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Therapeutic Application of Crisper CAS 9 for Sickle Cell Anemia : Current Progress and Future Prospects

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Abstract: *Sickle cell disease (SCD) is a congenital blood illness caused by faulty haemoglobinsynthesis, resulting in sickle-shaped red blood cells. This condition is caused by a single point mutation in the beta globin gene. SCD is primarily caused by a mutation in the gene that produces haemoglobin, the protein that transports oxygen in red blood cells. SCD patients may have chronic pain, exhaustion, anemia, stroke, organ damage, and increased infection risk. SCD treatment options focus on symptom management and preventing complications. Treatment options include supportive care, pharmacological therapies, hematopoietic stem cell transplantation, gene therapy, and gene editing. Gene editing can accurately fix inherited blood diseases like SCD.SCD focuses on symptom management and preventing complications. This covers supportive care, pharmacological therapies, hematopoietic stem cell transplantation, gene therapy, and gene editing. Gene editing is a promising treatment for genetic blood disorders like SCD. It can remove harmful variations, alleviate symptoms, and even cure the condition altogether. The CRISPR-Cas9 gene editing method is utilized to cure SCD. Sickle Cell Anemia SCA is a severe genetic disorder involving point mutations in the HBB gene encoding haemoglobin beta, which leads to abnormal haemoglobin and subsequent red blood cell sickling. Recently developed CRISPR-Cas9 gene editing has opened up newavenues for its targeted therapies. In the case of SCA, this CRISPR-Cas9 edits the HBB at precise locations. On the fronts of clinical success, two such next-generation treatments that are at the top of the race include Casegvy and Lyfgenia by CRISPR Therapeutics. These gene therapies are known to reprogram hematopoietic stem cells so that the production of healthy haemoglobin can be produced, hence potentially able to cure SCA and demonstrate the transformative power of CRISPR in precision medicine*

Keywords: Sickle Cell Disease (SCD); HBB gene; CRISPR-Cas9; gene editing; haemoglobin; hematopoietic stem cell therapy; Casgevy; Lyfgenia; precision medicine; genetic therapy

I. INTRODUCTION

Sickle cell disease (SCD) is a congenital blood illness where faulty hemoglobin molecules lead red blood cells to form a crescent or sickle shape[1]. This illness affects millions of people globally, especially those of African, Mediterranean, Middle Eastern, and South Asian herited[2].

SCD has a substantial impact on the health and wellbeing of those affected. Understanding the reasons, symptoms, and treatment choices is crucial for healthcare professionals, patients, and family members[3]. SCD is mostly caused by a mutation in the beta-globin gene (HBB), which produces hemoglobin[4].This gene mutation results in hemoglobin S (HbS), an aberrant haemoglobin with a unique molecular structure compared to normal adult HbA. HbS stiffens red blood cells, reducing their ability to flow via tiny capillaries. SCD-related health issues stem from altered red blood cell shape. Treatment for SCD aims to manage symptoms, prevent complications, and enhance patients' quality of life[5]. Effective pain management often requires the use of analgesics such as opioids, nonsteroidal anti-inflammatory medications, and adjuvant therapy. Adequate hydration, both orally and intravenously, is crucial for maintaining healthy hydration, both oral and intravenously, is crucial for maintaining healthy blood flow[6]. Blood transfusions may

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be necessary to enhance oxygen supply to tissues and lower the risk of consequences like stroke.Sickle[7]. Cell Anemia Overview: Sickle cell anemia is a hereditary blood disorder caused by a mutation in the β-hemoglobin gene, leading to the production of sickle hemoglobin (HbS)[8].

