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New insights into the pathophysiology and novel therapies for sickle cell disease

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ABSTRACT

Sickle cell disease (SCD) is one of the world's most common severe monogenic disorders affecting millions of people worldwide and represents a significant public health problem. The last two decades have seen a major increase in knowledge about the cascade of events that follow the polymerization of hemoglobin, the main pathophysiological event in SCD, including impaired biorheology and increased adhesion-mediated vaso-occlusion, hemolysis-mediated endothelial dysfunction, and inflammation. As a result, several distinctive therapeutic targets have been discovered, and a few drugs with innovative mechanisms of action are already on the Market. In contrast, several others are the focus of ongoing trials. This narrative review aims to describe some of the more recent data in the SCD literature regarding pathophysiology and novel treatments.

Keywords: Sickle cell disease; pathophysiology; perspective; treatment.

Introduction

Sickle cell disease (SCD) is a group of inherited disorders characterized by chronic hemolysis and vaso-occlusive episodes, resulting in tissue ischemia and severe pain, progressive multiorgan damage that predisposes patients to multiple chronic conditions, functional limitation, poor quality of life, reduced life span, and detrimental effects on the psychosocial well-being of those affected, their families, and communities[1-4].

Although SCD was the first monogenic disease described in the scientific literature over a century ago, it remains an illness with high morbidity and early mortality. SCD affects an estimated 30 million people; over 300,000 children are born with SCD yearly, 75% of whom are in Africa[3,5,6].

SCD is a group of inherited disorders, including sickle cell anemia [Hemoglobin (Hb) SS], responsible for the most common genotype and the most severe variant of SCD, and sickle-HbC disease (HbSC), sickle β^0-thalassemia (HbSβ^0), and sickle β^+-thalassemia (HbSβ^+). Less common Hb mutants, such as O^{Arab}, D^{Punjab}, or E, may be inherited in compound heterozygosity with β^S to result in SCD[3,4].

Vaso-occlusive crises (VOCs) are one of the hallmarks of SCD, the most frequent complication among children and adults, and a substantial cause of acute and chronic complications, hospitalization, and increased mortality[1-4]. In the last two decades, the availability of mouse models of SCD has allowed both the characterization of the pathogenesis of sickle cell-related organ damage(s) and the identification of new pathophysiology-based therapeutic options in addition to HU. This narrative review aims to describe some of the more recent data in the SCD literature regarding pathophysiology and novel treatments[1-4].

New insights into the pathophysiology of sickle cell disease

The polymerization of Hb under deoxygenation is the main pathophysiological event in SCD, described more than 70 years ago. The last two decades have seen a major increase in knowledge about the cascade of events that follow the polymerization of hemoglobin and the ensuing sickling of red blood cells. Based on current evidence, the main pathophysiological mechanisms of SCD encompass four significant processes: hemoglobin S polymerization, impaired biorheology and increased adhesion-mediated vaso-occlusion, hemolysis-mediated endothelial dysfunction, and inflammation, as shown in Figure 1. These processes collaborate to promote acute and chronic pain, end-organ injury, and failure in SCD[7-9].

Hemoglobin polymerization

SCD is a genetic disorder resulting from a point mutation in the sixth codon of the β-globin gene for hemoglobin (Hb), which results in the substitution of the hydrophilic glutamic acid residue (Glu) by a hydrophobic valine residue (Val). The mutant Hb, HbS, polymerizes upon deoxygenation, decreasing cell membrane fluidity and causing the erythrocytes to assume a sickle morphology[7,8,10].

The hallmark of sickle cell pathophysiology is the intraerythrocytic polymerization of deoxyhemoglobin S. Upon deoxygenation, the normal conformational change of the tetramer exposes on its external surface a hydrophobic β^6 valine (instead of the hydrophilic glutamate of HbA), resulting in decreased solubility and a tendency of deoxyhemoglobin S tetramers to aggregate or polymerize. The rate and degree of this polymerization determine the severity of sickle erythrocytes' rheologic impairment and the change in morphology for which the condition was named[8,10].

The polymerization rate and extent are correlated with the intracellular concentration of HbS, the type and fractional content of other Hbs present (particularly HbF), and the percent oxygen saturation. These variables correlate with the rate of hemolysis in SCD[7,8,10].

Vaso-occlusion

Vaso-occlusion leading to ischemia is the predominant pathophysiology responsible for

acute systemic painful vaso-occlusive crisis (VOC) and the requirement for emergency medical care by SCD patients. Increased plasma viscosity due to chronic hemolysis and reduced sickle erythrocyte deformability due to Hb polymerization and dehydration contribute to impaired blood flow through capillaries and postcapillary venules of tissues with high oxygen demand. Poorly deformable sickle erythrocytes may become mechanically sequestered in the microcirculation to promote transient vaso-occlusion. Epidemiological evidence indicates that an inflammatory or environmental stimulus, including infection, hypoxia, dehydration, acidosis, or other unidentified factors, often initiates VOC[4,7,8,11].

Sickle erythrocytes exhibit abnormally increased adherence to vascular endothelial cells and subendothelial extracellular matrix proteins. The sickling-dependent damage of erythrocyte membranes also promotes exposure to adhesion molecules and binding motifs not generally expressed on erythrocytes, such as phosphatidyl serine (PS) and intercellular adhesion molecule-4 (ICAM-4). As a result of chronic anemia, the bone marrow undergoes stress reticulocytosis and releases immature erythrocytes repleted of adhesion molecules such as α4β1 integrin (VLA-4) and CD36. 7,11,12 The overexpression of these cellular adhesion molecules causes the adhesive interactions of erythrocytes and reticulocytes with inflammatory and endothelial cells, promoting vaso-occlusion in SCD. Endothelial dysfunction and sterile inflammation, which are hallmarks of SCD, may contribute to upregulation of selectins (P- and E-), vascular-cell-adhesion-molecule-1 (VCAM-1), ICAM-1, and interleukin-8 (IL-8) on endothelial cells. The inflammatory milieu in SCD may also promote the activation of neutrophils, monocytes, and platelets, leading to increased adhesion to each other and activated endothelium[4,5,7-9].

