

CRISPR-Cas9 as a Curative Frontier: Precision Editing Strategies for Sick Cell Disease

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ABSTRACT

Sickle cell anemia (SCA) is an inherited hematological disorder caused by the polymerization of defective hemoglobin that results in chronic hemolysis and recurrent vaso-occlusive crises. Conventional therapies, such as hydroxyurea administration and blood transfusions, have primarily focused on symptom management and offer only partial clinical benefits. Clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated protein 9 (Cas9) technology has introduced a paradigm shift in the treatment of SCA by enabling precise and potentially curative genetic modification. This review outlines the molecular pathophysiology of SCA and elucidates the mechanisms of CRISPR-related genome modifications, highlighting the therapeutic use of correcting the causative rs334 mutation and modulating BCL11A expression. Recent advancements in preclinical and clinical research have been discussed, along with ethical, regulatory, and translational considerations. Targeting the genetic etiology of SCA through CRISPR-Cas9 offers a promising route towards a permanent cure, underscoring its potential as a transformative strategy in the field of precision genomic medicine.

Keywords: sickle cell anemia, CRISPR-Cas9, hemoglobin S, BCL11A, HbF, genome editing therapy.

1. INTRODUCTION

1.1 Overview of Sickle Cell Anemia

Despite decades of advancements in biomedicine, sickle cell anemia (SCA), one of the most researched monogenic illnesses, continues to be a significant health burden worldwide. The disorder is caused by a single A→T transversion in the *HBB* gene (rs334), which causes valine to replace glutamic acid at position six of the β -globin chain (Glu6Val) [1]. The physicochemical characteristics of hemoglobin are substantially changed by this seemingly insignificant amino acid shift, which encourages hydrophobic interactions that promote hemoglobin S (HbS) polymerization in deoxygenated environments [2].

Red blood cells (RBCs) lose their deformability, take on stiff, “sickle” shapes, and experience repeated cycles of injury as HbS polymers accumulate. This results in endothelial activation, chronic hemolysis, and recurrent vaso-occlusive crises (VOCs) [3], which are characteristic features that cause significant morbidity and early mortality [4]. SCA's worldwide spread is a result of past malaria-related screening. Sub-Saharan Africa has the highest disease burden, with more than 300,000 newborns suffering from SCA each year. According to epidemiological data, the frequency of SCA is 1 in 365 live births in African American and 1 in 16,300 live births in Hispanic neonates (Figure 1), and children aged 5 to 12 years in low-income environments are frequently underweight, which may be associated with increased disease severity [5,6].

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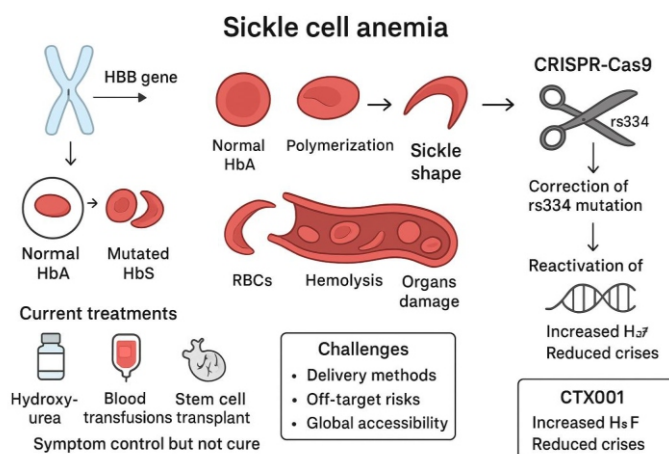


Figure 1: Sickle cell anemia and CRISPR Cas9

Additionally, there is a notable incidence of SCA in the Middle East, India, and some Mediterranean communities [7]. Most of the children in low- and middle-income countries (LMICs) still suffer from high early-life mortality because of delayed diagnosis, restricted access to antibiotics and immunizations, and poor care infrastructure, in contrast to advancements in newborn screening and supportive care in high-income regions [8]. Hematopoietic stem cell (HSC) transplantation, hydroxyurea, and continuous transfusion regimens are examples of current disease-modifying techniques that offer varied degrees of clinical benefit, but they fall short of a scalable, universal cure [9]. HSC transplantation is curative but restricted by donor availability, graft-versus-host disease, and prohibitive costs; hydroxyurea generates fetal hemoglobin (HbF) but is insufficient for patients with severe SCA; and transfusions cause iron overload problems [10]. In LMICs, where access to curative treatments is scarce and supportive care is variable, the therapeutic gap is especially noticeable [11] (Figure 2).

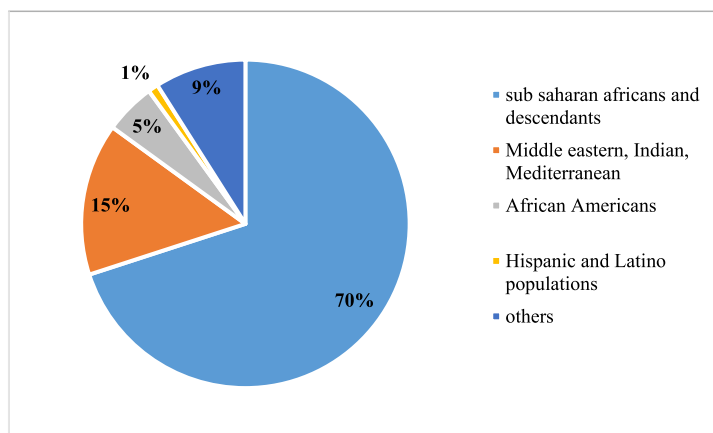


Figure 2: Global distribution of sickle cell anemia [12]

1.1.1 Clinical Burden and Complications of Sickle Cell Disease

According to the World Health Organization (WHO), approximately 5% of the global population carries the trait genes for hemoglobin disorders, primarily SCA and thalassemia. Hemoglobin disorders are genetic blood diseases caused by the inheritance of defective hemoglobin genes from perfectly healthy parents. Every year, more than 300,000 children are born with severe hemoglobin abnormalities. The health burden of hemoglobin problems can be significantly decreased by treatments and prevention programs. As per WHO, sickle cell disease (SCD) has been estimated to affect 7.74 million individuals worldwide in 2021, with 515,000 new cases, mostly in sub-Saharan Africa, that account for roughly 80% of all cases [13].

Table 1: Complications of sickle cell disease [15,16]

Complication	Definition	Age	Cause
Hand-foot syndrome	Painful swelling of one or more extremities	0–3 years	Bone marrow infarction secondary to vaso-occlusion
Painful crisis	Acute pain, most commonly in the extremities, chest, abdomen, and back	>6 months; occurs throughout life	Tissue damage and hypoxia
Acute chest syndrome	Symptoms similar to pneumonia	Increasing frequency from early childhood; occurs throughout life	Vaso-occlusion and bone marrow fat embolism
Stroke	Acute cerebrovascular accident	Increasing frequency with age	Infarctive stroke, vasculopathy of cerebral vessels, or hemorrhagic stroke
Heart failure	Most commonly left ventricular failure	Increasing frequency with age	Ventricular hypertrophy and high-output failure secondary to anemia
Kidney failure	Deteriorating kidney function, reduced concentrating ability, proteinuria, and progressive kidney failure	Increasing frequency with age	Tissue damage
Leg ulcer	Chronic skin ulcers, most commonly around the malleolar regions	Increasing frequency with age	Hemolysis with vasculopathy
Hypersplenism	Enlargement of the spleen with reduction in hemoglobin and multiple blood cell types	More common in childhood	Unknown, but commonly follows an episode of splenic sequestration

When considering the overall mortality burden, SCD ranks as the 12th most common cause of death in children under the age of 5 years, accounting for 81,000 fatalities in 2021. The impact of SCD is underestimated by traditional mortality recording; the actual deaths are 11 times higher than that indicated by specific statistics (376,000 vs. 34,400 deaths in 2021). The common consequences of SCD are acute pain crises, anemia, stroke, infections, kidney failure, and pregnancy-related concerns (Figure 3). Vaccinations to prevent infections and disease-modifying drugs like hydroxyurea are examples of effective therapies. Immediate action is required to ensure access to necessary medications and incorporation of SCD care into primary health systems [14] (Table 1).

1.2 Diagnostic Methods for SCD

1.2.1 Hematological and Molecular Diagnostics

Several diagnostic methods are available to identify SCA, which is a chromosomal genetic condition that is inherited rather than acquired. The diagnostic process is initiated by collecting and analyzing the patient's medical and family history. The first assessment of SCA is physical signs like jaundice, pale complexion, and swelling of the hands and feet. Blood tests play a significant role in the confirmation of SCD by using clinical indicators [17].

Blood test is the first diagnostic approach, in which a complete blood count from a blood sample is conducted to assess decreased hemoglobin levels and increased reticulocyte counts. Peripheral blood smearing is an advanced method used to identify SCD. This method allows the observation of distinct sickle-shaped RBCs. Another commonly used technique is high-performance liquid chromatography. This technique separates and identifies various forms of hemoglobin in the blood. Additional techniques include hemoglobin electrophoresis used to determine specific hemoglobin types, especially HbS, an important factor that causes SCA [18].

1.2.2 Prenatal and Imaging Techniques

The prenatal diagnostic methods used to detect SCD are of two types: chorionic villus sampling and amniocentesis (Figure 4). In chorionic villus sampling, placental cells are tested for genetic abnormalities, whereas amniocentesis is performed to test the amniotic fluid and identify the presence of the sickle cell gene. Transcranial doppler ultrasonography is a diagnostic technique used to measure cerebral blood flow, and it helps to determine stroke. A variety of imaging methods, including X-ray, ultrasonography, and magnetic resonance imaging [19], are used to check organ function and identify any related problems. These diagnostic techniques are important as they help us in early detection, which reduces the risks and improves the overall health of patients. Regardless, SCD is linked with undesirable maternal and prenatal outcomes, such as placental malformations related to vascular malperfusion [20].