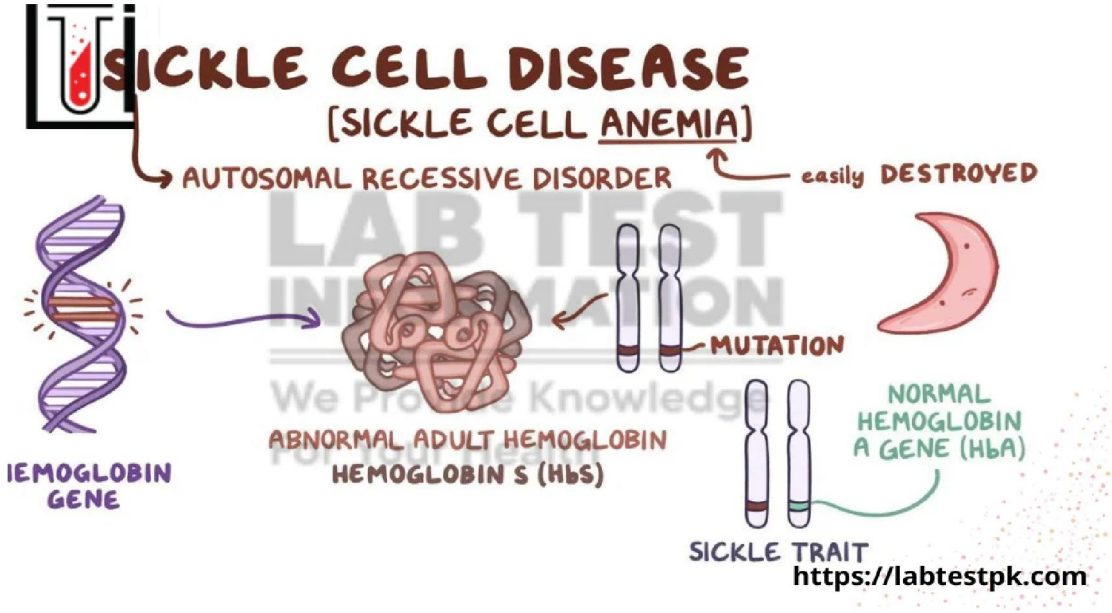

Fig .1: sickle cell anemia

This condition is characterized by the polymerization of deoxygenated HbS, which results in the sickling of red blood cells (RBCs), vasoocclusion, and hemolytic anemia[9]. The disease exhibits a diverse phenotype influenced by various genotypes, including homozygosity for HbS and compound heterozygosity with other hemoglobin mutations[5]. Key factors affecting the severity of sickle cell anemia include fetal hemoglobin (HbF) levels, which can inhibit HbS polymerization and mitigate symptoms. [10].

There is an urgent need for innovative therapies to improve patient outcomes in sickle cell disease, as there is currently a considerable lack of knowledge regarding determinants of SCD severity and few reliable objective laboratory tools for risk stratification in daily management[3]. Additionally, the existing treatments, such as hydroxyurea, have limitations, including significant side effects and the fact that approximately 40% of patients do not respond to it at all[11]. Therefore, the identification of new risk factors and objective markers for monitoring patients, as well as the development of safer and more widely applicable therapeutics, is essential. [12]

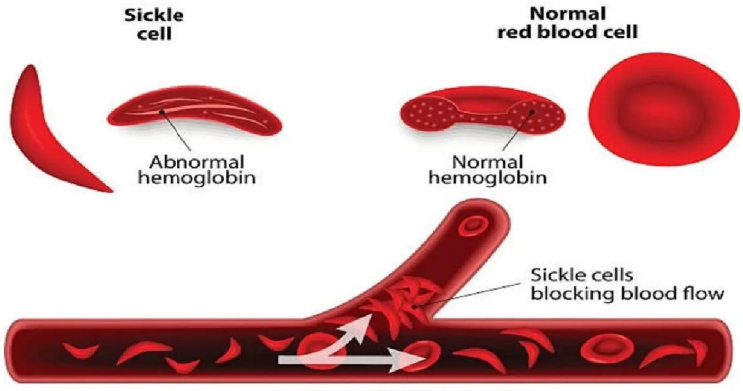

Fig .2: sickle cell shape

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Types :-

1. Sickle Cell Anemia (HbSS)

Homozygous HbS resulting in the most severe phenotype of SCD .

Characterized by chronic hemolysis, vaso-occlusive crises, and early organ damage .

2. Hemoglobin SC Disease (HbSC)

Compound heterozygosity for HbS and HbC, leading to a moderate clinical course with complications such as retinopathy andsplenomegaly .

3. Sickle β^0-Thalassemia (HbS/β^0-Thalassemia)

Coinheritance of HbS and β^0-thalassemia mutation, producing a clinical picture similar to HbSS due to absence of β-globin synthesis .

4. Sickle β^+-Thalassemia (HbS/β^+-Thalassemia)

Combination of HbS with a β^+ mutation permitting partialβ-globin production; results in milder anemia and fewer vaso-occlusive episodes .

5. RareSickle Cell Variants (HbSD, HbSE, HbSOArab, etc.)

These are compound heterozygous conditions involving HbS and other abnormal hemoglobins. Severity varies depending on the partner hemoglobin variant .