Hemolysis

Although the deoxygenation-polymerization-sickling axiom provides a basic understanding of SCD, there is an increasing appreciation that interactions of sickle cells with other cells and proteins contribute to the hemolytic and vaso-occlusive processes. The chronic hemolytic nature of SCD leads to chronic depletion of nitric oxide from the release of arginase and free heme, which is associated with impaired cleavage of large von Willebrand factor multimers by ADAMTS 13 and the activation of tolllike receptor 4 (TLR4)[4,5,7-9].

Blood coagulation activation, resulting in enhanced thrombin generation and evidence for platelet hyperreactivity, has been demonstrated in patients with SCD during steady-state and vaso-occlusive episodes. It has been suggested that the exposure of RBC membrane phosphatidylserine and circulating activated endothelial cells in SCD patients contributes to hypercoagulability by providing procoagulant surfaces. The correlation of elevated white blood cell counts to increased mortality and adverse outcomes identified by epidemiologic studies of SCD patients suggests that neutrophils also participate in vaso-occlusion[4,5,7,9-12].

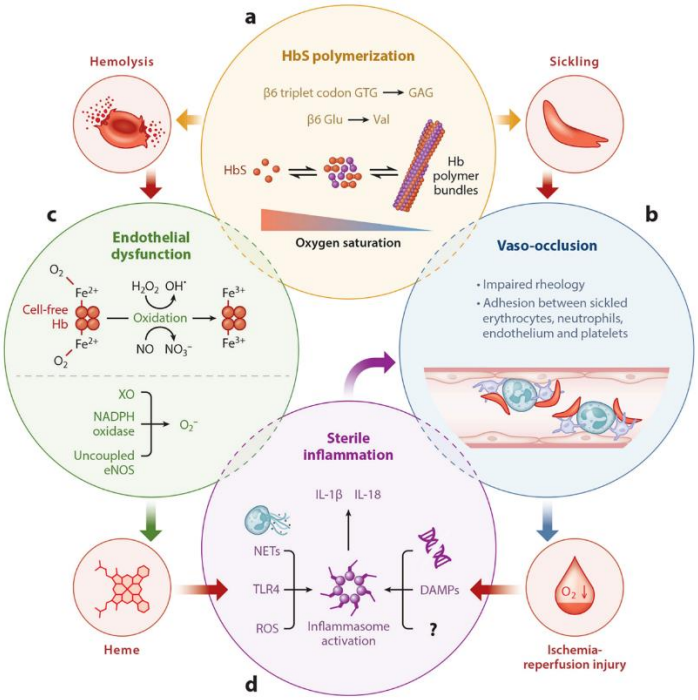

Figure 1. Molecular pathophysiology of sickle cell disease. (*a*) A single-nucleotide polymorphism in the βglobin gene leads to the substitution of valine for glutamic acid at the sixth position in the βglobin chain. Following deoxygenation, the mutated hemoglobin (HbS) molecules polymerize to form bundles. The polymer bundles result in erythrocyte sickling (*clockwise*), which in turn results in (*b*) impaired rheology of the blood and aggregation of sickle erythrocytes with neutrophils, platelets, and endothelial cells to promote stasis of blood flow, referred to as vaso-occlusion. Vaso-occlusion promotes ischemia-reperfusion (I-R) injury (*clockwise*). (*a*) Hemoglobin (Hb) polymer bundles also promote hemolysis or lysis of erythrocytes (*counterclockwise*), which (*c*) releases cell-free Hb into the blood circulation. Oxygenated Hb (Fe^{2+}) promotes endothelial dysfunction by depleting endothelial nitric oxide (NO) reserves to form nitrate (NO_3^-) and methemoglobin (Fe3+). Alternatively, Hb can react with H_2O_2 through the Fenton reaction to form hydroxyl free radical ($OH^•$) and methemoglobin (Fe^{3+}). Also, NADPH oxidase, xanthine oxidase (XO), and uncoupled endothelial NO synthase (eNOS) generate oxygen free radicals to promote endothelial dysfunction. Methemoglobin (Fe^{3+}) degrades to release cell-free heme (*counterclockwise*), which is a significant erythrocyte damage-associated molecular pattern (DAMP). (*d*) Reactive oxygen species (ROS) generation, Toll-like receptor 4 (TLR4) activation, neutrophil extracellular trap (NET) generation, release of tissue or cell-derived DAMPs, DNA, and other unknown factors (?) triggered by cell-free heme or I-R injury can contribute to sterile inflammation by activating the inflammasome pathway in vascular and inflammatory cells to release IL-1βFinally, sterile inflammation further promotes vaso-occlusion through a feedback loop by promoting the adhesiveness of neutrophils, platelets, and endothelial cells. (Sundd et al. 2019)

Phenotypic heterogeneity and sub-phenotypes

A single sickle mutation cannot explain the clinical heterogeneity of the disease phenotype within the same family in this multifactorial disorder. The clinical phenotype of SCD varies widely, influenced by additional cellular, genetic modifiers of disease severity, including factors that affect HbF level (African haplotypes of the HBB cluster; genetic variation of an erythroid-specific enhancer of BCL11A), co-inheritance of α-thalassemia; and nongenetic modifiers that include environmental factors (climate and air quality, altitude) as well as socioeconomic factors, which are assessed, for example, based on access to medical care, safe blood transfusions, and treatment of infections, infectious diseases[3]. These factors may explain much of the clinical variability, why some individuals have very severe diseases with frequent vaso-occlusive complications and early morbidity and death at a very young age, whereas others can go unnoticed until adulthood.

Based on the clinical heterogeneity of the disease, two sub-phenotypes of SCD have emerged, one attributed to "viscosity-vasoocclusion" and the other to "hemolysis-endothelial dysfunction"[14]. The vaso-occlusive subphenotype is manifested clinically by self-limited pain crises, acute chest syndrome and osteonecrosis, stroke, acute splenic sequestration, hepatic sequestration, and organ

failure such as renal disease and functional asplenia. Patients with the hemolytic sub-phenotype are more likely to develop a proliferative vasculopathy and organ dysfunction as they age, manifesting as pulmonary hypertension, diastolic left heart disease, renal dysfunction (proteinuria, albuminuria, and chronic kidney dysfunction), priapism, leg ulcers, elevated systolic systemic blood pressures, stroke, and sudden death. Although some overlapping between the sub-phenotypes is expected, clinical profiling to differentiate symptoms more typical for an individual patient with SCD is an innovative approach that may help direct personalized therapies for a specific sub-phenotype by targeting the predominant mechanism[3,14,15].

