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Advances in new drug therapies for the management of sickle cell disease

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Abstract

Introduction: Sickle cell disease (SCD) is an orphan disease in the United States, but is highly prevalent worldwide. Only two drugs, hydroxyurea and L-glutamine, are approved for this disease. With an improved understanding of the pathophysiology of SCD as well as the success of several recently approved drugs for other orphan diseases, there is an increased interest in the development of drugs for SCD.

Areas covered: This review summarizes published studies of drug therapies and ongoing trials of novel agents.

Expert opinion: The development of drugs with different mechanisms of action offers opportunities for combination and individualized therapy in SCD. In addition to acute pain crisis, the evaluation of other SCD-related complications, exercise capacity, patient reported outcomes and validated surrogate endpoints are necessary to advance drug development. It is important to involve sites in sub-Saharan Africa and India, which have the highest burden of SCD, in trials of novel therapies.

Keywords

Sickle cell disease; Treatment; Novel Drugs; Drug Development; Clinical Trials

Introduction

Sickle cell disease is a genetic disorder that occurs due to a single gene mutation and results in substitution of valine for glutamine in the beta globin chain. Although millions of individuals are affected worldwide, SCD is referred to an orphan disease in the United States (US), with a prevalence of approximately 100,000 individuals. An estimated 242,000 children with sickle cell anemia were born in sub-Saharan Africa in 2010, with approximately 40,000 children born in India [1]. Comparatively, the estimated number of

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Declaration of Interests

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children born in the Americas and Europe in 2010 were much lower, at 11,000 and 2,000, respectively [1]. The vast majority of children who reside in resource-rich countries live to adulthood [2, 3, 4], but there continues to be a high mortality rate associated with SCD in childhood in sub-Saharan Africa. Although they have an increased survival to adulthood, individuals with SCD in resource-rich nations have a shorter life expectancy compared with the general population [5, 6, 7]. Furthermore, despite the success of allogeneic bone marrow transplantation as a curative approach [8, 9] and the curative potential of gene therapy [10], these treatment modalities are not available to the vast majority of individuals with SCD, most of whom reside in resource-poor countries. As such, the availability of drug therapies that are safe, effective and affordable is highly desirable and warranted.

Pathophysiology

The development of effective therapies for SCD and its complications depends on an adequate understanding of its pathophysiology (Figure 1). The pathophysiology of SCD is complex and appears to be due to multiple pathways. The primary event is due to the insolubility of sickle hemoglobin (HbS) when it is deoxygenated, leading to intracellular red blood cell (RBC) polymer formation and subsequent erythrocyte deformity. The rate and extent of HbS polymerization is dependent on the extent and duration of hemoglobin deoxygenation, the presence of fetal hemoglobin, as well as the intracellular HbS concentration [11]. The intracellular HbS concentration can vary greatly in patients with SCD, due to both a high number of circulating reticulocytes, having some of the lowest intracellular hemoglobin concentrations, and a substantial number of very dense RBCs that arise following polymerization-induced membrane damage and dehydration. The dehydration of sickle RBC, following the loss of solute and osmotically obliged water, results from increased potassium efflux through two specific pathways: the calcium-activated potassium efflux (or Gardos) channel and the potassium-chloride co-transport pathway [11].

The clinical manifestations of SCD appear to be driven by two major pathophysiological processes: vaso-occlusion with ischemia-reperfusion injury and hemolytic anemia. Vaso-occlusive crisis likely occurs due to adhesive interactions of sickle RBCs and leukocytes with the endothelium causing vascular obstruction and tissue ischemia [12]. These episodes of microvascular obstruction are followed by the restoration of blood flow, which promotes further tissue injury by reperfusion. Reperfusion injury results in increased oxidant and inflammatory stress, with increased synthesis of inflammatory cytokines and increased expression of endothelial adhesion molecules. The inflammatory cascade from ischemia-reperfusion is amplified by the activation of CD1d-restricted invariant natural killer T (iNKT) cells [13].

Associations are reported between markers of hemolysis and complications of SCD, including cholelithiasis, lower extremity ulcers, priapism, increased echocardiography-derived tricuspid regurgitant jet velocity, chronic kidney disease, stroke and increased mortality [14]. Free plasma hemoglobin, released following intravascular hemolysis, reacts directly to scavenge nitric oxide (NO), and generates reactive oxygen species, such as the hydroxyl and superoxide radical, potent scavengers of NO [15, 16]. NO, normally produced

by the endothelium, regulates basal vasodilator tone, inhibits platelet and coagulation activation, and transcriptional expression of nuclear factor κB (NF κB)-dependent adhesion molecules, such as vascular cell-adhesion molecule-1, intercellular cell-adhesion molecule-1, and the selectins [17, 18]. Free plasma hemoglobin potently inhibits endothelial NO signaling, leading to endothelial cell dysfunction and NO resistance [12]. Arginase-1, released following hemolysis, metabolizes plasma arginine into ornithine, resulting in a decrease in the required substrate for NO synthesis and further decreases the bioavailability of NO [15]. Release of hemin following intravascular hemolysis also contributes to inflammation [19]. The contribution of inflammation, as well as platelet and coagulation activation to the pathophysiology of SCD have recently been extensively reviewed [20, 21].