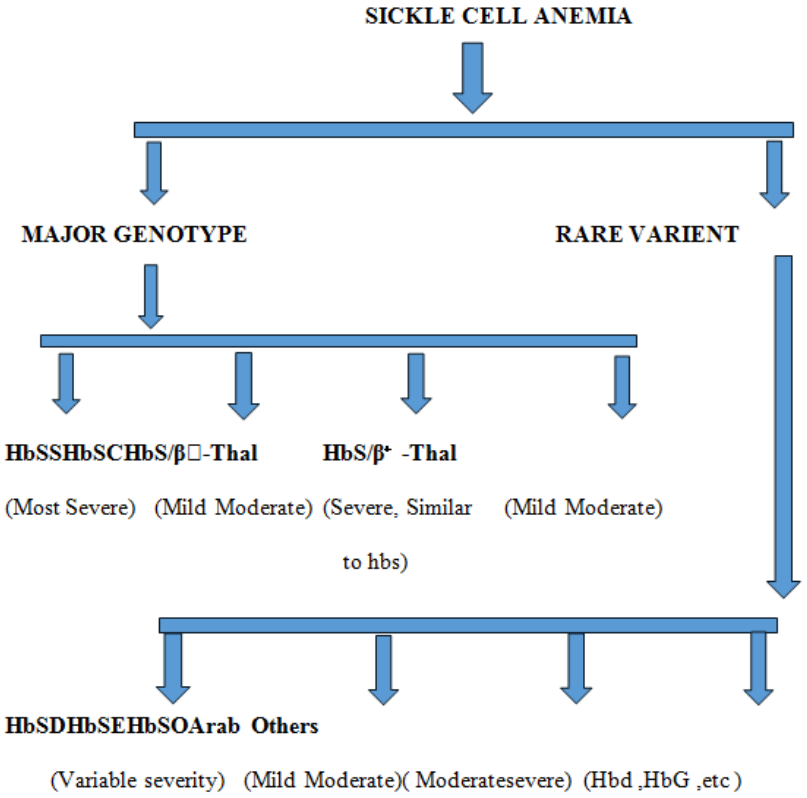

Fig .3:Types of Sickle Cell Anemia

Causes :-

1. autosomal recessive inheritance
2. Mutation in HBB gene.
3. Homozygous Mutation
4. Family History
5. Reduced oxygen levels

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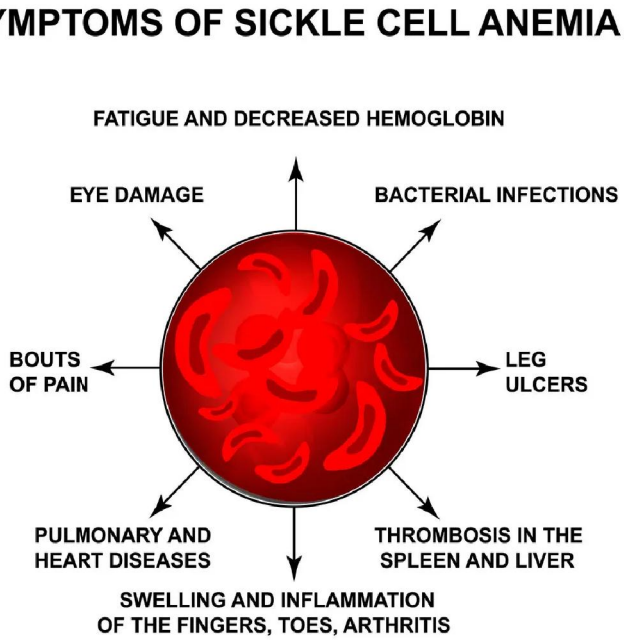
6. Dehydration
7. Genetic Variation [17–19]



Symptoms

Pathophysiology :-

Sickle cell disease (SCD) is driven by a complex interplay of biochemical triggers, hemoglobin polymerisation, cellular deformation, and downstream vascular complications. The diagram illustrates the sequential events leading from red blood cell injury to systemic organ damage (Figure 4).

1. Initiating Triggers: Acidosis, Hypoxia, and Dehydration:

Under physiological stress such as acidosis, tissue hypoxia, and red cell dehydration, hemoglobin S (HbS) becomes prone to polymerisation within erythrocytes [9]. These triggers accelerate intracellular HbS fibre formation, altering erythrocyte deformability.

2. HbS Polymerisation and Sickle Cell Formation:

Polymerised HbS causes the red blood cell (RBC) to transform into the characteristic sickle shape [13]. This deformation makes cells rigid and prone to mechanical destruction. In addition, sickled RBCs show altered expression of adhesion molecules, increasing their affinity for vascular endothelium and leukocytes [14].

3. Hemolysis and Vaso-occlusion:

Sickled cells undergo hemolysis, releasing free hemoglobin and inflammatory mediators that further worsen endothelial injury [15]. Simultaneously, rigid RBCs obstruct the microcirculation, resulting in vaso-occlusion, the central pathological hallmark of SCD [16]. These vaso-occlusive events trigger acute pain crises and drive chronic organ damage.

4. Downstream Pathological Consequences :

Vaso-occlusion initiates four major pathologicalpathways:Vasculopathy due to chronic endothelial dysfunction and nitric oxide depletion [17].Tissue infarction, particularly affecting bone, spleen, and other highly vascular organs [18].Chronic hemolyticanemia, resulting from continuous RBC destruction and impaired erythropoiesis [10].Inflammation, driven by leukocyte activation, cytokine release, and oxidative stress [19].These processes are interlinked and mutually reinforcing, accelerating disease progression.