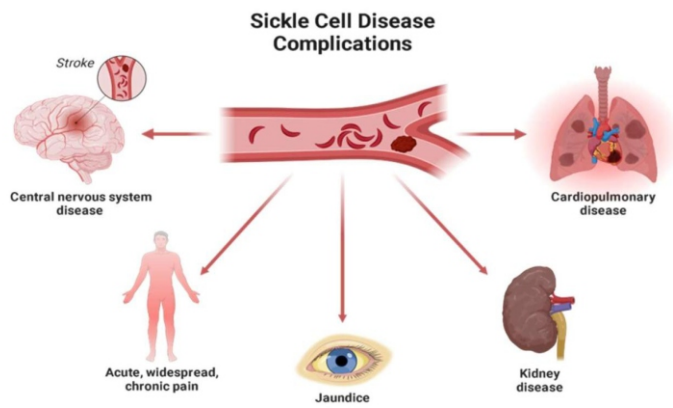


Figure 3: Complications of sickle cell anemia

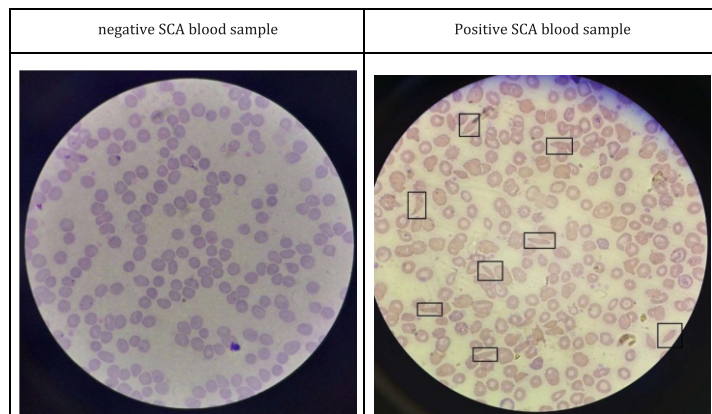


Figure 4: Normal RBCs vs Sickle RBCs from Blood sample

1.3 Current and Emerging Treatments

1.3.1 Hydroxyurea and Blood Transfusions

Historically, treatment options for SCA were largely restricted to blood transfusions administered primarily in cases such as acute chest syndrome or severe anemia, with the use of opioids limited to instances of intense pain. Over time, therapeutic strategies expanded to include prolonged transfusion regimens with iron removal therapy, including the administration of hydroxyurea for patients with severe or complex presentations. Recent information on the pathophysiological and molecular causes of SCA have sparked the creation of several novel treatment approaches. These new treatments and drugs offer the potential to not only manage symptoms more effectively but also alter or reverse the course of the disease [21]. Hydroxyurea remains one of the limited therapies capable of modifying the clinical course of SCA and approved by the U.S. Food and Drug Administration (FDA). Its efficacy in managing SCA was recognized more than 30 years ago, primarily because of its capacity to enhance HbF levels, which contributes to reduced disease severity. Originally designed and used as an anticancer agent, hydroxyurea functions by inhibiting ribonucleotide reductase, a critical enzyme necessary for the synthesis of deoxyribonucleotides and subsequent DNA replication processes [22].

Hydroxyurea can affect a number of pathological processes, so it is considered an important therapeutic drug in the treatment of SCA [23]. It efficiently decreases platelet, reticulocyte, neutrophil, and inflammatory cytokine levels when taken as directed. These factors are all linked to the promotion of inflammation and vascular blockages. By decreasing the levels of these contributors, hydroxyurea significantly helps to prevent VOCs.

Furthermore, the ability of hydroxyurea to induce HbF production remains one of its most important clinical benefits, offering a proven approach to reducing the severity of SCA symptoms [24]. While hydroxyurea offers substantial therapeutic benefits, it is also associated with notable adverse effects that warrant careful consideration. It may suppress bone marrow function, leading to reduced blood cell counts and decreased overall hemoglobin production [25]. *Plasmodium falciparum* schizont stages in normal and sickle RBCs can be killed in vitro by using hydroxyurea and other ribonucleotide reductase inhibitors. Hydroxyurea for SCA treatment in areas where malaria is widespread, offers a novel biological model for treating a genetic illness. It also helps to treat a potentially fatal infection concurrently, which is necessary in global health settings [26,27].

1.4 Gene Editing as a Curative Option

1.4.1 CRISPR-Based Approaches

The development of precise genome-editing tools over the past decade, particularly clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated protein 9 (Cas9), has substantially expanded the therapeutic potential for SCA. CRISPR-Cas9 enables programmable, sequence-specific modification of genomic loci by using a single guide RNA. SCA represents an important model for therapeutic genome editing because it is attributable to a well-characterized pathogenic variant in the *HBB* gene. Three major CRISPR-based strategies have been explored: direct correction of the rs334 mutation, disruption of the BCL11A erythroid-specific enhancer to reactivate HbF, and targeted engineering of HSCs.

Direct correction of the rs334 mutation aims to restore the normal *HBB* sequence through precise genome editing. Preclinical studies have demonstrated the potential to correct the Glu6Val mutation in patient-derived HSCs and improve hemoglobin production and sickling-related phenotypes [28]. However, off-target editing, large deletions, chromosomal rearrangements, and other structural variants remain important safety considerations that require further investigation.

The second strategy involves disruption of the erythroid-specific BCL11A enhancer to reactivate HbF (Figure 5). Because HbF has anti-sickling properties, increasing its expression can reduce HbS polymerization and the clinical manifestations of SCA. CRISPR-mediated BCL11A enhancer editing has demonstrated substantial HbF induction and progressed to clinical application. The successful CTX001/Exa-cel studies have demonstrated sustained clinical benefits, including reductions in VOCs and prolonged transfusion independence in the treated patients [29,30].

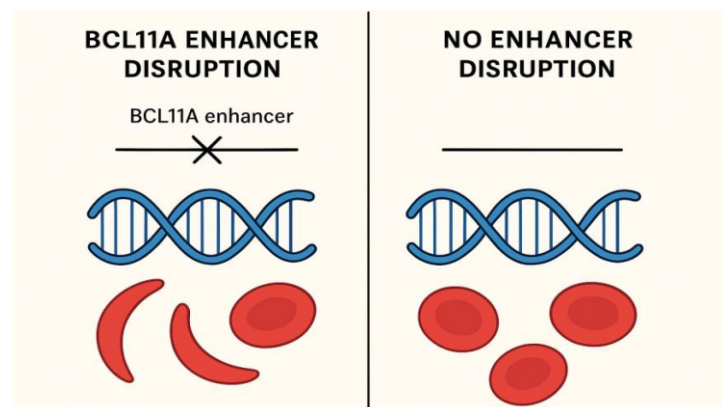


Figure 5: BCL11A enhancer disruption vs No enhancer disruption

The third approach focuses on the precise engineering of autologous HSCs, which can repopulate the hematopoietic system after transplantation. Advances in high-fidelity Cas9 variants, electroporation, and ribonucleoprotein (RNP) delivery have improved editing efficiency while potentially reducing genotoxicity [31]. These developments have facilitated the translation of CRISPR-Cas9-based approaches into clinical studies for SCA (Figure 6).

Other genome-editing platforms, such as zinc finger nucleases, transcription activator-like effector nucleases, base editing, prime editing, Cas12f, and CasMINI, are also being investigated as alternative or next-generation approaches. These technologies provide opportunities to improve editing precision and expand the range of achievable genomic modifications. However, unintended off-target effects and other genomic alterations remain important considerations that require comprehensive safety assessments [33,34] (Table 2).

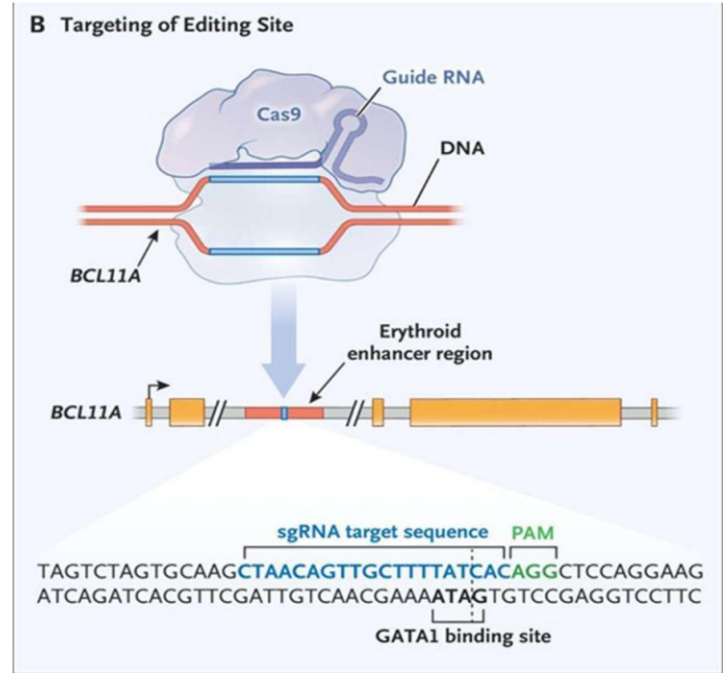


Figure 6: CRISPR Cas 9 editing the BCL11A gene [32]

Table 2: Comparison of Different Gene Editing Tools [35]

Feature	ZFNs (Zinc finger nucleases)	TALENs (Transcription Activator like effector nucleases)	CRISPR Cas9
Precision	Moderate – DNA recognition via protein-DNA interaction; can have off-target effects	High – More customizable and specific to target sequence	Very High – RNAguided; can be engineered for high specificity
Efficiency	Moderate – Laborintensive design and construction	Moderate to High – Easier to design than ZFNs	Very High – Rapid design and implementation
Ease of Use	Complex – Requires designing specific proteins for each target	Easier than ZFNs, but still protein-based	Simple – Uses guide RNA (gRNA), no protein engineering needed
Cost	High – Custom proteins are expensive to produce	High – But less than ZFNs	Low – Widely available and affordable
Delivery	Viral vectors or electroporation	Similar to ZFNs, sometimes more difficult due to larger size	Versatile – Compatible with many delivery methods
Applications	Gene knockout/knockin in research; early clinical trials	Used in disease modeling and some therapies	Widely used in research, diagnostics, agriculture, and human therapy (e.g., sickle cell, cancer immunotherapy)

2. MOLECULAR PATHOPHYSIOLOGY OF SCA

2.1 HbS Polymerization and Sickling

A single nucleotide change in the β -globin gene (Glu6→Val) causes SCA by changing the physicochemical profile of hemoglobin and increasing the likelihood that deoxygenated hemoglobin will polymerize into stiff, insoluble fibers. This polymerization is the main molecular process responsible for erythrocyte sickling, mechanical stiffness, and decreased deformability [36]. RBCs restore their biconcave shape during initially reversible cycles of deoxygenation and reoxygenation, yet accumulated membrane and cytoskeletal damage eventually results in an irreversibly sickled, dense population of erythrocytes with a shorter lifespan [37].