Drug Development in Sickle Cell Disease

Despite an improved understanding of the pathophysiology of SCD, available drug treatments remain limited. Although SCD is referred to as the first molecular disease [22], to date, only two drugs, hydroxyurea and L-glutamine, are approved by regulatory agencies including the Food and Drug Administration (FDA) and European Medicines Agency specifically for this disease. This compares unfavorably with many other prevalent and orphan diseases in the US. First described relatively recently, there were 25 FDA approved individual drugs and 14 approved combination drugs for the treatment of HIV as at November, 2017 (<https://aidsinfo.nih.gov/education-materials/fact-sheets/21/58/fda-approved-hiv-medicines>). Similarly, there are at least 10 drugs approved for pulmonary arterial hypertension and 9 drugs, including 2 FDA-approved transmembrane conductance regulator modulators, for cystic fibrosis. The disparity in approved drug therapies for SCD compared to many other disease conditions is likely due to multiple factors. There is disparate funding per individual affected by SCD and cystic fibrosis from both the National Institutes of Health and national foundations [23]. With the low prevalence of SCD in resource-rich countries and the limited economic power of the affected patients, there was no great incentive to invest in the development of new drugs for many years. However, there is now a growing interest in the development of new drugs and the repurposing of existing drugs for SCD. Government incentives including the Orphan Drug Act of 1983 and the Rare Diseases Act of 2003, both in the U.S., and the Orphan Regulation adopted by the European Union in 1999 have provided organizational support and financial incentives to defray the costs of drug development [24]. Furthermore, successes in the development of drugs for orphan indications (e.g. eculizumab for paroxysmal nocturnal hemoglobinuria) show that the market for orphan diseases can be lucrative (<http://www.pharmaceutical-journal.com/news-and-analysis/news-in-brief/eculizumab-with-340200-price-tag-gets-go-ahead-from-nice/20067720.article>). Despite the increased funding since the passage of the National Sickle Cell Anemia Control Act of 1972 and the Sickle Cell Treatment Act of 2003 in the U.S., research funding for SCD remains inadequate. Finally, the successful accrual into several relatively large, multicenter trials in SCD [25, 26, 27, 28, 29] suggests that, given the opportunity, patients are willing to participate in well-designed clinical trials.

Hemoglobin F Inducing and Anti-Sickling Agents

There are several therapeutic approaches to inhibiting HbS polymerization, including blocking intermolecular contacts in the sickle fiber, induction of hemoglobin F (HbF) synthesis, reduction of intracellular hemoglobin S concentration, increase in oxygen affinity and reduction of concentration of 2,3,-diphosphoglycerate [30]. Ongoing studies of anti-sickling agents are shown in Table 1.

Hemoglobin F Inducing Agents

It is well recognized that high levels of HbF ameliorates the severity of SCD [7, 31, 32, 33]. Hydroxyurea is approved by the FDA, as well as the European Medicines Agency, for treatment of individuals with sickle cell anemia. Hydroxyurea is a potent inducer of HbF [34], although the mechanisms for HbF induction are not fully understood [34, 35, 36, 37]. In addition, it is an NO donor [38, 39], improves erythrocyte deformability, reduces hemolysis, lowers circulating leukocytes and reticulocytes [40], and reduces surface expression of adhesion receptors [41, 42, 43, 44]. A randomized, placebo-controlled, phase 3 trial of adult patients with severe sickle cell anemia showed significant reductions in the frequency of vaso-occlusive crises, acute chest syndrome, need for blood transfusion, and delayed onset of first vaso-occlusive crisis with hydroxyurea treatment [26]. Another phase 3 clinical trial of hydroxyurea (BABY HUG), involving infants with sickle cell anemia randomized to either hydroxyurea or placebo similarly showed significant decreases in pain episodes, acute chest syndrome, hospitalizations, and RBC transfusions, although no benefits were observed on the primary endpoints, splenic function and glomerular filtration rate [28]. By decreasing the transcranial Doppler velocity and albuminuria, hydroxyurea may decrease the risk of stroke and chronic kidney disease in sickle cell anemia [45, 46]. Long-term use of hydroxyurea also appears to reduce mortality in patients with SCD [47, 48, 49, 50]. Recent data demonstrate that treatment with low, fixed weight-based dosing of hydroxyurea (10 mg/kg daily) decreases the rates of acute painful events, hospitalization, and blood transfusions in both children and adults with low toxicity [51, 52], suggesting that limited monitoring for hematological toxicities may be feasible in resource-limited areas. Despite its multiple beneficial effects, a substantial number of patients on hydroxyurea therapy may not obtain an adequate clinical response. In addition, despite its overall favorable safety profile, some patients remain reluctant to take this medication due to its effect on decreasing spermatogenesis [53] and their concerns regarding increased risk for leukemogenesis and teratogenicity.