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5. Clinical Complications:

The combined effects of vasculopathy, infarction, anemia, and inflammation manifest as a spectrum of systemic complications:Stroke, associated with cerebrovascular occlusion and endothelial injury [20].Infection, due to functional asplenia and impaired immune clearance [21].Pain crises, caused by ischemia and nerve sensitisation within affected tissues [22].Leg ulcers, driven by microvascular disease and chronic hemolysis [23].Chronic kidney disease, resulting from repeated renal ischemia and medullary hypoxia [24].These complications contribute significantly to morbidity, mortality, and reduced quality of life in individuals with SCD.

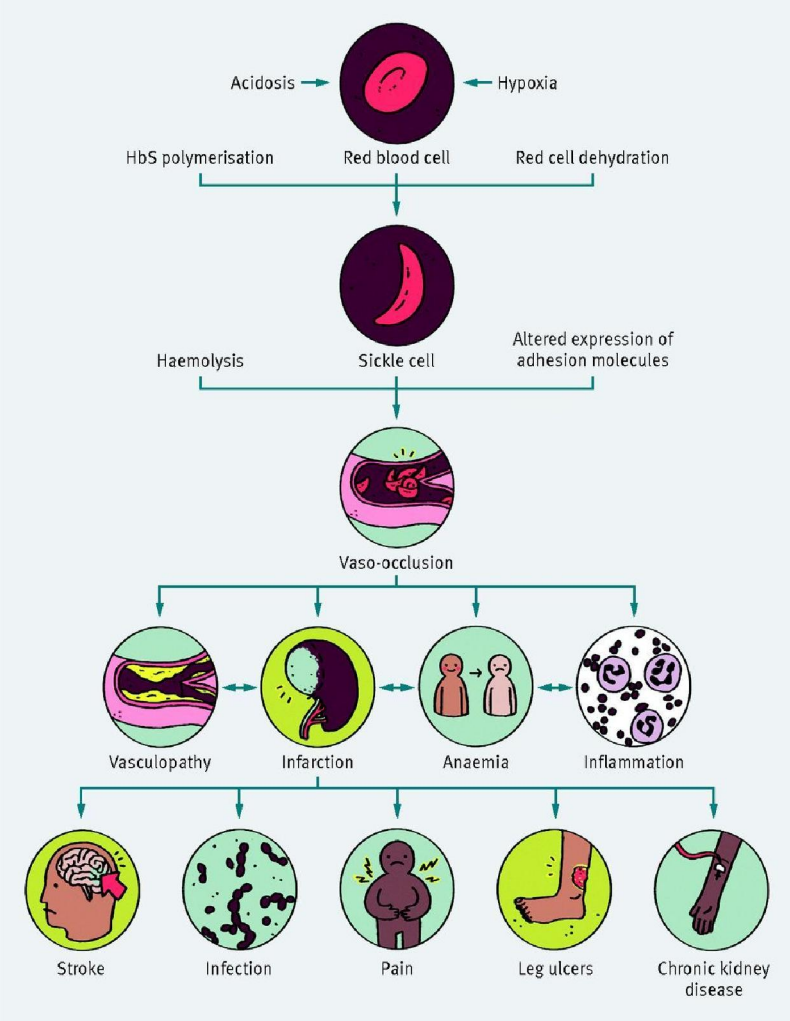

Fig.4:Pathophysiological cascade in sickle cell disease, illustrating progression from HbS polymerisation to clinical complication

Symptomatic and Supportive Interventions:-

1. Pain Management:

Pain crises represent one of the most common clinical manifestations of SCD. They are managed through pharmacological agents such as nonsteroidal anti-inflammatory drugs (NSAIDs), opioids, and patient-controlled analgesia. Adjunctive non-pharmacological interventions including heat therapy, relaxation, and distraction techniques further contribute to effective pain management and patient comfort [25].

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2. Hydration Therapy:Adequate hydration plays a vital role in preventing vaso-occlusive crises by reducing blood viscosity and minimizing erythrocyte sickling. Patients are advised to maintain optimal fluid intake, especially during infection, fever, or exposure to extreme temperatures [26].

3. Blood Transfusion Therapy:

Red blood cell transfusions are recommended for severe anemia, acute chest syndrome (ACS), or cerebrovascular events. These transfusions improve oxygen delivery, reduce hemoglobin S (HbS) concentration, and minimize the frequency of vaso-occlusive episodes [26].

4. Comprehensive Supportive Care Measures:

Comprehensive management of SCD requires an integrative, multidisciplinary approach comprising regular medical monitoring, psychosocial counseling, patient education, and genetic guidance. Such programs have been shown to improve clinical outcomes and enhance the quality of life of affected individuals and their families [27].Patient education regarding the recognition of early symptoms, identification of pain crisis triggers, and adherence to hydration guidelines is essential. Preventive strategies—such as vaccination, infection control, and avoiding extreme temperatures—play a pivotal role in reducing complications [28]. Long-term management should be personalized according to disease severity, patient-specific factors, and clinical progression, under the supervision of healthcare professionals experienced in SCD care.