Recent studies have identified a range of intracellular modulators that shorten the nucleation delay time and speed up HbS polymerization [38]. The deoxygenation mechanism that speeds up HbS polymerization results in increased cytosolic 2,3-diphosphoglycerate levels that promote the production of deoxy-HbS via reducing hemoglobin-oxygen affinity and increasing sphingosine-1 activity. The Ca^{2+} -dependent Gardos channel is activated by elevated intracellular calcium, leading to fast potassium efflux and cellular dehydration and repeated erythrocyte membrane damage, which increases the concentration of mean corpuscular hemoglobin and amplifies polymer production [39].

Sphingosine-1-phosphate-mediated metabolic dysregulation has also been demonstrated to exacerbate sickling by modifying RBC ionic balance and increasing oxidative stress [40]. Recently, the persistence of mitochondria in adult sickle erythrocytes has been identified as an unexpected factor in the production of ROS [41], increase in intracellular Ca^{2+} , and irreversible membrane stiffness. These elements work together to produce an intracellular milieu that is highly polymerization-permissive, which shows that even mild hypoxia or acidosis can cause sickling crises.

2.2 Hemolysis, Inflammation, and Vaso-occlusion

Although HbS polymerization is the initial defect, the resulting cascade of hemolysis and vaso-occlusion is mostly responsible for the severity of SCA [42]. Intravascular hemolysis releases free hemoglobin and heme into the bloodstream, where they quickly scavenge nitric oxide, limit vasodilation, and cause oxidative stress and endothelial damage [43]. This hemolysis-related vasculopathy promotes a pro-adhesive, pro-thrombotic condition and primes the vascular wall for leukocyte and platelet activation. Vaso-occlusion is not caused by simple mechanical blockage but rather by multilevel cellular cross-talk [44]. Heterocellular aggregates of activated RBCs, neutrophils, monocytes, and platelets stick to the inflammatory endothelium via integrins, selectins, and erythrocyte adhesion molecules.

A persistent cycle of tissue damage is sustained by the subsequent microvascular obstruction, which causes ischemia and reperfusion-driven bursts of ROS and inflammatory cytokines. Long-term morbidity and mortality in SCA are ultimately determined by persistent inflammation, which propels progressive organ damage, especially in the spleen, lungs, kidneys, and central nervous system [45]. Long strands are easily formed by HbS polymers. The affected erythrocytes are deformed by the lengthening polymer fibers, which reduces their flexibility and affects rheology. This erythrocyte distortion causes hemolytic anemia and vaso-occlusion, which obstructs blood flow and causes ischemic episodes called crises (Figure 7). The brain, bones, lungs, spleen, and liver are among the organs that sustain significant damage during crises, leading to significant morbidity and mortality.

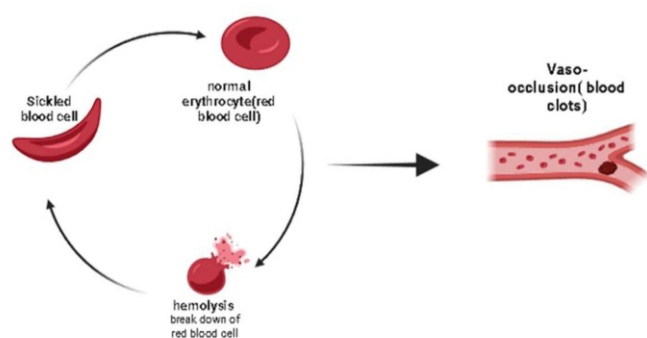


Figure 7: Sickle cell anemia cycle

3. EVOLUTION OF GENE THERAPY FOR SCA

3.1 From Viral Vectors to Genome Editing: An Era of Rapid Transition

In the past, lentiviral vectors were used extensively in gene therapy for hemoglobinopathies such as SCD and β -thalassemia, for example, LentiGlobin (also known as lovotibeglogene autotemcel/Lyfgenia). Using this method, functional globin genes were transferred to HSCs generated from patients. Although these methods resulted in continuous hemoglobin production, they had intrinsic drawbacks, such as the possibility of insertional mutagenesis, fluctuating transgene expression, and silencing or dysregulation due to the vector. However, since 2020, the field has experienced an important change, shifting from “gene addition” to “gene correction and regulation” with the use of gene-editing technologies and programmable nucleases [46].

3.2 Rationale for Choosing Genome Editing

Safe and precise genome editing reduces the dangers of insertional mutagenesis and unexpected expression by precisely correcting (or modulating) the endogenous locus instead of randomly inserting a transgene [47]. Sturdy physiological expression in an erythroid environment and editing the native globin gene or its regulatory elements maintain the natural control of transcription and regulation, perhaps providing more stable, regulated expression than exogenous vectors [48]. With respect to ease of use and scalability, when compared with viral vector generation, transduction, and long-term transgene maintenance, genome editing may eventually be less complicated and expensive because of the success of ex vivo editing and encouraging developments toward in vivo editing [49].

3.3 Implementation of ex vivo CRISPR-Cas 9 Editing in SCA

3.3.1 Correction of the Defective Gene

CRISPR-Cas9-based genome editing has been investigated as a strategy for directly correcting the pathogenic rs334 mutation in the *HBB* gene responsible for SCA. Compared with conventional lentiviral gene-addition approaches, CRISPR-Cas9 offers the potential for targeted modification of the endogenous *HBB* locus while preserving its native genomic context [50,51]. In this approach, the pathogenic sequence is targeted for precise genome modification, with homology-directed repair (HDR) enabling sequence correction when an appropriate donor DNA template is available. Thus, direct genome editing of the rs334 mutation represents a potential strategy for restoring functional β -globin production and addressing the underlying genetic defect in SCA [52].

3.3.2 BCL11A Editing to Induce HbF

HDR uses a donor DNA template to facilitate precise correction of a Cas9-induced double-strand break (DSB) and can therefore support restoration of normal hemoglobin production [53]. An alternative genome-editing strategy is to reactivate HbF by targeting BCL11A, a key regulator of the developmental transition from HbF to adult hemoglobin. Disruption of the erythroid-specific regulatory region of BCL11A can relieve repression of γ -globin expression and increase HbF production [54]. Because HbF is less prone to polymerization than HbS under deoxygenated conditions, increased HbF levels can reduce erythrocyte sickling and alleviate the clinical manifestations of SCA [55]. Consequently, BCL11A editing represents an important therapeutic strategy for reducing disease severity in affected individuals [56].

Rather than directly modifying the pathogenic *HBB* allele, CRISPR-Cas9-mediated editing of the BCL11A enhancer provides an alternative approach for increasing HbF expression [57-59]. After ex vivo editing, autologous HSCs are reinfused into the patient, where the modified cells can contribute to hematopoiesis and sustained production of HbF [60,61].

Reactivation of HbF through BCL11A enhancer editing has progressed from a mechanistic strategy to a clinically validated therapeutic approach. In patient-derived CD34+ HSCs, CRISPR-Cas9-mediated editing of the erythroid-specific BCL11A enhancer can relieve repression of the γ -globin genes (*HBG1/HBG2*), thereby increasing HbF production. Exagamglogene autotemcel (Exa-cel; brand name Casgevy), approved in 2023 for SCD and transfusion-dependent β -thalassemia (TDT), is based on this genome-editing strategy [62]. Clinical studies of Exa-cel have demonstrated substantial reductions in VOCs and transfusion requirements, together with sustained HbF induction and durable therapeutic benefit [63-65].

3.4 Implementation of in vivo CRISPR-Cas9 Gene Editing in SCA

Until recently, ex vivo treatment of patient-derived HSCs, including extraction, editing, conditioning, and reinfusion, was the main gene-editing therapy for SCD and β -thalassemia [66]. This is a complicated, costly, and infrastructure-intensive technique. The trend towards in vivo HSC gene therapy, which involves introducing gene editing agents directly into the body (for example, through targeted vectors or nanoparticles) to modify HSC in situ, is becoming more prominent in recent years.

