unmet medical needs, improve quality of life, and cure genetic diseases, while also acknowledging and addressing ethical and safety concerns. Tailored interventions, such as workshops for healthcare workers, seminars for policymakers, and school-based programs, can ensure that different demographics receive relevant and comprehensible information [198, 199]. It is also beneficial to have public discussion platforms where people may voice their concerns, ask questions, and get direct answers from professionals. These initiatives can produce a better-informed society that is better able to assess and encourage the responsible use of gene therapies.

## 8. Opportunities for CRISPR-Cas9 Adoption in Africa

CRISPR-Cas9 technology offers Africa a transformative opportunity to strengthen its capacity for genetic medicine, addressing the continent’s high burden of genetic diseases like SCD [192, 200]. With sub-Saharan Africa having a notably high prevalence of SCD, creating CRISPR-based treatments specifically for this region could drastically lower rates of morbidity and mortality [57]. Implementing CRISPR-based treatments requires expanding infrastructure, such as providing laboratories with cutting-edge genomic editing tools and bioinformatics capabilities. Moreover, the integration of genetic medicine into current

healthcare systems can be improved by supporting interdisciplinary training for medical professionals, such as geneticists, molecular biologists, and clinicians [201]. Investing in genetic counseling is also necessary to prepare communities for the ethical, social, and clinical implications of CRISPR therapies [198].

Developing local expertise in genome editing and biotechnology is an important foundation for sustainable CRISPR-Cas9 adoption in Africa [198, 202]. The establishment of graduate and postgraduate programs in molecular biology, bioinformatics, and biotechnology in African universities can result in a workforce with the necessary skills to advance research on gene editing. Additionally, measures like mentorship programs and partnerships with leading research institutions globally can accelerate knowledge transfer and innovation [203]. Local knowledge guarantees culturally appropriate responds to genetic health issues and reduces dependency on external support [204]. Also, because of Africa's genetic diversity, local researchers have a rare chance to investigate how CRISPR affects different populations, which could lead to the discovery of new treatment approaches and increase the technology's global applicability [205, 206].

CRISPR-Cas9 technology can be incorporated into Africa's healthcare and research ecosystems through collaborative research projects [192]. International research organizations and African universities can collaborate to share resources, exchange knowledge, and create gene-editing applications tailored to a specific region. For example, genome-editing projects in SCD treatments and malaria vector control have been made possible by initiatives supported by institutions such as the Bill & Melinda Gates Foundation and the Wellcome Trust [187]. African nations can also benefit from regional collaborations, such as the African Union's scientific research initiatives, to establish centralized research hubs for CRISPR technology. Such partnerships have the potential to increase CRISPR-Cas9 adoption throughout the continent by facilitating funding acquisition, streamlining regulatory frameworks, and raising public awareness.

## CONCLUSION

CRISPR-Cas9 presents a transformative opportunity in the fight against sickle cell disease, as it offers the potential for a long-term solution. Preclinical and clinical studies show that CRISPR-Cas9 has the potential to be a revolutionary and effective treatment for sickle cell disease. In contrast to traditional symptomatic treatments, CRISPR-Cas9 addresses the genetic basis of the disease, offering a more sustainable and comprehensive solution. However, realizing this potential in Sub-Saharan Africa requires addressing significant challenges, including inadequate healthcare infrastructure, high treatment costs, limited expertise, and societal and ethical concerns about gene-editing technologies. While CRISPR-Cas9 is not an immediate solution, it represents a significant step toward addressing the high burden of sickle cell disease in Sub-Saharan Africa. With sustained commitment, strategic investments, and strong collaborative efforts, CRISPR-based therapies could become accessible, affordable, and widely implementable, paving the way for a future where sickle cell disease is no longer a life-limiting condition in Sub-Saharan Africa.

Ethical Approval and Consent:

As per international standards or university standards written ethical approval has been collected and preserved by the author(s).

As per international standards or university standards, patient(s) written consent has been collected and preserved by the author(s).

## Disclaimer (Artificial intelligence)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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