5. Pharmacological Therapeutics:

Although significant progress has been made in understanding SCD pathogenesis, most existing pharmacotherapies primarily focus on preventing HbS polymerization and reducing the frequency of complications rather than curing the disease [3].

6. Hydroxyurea (Hydroxycarbamide):

Hydroxyurea is an FDA-approved agent that induces fetalhemoglobin (HbF) synthesis and remains the cornerstone of disease-modifying therapy in SCD . It acts by inhibiting ribonucleotide reductase, leading to cell cycle arrest and stimulating erythroid progenitor cells to increase HbFproduction .

Furthermore, it modulates transcriptional activity at the globin gene locus by interfering with repressor–cofactor complexes [29].

Hydroxyurea therapy results in decreased neutrophil and reticulocyte counts, enhanced erythrocyte hydration and deformability, and increased nitric oxide (NO) bioavailability, thereby reducing vaso-occlusive crises and mortality .

It is generally safe and effective across age groups, with up to 70% response rates reported . However, variability in patient response may be attributed to non-adherence or inadequate dosing . Hydroxyurea remains the most accessible and effective disease-modifying treatment for SCD worldwide [32,33].

7. Crizanlizumab:

Crizanlizumab is a monoclonal antibody directed against P-selectin, a cell adhesion molecule expressed on activated endothelial cells and platelets. It functions by preventing the adhesion of sickled erythrocytes and leukocytes to the endothelium, thereby reducing vaso-occlusive events [34]. This drug represents the first FDA-approved biologic therapy targeting cell adhesion pathways not addressed by hydroxyurea.However, following the results of the STAND trial, which demonstrated no significant reduction in vaso-occlusive episodes compared to placebo, the European Medicines Agency revoked its authorization in 2023 [35].

8. Voxelotor (GBT440/Oxbryta):

Voxelotor is an oral small-molecule drug designed to stabilize hemoglobin in its oxygenated form. By increasing oxygen affinity and preventing HbS polymerization, it effectively decreases red cell sickling and hemolysis . The drug enhances hemoglobin stability and oxygen availability, thereby mitigating anemia severity and improving hematological parameters [36,37]. Phase 3 clinical trials confirmed significant improvements in hemoglobin concentration and hemolytic indices with minimal side effects . However, it is contraindicated in patients receiving CYP3A4 inhibitors or those with known hypersensitivity. Additionally, voxelotor may interfere with Hb subtype identification in high-performance liquid chromatography (HPLC) analysis [38].

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9. L-Glutamine:

L-glutamine, approved by the FDA, functions by enhancing nicotinamide adenine dinucleotide (NAD) synthesis, thereby reducing oxidative stress in erythrocytes. This mechanism helps lower the frequency of vaso-occlusive crises .

Despite a decrease in pain crisis frequency, Phase 3 trials showed no significant improvement in hemoglobin or reticulocyte counts [39].

Although L-glutamine demonstrates clinical benefits, its cost and limited availability hinder its widespread use, and its efficacy remains comparatively lower than hydroxyurea [40].

10. Advances in Combination Drug Therapies:

Recent advancements in SCD management have introduced combination drug therapy, an approach integrating multiple pharmacological agents to target various aspects of the disease pathophysiology—such as HbF induction, anti-inflammatory modulation, and anti-adhesion mechanisms [41].

Studies by Atweh et al. demonstrated the synergistic effects of combining hydroxyurea with butyrate, which collectively enhance HbF production and inhibit HbSpolymerization . However, the optimal regimen and clinical phenotypic correlations remain under investigation. Future studies and clinical trials are necessary to standardize these strategies, facilitating their application in precision and personalized medicine [41].

11.Examples of Combination Therapy Include:

Hydroxyurea + Folic Acid: Enhances fetalhemoglobin production and supports erythropoiesis.