Clinical manifestations

ANEMIA

In SCD patients, the erythrocyte lifespan is shortened from the standard 120 days to approximately 10 to 25 days, resulting in moderate to severe hemolytic anemia (ranging from 6 to 11 g/dL). The anemia is generally well tolerated because of compensatory cardiovascular changes and increased levels of 2,3-DPG[1,2].

HbSS is associated with the most severe anemia, most frequent pain, and shortest life expectancy, although there is tremendous heterogeneity in these variables even within this genotype.

HbSβ^0-thalassemia can closely mimic HbSS despite the smaller red blood cells, lower MCH concentrations, and higher levels of HbF and HbA_2 associated with this genotype[3,4].

Patients with HbSC generally live longer and have less severe anemia (~20% are not anemic), higher MCH concentrations, and less frequent pain. Still, they have more frequent ocular and bone complications that may be explained by the increased hematocrit combined with the higher MCH concentration (MCHC) and cellular dehydration. This results in higher whole-blood viscosity, which may increase the likelihood of vaso-occlusion. Patients with HbSβ^+-thalassemia have less severe anemia and pain than patients with HbSβ^0-thalassemia. This results from smaller cells, lower MCHC, increased content of HbF and HbA_2, and, most importantly, the presence of significant amounts (10% to 30%) of HbA that interfere with polymerization[3,4].

Although patients have chronic anemia, several conditions can exacerbate this anemia and lead to acute symptomatic anemic events. The transient aplastic crisis resulting from erythroid aplasia is often caused by human parvovirus infection as well as other infections, which may result in severe or life-threatening anemia. Lesser degrees of bone marrow "suppression" are associated with different infections. Sudden anemia may be caused by acute splenic sequestration in children with HbSS or Sβ^0 (and in adults with HbSC or Sβ^+-thalassemia) or, less frequently, hepatic sequestration, concomitant glucose-6-phosphate dehydrogenase (G6PD) deficiency, or superimposed autoimmune hemolysis. Chronic exacerbations of anemia may result from folate, iron deficiency, or reduced erythropoietin levels caused by chronic renal insufficiency[1-4].

ACUTE AND CHRONIC COMPLICATIONS

SCD is a multisystem disorder. Vaso-occlusion typically causes acute complications, including ischemic damage to tissues, resulting in severe pain or organ failure. Acute chest syndrome is a typical example of organ failure in SCD and one of the leading causes of hospitalization and death among SCD patients. The usual clinical complications of SCD are shown in Figure 2[1-4].

Chronic complications fall into two main groups: those related to large vessel vasculopathy (cerebrovascular disease, pulmonary hypertension, priapism, leg ulcer, and retinopathy) and those caused by progressive ischemic organ damage (hyposplenism, renal failure, heart failure, bone disease, and liver damage). The risk of life-threatening septicemia and meningitis because of encapsulated organisms, such as Streptococcus pneumoniae, is markedly increased in children with SCD. This susceptibility is related to functional and

anatomic asplenia and decreased opsonization because of deficient production of natural antibodies. The risk for such infections persists into adulthood[1-4].

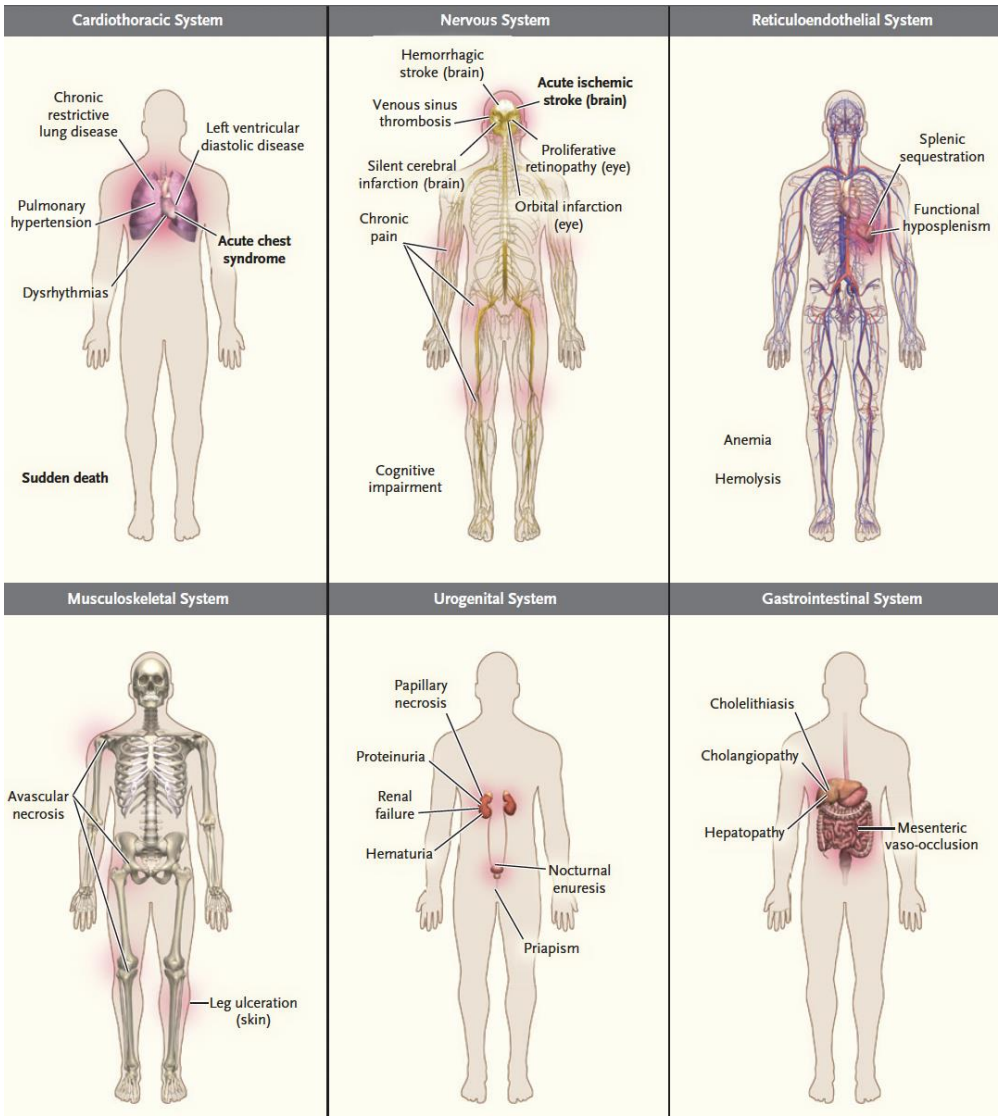

Figure 2. Common Clinical Complications of Sickle Cell Disease (Piel et al., 2017)