Decitabine (5-aza-2'-deoxycytidine), a DNA hypomethylating agent which depletes DNA methyltransferase (DNMT1), has been studied as a potential diseasemodifying agent for SCD. Administered via a subcutaneous route, at demonstrably non-cytotoxic doses, decitabine increased HbF and total hemoglobin levels in patients who had no response to hydroxyurea [54]. However, decitabine has a very short plasma half-life of only minutes, and negligible solid tissue distribution and oral bioavailability due to its rapid inactivation to uridine degradation products by cytidine deaminase (CDA), an enzyme which is highly expressed in the intestines and liver [55, 56, 57, 58, 59]). In a recent study combining escalating doses of oral decitabine with an oral CDA inhibitor, tetrahydrouridine (THU),

patients were treated twice per week for 8 weeks, with 4 weeks of follow-up [60]. The primary endpoint in this early phase study of grade 3 non-hematologic toxicity was not reached, and adverse events were not significantly different in patients treated with THU-decitabine compared with placebo [60]. However, the highest tested dose decreased DNMT1 protein in peripheral blood mononuclear cells, decreased repetitive element CpG methylation, and increased HbF, doubling F- cells up to approximately 80% of total RBCs, and increased total hemoglobin as reticulocytes simultaneously decreased. Furthermore, biomarkers of hemolysis, coagulation activation, and inflammation improved.

Histone deacetylase inhibitors increase levels of HbF in SCD [61, 62, 63, 64, 65, 66, 67]. A small study of sodium butyrate showed that treatment with intermittent doses, rather than continuous therapy, resulted in sustained increases in the levels of HbF, F-cells and total hemoglobin [61]. Despite initially promising results, the orally available sodium dimethyl butyrate (HQQ-1001) did not produce a significant increase in HbF levels, but appeared to result in more pain episodes compared with placebo [67]. Pomalidomide, a third-generation immunomodulatory drug, induces a fetal-like erythroid differentiation program, leading to a reversion of γ -globin silencing in adult human erythroblasts. The transcription networks involved in γ -globin repression, including BCL11A, SOX6, IKZF1, KLF1, and LSD1 are selectively and differentially affected by pomalidomide [68]. In a transgenic mouse model of SCD, 8-weeks of treatment with pomalidomide induced modest increases of HbF, augmented erythropoiesis and preserved bone marrow function [69]. A dose-finding study of pomalidomide in patients with SCD, only available in abstract form, reported increases in HbF and total hemoglobin only at the highest dose or with greater than 6 months of exposure [70].

Other Antisickling and Hemoglobin Modifying Agents

Senicapoc, selectively blocks the calcium-activated, potassium efflux (Gardos) channel, and improves anemia and hemolysis in patients with SCD [71, 72]. However, a phase 3, randomized, placebo-controlled trial, showed no significant improvement in the rate of painful crises in patients treated with senicapoc compared to those on placebo [71]. It was suggested that senicapoc did not decrease the frequency of painful episodes in SCD due to the shift to a “more vaso-occlusive phenotype” with increased hemoglobin and possibly increased whole blood viscosity [73]. However, post-hoc analyses from the phase 3 trial showed no significant correlation between total hemoglobin or change from baseline hemoglobin and the crisis rate experienced by patients receiving senicapoc [71]. Magnesium decreases potassium and water loss by blocking the potassium-chloride co-transport channel in RBCs [11]. A phase 2 trial of hydroxyurea and magnesium pidolate, alone and in combination, in HbSC patients showed no effects of magnesium pidolate on its own [74]. In a multicenter, double-blind, placebo-controlled study of patients with HbSS or HbS β^0 thalassemia, intravenous magnesium sulfate did not shorten the length of stay, lessen opioid use or improve the quality of life in children who required inpatient hospitalization after failing emergency department management for pain [75].