Such a breakthrough could significantly reduce expenses; increase accessibility, particularly in locations with limited resources where hemoglobinopathies are common; and simplify treatment. Concurrently, safety, immunogenicity, delivery efficiency, and off-target editing remain major challenges that ongoing research aims to overcome [67]. Between 2023 and 2025, significant preclinical development brought in vivo genome editing for SCD closer to clinical translation. Notably, a 2023 study found that vectorized prime editing delivered directly to HSCs in vivo rescued the SCD phenotype in a mouse model, correcting the sickle cell *HBB* mutation without requiring ex vivo manipulation, transplantation, or myeloablation [68]. Parallel studies have concentrated on developing innovative delivery vehicles, like targeted lipid nanoparticle (LNP) systems capable of delivering RNA gene writing payloads to long-term HSCs in both humanized mice and non-human primates with a single intravenous infusion [69]. In a 2025 non-human primate study, specially developed LNPs that delivered editing components to HSCs achieved therapeutically relevant editing levels in γ -globin (*HBG1/2*) promoters, a strategy intended to upregulate HbF, with a mean of 58% on target editing at 5 months after just one injection [70]. In addition to these delivery breakthroughs, improved gene-editing modalities, such as base editors that avoid DSBs, are being recognized as safer and more precise for HSC gene therapy, making them appropriate for long-term correction of monogenic blood diseases such as SCD. Overall, these results point to a feasible near-term approach to first in-human in vivo editing trials targeting HSCs for SCD or related hemoglobinopathies [71].

3.4.1 Challenges of in vivo CRISPR-Cas9 Gene Editing

Despite these improvements, several significant difficulties and safety or translation barriers remain before in vivo CRISPR-based therapeutics for SCD can become conventional clinical practices. First, while high editing efficiency in animal models (mice and non-human primates) is exciting, its applicability to humans, particularly targeting long-term repopulation of HSCs at therapeutically adequate quantities, is unknown [72]. Differences in HSC biology, marrow microenvironment, immunological responses, and vector biodistribution between species may make it difficult to replicate results in humans [73]. Second, there are still concerns about off-target effects, significant genomic rearrangements, and long-term genotoxicity, particularly if editing entails DSBs, even with base editors or prime editors, and uncommon off-target or unintentional changes need to be carefully characterized over an extended period. Prior to clinical trials, thorough preclinical genomic-integrity evaluation, clonal monitoring, and structural variant analysis are required [74]. Third, production must adhere to strict regulatory and quality (GMP) requirements to manufacture and scale clinical-grade delivery vehicles such as LNPs that are safe, repeatable, and provide uniform dosages in humans [75,76]. Finally, because SCD disproportionately impacts low-resource areas, ethical, legal, and access issues must be considered. In vivo editing raises concerns about cost, informed consent, long-term monitoring, and equitable availability, especially in global contexts [77]. In the future, it will be crucial to continue improving delivery mechanisms more marrow-tropic LNPs, potentially tissue-specific targeting ligands in conjunction with precision editing tools (base or prime editors), strict safety validation, and scalable production pipelines [78].

If these coincide, in vivo HSC editing may provide a potentially durable therapeutic approach for SCD by eliminating the requirement for ex vivo manipulation, conditioning regimes, and transplantation logistics. However, preclinical validation, regulatory strategy, ethical governance, and global access planning must all work together to accomplish this.

3.4.2 Improving CRISPR Treatments for SCD

Beam Therapeutics and CRISPR Therapeutics are working on in-vivo editing techniques for TDT and SCD. Currently, all treatments are ex vivo, meaning that after the patient receives rigorous chemotherapy, the cells are removed from the body, altered and quality-checked in a specialized laboratory, and then reintroduced into the body. Delivering the genome editing medication straight into the patient's body, where the cells will be altered, is known as in vivo therapy [79], in which antibodies are used to target chemotherapy to particular cells, find ways to lessen the impact of chemotherapy conditioning, and eventually enable the use of lower doses [80]. This research is in its early stages when compared with that being conducted by CRISPR Therapeutics. However, if effective, these advancements would lessen the burden on patients, and in vivo editing could greatly expand access to treatment.

4. Transition beyond CRISPR-Cas 9: The Rise of Base Editing, Prime Editing, and Next-Generation Nucleases

4.1 CRISPR as a Bacterial Immune System

CRISPR and Cas proteins constitute an adaptive immune system found in bacteria and archaea that provides defense against invading genetic elements, including bacteriophages and plasmids [81]. CRISPR loci contain repetitive DNA sequences interspersed with spacer sequences derived from previously encountered foreign genetic material. These spacer sequences provide a molecular memory of prior infections, whereas Cas proteins participate in the recognition and degradation of foreign nucleic acids [82,83].

Among the different CRISPR systems, the type II CRISPR-Cas9 system has been widely adapted for genome editing because Cas9 can be directed to specific DNA sequences by a guide RNA (gRNA) and requires recognition of a protospacer adjacent motif (PAM) for target binding and cleavage [84–86]. The gRNA-Cas9 complex recognizes a complementary DNA sequence and facilitates site-specific DNA cleavage, after which cellular DNA-repair pathways determine the resulting editing outcome [87–89].

4.2 Mechanism of Cas9-Mediated DNA Editing

In therapeutic genome editing, CRISPR-Cas9 uses a gRNA to direct the Cas9 nuclease to a complementary genomic sequence adjacent to an appropriate PAM. Target recognition is followed by Cas9-mediated cleavage of the DNA, generating a site-specific DSB [90,91]. The DSB is repaired primarily through endogenous DNA repair pathways, particularly non-homologous end joining (NHEJ) or HDR [92–95]. NHEJ can introduce insertions or deletions (indels) that disrupt gene function, whereas HDR can facilitate precise sequence correction when a suitable repair template is available. In SCD, these repair mechanisms support different therapeutic strategies, including direct correction of the pathogenic *HBB* mutation and disruption of regulatory elements involved in HbF expression. In particular, CRISPR-Cas9-mediated editing of the erythroid-specific *BCL11A* enhancer can reactivate HbF production and thereby reduce HbS polymerization and sickling.

The selection of the editing strategy depends on the target locus, desired genetic outcome, editing efficiency, and associated safety considerations [96-98] (Figure 8).

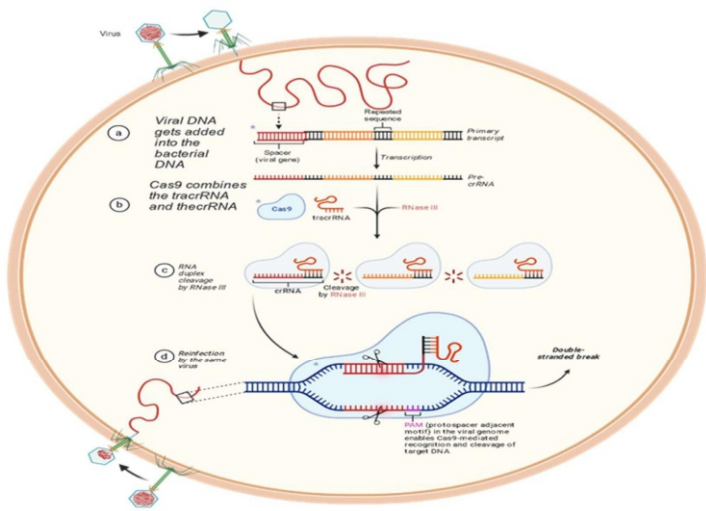


Figure 8: CRISPR in Bacteria [99]

4.3 Limitations

However, a major CRISPR-Cas9 disadvantage is the use of DSBs [100], which may have unforeseen consequences even though their introduction is necessary to induce repair. At the site of the cut, the NHEJ repair mechanism may add insertions or deletions that interfere with coding sequences or regulatory regions, occasionally resulting in changes that cause loss of function.

HDR is practically inactive in non-dividing cells, making precise correction impossible. Off-target activity is another major problem. The Cas9 enzyme can bind to and cleave DNA at unwanted genomic locations that are highly similar to the target sequence, despite well-designed gRNAs. This may result in off-target mutations that affect important genes or oncogenes and have unforeseen consequences [101].

4.4 Improving Delivery Systems and Reducing Off-Target Effects

Successful and secure delivery of reagents to target cells is still a significant challenge. Existing ex vivo editing methods for SCD are based on electroporation of Cas9 gRNA RNPs into cultured hematopoietic stem and progenitor cells (HSPCs). Although this is effective, it involves myeloablative conditioning and specialized facilities, so it is not readily accessible in resource-limited settings. New delivery strategies such as LNPs are presently utilized in mRNA vaccines, and LNPs have the capacity to deliver Cas9 mRNA as well as gRNA into target cells without integration risks. Non-integrating viral vectors, like adenovirus and integrase-deficient lentivirus, allow transient expression and reduced genotoxicity risk. Cell-penetrating peptides and extracellular vesicles provide non-invasive delivery for in vivo uses. The expression period of genome editors affects vectors that are used for in vivo genome editing [102,103]. Currently, the principal vectors for this kind of therapy are adeno-associated virus and LNPs. AAV-delivered gene expression can endure for a long period in terminally differentiated cell types [104-106] (Table 3).