Novel therapies for sickle cell disease

Despite remarkable achievements in epidemiological and pathophysiologic knowledge, life expectancy for patients with SCD is reduced by about 30 years, even with the best medical care, and the quality of life is often poor. Fortunately, the current understanding of the cellular, molecular, and biophysical pathobiology of SCD has inspired several current and potential future therapeutic approaches to prevent disease morbidity[2,3,8,16].

The management of SCD patients may include hydroxyurea (HU), folic acid, blood transfusion, iron chelation, antibiotic therapies, vaccination, hematopoietic stem cell transplantation (HSCT), and gene therapy. In the last two decades, the availability of mouse models of SCD has allowed both the characterization of the pathogenesis of sickle cell-related organ damage(s) and the identification of new pathophysiology-based therapeutic options, in addition to HU. These include agents such as l-glutamine, crizanlizumab, and voxelotor. (Figure 2)[7,8,16-20]

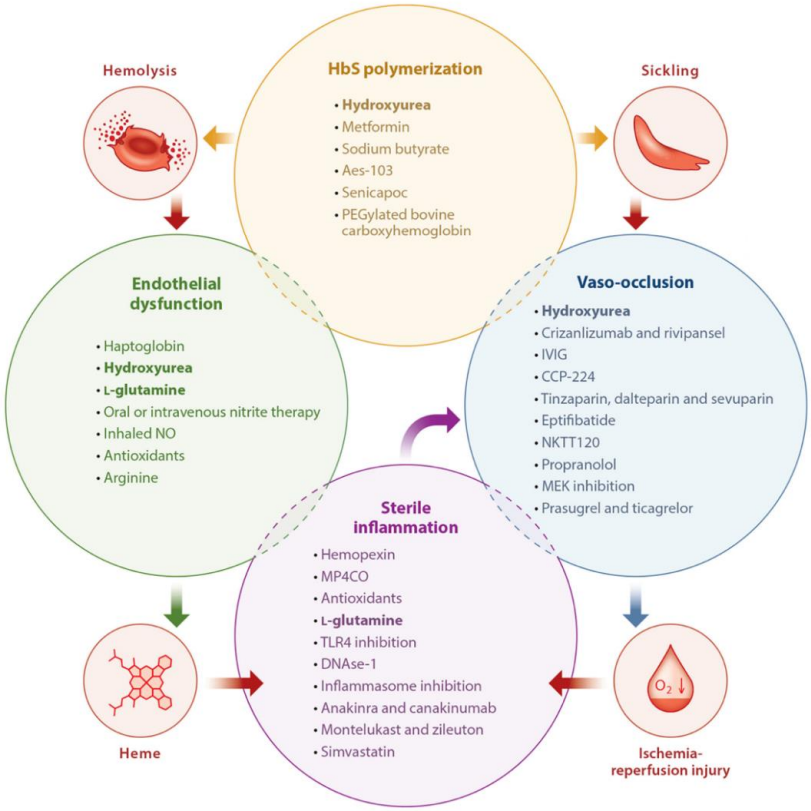

Figure 3. Disease-modifying treatments to prevent vaso-occlusive morbidity in SCD

The HU is an inhibitor of the ribonucleotide reductase drug with many beneficial effects for treating people with SCD, including increasing fetal Hbconcentration in RBCs, improving nitric oxide metabolism, and reducing red cell-endothelial interaction and erythrocyte density. Such disease-modifying effects have been shown to decrease VOCs, acute chest syndrome (ACS), the number/length of hospitalizations, and the need for transfusions, noticeably reducing the mortality rate and improving overall survival. In the last two decades, the availability of mouse models of SCD has allowed both the characterization of the pathogenesis of sickle cell-related organ damage(s) and the identification of new pathophysiology-based therapeutic options, in addition to HU. These include agents such as l-glutamine, crizanlizumab, and voxelotor. Blood transfusion currently represents a supportive therapy to manage anemia, vasculopathy, and VOCs and to prevent serious complications, particularly the risk of primary and secondary stroke. The HSCT is a potentially curative procedure, although its use is restricted to a few patients due to the high cost, toxicity, and limited availability of suitable donors. In addition to HSCT, gene therapy is being explored as a curative option for SCD. Gene therapy aims to replace a patient's abnormal gene with new genetic material; it offers a potential cure for SCD without the need for a bone marrow donor or the toxicity associated with traditional conditioning regimens for HSCT. (Sundd et al. 2019)

Hydroxyurea

The HU is an inhibitor of the ribonucleotide reductase drug with many beneficial effects for treating people with SCD, including increasing fetal Hb concentration in RBCs, improving nitric oxide metabolism, and reducing red cell-endothelial interaction and erythrocyte density. Such disease-modifying effects have been shown to decrease VOCs, acute chest syndrome (ACS), the number/length of hospitalizations, and the need for transfusions, noticeably reducing the mortality rate and improving overall survival for adults and children with SCD[2,3,8,21].

The main indications for HU are patients with three or more VOCs per year, two or more acute chest

syndrome events per year, symptomatic anemia in all immunized patients, organ damage such as chronic leg ulcers, priapism, pulmonary hypertension, hemolysis with high LDH, and transcranial Doppler velocities between 160 and 200 cm/s. HU is dispensed free of charge to patients who meet these criteria. For patients with indication, HU is recommended for individuals older than nine months, at 15 mg/kg/day for the initial dose and up to 35 mg/kg/day, depending on haematologic toxicity. The optimal dose is defined as a stable daily dose that causes mild myelosuppression without other laboratory toxicities. Most HU benefits are usually observed after 2-3 months of treatment, and the results remain stable once a stable dose is reached and taken regularly[8,11-24].