Sanguinate (pegylated bovine carboxyhemoglobin) is a carbon monoxide releasing/oxygen transfer agent that was developed for the treatment of anemic and ischemic hypoxia. Due to

its modification by polyethylene glycol and the antivasoconstrictive activity of carbon monoxide, sanguinate has demonstrated no vasoactivity in vivo [76], and appears to be safe in healthy individuals [77] and patients with stable sickle cell anemia [76]. Sanguinate has been granted orphan drug status and two randomized, double-blind placebo-controlled phase 2 studies evaluating its safety and efficacy in individuals with SCD experiencing vaso-occlusive crisis are ongoing (NCT02672540; NCT02411708). MP4CO (pegylated carboxyhemoglobin) markedly induced hepatic heme oxygenase-1 activity and inhibited NF- κ B activation and microvascular stasis in sickle mice [78]. Although a phase 1 safety study has been completed (NCT01356485), a planned phase 2 study was withdrawn before enrollment, and the sponsor has ceased operations.

Voxelotor (previously called GBT440) is a novel, orally bioavailable small molecule which binds specifically to hemoglobin, with an approximately 150:1 RBC:plasma partition in various animal species, increases hemoglobin oxygen affinity and stabilizes the oxyhemoglobin state [79]. Voxelotor delays in vitro HbS polymerization and prevents sickling of RBCs. In a murine model of SCD, GBT440 extended the half-life of RBCs, reduced reticulocyte counts and prevented ex vivo RBC sickling [79]. Preliminary results suggest that voxelotor at 900 mg is well tolerated, with marked improvement in hemoglobin and reduction in clinical measures of hemolysis in adolescents with SCD [80]. These hematologic improvements were observed in patients on maximum tolerated dose of hydroxyurea. A phase 3, double-blind, randomized, placebo-controlled trial of voxelotor in patients with SCD is ongoing (NCT03036813). The primary endpoint is a change in hemoglobin, with key secondary outcomes, based on patient-reported outcomes. These endpoints are novel in the setting of SCD. In addition, a phase 2a, open-label, study in pediatric patients is ongoing (NCT02850406).

5-hydroxymethyl-2-furfural (5HMF, also called Aes-103) interacts allosterically with HbS, thereby increasing oxygen affinity and decreasing HbS polymerization and RBC sickling during hypoxia. Short-term administration of high doses in healthy volunteers and lung surgery patients was well tolerated [81, 82]. In addition, a phase 1 trial with oral doses up to 4,000 mg, in patients with SCD, some treated with hydroxyurea, showed no significant side effects [83]. However, a phase 2 clinical trial of Aes-103 in SCD was terminated early by the Sponsor due to unblinding between the study drug and placebo groups at the subject, site and Sponsor levels.

Anti-Adhesion Agents

Several drugs targeting adhesion of blood cells to the endothelium are being investigated as treatments for SCD (Table 2). Purified poloxamer 188, a nonionic block copolymer surfactant with hemorheologic and antithrombotic properties, was shown to significantly decrease the duration of pain episodes, especially in children and patients on hydroxyurea [84]. However, the decrease in the duration of pain episodes did not appear to be clinically meaningful. In another phase 3 study in children and adults with vaso-occlusive crisis, treatment with purified poloxamer 188 (vepoloxamer or MST-188) did not significantly reduce the duration of vaso-occlusive crises compared with placebo (NCT01737814)

(<https://www.prnewswire.com/news-releases/mast-therapeutics-reports-top-line-results-from-phase-3-study-in-sickle-cell-disease-300331289.html>).

Crizanlizumab, an antibody against the adhesion molecule P-selectin, was evaluated in a double-blind, randomized, placebo-controlled, phase 2 trial, in 198 patients with SCD [25]. Treatment with high-dose crizanlizumab resulted in a significantly lower median crisis rate, significantly longer median times to the first and second crises, and a significantly lower median rate of uncomplicated crises per year compared with placebo. The adverse events that occurred in 10% or more of the patients in either active-treatment group and at a frequency that was at least twice as high as that in the placebo group were arthralgia, diarrhea, pruritus, vomiting, and chest pain. A phase 2 study to further evaluate the pharmacokinetics and pharmacodynamics of crizanlizumab (SEG101) in adult patients is ongoing (NCT03264989). Rivipansel sodium (formerly called GMI-1070), a small-molecule pan-selectin inhibitor that binds to E-, P- and L-selectin is being evaluated as treatment for acute vaso-occlusive episodes (NCT02187003). In a randomized, double-blind, adaptive, phase 2 trial, rivipansel was shown to shorten the time to resolution of vaso-occlusive crisis compared with placebo [85]. Although the absolute decreases in times to resolution of vaso-occlusive crisis (mean of 41 hours and median of 63 hours) appeared to be clinically meaningful, the differences were not statistically significant. However, treatment with rivipansel resulted in a large and statistically significant reduction in the mean cumulative intravenous opioid analgesic use compared with placebo.