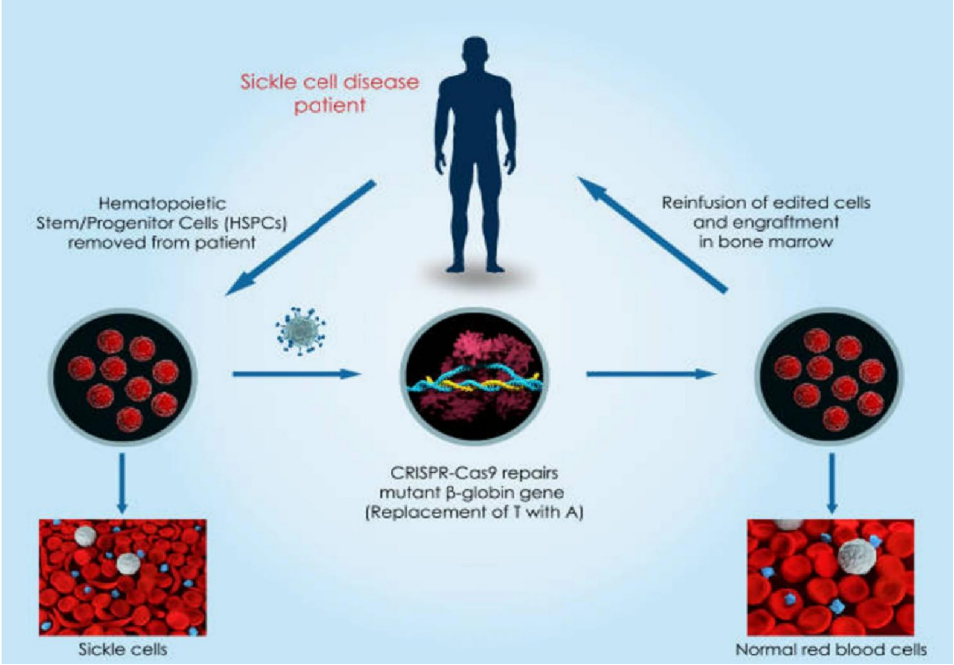
Hydroxyurea + Erythropoietin: Stimulates erythropoiesis in patients with severe anemia.

Penicillin + Vaccination: Reduces infection-related complications in SCD patients.

Overall Benefits:

Combination therapy offers a multifaceted advantage, including improved symptom control, reduced frequency of complications, enhanced quality of life, and increased survival outcomes.

Crisper cas 9 mechanism for sickle cell anemia :



1.Sickle Cell Disease Background:

The diagram represents the molecular therapeutic workflow for sickle cell disease (SCD), a monogenic disorder caused by a single A→T substitution in the β-globin (HBB) gene, resulting in the formation of hemoglobin S (HbS) and sickled erythrocytes [1].

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2. Collection of Patient HSPCs:

Hematopoietic stem/progenitor cells (HSPCs) are mobilized from the patient and collected through leukapheresis (Figure, left side). These $CD34^+$ cells carry the pathogenic HBB mutation seen in SCD individuals [42].

3. Ex Vivo CRISPR-Cas9 Editing:

The collected HSPCs undergo ex vivo genome editing using the CRISPR-Cas9 nuclease complex. Cas9 introduces a targeted double-strand break at the mutated HBB site, guided by a single-guide RNA specific to the sickle mutation [43].

4. Correction of the β-globin Mutation:

A donor repair template facilitates homology-directed repair (HDR), correcting the disease-causing thymine (T) back to adenine (A), thereby restoring the normal β-globin coding sequence (depicted in the central part of the diagram) [43].

5. Generation of Corrected Stem Cells:

Post-editing, the HSPCs now carry a corrected β-globin gene and are expanded in controlled laboratory conditions, ensuring edited cells maintain self-renewal and differentiation capacity [44].

6. Reinfusion into the Patient:

After conditioning therapy, the corrected autologous HSPCs are reinfused back into the patient's bloodstream. These cells home to the bone marrow and undergo long-term engraftment [44].

7. Restoration of Normal Hemoglobin Production:

Upon successful engraftment, the edited stem cells differentiate into erythroid cells capable of producing normal hemoglobin A instead of HbS, resulting in normal, biconcave red blood cells rather than sickle-shaped cells [44].

8. Clinical Impact:

This approach reduces hemolysis, vaso-occlusive events, and overall disease burden, demonstrating the therapeutic promise of CRISPR-based autologous gene correction in SCD management [1,44].

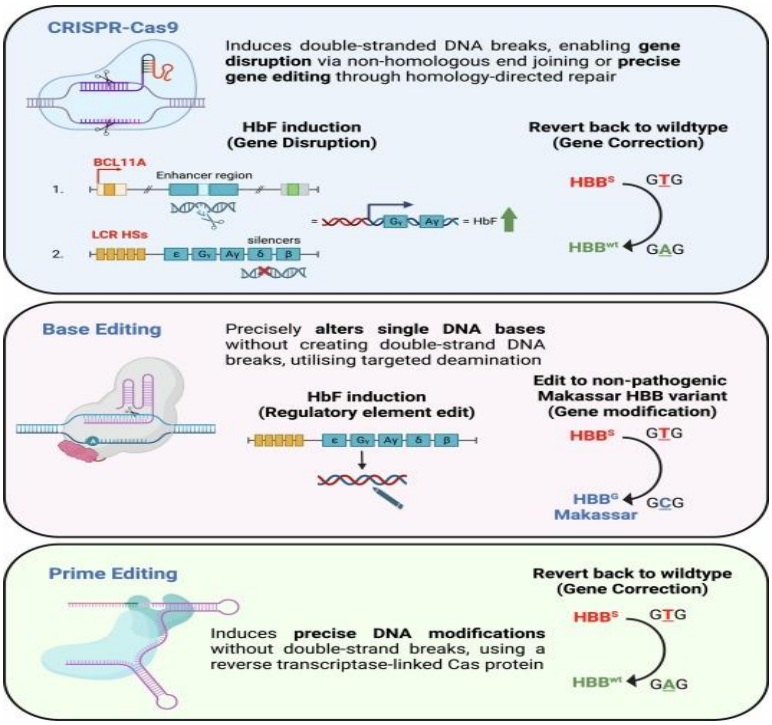

Fig. Crisper cas 9 Gene editing steps

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Crisper cas 9 Gene Editing:

1. CRISPR-Cas9 Editing Strategy

Mechanism of Action:CRISPR-Cas9 introduces double-stranded DNA breaks (DSBs) at the target locus, enabling either gene disruption through non-homologous end joining (NHEJ) or precise gene correction via homology-directed repair (HDR) [45].

HbF Induction (Gene Disruption):The upper panel shows disruption of regulatory elements such as the BCL11A erythroid enhancer, which normally represses fetalhemoglobin (HbF) expression.

CRISPR-mediated editing :of this enhancer derepresses γ-globin genes, increasing HbF, which ameliorates sickling by inhibiting HbS polymerization [46].

Modification of LCR and Silencer Elements:Targeting locus control region (LCR) sequences reduces silencing of γ-globin, further strengthening HbF induction as a therapeutic strategy [47].

Direct Gene Correction of HBB Mutation:The disease-causing HBB^S mutation (GTG → GAG) is corrected by HDR following Cas9-induced cleavage, restoring normal β-globin and normal hemoglobin A (HbA) production [48].

2. Base Editing Approach

Mechanism of Action:Base editors catalyze single-nucleotide conversions without generating DSBs, using cytosine or adenine deaminases fused to Cas proteins

This reduces risks of chromosomal rearrangements or large indels[49].

Regulatory Element Editing for HbFInduction:Base editors can modify transcriptional repressors or enhancer sequences to increase γ-globin expression, enhancing HbF levels in erythroid cells [50].

Conversion to Benign HBB Variant (Makassar Variant):The base-editing panel shows modification of the mutantHBB^S codon (GTG) to produce a Makassar (GCG) variant.

This variant eliminates the sickling phenotype without reverting to wild-type, making it a safe gene-modification approach [51].

3. Prime Editing Strategy

Mechanism of Action:Prime editing uses a Cas9 nickase fused to reverse transcriptase, guided by a prime editing guide RNA (pegRNA), enabling precise edits—including insertions, deletions, and base substitutions—without DSBs or donor templates [52].

Correction of the Sickle Mutation:The diagram shows correction of the HBB^S mutation (GTG) directly back to the wild-type sequence (GAG).

This method has high editing precision and low off-target mutagenesis, offering advantages over classic CRISPR-Cas9 HD

Comprehensive Framework of Gene Editing Cargo, Delivery Systems, and Application Routes

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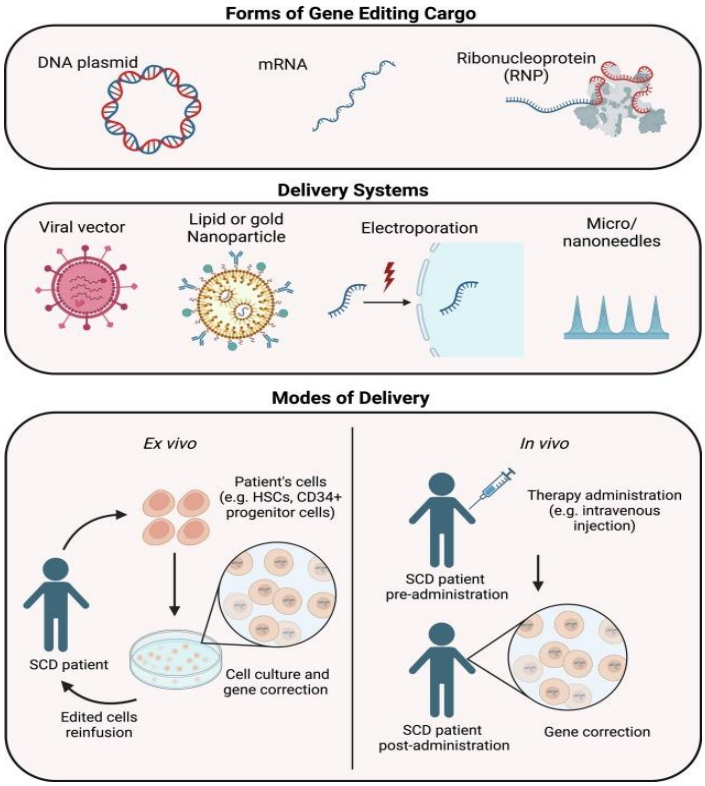

Fig .Comprehensive Framework of Gene Editing Cargo, Delivery Systems, and Application Routes

1. Forms of Gene-Editing Cargo

DNA Plasmid:

Plasmid-based systems deliver CRISPR components as DNA templates, enabling prolonged intracellular expression. However, risks of integration and reduced editing precision limit their clinical utility for SCD [53].

mRNA:

mRNA encoding Cas9 or base editors provides transient, non-integrating expression with a favorable safety profile. mRNA-based delivery minimizes risks of off-target activity due to short degradation time [54].