Irrespective of the question of optimal dosage, HU is underutilized both in low- and high-resource countries because of healthcare infrastructure deficiencies, poor compliance, and fear of toxicity, particularly worries about the toxic effect on spermatogenesis.25 HU's benefit/risk ratio in SCD strongly argues for its wider use. Approximately one-third of adults will have a poor reaction, demonstrating the need for additional agents[8,9,20].

Modulation of oxidative stress

L-GLUTAMINE

The role of oxidative stress in the pathophysiology of SCD is complex. Reactive oxygen species generation affects the integrity of the red blood cell membrane, with a dose-dependent effect on membrane rigidity and decreased elasticity. Both red cell and leukocyte adhesion have been shown to increase with superoxide production in SCD[8,26].

Sickle red cells have a lower redox ratio than normal red cells, contributing to the disease's pathophysiology. Glutamine is a precursor for synthesizing glutathione (GSH), nicotinamide adenine dinucleotide (NAD), and arginine, which protect erythrocytes from oxidative damage and indirectly maintain vascular tone[8,26].

L-glutamine therapy, which increases the proportion of reduced nicotinamide adenine dinucleotide in sickle red blood cells and presumably reduces oxidative stress and potentially painful events, was tested in a randomized controlled clinical trial. The randomized study included 230 patients (aged 5 to 58 years) with either HbSS or $S\beta^0$- thalassemia with a history of 2 or more crises during the previous year. Patients randomized to l-glutamine had significantly fewer sickle cell crises than patients receiving placebo (median 3.0 versus 4.0 crises). Fewer hospitalizations (median 2.0 versus 3.0) and episodes of acute chest syndrome occurred in the study group. The majority of subjects on both arms were on hydroxyurea. L-Glutamine (Endari) was approved by the United States Food and Drug Administration (FDA) in 2017. Endari is available in powder form and is mixed with room temperature food at doses of 5 to 15 grams based on weight and given twice daily[8,26].

Oral administration of L-glutamine raised the redox ratio within sickle cells. It was associated with patient-reported clinical improvement, although its mechanism of action is not entirely understood[26,27].

Antisickling agents

VOXELOTOR

Voxelotor is an oral HbS polymerization inhibitor that reversibly binds to Hb's alpha chain and modulates its affinity for oxygen, preventing HbS sickling[8]. Voxelotor is an oral therapy that modifies Hb to increase the affinity between Hb and oxygen. The modified Hb is thought to avoid polymerization and sickling of HbS. In the pivotal phase 3 HOPE trial, voxelotor increased hemoglobin and reduced markers of chronic hemolysis. While subsequent abstracts report improvement in pain episodes, recent publications failed to show a consistent improvement in pain with voxelotor therapy[28]. Treatment side effects include gastrointestinal complaints reported to respond to dose reductions. Voxelotor was approved by the FDA in 2019 for children over 12 years old and adults. In 2021, the FDA approved voxelotor for children over four years. The

recommended dose for patients over 40 kg or adults is 1500 mg/day[28,29].

GBT021601

GBT021601, a next-generation HbS polymerization inhibitor, has an increased oxygen affinity compared to Voxelotor, leading to higher Hb occupancy. 19,20 GBT021601 is being investigated in a phase 2/3 clinical trial for adult and pediatric sickle cell disease patients (NCT05431088). Preliminary results showed a mean (standard deviation) increase in Hb from baseline was 2.67 (1.52) g/dL for the 100- mg group (n = 12) and 3.17 (1.82) g/dL for the 150-mg group (n = 11) after 12 weeks of treatment. A favorable trend toward reduction from baseline was observed in markers of hemolysis. For 27 patients with ≥ 1 VOC at baseline, the baseline annualized VOC rate was 2.30 (95% confidence interval (CI) 1.81– 2.92), and the on-study annualized VOC rate was 1.16 (95% CI 0.55–2.43) with a median (range) on-study duration of 0.4 (0.03–0.41) years[30].

Antiadhesion molecules

CRIZANLIZUMAB

Crizanlizumab is a humanized immunoglobulin monoclonal antibody produced using recombinant DNA technology. It binds to and inhibits P-selectin, blocking the interaction with its ligand, PSGL-1.19,20 The safety and efficacy of crizanlizumab in preventing SCD-related pain crises were evaluated in a double-blind, randomized, placebo-controlled, phase 2 trial (SUSTAIN); this trial included 198 randomized patients 16 to 65 years of age who received 2.5 mg/kg, 5 mg/kg or placebo intravenously, with two loading doses two weeks apart, and then every four weeks for 52 weeks. In the intent-to-treat analysis, the median annual rate of painful crises was 1.63 in the high-dose crizanlizumab group compared to 2.98 in the placebo group (a 45.3% lower rate with the drug) and 2.01 in the low-dose crizanlizumab group. The reduction in the annual crisis rate was more significant for patients not receiving HU (32.1% vs. 50%) and for patients with a genotype other than HbSS (34.6% vs. 50.5%)[8,31].

The overall incidences of severe and adverse events among the patients treated with crizanlizumab were similar to those among patients who received a placebo. The incidence of AEs leading to discontinuation was generally low in both dose groups (0–8.0% in the crizanlizumab arm vs. 0–8.7% in the placebo arm across all subgroups). No treatment-related AEs led to death[8,31].

A post hoc analysis of the SUSTAIN study evaluated only patients receiving 5 mg/kg crizanlizumab versus placebo. Efficacy endpoints were the proportion of VOC event-free patients, time-to-first VOC, time-to-second VOC, and types of VOC, including uncomplicated crises and acute chest syndrome. A more significant proportion of patients in the crizanlizumab group (n.24/67; 35.8%) than patients in the placebo group (n.11/65; 16.9%) did not experience a VOC, independent of the number of events in the year before the study, genotype and HU use. Time-to-first VOC was also increased in the treatment group, with a more significant effect in the group of patients not using HU (HR: 0.40; 95% CI 0.17–0.93) and in the non-HbSS genotype subgroup (HR: 0.30; 95% CI 0.11–0.81). Finally, the median rate of uncomplicated VOCs was lower for crizanlizumab 5 mg/kg than placebo across all subgroups, but no statistical analysis was shown for this endpoint. The rates of treatment-related AEs and SAEs, determined according to the investigator's judgment, were higher in patients treated with crizanlizumab than in patients treated with a placebo across all subgroups. However, the incidence of AEs leading to discontinuation was generally low in both dose groups (0%-8.0% in the crizanlizumab arm vs. 0–8.7% in the placebo arm)[8,32].