In addition to their anticoagulant effects, heparins are known to inhibit adhesive interactions via P-selectin [86, 87]. Sevuparin is a derivative of low-molecular-weight heparin that retains the P-selectin-binding domain of heparin but largely lacks anticoagulant properties [88]. Sevuparin binds to P- and L-selectins, thrombospondin, fibronectin and von Willebrand factor and inhibits the adhesion of sickle RBCs to stimulated cultured endothelial cells in vitro [88]. In addition, sevuparin prevents vaso-occlusion and normalizes blood flow in a mouse model of SCD vaso-occlusion [88]. A phase 2 clinical trial of sevuparin for the management of acute vaso-occlusive crisis is ongoing (NCT02515838), the primary endpoint being the time to resolution of vaso-occlusive crisis. In a multicenter study with 253 patients, tinzaparin at its usual therapeutic dose was reported to decrease the total number of hospital days, the overall number of days with pain crisis and the number of days of the most severe pain scores compared with placebo [89]. However, it is uncertain whether the observed benefit of tinzaparin was due to its anticoagulant or its anti-adhesive effect. Furthermore, issues regarding standard of care and potential study bias make the study difficult to interpret [90]. A phase 2, pilot feasibility study of unfractionated heparin (NCT02098993) and a phase 3, randomized, placebo-controlled study of tinzaparin in acute chest syndrome are ongoing (NCT02580773).

Intravenous immunoglobulin (IVIG) reverses vaso-occlusion in sickle mice by rapidly inhibiting neutrophil adhesion to the endothelium and blocking interactions between neutrophils and RBCs [91]. IVIG blocks $FC\gamma RIII$ receptors, leading to inhibition of neutrophil adhesion, reduction of RBC capture by leukocytes, and reduced Mac-1 activity as a result of recruitment of Src homology 2-containing tyrosine phosphatase-1 [92]. Administration of IVIG to patients with SCD during acute vaso-occlusive crises in a phase 1 study resulted in

decreased Mac-1 function from baseline and produced no significant adverse events at doses up to 800 mg/kg [93]. A phase 2 clinical trial of IVIG in patients during acute pain episodes is ongoing (NCT01757418).

Anti-Oxidant Agents

Increased oxidant stress plays a major role in the pathophysiology of SCD, and leads to disturbances of cell membranes, expression of adhesion molecules and hemolysis. In a phase 3, multicenter, double-blind, placebo-controlled trial, 230 patients with HbSS or HbS β^0 -thalassemia were randomized to receive oral L-glutamine or placebo at a ratio of 2:1 for 48 weeks followed by a 3 week taper. Treatment with L-glutamine produced a significantly lower number of pain crises, hospitalizations, cumulative hospital days and number of patients affected by acute chest syndrome compared with placebo [94]. Adverse events in the L-glutamine treatment arm were similar to those observed in the placebo arm. Based on these data, L-glutamine was recently approved by the FDA for the reduction of acute complications of SCD in adult and pediatric patients 5 years and older (<https://www.fda.gov/newsevents/newsroom/pressannouncements/ucm566084.htm>).

In an open label pilot study of oral N-acetylcysteine (NAC), 11 consecutive patients were randomly assigned to receive 1,200 mg or 2,400 mg NAC daily for 6 weeks [95]. There was an increase in whole blood glutathione levels and a decrease in erythrocyte outer membrane phosphatidylserine exposure, plasma levels of advanced glycation products and cell-free hemoglobin after 6 weeks of treatment in both groups. No patients experienced painful crises or other significant SCD-or NAC-related complications during the trial. In a randomized, placebo-controlled, double-blind trial, treatment with NAC at a dose of 600 mg twice daily for 6 months did not decrease the rate of SCD-related pain days per patient year compared with placebo [96]. In addition, there were no differences in the days with vaso-occlusive crises, admission days, number of admissions or days with home analgesic use. However, a per-protocol analysis performed in adherent patients revealed a significantly lower rate of vaso-occlusive crisis days in the NAC arm compared with placebo. Another phase 1/2 pilot study of NAC in patients with vaso-occlusive crisis is ongoing (NCT01800526). Ongoing studies of anti-oxidants in SCD are shown in Table 3.