Table 3: Comparative Gene-Editing Approaches for Sickle Cell Disease [104,105]

Gene editing approaches	Mechanism	Application in sickle cell disease	Reported editing efficiency in HSCs	Major genomic concerns	Delivery and scalability
CRISPR Cas 9 (spCas9, DSB based)	sgRNA guided nucleases creates double stranded break (DSB), repaired by NHEJ (knockout) or HDR (precise repair)	BCL11A erythroid enhancer knockout to induce HbF, Homology directed repair HDR based correction of rs334	Clinical ex-vivo BCL11A editing (Exa-cel or CTX001) delivers significant HbF induction and clinical benefit, large cohorts demonstrate sustained functional cure rates in many patients treated real world clinical follow ups 2023-2024	Large deletions, translocations, p53 activation can result from double stranded break, HDR is ineffective in quiescent long term hematopoietic stem cells, conditioning toxicity is still necessary for transplantation	The most effective ex-vivo method for HSCs at the moment is autologous transplantation along with RNP electroporation. High infrastructure and production requirements, high cost per patient
Adenine Base Editing	Fusion of deaminase (A to G) conversions) to nCas or dCas, edits single base without double stranded break	Direct correction of rs334 to benign variants, editing of HBG regulatory sites to reactivate HbF	High ex-vivo rectification rates have been documented in refined methods. Preclinical and HSC engraftment findings from 2024-2025 show that base edited HSCs have strong long term persistence (with several groups moving towards to investigational new drugs IND)	Bystander edits are restricted to transition substitutions within the editing window. Low level RNA off -target editing is possible (though this is lessened by engineering)	Good ex-vivo characteristics (mRNA or RNP electroporation), in vivo delivery more challenging but possible with LNP or AAV split systems. Near clinical translation
Prime Editing (nCas9-RT + prime editing guide RNA pegRNA)	Cas9 nickase fused to reverse transcriptase uses pegRNA template to write precise edits (all base to base and small indels without double stranded break)	Precise correction of rs334 (A to T transversion) to wild type HBB, multiplex rewriting of γ -globin promoters	In hematopoietic stem and progenitor cells, ex-vivo correction frequencies ranged from 15 to 41%, and further translational research in 2024-2025 demonstrated long lasting engraftment and in vivo rescue in animal models.	In-vivo delivery is complicated by substantial editor components, pegRNA design complexity, and sometimes lower efficiency than the best non homologous end joining (NHEJ) technique	Currently best suited for ex-vivo HSC editing, split systems or small editors are needed for LNP or AAV distribution, early phase of translation
Compact Cas systems (Cas12f,CasMINI, engineered hpCasMINI))	Naturally small CRISPR effectors (type V to F) engineered for nuclease, base or prime fusions, enable single Adeno associated virus packaging	Potential for in vivo Hematopoietic Stem Cells HSC editing (Adeno associated viruses AAV or Lipid Nanoparticles LNPs), base or prime fusions for direct correction or regulatory edits	Engineering of these Cas systems significantly increases activity (hpCasMINI,exoCasMINI has greater editing rates, recent preclinical experiments demonstrate productive editing when packed in adeno associated viruses	Lower intrinsic activity than SpCas9 in the past, specificity and off-target profiling of produced variants is still ongoing	A promising delivery profile that fits a single AAV, could allow for in vivo HSC editing without the need for a transplant, assuming activity and safety are equal, this could be a significant translational benefit.

ZFNs (Zinc finger nucleases), TALENs (Transcription Activator like effector nucleases) protein guided	Designer DNA binding proteins fused to FokI nuclease create double stranded break (protein redesign per target)	Early clinical knockout or repair approaches, historically used in gene editing trials	Illustrated proof of concept in previous hemoglobinopathy research, CRISPR has mainly replaced it for new initiatives	Double stranded break genotoxicity is similar to other nuclease techniques, the design is complicated and expensive	Compared to CRISPR, the present clinical pipeline is more constrained and less adaptable for quick target changes.
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Minimizing off-target effects is essential for clinical safety. High-fidelity Cas9 derivatives, gRNA truncations, and better computational prediction software greatly reduce the rates of unwanted editing [107,108]. Orthogonal methods using gene editing tools allow single-nucleotide changes, with fewer DNA DSBs significantly lowering genotoxic risks. In the long term, the best SCD therapy would be a nonmyeloablative in vivo CRISPR delivery system that edits HSPCs in situ within the bone marrow without requiring cell harvesting and transplantation and sacrificing of the patient's quality of life [109]. Overcoming challenges in CRISPR technology involves solving issues that include off-target effects. The specificity of CRISPR is being improved by scientists through the development of new Cas proteins and gRNA. Additionally, bioinformatics techniques are being used to forecast and reduce off-target effects. Next-generation viral vectors with efficient cargo capacity, very low immunogenicity, and non-viral systems have led to improved delivery efficacy. With respect to non-viral system advancements, exploits nanoparticles and peptides to strengthen ability and safety and involve attempts to improve the remedial applications of CRISPR-like gene therapy [110]. CRISPR-Cas9 is a potent medical therapy for SCA, as it directly fixes the *HBB* mutation or helps to reactivate HbF. The study of CTX001 has illustrated that modified stem cells can have potentially durable therapeutic benefits. Although advances like base editing and prime editing provide better precision and lower off-target hazards, issues remain in the delivery, editing efficiency, engraftment, and long-term safety. Overall, CRISPR is a transformational technique for hematological illnesses, with SCD at the forefront of clinical development [111-114]. Although advancements in Cas9 engineering, such as shortened gRNAs and high-fidelity variants, have significantly reduced off-target effects [115], the risks still pose a barrier to clinical usage, particularly in tissues in which even minor abnormalities may have serious consequences.

4.5 Role of High-Fidelity Cas Variants in Treating SCD

Compact Cas research has become increasingly important for SCD, as the ultimate treatment objective is to enable in vivo editing of bone marrow HSCs and fix the rs334 mutation or reawaken HbF without transplantation [116]. Although nuclease activity in HSCs remain low, early Cas12f investigations in recent years have shown that its incredibly small size allows AAV and LNP administration directly to hematopoietic regions [117,118], establishing proof of feasibility for in situ genome-editing techniques in SCD. Recently, a significant advancement was made with the creation of hyper-engineered compact variants (hpCasMINI) [119].

Without requiring myeloablative conditioning or ex vivo cell manipulation, these constructs demonstrated multifold increases in mammalian editing activity while maintaining high specificity, making hpCasMINI an option to support future in vivo BCL11A enhancer disruption or HBB correction in SCD [120]. All these advancements indicate compact Cas systems as a long-term translation pathway towards accessible, non-transplant gene therapy for SCD, which is especially pertinent in situations with low resources [121,122]. High-fidelity SpCas9 variants continue to be the most feasible for ex vivo autologous HSC editing in SCD for near-term clinical translation [123]. From 2023 to 2025, variants like Sniper2L [124] and other high-fidelity Cas9 versions underwent extensive optimization, resulting in high editing efficiency at the BCL11A enhancer with a markedly lower off-target burden while maintaining colony-forming and engraftment potential in CD34+ cells [125]. Because of their high HbF induction efficiency, low genotoxic footprint, and compatibility with GMP-grade RNP manufacturing workflows, high-fidelity Cas9 (R691A) and similar high-fidelity variants currently have the strongest translational pedigree in SCD and have been frequently highlighted in Investigational New Drug enabling process development [126]. Therefore, compact Cas nucleases, particularly hpCasMINI [127], represent platforms with the most potential for the long-term aim of a single dose in in-vivo gene editing cure for SCD, whereas high-fidelity SpCas9 variants represent the current and near-future path towards curative SCD gene therapy [128].

5. CRISPR in Clinical Trials
5.1 CTX001 and CLIMB Studies

In this review article, an elaborate estimation from preliminary findings of the CLIMB THAL-111 and CLIMB SCD-121 studies has been described [129,130]. A novel CRISPR-Cas9-based treatment called CTX001 has been examined for SCD and TDT. By altering the BCL11A erythroid enhancer in autologous CD34+ HSCs, CTX001 aims to reduce the symptoms of various hereditary diseases and increase the generation of HbF [131-136]. This method provides an effective means to address genetic alterations causing SCA and TDT [137]. CTX001 therapy resulted in transfusion independence in patients with TDT. In patients with SCD, CTX001 treatment resulted in a noticeable reduction in severe VOCs. Despite certain challenges, such as the adverse effects linked to the conditioning regimen needed for stem cell transplantation, the initial results highlight the potential of CTX001 as a curative approach. While additional long-term data is still being collected, these encouraging findings represent a major advancement in genetic medicine [138] (Table 4).

Table 4: Cas variants in sickle cell disease gene editing [135,136]

Cas variants	Editing application in SCD	Efficiency in HSCs	Safety and off target considerations	Translational status in SCD
SpCas9	Highly efficient for BCL11A enhancer disruption, robust HbF reactivation	Very high	Higher baseline off-target risks and requires monitoring	Clinically validated, used in Exa-cel or CTX001, durable VOC free outcomes
High Fidelity Cas9 (R691A)	Same BCL11A editing with reduced genotoxicity	High	Markedly reduced off- target edits, preserves HSC viability	IND enabling standard , considered best choice for ex-vivo SCD therapy pipelines
SpCas9-HF1,eSpCas9,HypaCas9	Can perform rs334 HDR correction or BCL11A editing	Moderate to high (guide dependent)	Strong fidelity but some loss of activity on specific sgRNAs	Used when maximum specificity is required but requires guide screening
Sniper2L, Next generation HF Cas9 variants	Same intended edits with retained activity and high fidelity	High	Very low off-target risk	Rising alternative to High Fidelity Cas9, requires empirical optimization for each SCD sgRNA
Cas12f (natural compact)	Enables in-vivo HSC editing via BCL11A or HBB	Low to moderate	Low off-target effect	Promising for delivery but not yet optimal for durable HSC correction
CasMINI	Compact delivery option for SCD gene reactivation strategies	Moderate	Low	Proof of concept, requires further activity enhancement for HSCs
hpCasMINI	Emerging option for direct in-vivo correction of rs334 or BCL11A enhancer	High (post engineering)	Low, maintains specificity while boosting activity	Most promising future candidate for non-transplant in-vivo HSC editing in SCD

5.1.1 Efficacy and Early Results

The ability of CRISPR-based treatments such as CTX001 has been measured using hematological markers, safety improvement, and betterment of life [139]. The balance in organ function is an early clue that holds up prevention of further organ damage, and the reversal of damage is uncertain. According to the first trial outcomes of HbF induction, the mean HbF was greater than 40% and pancellular HbF expression was greater than 90% in certain individuals. VOC elimination observed during the follow up is the solution for VOC in patients treated for SCD.