Subsequently, the STAND trial (NCT03814746), which is a phase 3, multicenter, randomized, double-blind study to assess the efficacy and safety of two doses of Crizanlizumab versus placebo, with or without hydroxyurea therapy in adolescent and adult SCD patients with frequent VOCs, showed no statistically significant difference between Crizanlizumab 5 mg/kg or Crizanlizumab 7.5 mg/kg

and placebo in annualized rates of VOCs (pain crises) leading to a healthcare visit over the first-year post-randomization. These findings were inconsistent with previous trial results from SUSTAIN. STAND trial results did not suggest new safety concerns with Crizanlizumab. EMA withdrew Crizanlizumab from use in the European Union in 2023. However, the FDA continues to approve it for use in adults and pediatric patients aged 16 years or older with sickle cell disease to reduce the frequency of vaso-occlusive crises[20].

INCLACUMAB

Inclacumab is another anti-P selectin monoclonal antibody developed for treating and preventing cardiovascular diseases. Three studies are registered at clinicaltrials.gov evaluating inclacumab for preventing VOCs in SCD patients[2,3,33]. NCT04927247 is a randomized, double-blind, multicenter study of a single dose of inclacumab compared to a placebo. The study aims to evaluate the number of readmissions in 90 days due to VOCs in SCD patients aged 12 years or older. Recruiting ends in December 2023, and follow-up will be completed in 2024. The second study already recruiting patients is NCT04935879, a randomized, double-masked, placebo-controlled trial evaluating inclacumab infusions every 12 weeks for 48 weeks. The primary outcome measure is the rate of VOCs during the treatment period. This study will recruit patients over 12 years old and is expected to be completed in October 2023. Participants in both studies can participate in an open-label extension study (NCT05348915) of inclacumab every 12 weeks to evaluate its safety (primary outcome) and efficacy over five years[33].

Activators of pyruvate kinase activity

MITAPIVAT

Mitapivat (AG-348) is a novel, first-in-class oral small molecule allosteric activator of the pyruvate kinase enzyme. Mitapivat has been shown to significantly upregulate both wild-type and numerous mutant forms of erythrocyte pyruvate kinase (PK), increasing adenosine triphosphate (ATP) production and reducing levels of 2,3-diphosphoglycerate. Mitapivat has been an FDA-approved PK deficiency treatment since 2022 and is well tolerated in this patient group[7-9].

A phase 1 proof of concept study showed a mean hemoglobin increase of 1.2 g/dL at the 50 mg BID dose level, with 9 of 16 (56.3%) patients achieving a hemoglobin response of a ≥1 g/dL increase compared with baseline in patients with SCD[34]

The ESTIMATE study was a phase 2, investigator-initiated, open-label, single-center study that evaluated the safety plus efficacy and provided proof of concept for Mitapivat treatment in SCD patients. Van Dijk et al. showed that treatment with Mitapivat improves anemia, Hb-oxygen affinity, and sickling parameters and reduces hemolysis in patients with SCD in the 8-week dose-finding period. An increase in Hb level of ≥1 g/dL from baseline was achieved in 75% (6/8) of participants. Mitapivat was well tolerated[35].

RISE UP (NCT05031780) is a phase 2/3 study that aims to determine the recommended dose of Mitapivat and evaluate its efficacy and safety in sickle cell disease patients. The study is expected to be completed in 2025. The FDA has granted Mitapivat orphan drug designation in the U.S.A.

A second-generation molecule AG-946 has completed a phase 1 trial (NCT04536792)[36].

ETAVOPIVAT

Etavopivat (FT-4202) is another selective activator of PKR, which increases PKR activity, resulting in decreased 2,3-DPG and increased ATP.[37] Data from the phase 1 study showed that Etavopivat 400 mg once daily was generally well tolerated. A subsequent phase 2/3 trial (HIBISCUS, NCT04624659) will identify doses and confirm efficacy and safety in sickle cell disease patients.[38] The study completion date is the end of 2026.

Hematopoietic stem cell transplantation

Allogeneic transplantation is a curative therapy for people with sickle cell disease.20 In a recent

assessment of outcomes from 3 transplant registries that included 1000 recipients of HLA-identical sibling transplants performed between 1986 and 2013, the 5-year overall survival for children under 16 years was 95%, with an event-free survival of 93%. For those over 15, the overall survival and event-free survival were 81%[7-9,39].

The indications for HSCT can be classified into clinical, laboratory, and radiological markers of disease severity plus end organ damage.35 Alternative sources such as umbilical cord blood, unrelated matched, and haplotype donors are now being investigated. These alternative donor options and nonmyeloablative conditioning regimens have shown some promise but remain investigational. As these efforts are undergoing further development, consideration of long-term effects of transplants, such as loss of fertility and secondary malignancies, should also be considered[40].

Finally, the availability of HSCT is limited for various reasons, including infrastructure, e.g., the number and capacities of centers delivering transplants in different countries around the world. There are efforts to improve the global availability of HSCT shortly. Other limitations include the cost of treatment, acceptability, and the availability of suitable donors[39,40].

Gene therapy

Gene therapy includes transplantation of genetically modified autologous hematopoietic stem cells. Stem cells get mobilized from the SCD patient, i.e., the recipient, from peripheral blood. The stem cells get altered genetically after the recipient undergoes conditioning chemotherapy followed by infusion of the modified stem cells. 8 Different Technologies are used to modify genes genetically: 1) gene addition, 2) gene silencing, 3) gene editing, and 4) gene correction. Examples include 1) Lentiglobin BB305, 2) Suppression of BCL11A, 3) Gene therapy targeting BCL11A, and 4) Introducing non-sickling hemoglobin, respectively[7-9].

We kindly refer to two excellent in-depth reviews discussing gene therapy in SCD published recently[41,42]. We have selected two gene therapy products that have recently been approved.