Anti-Inflammatory Agents

Vaso-occlusion promotes tissue injury by reperfusion following ischemia-reperfusion, with the resultant inflammatory cascade amplified by the activation of CD1d-restricted iNKT cells. In addition, leukocytes, platelets, and multiple proinflammatory pathways appear to contribute to the pathophysiology of SCD. Several approaches to downregulate inflammatory pathways are being evaluated in patients with SCD (Table 3). Treatment of children and adolescents during acute pain episodes with methylprednisolone resulted in significantly shorter duration of inpatient analgesic therapy compared with placebo, although the patients who received methylprednisolone had more “rebound” pain episodes after treatment was discontinued [97]. Intravenous dexamethasone resulted in a significantly shorter hospital stay, prevented clinical deterioration and reduced the need for blood transfusion in patients with mild to moderately severe cases of acute chest syndrome when

compared with placebo [98]. However, more patients treated with dexamethasone were re-admitted within 72 hours of discharge. A more recent study using tapered oral dexamethasone was terminated early due to slow accrual [99].

iNKT cell activation is downregulated by activation of the adenosine A_{2A} receptor (A_{2A}R), and adenosine reduces the activation of iNKT cells as well as cytokine production by iNKT cells through this receptor [100, 101, 102]. Sick mice and patients with SCD have more activated iNKT cells than controls [103]. Blockade or depletion of iNKT cells with an A_{2A}R agonist or an anti-iNKT cell antibody in mice decreased inflammation and tissue injury following ischemia-reperfusion [102, 103, 104]. Regadenoson, a partially selective A_{2A}R agonist, was safe in children and adults in the steady state and during vaso-occlusive crises in a phase 1 study [105]. iNKT cells were more activated in patients with SCD in steady state than in control subjects, with more marked activation during vaso-occlusive crises. A 24-hour infusion of regadenoson during pain episodes significantly decreased phospho-NF- κ B p65 activation in iNKT cells [105]. However, in a recently completed phase 2 study in children and adults experiencing pain crises, the proportion of subjects who demonstrated a reduction of greater than 30% in activated iNKT cells was not significantly different between the regadenoson and placebo arms [106]. Furthermore, no differences were observed in the length of hospital stay, mean total opioid use, or pain scores in both treatment groups. In an open-label, multi-center, single-ascending-dose study, treatment with NKTT120, an anti-iNKT cell monoclonal antibody, produced rapid, specific and sustained iNKT cell depletion without any infusional toxicity or attributed serious adverse events [106]. A randomized, placebo-controlled clinical trial of NKTT120 will be required to determine the safety and efficacy of long-term iNKT cell depletion.

Statins exhibit benefits independent of their cholesterol lowering effect. SCD is characterized by progressive vascular injury and endothelial dysfunction similar to that observed in atherosclerosis [107, 108, 109]. A pilot study showed that treatment with simvastatin was well tolerated and increased levels of nitric oxide metabolites and decreased levels of both C-reactive protein (CRP) and interleukin-6 in patients with SCD [110]. Treatment of 19 patients with a single daily dose of simvastatin for 3 months in an open label study resulted in a significant reduction in the frequency of pain episodes, oral analgesic use, and levels of circulating high sensitivity CRP, soluble E-selectin, soluble ICAM-1, soluble ICAM-3 and VEGF [111]. These results provide supporting clinical data for the conduct of a randomized, placebo-controlled trial of simvastatin in sickle cell anemia. A study of the effect of atorvastatin on endothelial dysfunction and albuminuria in sickle cell anemia is ongoing (NCT01732718).

The cysteinyl leukotrienes (CysLTs) are lipid inflammatory mediators that cause bronchoconstriction and play a role in leukocyte recruitment, endothelial cell adhesion, vasoconstriction, increased vascular permeability, and smooth muscle proliferation [112]. These pro-inflammatory and vascular effects provide biologic plausibility for a role of CysLTs in the process of vaso-occlusion [113]. Elevated urinary levels of leukotriene E₄ are observed during the steady state, and are associated with an increased risk of pain crisis and acute chest syndrome [114, 115, 116]. The leukotriene receptor antagonist, montelukast, is being tested in a phase 2, randomized, placebo-controlled trial to evaluate its effect, in

combination with hydroxyurea, on the level of soluble VCAM-1, lung function and forearm microvascular flow (NCT01960413). Zileuton targets leukotriene synthesis by inhibiting 5-lipoxygenase (5-LO). 5-LO causes increased production of leukotrienes, including leukotriene B₄, a very potent chemoattractant and neutrophil and endothelial cell activator, and leukotriene E₄, which is associated with lung inflammation. Zileuton has been shown to reduce activated neutrophil-mediated sickle RBC adhesion to rat pulmonary vasculature [117], and to reduce IL-13 production by murine splenic lymphocytes [118]. Zileuton has also been reported to increase HbF in erythroid progenitor cells via an L-arginine/NO-dependent mechanism [119]. A phase 1 trial of zileuton to examine its safety, pharmacokinetics and effect on inflammatory biomarkers in patients with SCD has been completed (NCT01136941), but the results have not yet been published.