Nevertheless, the success of these studies has firmly entrenched CRISPR-based editing as an acceptable clinical tool for hemoglobinopathies [140]. Nanomaterials also bear significant responsibility for enhancing the delivery and efficiency of CRISPR-Cas9 [141]. Proper assembling of the components of CRISPR-Cas9 is significant for potential genome alterations [142]. Nanomaterials have proven to be strong contenders, with regulated and targeted delivery capabilities. Nanomaterials possess distinctive features such as a large surface area, reversible surface modification, and multifunctionality, thus qualifying as perfect candidates for the enhancement of CRISPR-Cas9 delivery efficiency [143-145].

5.2 What is Casgevy and How Does it Work for SCD?

For individuals aged 12 years and older with SCD or TDT, exagamglogene autotemcel (Exa-cel; formerly CTX001), marketed as Casgevy, is an autologous CRISPR-Cas9 gene-edited HSC therapy. The treatment involves collecting a patient's HSCs and modifying them ex vivo by using CRISPR-Cas9 gene editing to disrupt the erythroid-specific enhancer of the *BCL11A* gene, thereby increasing HbF production [146]. Normally, HbF production declines after birth; however, increased HbF expression can compensate for the defective hemoglobin associated with SCD and TDT. In SCD, increased HbF reduces the tendency of RBCs to sickle, thereby potentially reducing vaso-occlusive events and hemolytic anemia. In TDT, increased HbF production may improve hemoglobin levels and reduce dependence on regular RBC transfusions [147].

5.2.1 Blood Disorders and Approval for Casgevy

SCD and TDT were among the early targets for the clinical development of CRISPR-based therapies because both are inherited disorders involving hemoglobin production.

The therapeutic strategy used in the development of Exa-cel by CRISPR Therapeutics and Vertex Pharmaceuticals involves increasing HbF expression by disrupting the erythroid-specific enhancer of BCL11A. Increased HbF can compensate for defective hemoglobin production and reduce the clinical manifestations associated with SCD and TDT [148,149].

The United Kingdom's Medicines and Healthcare Products Regulatory Agency (MHRA) authorized Casgevy in November 2023 for eligible patients aged 12 years and older with SCD and TDT. Subsequently, the FDA approved Casgevy in December 2023 for eligible patients with SCD and, later, for TDT. These approvals represented major milestones in the clinical translation of CRISPR-based gene-editing therapies [150]. The clinical data from studies of Exa-cel demonstrated substantial reductions in vaso-occlusive events among patients with SCD and reduced transfusion dependence among patients with TDT [151].

5.2.2 Efficacy and Results of the Treatment

The clinical studies of Exa-cel have demonstrated substantial therapeutic benefits in patients with SCD and TDT. In patients with TDT, 25 of 27 treated participants achieved transfusion independence for a sustained period, whereas the remaining participants experienced substantial reductions in transfusion requirements. Among patients with SCD, 16 of 17 participants achieved freedom from severe vaso-occlusive events during the reported assessment period [152]. HbF levels increased following treatment, supporting the proposed mechanism of BCL11A enhancer disruption and subsequent reactivation of HbF production. These findings indicate that Exa-cel may provide durable clinical benefits and substantially reduce disease burden in patients with SCD and TDT, although continued long-term follow-up is required to establish the durability of treatment efficacy and safety [153].

5.2.3 Challenges in Casgevy Treatment

The first problem is access to technology, as the therapy is difficult to produce and administer. There are few places in the US or overseas with the technological capability. The second issue is financial access. The cost of Casgevy is approximately \$12 million per patient. Risk consideration exists in addition to concerns about pricing and accessibility. Before receiving the CRISPR treatment, patients must undergo chemotherapy, which may have major adverse consequences [154].

The research on next-generation medicines is being driven by the desire to lower costs while simultaneously improving safety and patient experience.

5.3 Treating SCD with CRISPR-Cas Proteins

Editas Medicine is using a CRISPR system with Cas12a protein instead of the more well-known Cas9 protein in phase 1/2 trials for patients with severe SCD and TDT. Their strategy is to make changes that activate HbF, like in the case of Casgevy. Cas12 is being used in a clinical experiment for the first time. *Streptococcus pyogenes* Cas9, also known as SpCas9, is a gene-altering workhorse and new-generation Cas protein that provides novel benefits for therapeutic use. Cas12a (Cpf1) has a class 2 type V nucleus along with T-rich PAM recognition (TTTV), increasing the number of targetable genomic sites beyond the Cas9 NGG PAM requirement. Cas12a creates staggered breaks with 5' overhangs, which could allow for more efficient HDR and lower rates of indel formation than the blunt cut of Cas9.

Table 5: Comparison of Cas proteins [163]

Feature	Cas9	Cas12 (Cpf1)	Cas13
Target Type	DNA	DNA	RNA
Nuclease Activity	Double-stranded DNA cleavage	dsDNA cleavages	ssRNA cleavage
Guide RNA Requirement	crRNA + tracrRNA (or single sgRNA)	crRNA only	crRNA only
PAM Sequence	NGG (commonly)	T-rich (e.g., TTTV)	Not PAM-dependent, but PFS required
Cutting Style	Blunt ends	Sticky ends (5' overhangs)	No DNA cutting, targets RNA
Collateral Cleavage Activity	No	No	Yes (used in diagnostics)
Use in Diagnostics	Limited	Some use	Extensive (e.g., SHERLOCK)
Size	~160 kDa	Smaller than Cas9	Variable, typically similar to Cas9
Applications	Genome editing, gene knockout	Genome editing, improved targeting	RNA knockdown, diagnostics, epitranscriptomics

5.3.1 Efficacy and Results of the Treatment

To date, 17 participants have been treated. Eleven patients with SCD exhibited significant increases in HbF levels and have been free of vaso-occlusive events since receiving the medication (at least 5 months), suggesting that the efficacy is as strong as that of Casgevy. No significant side effects have been noted. Strong hemoglobin gains that eliminate the requirement for transfusion have also been observed in 6 patients with TDT. The FDA has designated the treatment as an RMAT and orphan medication [158].

5.4 Clinical Trials of Base Editing Therapy for SCD

In January 2024, Beam Therapeutics reported that the first patient with severe SCD has been dosed in its US-based phase 1/2 trial of base editing treatment. Beam Therapeutics is activating HbF by base modification, which is a variation of CRISPR-Cas9 technology known as "base editing;" this decreases some safety hazards by altering individual DNA nucleotides without causing DSBs in the DNA [159].

6. A Somatic and Germline Editing Approach towards SCD Treatments

While germline editing remained morally and scientifically unfeasible between 2023 and 2025, somatic genome editing techniques for SCD made quick progress towards clinical translation [160]. In late 2023, somatic approaches, mainly ex vivo editing of autologous HSCs, reached a significant regulatory milestone when CRISPR-based HSC therapies were authorized for clinical use after the treated patients experienced sustained HbF induction and long-lasting reductions in VOCs (Figure 9) [161].

It also self-processes CRISPR RNAs (crRNAs) from a precursor transcript, allowing for easier multiplex editing [155]. Cas13 RNA-guided nuclease can cleave single-stranded RNA sequences based on a crRNA guide. Although it cannot be used directly for permanent DNA modification, Cas13 can perform transient knockdown of disease-related transcripts, RNA editing by fusion to ADAR deaminases, and treatment of RNA viruses. For SCD, Cas13 would ideally be used to destroy mutant HBB mRNA or adjust the expression of regulatory factors without genome change [156]. Cas14 and other small nucleases have lower molecular weights for easier delivery through viral vectors with limited capacity (e.g., adeno-associated virus). The nucleases may have lesser off-target activity, enhancing safety profiles. The continual discovery and engineering of novel Cas effectors promise to expand the therapeutic arsenal for SCD, enabling more targeted, efficient, and safe gene treatments [157] (Table 5).

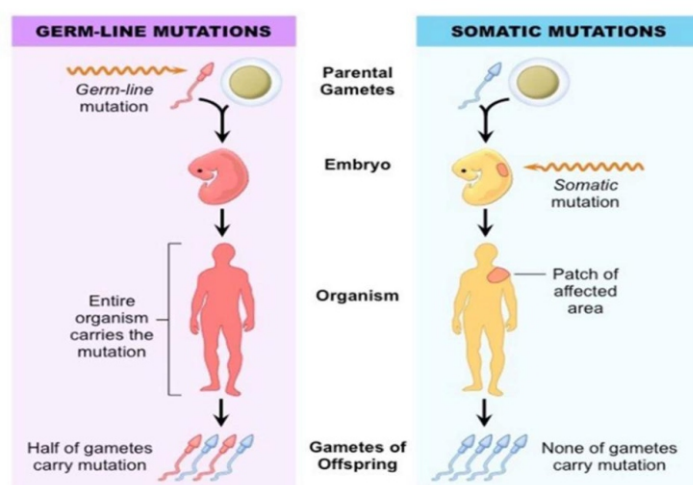


Figure 9: Comparison of germline and somatic gene editing

Significant long-term clinical benefits have been established by ongoing studies, and new cost and access issues were brought to light by implementation initiatives, including NHS England adoption. In vivo editing may be the next stage of somatic therapy development, according to parallel preclinical progress [162]. In 2023, Li *et al.* demonstrated that in vivo prime editing of HSCs corrected the sickle mutation and functionally rescued the disease in a mouse model, establishing proof of concept for direct mutation reversal. Targeted LNP delivery allowed for approximately 58% mean editing of the HBG1/2 promoter in non-human primates following a single dosage, according to a 2025 study by Editas Medicine [163]. This suggests that clinically significant in vivo HSC editing for HbF induction is feasible (Table 6).

Table 6: Somatic vs Germline editing

CONSIDERATIONS	SOMATIC GENE EDITING	GERMLINE GENE EDITING
EDITS	Somatic therapies target genes in specific types of cells (blood cells, for example).	Germline modifications are made so early in development that any changes is copied into all of the new cells.
COPY	The edited gene is contained only in the target cell type. No other types of cells are affected.	The edited gene is copied in every cell, including sperm or eggs.
RISKS AND NEXT GENERATION	Any changes, including potential off-target effects, are limited to the treated individual. The edited gene is not passed down to future generation.	The edited gene is passed on to future generations.
CONSENSUS	Somatic cell therapies have been researched and tested for more than 20 years and are highly regulated.	Human germline editing is new. Heritability of germline changes presents new legal and societal considerations.