Exagamglogene autostemcel (Exa-cel) was the first gene therapy for SCD approved by the MHRA in England in November 2023 and FDA approved in December 2023. Exa-cel uses a CRISPR-Cas9 to disrupt the BCL11A erythroid enhancer to increase HbF. A mean proportion of HbF > 20% and Hb level >11 g/L were found three months post-infusion. Most patients (n = 29/30, 96.7%) in the primary efficacy group did not experience a VOC for 12 months after the Exa-cel infusion. All patients remained free from hospitalization at 12 months. The mean VOC-free duration was 22.4 months (14.8 to 45.5 months). The study reported rapid, robust, and durable increases in HbF levels[43].

Lovotibeglogene autostemcel (lentiglobin bb1111, Lovo-cel) adds functional copies of a modified beta-globin gene (B T87Q) in stem cells. It showed a high and sustained level of HbA expression, with a reduced HbS resulting in the resolution of VOCs and hemolysis markers[44,45]. There was one acute myeloid leukemia case reported, which was not related to the gene therapy itself but due to a combination of causes, including the genetically predisposition abnormal bone marrow niche in SCD[46] and the conditioning chemotherapy[47]. FDA approval was granted for Lovo-cel in December 2023. In conclusion, gene therapies promise to cure sickle cell disease, but long-term information on safety and efficacy is needed. Currently, this treatment is not yet accessible for SCD patients.

Discussion

The polymerization of intracellular hemoglobin in SCD leads to a complex cascade that includes vaso-occlusion, ischemia, reperfusion injury, anemia, pain, end-organ damage, and reduced life expectancy. For over three decades, HU was the only available option for SCD treatment with a disease-modifying impact. In the last few years, a better understanding of SCD pathophysiology has led to developing several drugs that may prevent end-organ damage and increase life expectancy in

SCD patients. The long-term outcomes of these treatments, the role of real-life observation, and post-marketing studies are needed to understand the potential of each novel therapy better and, possibly, to design multi-agent trials of drugs with different mechanisms of action and nonoverlapping toxicities. Despite the increase in therapeutic options, the paramount challenge will be making the likely benefits of new treatments available globally since 95% of SCD patients are in countries with limited resources.

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Refrences:

1. Rees DC, Williams TN, Gladwin MT. Sickle-cell disease. Lancet. 2010; 376:2018–31.

2. Ware RE, de Montalembert M, Tshilolo L, Abboud MR. Sickle cell disease. Lancet. 2017; 390:311–23.

3. Piel FB, Steinberg MH, Rees DC. Sickle cell disease. Nat Rev Dis Primers. 2018;4:18010.

4. Kato GJ, Piel FB, Reid CD, Gaston MH, Ohene-Frempong K, Krishnamurti L, et al. Sickle cell disease. Nat Rev Dis Primers. 2018;4:18010.

5. Piel FB, Patil AP, Howes RE, Nyangiri OA, Gething PW, Dewi M, et al. Global epidemiology of sickle haemoglobin in neonates: A contemporary geostatistical model-based map and population estimates. Lancet. 2013;381:142–51.

6. GBD 2021 Sickle Cell Disease Collaborators: Global, regional, and national prevalence and mortality burden of sickle cell disease, 2000-2021: a systematic analysis from the global burden of disease study 2021. Lancet Haematol. 2023, 10.1016/s2352-3026(23)00118-7

7. Sundd P, Gladwin MT, Novelli EM. Pathophysiology of Sickle Cell Disease. Annu Rev Pathol. 2019; 14: 263–292.

8. Araújo ADS, Silva Pinto AC, Lobo CLC, Figueiredo MS, Menosi Gualandro SF, Olalla Saad ST, Cancado RD. Novel Insights into the Pathophysiology and Treatment of Sickle Cell Disease. Hemoglobin. 2023 Nov;47(2):71-79.

9. Moerdler S, Manwani D. New insights into the pathophysiology and development of novel therapies for sickle cell disease. Hematology Am Soc Hematol Educ Program. 2018 Nov 30;2018(1):493-506.

10. Brittenham GM, Schechter AN, Noguchi CT. Hemoglobin S polymerization: primary determinant of the hemolytic and clinical severity of the sickling syndromes. Blood. 1985; 65:183–89.

11. Manwani D, Frenette PS.Vaso-occlusion in sickle cell disease: pathophysiology and novel targeted therapies. Blood.2013; 122:3892–98.

12. Zhang D, Xu C, Manwani D, Frenette PS. Neutrophils, platelets, and inflammatory pathways at the nexus of sickle cell disease pathophysiology. Blood. 2016; 127:801–9.

13. Steinberg MH, Sebastiani P. Genetic modifiers of sickle cell disease. Am J Hematol 2012; 87: 795-803.

14. Kato GJ, Gladwin MT, Steinberg MH. Deconstructing sickle cell disease: reappraisal of the role of hemolysis in the development of clinical subphenotypes. Blood Rev 2007; 21: 37-47.

15. Tewari S, Brousse V, Piel FB, Menzel S, Rees DC. Environmental determinants of severity in sickle cell disease. Haematologica. 2015;100: 1108–16.

16. Salinas Cisneros G, Thein SL. Research in sickle cell disease: From bedside to bench to bedside. Hemasphere. 2021;5:e584.

17. da Silva Araújo A, Silva Pinto AC, de Castro Lobo CL, Figueiredo MS, Menosi Gualandro SF, Olalla Saad ST, Cançado RD. Sickle Cell Disease in Brazil: Current Management. Hemoglobin. 2024 Apr 25:1-13.

18. Telen MJ. Beyond hydroxyurea: new and old drugs in the pipeline for sickle cell disease. Blood 2016; 127: 810-9.

19. Guarino S & Lanzkron S. How to utilize new therapies for sickle cell disease. Hematology Am Soc Hematol Educ Program. 2022 Dec 9;2022(1):283-285.

20. Lugthart S, Ginete C, Kuona P, Brito M, Inusa BPD. An updated review of new therapies in sickle cell disease: the prospects for drug combinations. Expert Opin Pharmacother. 2024; 25(2):157-170.

21. Voskaridou E, Christoulas D, Bilalis A, Plata E, Varvagiannis K, Stamatopoulos G, et al. The effect of prolonged administration of hydroxyurea on morbidity and mortality in adult patients with sickle cell syndromes: results of a 17-year, single-center trial (LaSHS) Blood. 2010;115:2354–63.