Long-chain omega-3 polyunsaturated fatty acids, such as eicosapentaenoic acid (EPA [n-3]) and docosahexaenoic acid (DHA [n-6]), have beneficial effects in chronic inflammatory disorders. Patients with SCD have a high n-6/n-3 ratio in blood cells and plasma and this fatty acid abnormality may contribute to the chronic inflammatory state observed in SCD [120, 121]. Supplementation with omega-3 fatty acids improve cell membrane abnormalities, and decrease markers of coagulation activation, oxidative stress, pro-inflammatory cytokines and adhesive molecules [122, 123, 124, 125]. Furthermore, in a randomized, single center study, treatment with daily omega-3 capsules containing 277.8 mg DHA and 39.0 mg EPA significantly reduced the median rate of clinical vaso-occlusive events, severe anemia, blood transfusion requirements, and absence from school compared with placebo [126]. Top line results from a phase 2, randomized, double-blind, placebo-controlled study of children with SCD, showed that treatment with DHA resulted in a statistically significant change from baseline in blood cell membrane fatty acids concentration within 4 weeks (<https://www.sancilio.com/altemia-achieves-successful-clinical-results-in-pediatric-patients-with-sickle-cell-disease-scd/>). In addition, DHA was reported to produce a clinically meaningful reduction of pain crises, with statistically significant improvements in markers of coagulation, inflammation and adhesion after 8 weeks of treatment. No treatment-related serious adverse events were reported.

Anticoagulant Agents

There is abundant evidence that SCD represents a hypercoagulable state. In addition to an increased risk of thrombotic complications, patients exhibit increased thrombin generation, abnormal activation of fibrinolysis, decreased levels of natural anticoagulant proteins, activation of platelets, as well as increased tissue factor (TF) antigen and TF procoagulant activity in the non-crisis, “steady state” [21]. Increasing evidence suggest that coagulation activation may play a role in the pathophysiology of SCD. Data from mice that express hemoglobin S show that inhibition of TF activity effectively prevented the accelerated thrombus formation in a light/dye-induced model of cerebral microvascular thrombosis [131]. Inhibition of TF in another mouse model of SCD significantly reduced plasma levels of TAT, interleukin-6, soluble VCAM-1, and serum amyloid protein, as well as neutrophil infiltration in the lung [132]. More recently, reduction of prothrombin level to approximately 10% activity in sickle mice not only resulted in lower plasma levels of coagulation activation and inflammatory biomarkers, but also diminished early mortality, as well as damage to

organs, including the lung, kidney, heart and liver, with no increase in bleeding complications [134].

Most of the published clinical studies of anticoagulant agents in SCD have been small, poorly controlled and of relatively low quality [135, 136, 137]. With the current availability of direct oral anticoagulants, an ongoing study is evaluating the effect of rivaroxaban in SCD (NCT02072668) (Table 4). A randomized, double-blind, placebo controlled study of SCD patients during acute pain episodes, reported clinical benefit for the low molecular weight heparin, tinzaparin, compared with placebo [89]. However, it is uncertain whether these reported beneficial effects were a result of the anticoagulant effect or the P-selectin blocking effect of tinzaparin. Two studies in acute chest syndrome, one a phase 2 feasibility study of therapeutic dose unfractionated heparin (NCT02098993) and another a phase 3 study of tinzaparin (NCT02580773), are ongoing.

Antiplatelet Agents

Platelets are activated in SCD and may contribute to disease pathophysiology [138, 139]. Multiple relatively small studies have evaluated the clinical effects of aspirin in SCD [140, 141, 142]. Although one study reported an increase in oxygen affinity, hemoglobin level and RBC life span [141], other studies showed only modest or no benefit for aspirin on hemoglobin levels or acute pain episodes [135, 140, 142]. A pilot study of eptifibatide showed that this glycoprotein IIb/IIIa inhibitor was safe when administered during acute pain episodes, but did not reduce the time to resolution of such pain episodes [143]. Ticlopidine, a P2Y₁₂ ADP-receptor antagonist, reportedly decreased the number of pain episodes, the mean duration of pain episodes and the severity of such episodes in a multicenter study of 140 sickle cell anemia patients [144]. However, a more recent phase 3 trial of prasugrel, a newer generation P2Y₁₂ receptor blocker, in children with SCD showed no significant reduction of the frequency of pain episodes [29]. A phase 2 study to investigate the efficacy of two different doses of ticagrelor has recently been completed and the results are awaited (NCT02482298).

Nitric Oxide and Related Agents

With the role of hemolysis in NO scavenging and subsequent endothelial dysfunction in SCD, several studies have evaluated the effect of NO and related agents as treatments for SCD. Inhaled NO gas for up to 72 hours did not decrease the median time to resolution of pain crisis, length of hospitalization, visual analog pain scale scores, cumulative opioid usage, or rate of acute chest syndrome compared with placebo in an adequately powered placebo-controlled trial [145]. Treatment of children hospitalized for acute pain episodes with L-arginine for 5 days or until discharge significantly decreased total parenteral opioid use and resulted in lower pain scores at discharge compared to placebo, although no significant difference in hospital length of stay was observed [146]. Sildenafil, a phosphodiesterase 5 inhibitor, increases NO-mediated effects by inhibiting cyclic guanosine monophosphate (cGMP) degradation. A double-blind, placebo-controlled trial of sildenafil was stopped early due to a higher percentage of subjects experiencing serious adverse events in the sildenafil arm, predominantly hospitalization for pain, compared with placebo [147].