6.1 Ethical, Legal, and Regulatory Perspectives and Public Debate

The safety and consequences of germline editing are still largely unpredictable, especially when it involves human embryos. The alteration of human DNA can be a slippery slope, and medical interventions might quickly shift toward enhancement practices that are challenging to oversee or regulate [164]. The absence of standardized international regulations for CRISPR-Cas9 technology contributes to the complexity of its ethical and social impacts [165]. Different countries have taken diverse approaches to genome editing, with some enforcing strict laws or complete prohibitions and others permitting its use for scientific research or clinical applications within defined regulatory frameworks [166]. In nations with an established legislative framework, it may be suitable to let CRISPR-based research advance with adequate safeguards [167,168]. In contrast, clinical germline gene editing for SCD is still generally discouraged by global governance bodies because of multigenerational safety risks, mosaicism, off-target heritability, and unresolved consent concerns [169,170]. No responsible clinical germline program for SCD has been started to date, despite policy debate and increased bioethical discourse [171,172]. Germline editing is still limited to theoretical and policy frameworks rather than clinical development, whereas somatic editing, especially HSC-directed ex vivo and next-generation in vivo platforms, represents the practical and morally sound path for SCD intervention in the near future according to current scientific and regulatory trajectories [173,174].

There is an ongoing debate between the scientific and global communities regarding the practical applications of human germline engineering. Globally, human germline engineering has been banned in many countries for ethical reasons. This topic is highly controversial because of concerns that the techniques will create humans with “perfect” traits and qualities, leading to a widening social gap [175].

7. Challenges and Solutions in LMICs

Most of the SCD cases worldwide occur in LMICs; however, the patients encounter substantial obstacles when trying to acquire curative treatments like HSC transplantation and new gene-editing techniques [176,177]. Equitable access to advanced care is restricted by a lack of treatment infrastructure, high procedural and medication costs, reliance on apheresis and myeloablative conditioning platforms, and disjointed regulatory routes [178-180]. According to recent analyses, the gap between therapeutic innovation and practical accessibility is increasing; however, specific approaches are being developed to help close this gap, including tiered pricing, regional GMP and transplant centers, increased apheresis capacity, simplified regulatory harmonization, and use of low toxicity or in vivo editing techniques [181-185]. WHO's Sickle Cell Disease Strategy (2023) and the WHO Africa SCD action plan (2024) call for subsidized pricing structures, regional HSCT or GMP centers, uniform regulatory pathways, and investment in workforce training to increase implementation capacity across Africa and South Asia. These specific initiatives have started addressing these disparities since 2023 (WHO, 2023; WHO AFRO, 2024) (Table 7) (Figure 10).

Table 7: Challenges faced by LMICs and Strategies to Overcome challenges

LMIC Challenges	Description	Emerging Solutions
High cost of curative SCD therapies	For the majority of LMIC patients, hematopoietic stem cell transplantation (HSCT) and gene editing therapies are still too expensive.	Tiered or subsidized structures; universal health care for the SCD medications.
Limited transplant and GMP infrastructure	Few facilities that can produce cell or gene therapies or perform HSCT.	Creation of regional GMP and HSCT centres throughout South Asia and Africa.
Shortage of trained clinical and laboratory personnel	Insufficient knowledge of genome editing, apheresis, HSCT, and post – transplant care.	Partnerships for south-south and workforce capacity building.
Apheresis and conditioning constraints	Eligibility is restricted by a strong reliance on high myeloablation.	Adoption of in vivo and low toxicity gene editing techniques lowering the need for conditioning.
Regulatory barriers and slow clinical trial approvals	Access to novel therapies is delayed by scattered regulatory channels.	Regulatory framework standardization and the establishment of the African Medicines.
Weak long term patient monitoring systems	Limited survival and follow-up information following sophisticated treatments.	WHO sponsored national monitoring platforms and digital SCD registries

8. Future Directions

Encouraging results from preclinical and early-phase clinical studies utilizing CRISPR-Cas9 to target the HBG1/HBG2 promoters or BCL11A enhancer have paved the way for continued refinement of gene editing approaches. Ongoing research aim to boost editing efficiency and optimize gRNA configurations and maintain long-term HbF expression in RBCs. To achieve sustained therapeutic benefits, future strategies must also focus on enhancing editing outcomes in repopulating hematopoietic and progenitor stem cells [186,187]. Their potential applications extend beyond SCD and β -thalassemia and could encompass other rare hemoglobinopathies and genetic conditions characterized by abnormal hemoglobin production [188,189]. A major focus of ongoing research is the reduction of conditioning regimen toxicity. Current strategies aim to optimize busulfan-based myeloablative protocols and enhance therapeutic outcomes. Upcoming clinical trials are expected to explore the use of nongenotoxic antibody–drug conjugates and less-intensive conditioning approaches to enable safer and more efficient HSC transplantation (Table 8) [190].

Table 8: Sickle Cell Disease (SCD) Implementation Scenarios in LMICs [200-202]

Scenario Component	Near-Term Feasibility (Immediate Action)	Long-Term Aspirational Strategies
Financial Management	Targeted Subsidies: Reducing hydroxyurea costs from €100–200 to €50 per patient through government and donor partnerships	Sustainable Financing: Implementing Results-Based Financing (RBF) and tiered co-payment models based on household income
Service Delivery	Decentralization: Moving care from tertiary centers to district hospitals and establishing satellite clinics within 10 km of rural patients	Digital Integration: Widespread use of telemedicine and mobile health apps for crisis management and adherence tracking
Medication Strategy	Optimized Dosing: Prioritizing Maximum Tolerated Dose (MTD) over fixed doses to maximize DALYs averted and blood savings	Local Production: Establishing local generic manufacturing to lower drug prices to approximately US\$ 0.09 per 500 mg
Clinical Monitoring	Simplified Protocols: Reducing laboratory monitoring frequency after the initial dose-escalation phase to lower personnel and transport costs	Integrated Care: Managing SCD alongside other chronic NCDs (diabetes, heart failure) using shared infrastructure to reduce per-patient costs
Human Resources	Task-Shifting: Training nurses and community health workers to handle longitudinal care, reducing reliance on scarce specialized physicians	Specialized Workforce: Upskilling broad primary healthcare teams and integrating SCD management into mandatory national health training.
Detection & Awareness	Stigma Reduction: Community-led education campaigns to increase the understanding of hydroxyurea benefits beyond the current 40%	Universal Screening: Implementing mandatory newborn screening (NBS) in all malaria-endemic regions to start preventive care at birth

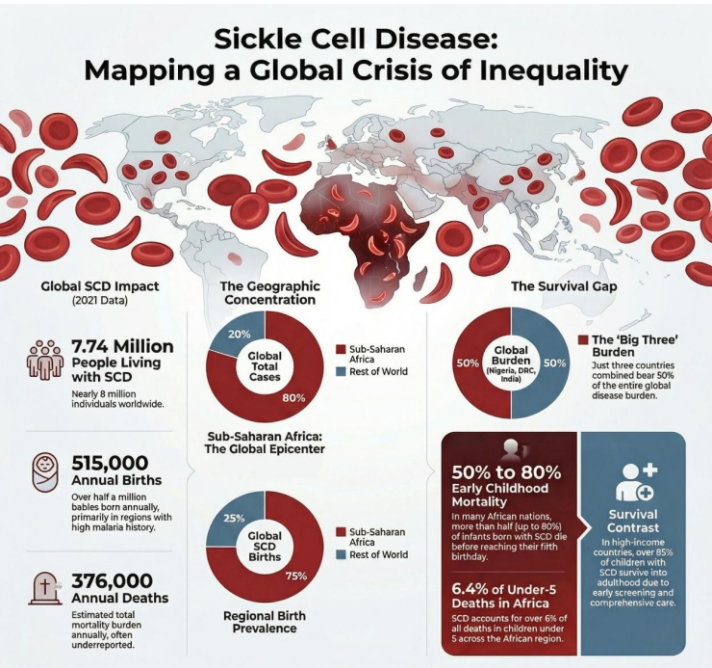


Figure 10: Quantitative data on Sickle cell disease in Low and middle income countries [191-195]

Simultaneously, the genetic diversity inherent in β -hemoglobinopathies underscores the need to assess gene-editing approaches across a wider array of patient-specific genotypes. To maximize therapeutic effectiveness, treatment strategies must be tailored to accommodate individual genetic variations. To reduce off-target effects and increase whole-genome stability, research is also being conducted to increase the precision of gene-editing tools and improve the efficiency of DNA repair pathways like HDR [191]. In addition to treating hemoglobinopathies, CRISPR-Cas9 demonstrates significant promise for addressing various genetic disorders, including cancers, neurodegenerative diseases, and metabolic syndromes. Its ability to selectively edit or eliminate specific genes underscores its versatility as a powerful tool for developing precise, individualized treatment strategies [192]. In the future, extensive and global implementation of rapid RBC genotyping for patients and donor populations, along with an open national database for data storage, could make an improved RBC genetic matching technique viable for individuals with SCD [193]. This technology has changed the area of genetic modifications with its extraordinary accuracy and efficacy in changing DNA sequences.