22. John CC, Opoka RO, Latham TS, Hume HA, Nabaggala C, Kasirye P, et al. Hydroxyurea dose escalation for sickle cell anemia in Sub-Saharan Africa. N Engl J Med. 2020;382:2524–33.

23. Power-Hays A, Ware RE. Effective use of hydroxyurea for sickle cell anemia in low-resource countries. Curr Opin Hematol. 2020;27:172–80.

24. Wong TE, Brandow AM, Lim W, Lottenberg R. Update on the use of hydroxyurea therapy in sickle cell disease. Blood 2014; 124: 3850-3857, quiz 4004.

25. Joseph L, Jean C, Manceau S, Chalas C, Arnaud C, Kamdem A, et al. Effect of hydroxyurea exposure before puberty on sperm parameters in males with sickle cell disease. Blood. 2021;137: 826–9.

26. Niihara Y, Miller ST, Kanter J, et al; Investigators of the Phase 3 Trial of l-Glutamine in Sickle Cell Disease. A phase 3 trial of L-glutamine in sickle cell disease. N Engl J Med. 2018;379(3) :226-235.

27. Ogu UO, Thomas M, Chan F, et al. L-glutamine use in adults with sickle cell disease: clinical trials where success meets reality. Am J Hematol. 2021;96(1):E38-E40.

28. Vichinsky E, Hoppe CC, Ataga KI, et al. HOPE Trial Investigators. A phase 3 randomized trial of voxelotor in sickle cell disease. N Engl J Med. 2019;381(6):509-519.

29. Zaidi AU, Lipato T, Alvarez OA, et al. Real-world effectiveness of voxelotor for treating sickle cell disease in the US. Blood. 2020;136(suppl 1):25.

30. Saraf SL, Abdullahi SU, Akinsete AM, et al. Preliminary results from a multicenter phase 2/3 study of next-generation HbS polymerization inhibitor GBT021601 for the treatment of patients with sickle cell disease. Blood. 2023;142 (Supplement 1):274.

31. Ataga KI, Kutlar A, Kanter J, Liles D, Cancado R, Friedrisch J, Guthrie TH, Knight-Madden J, Alvarez OA, Gordeuk VR, Gualandro S, Colella MP, Smith WR, Rollins SA, Stocker JW, Rother RP. Crizanlizumab for the Prevention of Pain Crises in Sickle Cell Disease. N Engl J Med. 2017 Feb 2;376(5):429-439.

32. Kutlar A, Kanter J, Liles DK, et al. Effect of crizanlizumab on pain crises in subgroups of patients with sickle cell disease: a SUSTAIN study analysis. Am J Hematol. 2019;94(1):55-61.

33. Andemariam B, Inati A, Colombatti R, Minniti C, Brown C, Hottmann M et al. Trial in progress: the thrive studies evaluating the efficacy, safety, and long-term treatment with inclacumab, a p-selectin inhibitor, in patientes with sickle cell disease. HemaSphere, 2023; 7(S1):23-24.

34. Xu JZ, Conrey A, Frey I, et al. A phase 1 dose escalation study of the pyruvate kinase activator mitapivat (AG-348) in sickle cell disease. Blood. 2022;140(19):2053–2062

35. van Dijk MJ, Rab MAE, van Oirschot BA, et al. Safety and efficacy of mitapivat, an oral pyruvate kinase activator, in sickle cell disease: a phase 2, open-label study. Am J Hematol. 2022;97(7):E226–E229.

36. Study details | a study evaluating the efficacy and safety of mitapivat (AG-348) in participants with sickle cell disease (RISE UP) | ClinicalTrials.Gov [Internet]. [cited 2023 Dec 10]. Available from: https://clinicaltrials.gov/study/NCT05031780

37. Forsyth S, Schroeder P, Geib J, et al. Safety, pharmacokinetics, and pharmacodynamics of etavopivat (FT-4202), an allosteric activator of pyruvate kinase-R, in healthy adults: a randomized, placebo-controlled, double-blind, first-in-human phase 1 trial. Clin Pharmacol Drug Dev. 2022;11(5) :654–665.

38. Study details | a study of Etavopivat in adults and adolescents with sickle cell disease (HIBISCUS) | ClinicalTrials.Gov [Internet]. [cited 2023 Dec 10]. Available from: https://www.clinicaltrials.gov/study/NCT04624659

39. Bernaudin F, Dalle JH, Bories D, et al. Long-term event-free survival, chimerism and fertility outcomes in 234 patients with sickle-cell anemia younger than 30 years after myeloablative conditioning and matched-sibling transplantation in France. Haematologica. 2020;105(1):91–101. doi: 10.3324/haematol.2018.21320786

40. Guilcher GMT, Truong TH, Saraf SL, Joseph JJ, Rondelli D, Hsieh MM. Curative therapies: Allogeneic hematopoietic cell transplantation from

matched related donors using myeloablative, reduced intensity, and nonmyeloablative conditioning in sickle cell disease. Seminars in Hematology 55 (2018) 87–93.

41. Inam Z, Tisdale JF, Leonard A. Outcomes and long-term effects of hematopoietic stem cell transplant in sickle cell disease. Expert Rev Hematol. 2023;16(11):879–903.

42. Lidonnici MR, Scaramuzza S, Ferrari G. Gene therapy for hemoglobinopathies. Hum Gene Ther. 2023;34(17–18):793–807.

43. Frangoul H, Locatelli F, Bhatia M, et al. Efficacy and safety of a single dose of exagamglogene autotemcel for severe sickle cell disease. Blood. 2022;140(Supplement 1):29–31.

44. Kanter J, Thompson AA, Pierciey FJJ, et al. Lovo-cel gene therapy for sickle cell disease: treatment process evolution and outcomes in the initial groups of the HGB-206 study. Am J Hematol. 2023;98 (1):11–22.

45. Kanter J, Walters MC, Krishnamurti L, et al. Biologic and clinical efficacy of LentiGlobin for sickle cell disease. N Engl J Med. 2022;386(7):617–628.

46. Spencer Chapman M, Cull AH, Ciuculescu MF, et al. Clonal selection of hematopoietic stem cells after gene therapy for sickle cell disease. Nat Med. 2023;29(12):3175–3183.

47. Goyal S, Tisdale J, Schmidt M, et al. Acute myeloid leukemia case after gene therapy for sickle cell disease. N Engl J Med. 2022;386 (2):138–147.