Ribonucleoprotein (RNP) Complexes:

Cas9 protein pre-complexed with sgRNA is the most clinically advanced gene-editing cargo for SCD. RNPs act immediately upon entering the cell and degrade rapidly, offering high on-target efficiency and minimal genomic toxicity [55].

2. Delivery Systems for Gene Editing

Viral Vectors:

Adeno-associated viruses (AAV6) or lentiviral systems can deliver CRISPR elements or donor templates. AAV6 is widely used for ex vivo editing of hematopoietic stem cells (HSCs) due to its high tropism and HDR support [56].

Lipid or Gold Nanoparticles:

Nanoparticles can encapsulate mRNA, RNPs, or CRISPR components. Lipid nanoparticles (LNPs) are non-viral, non-integrating carriers with high biocompatibility and potential for in vivo HSC targeting [57].

Electroporation:

Electroporation is a physical method that transiently permeabilizes the cell membrane to deliver RNPs or DNA templates. It is the standard clinical method for ex vivo editing of $CD34^+$ HSCs in SCD trials [58].

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Micro/Nanoneedles:
These emerging technologies enable minimally invasive, localized delivery of CRISPR cargo. They provide controlled dosing and reduced systemic exposure, though still experimental for hematopoietic disorders [59].

3. Modes of Delivery
A. Ex Vivo Gene Editing
Overview:
Ex vivo editing is currently the gold standard for SCD clinical trials. $CD34^+$ hematopoietic stem and progenitor cells (HSPCs) are harvested from the patient, edited outside the body, expanded, and reinfused after conditioning [60].
Workflow:
SCD patient undergoes HSPC mobilization and collection.Cells are cultured and corrected using CRISPR-Cas9 RNPs or other editing cargo.Edited cells are reinfused to reconstitute lifelong, corrected hematopoiesis.This strategy results in high editing efficiency and controlled assessment of edited cells prior to infusion.
B. In Vivo Gene Editing
Overview:
In vivo delivery involves direct administration of gene-editing components (via IV injection), targeting HSCs inside the bone marrow. This method removes the need for ex vivo cell manipulation but poses targeting and safety challenges [61].
Process:
CRISPR therapy is administered to the SCD patient.Editing occurs directly in hematopoietic cells within the body.This approach is under development, with nanoparticle-based systems showing early potention

Future perspective :
1.Advancement toward in vivo gene editing is expected to eliminate the need for stem cell harvest and high-intensity conditioning, significantly improving accessibility.
2.Next-generation tools, such as base editing, prime editing, and high-fidelity Cas variants, will likely enhance precision and reduce off-target DNA damage.
3.Development of safer conditioning strategies, including antibody-mediated or non-myeloablative regimens, will expand eligibility and reduce toxicity.
4.Optimization of delivery platforms—such as lipid nanoparticles, viral vectors, or engineered RNP systems—will be crucial for broad clinical translation.
5.Automated, decentralized manufacturing systems may reduce treatment cost and enable widespread availability in low- and middle-income regions.
6.Integration of AI-driven sgRNA design and off-target prediction tools will support individualized editing strategies and enhance genomic safety.
7.Robust long-term follow-up frameworks will be essential to monitor edited stem cell behavior, genomic stability, and potential late-emerging effects.
8.Global health policies must evolve to ensure equitable distribution of gene-editing therapies, addressing the disproportionate disease burden in Africa and South Asia.
9.In the long term, CRISPR therapeutics may evolve into a single-dose, minimally invasive, globally accessible intervention, redefining curative care for hemoglobinopathies

II. CONCLUSION

CRISPR-Cas9 therapy has emerged as a transformative approach for sickle cell anemia, offering the possibility of a durable, one-time cure by directly correcting or reprogramming the genetic defect underlying the disease. Early clinical studies have demonstrated sustained increases in fetal hemoglobin, reduced vaso-occlusive complications, and overall improvement in hematologic function, validating the therapeutic potential of genome editing. However, the reliance on ex vivo stem cell manipulation, intensive conditioning regimens, and highly specialized infrastructure continues to limit

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widespread applicability, especially in regions with the highest disease burden. Moreover, concerns related to long-term genomic safety, off-target effects, and the high cost of treatment highlight the need for continued refinement and monitoring. Despite these challenges, CRISPR-Cas9 remains a promising and potentially curative modality, marking a significant shift toward precision genetic therapy for hemoglobinopathies.

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