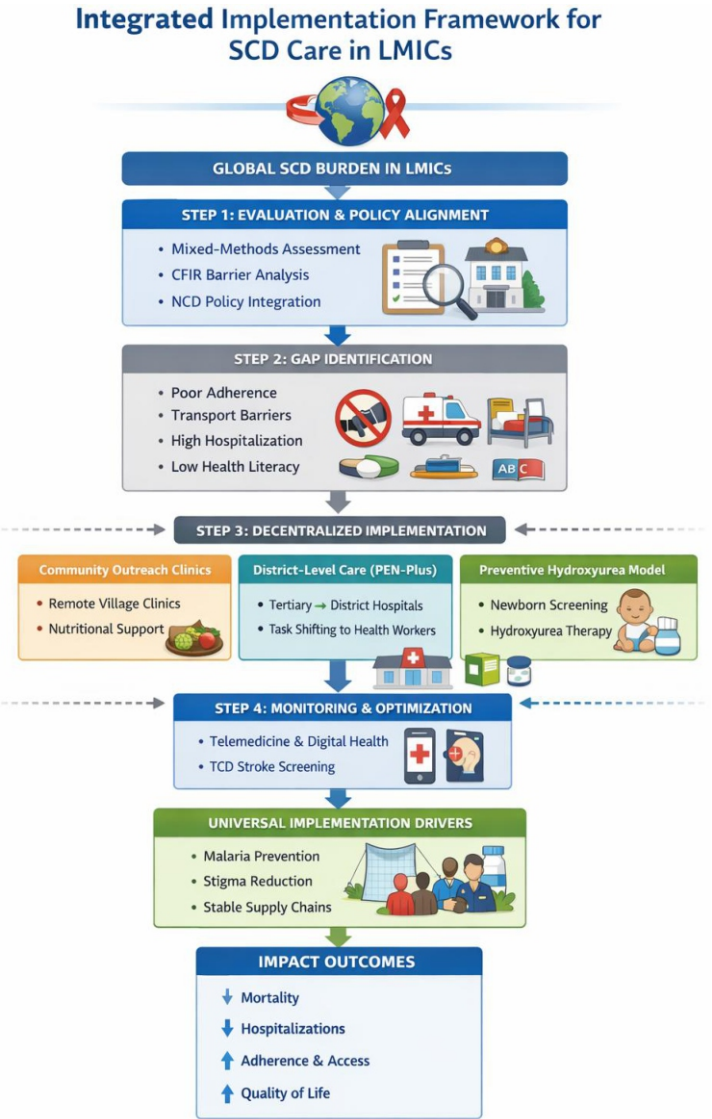


Figure 11: concrete implementation models on sickle cell disease in LMIC [201]

This sophisticated gene-modifying technology has enormous applications in health, agriculture, as well as fundamental research. Current research is looking into new directions to improve the capabilities of CRISPR-Cas9 and overcome any limitations [194-197]. Future developments in SCD gene therapy are anticipated to move away from intricate ex vivo HSC programming and towards single-dose, in vivo editing platforms that remove transplanting and conditioning obstacles [198].

This shift is probably going to be driven by next-generation compact nucleases like hpCasMINI, enhanced HSC tropic LNP systems, and AAV-free non-viral delivery frameworks, which will allow direct editing of bone marrow stem cells in situ while lowering the toxicity and expense [199]. By correcting the rs334 mutation or reactivating γ -globin without DSBs, parallel advancements in precision editors such as adenine base editors and prime editors optimized for HSC persistence aim to reduce genotoxic risks and enhance long-term safety. Moreover, the future of CRISPR-Cas9 as a potent tool for modifying the plant genome and precise gene insertion through HDR in CRISPR-Cas9-mediated gene editing offer previously unheard of control over genetic changes [200].

In the short term, therapeutic pipelines will continue to be shaped by high-fidelity nucleases (HiFi Cas9, Sniper2L), whereas, in the long term, the focus will be on long-lasting, conditioning-free editing with little off-target footprint. Global accessibility frameworks, including tiered pricing, regional GMP centers, WHO-aligned regulatory standardization, and LMIC-focused clinical trials, are crucial, in addition to these technological advancements, to stop treatment disparities from increasing [201]. In general, biological accuracy, streamlined delivery, and equitable scaling will be included in the next stage of SCD treatments, with the practical objective of creating a one-time curative treatment that is safe, inexpensive, and deployable globally [202] (Tables 9–11).

Table 9: Comparison of CRISPR-Cas9 mediated strategies.

Parameter	rs334 Mutation Correction (HDR-based CRISPR-Cas9)	BCL11A Enhancer Disruption (CRISPR-Cas9 NHEJ)	Base Editing (ABE/CBE)	Prime Editing
Editing Target	β -globin (<i>HBB</i> , rs334 mutation)	<i>BCL11A</i> erythroid enhancer	<i>HBB</i> or regulatory elements	Precise <i>HBB</i> mutation correction
Mechanism of Action	HDR-mediated correction restores HbA	NHEJ disruption reactivates HbF	Direct base conversion without DSB	Reverse transcriptase-guided DNA rewriting
Type of Editing	DSB-dependent (HDR)	DSB-dependent (NHEJ)	DSB-free editing	DSB-free editing
Therapeutic Outcome	Restoration of adult hemoglobin (HbA)	HbF induction compensates HbS	Enhanced HbF or corrected base	Direct mutation correction to normal sequence
Editing Efficiency (HSCs)	Low–moderate (HDR limited in HSCs)	High efficiency in HSCs	Moderate–high efficiency with durable HbF induction	Low–moderate (requires optimization)
Clinical Development Stage	Preclinical–early clinical	Clinically advanced; FDA-approved therapy (Casgevy)	Preclinical to early clinical	Early preclinical stage
Advantages	Curative, mutation-specific	Clinically validated, high efficiency, HDR-independent	No DSB, reduced genotoxicity; high HbF induction	Highly precise; enables all mutation types
Limitations	Low HDR efficiency; donor template required	Does not correct mutation; relies on HbF compensation	Limited base conversion scope; potential bystander edits	Low efficiency; complex delivery
Off-target / Safety Concerns	DSB-induced genotoxicity, chromosomal rearrangements	DSB-associated risks; p53 activation	Off-target deamination; RNA edits possible	Lower DSB risk but unintended edits possible
Long-term Risks	Clonal expansion; genomic instability	Unknown long-term enhancer effects	Accumulated base changes	Long-term safety not established
Translational Feasibility	Limited due to HDR dependency	Highly feasible (ex vivo clinical success)	Promising; improved safety and durability	Currently limited; requires optimization
Overall Clinical Potential	Definitive cure but technically constrained	Most clinically advanced and practical approach	Strong next-generation candidate	Future precision therapy with high potential

Table 10: Comparison of primary CRISPR-based genome editing and regulation techniques

Method	Mechanism	Primary Application	Key features
NHEJ	Error-prone repair (no template)	Gene Knockout	Highest efficiency
HDR	Precise repair (with template)	Gene Knock-in	High precision, low efficiency
Base editing	Deamination (no DSB)	Point Mutation Correction	Safe, precise, no indels
CRISPR (i/a)	Steric hindrance (dCas9)	Transcription Regulation	Reversible, no DNA damage

Table 11: systematic summary of the cell-based gene therapies for Sickle Cell Disease (SCD)

Parameter	Casgevy (CTX001/CLIMB)	Reni-cel (Editas/Cas12a)	Risto-cel (Beam/Base Editing)
Target	Erythroid-specific enhancer region of the BCL11A gene	HBG1 and HBG2 (γ -globin) gene promoters	HBG1 and HBG2 gene promoters
Edit Type	CRISPR-Cas9 (introducing a precise double-strand break)	CRISPR-AsCas12a nuclease	Adenine Base Editor (A-to-G substitutions; no double-strand breaks)
Status	Ex vivo (autologous CD34+ cells)	Ex vivo (autologous CD34+ cells)	Ex vivo (autologous CD34+ cells)
Conditioning	Myeloablative busulfan (pharmacokinetically adjusted)	Myeloablative busulfan	Myeloablative busulfan (pharmacokinetically guided)
Outcomes	93% of patients free from severe VOCs for 12+ months; ~40% HbF; normalization of total Hb	96% (27/28) free of VOCs; $\geq 40\%$ HbF; mean total Hb increased to 13.8 g/dL at 6 months	No reported severe VOCs post-engraftment; $>60\%$ HbF; resolution of anemia
Follow-up	Median 33.2 months (up to 59.6 months)	Median 9.5 months (up to 25.2 months)	Mean 6.6 months (range 0.3 to 20.4 months)
Major Adverse Events	Stomatitis, febrile neutropenia, and thrombocytopenia (conditioning-related)	Majority occurred within 30 days of infusion; consistent with busulfan (stomatitis, nausea)	Stomatitis, febrile neutropenia; one death from idiopathic pneumonia syndrome (IPS) related to busulfan

9. Conclusion

Driven by the clinical validation of ex vivo CRISPR-Cas9 editing of autologous HSCs, in which durable HbF reactivation eliminates VOCs and transfusion dependence in many treated individuals, SCD has evolved from a lifelong progressive disorder with few therapeutic options to the leading success case of modern genome editing. The most compelling success story of genome editing is highlighted by the approval of the CRISPR-based therapy Exa-cel (Casgevy). Although this milestone shows the therapeutic potential of precise editing, endogenous globin regulation, conditioning toxicity, high manufacturing costs, specialized transplant infrastructure, and restricted availability in LMICs continue to limit current treatment. These difficulties have accelerated the transition to safer, more scalable next-generation platforms, where single-dose in vivo HSC editing without transplantation may be possible when high-fidelity Cas9 variants, base and prime editors, and compact nucleases like Cas12f, CasMINI, and hpCasMINI are combined with marrow-tropic LNPs and non-viral vectors. In the end, equitable translation through regulatory standardization, regional GMP capacity, tiered pricing, and LMIC-focused clinical trials will be just as important to the future of SCD gene therapy as molecular and delivery innovation. CRISPR-based therapy has a realistic chance of developing into a one-time SCD cure that is safe, inexpensive, and broadly available, if these technology and global-health approaches can be collated.

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Conflict of Interest

The authors have no competing interest.

Data Availability

Not Applicable.

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