Furthermore, no evidence of a treatment effect of sildenafil was observed on 6-minute walk distance, echocardiography-derived tricuspid regurgitant jet velocity, or N-terminal pro-brain natriuretic peptide. Although myalgia and back pain are recognized side effects of chronic administration of sildenafil, evidence of a role for NO and cGMP in the processing of inflammatory and neuropathic pain suggests that the increased pain may be due to inhibition of cGMP degradation [148, 149]. A phase 2 multicenter study of riociguat, a soluble guanylate cycle stimulator, is ongoing (NCT02633397) (Table 5).

Clinical Endpoints and Biomarkers in Sickle Cell Disease

Despite the fact that SCD involves multiple organs and systems, most drug trials have focused on acute pain episodes as the primary clinical endpoint. This is not surprising as these painful episodes represent the most frequent reason for medical contact by patients with SCD. However, with the complex pathophysiology of vaso-occlusive crises, combined with the difficulty in accurately quantifying these episodes, the emphasis on pain crisis as a clinical endpoint may be severely limiting. Most studies of acute pain episodes have evaluated only those episodes requiring healthcare utilization, i.e. episodes requiring visits to emergency departments, hospitalizations or other physician contact at which time patients are treated with parenteral or oral analgesics [25, 26, 71]. With the evidence that the bulk of pain episodes are treated at home [150], it is important that studies incorporate these episodes which substantially contribute to disease morbidity and decreased quality of life in SCD [29]. These pain episodes that do not require healthcare utilization may be measured using patient-reported instruments, such as in the ongoing phase 3 trial of voxelotor.

Clinical trials in SCD should also include such complications as ischemic stroke, chronic kidney disease, priapism, pulmonary hypertension, leg ulcers and bone disease as clinical endpoints. However, as these events usually occur less frequently than acute pain episodes, studying each of these complications may require larger numbers of study subjects and/or a longer duration in order to observe and quantify treatment effects. Multiple relatively large studies have evaluated overt and silent stroke as clinical endpoints in SCD in the setting of RBC transfusion therapy [27, 151, 152]. Despite the challenge of drug trials in a setting where chronic RBC transfusion therapy is the accepted standard of care, the successful conduct of the SWITCH and TWITCH trials [45, 153], as well as the ongoing SPRING trial (NCT02560935) in sub-Saharan Africa suggests that the utility of stroke or clinical endpoints other than acute pain episodes is feasible.

Patient-reported outcomes, including health-related quality of life (HRQoL), are suitable endpoints in drug development [154]. Significant impairment of HRQoL is present in SCD patients at baseline and in association with multiple complications [155]. Low hemoglobin and HbF levels are also associated with impaired HRQoL in adult patients [156]. The use of hydroxyurea is associated with improved HRQoL in individuals who are high HbF responders [157]. SCD-specific HRQoL instruments have been developed and provide opportunities to explore the impact of the disease and its treatment on patients [156]. Exercise capacity is also a clinical endpoint for drug development [158, 159]. Decreased exercise capacity is common in SCD due to anemia, cardiac disease, restrictive and obstructive lung disease, pulmonary vascular disease, as well as bone and muscle

abnormalities. The 6-minute walk test is a useful measure of functional capacity, and is decreased with elevated tricuspid regurgitant jet velocity and pulmonary hypertension in SCD [160, 161, 162].

Surrogate endpoints may be targets for drug development. A biomarker is defined as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention [163]. Although multiple laboratory biomarkers are described in SCD [164], many of these are of limited clinical value and require further assessment in prospective studies to validate their prognostic importance before they are acceptable as surrogate endpoints. Increased transcranial Doppler ultrasound (TCD) velocity of at least 200 cm/s is associated with an increased risk of stroke in children with sickle cell anemia [152, 165], and is a validated surrogate endpoint in children [151, 152]. Increased tricuspid regurgitant jet velocity is associated with increased mortality and identifies adult patients at higher risk of having pulmonary hypertension defined by right heart catheterization [7, 162, 166, 167, 168], but it has not been validated as a suitable surrogate endpoint in SCD. Although SCD is characterized by a chronic anemia, increase in hemoglobin may not always be associated with clinical benefit as was observed in the phase 3 trial of senicapoc [71]. The increase in hemoglobin following hydroxyurea therapy is associated with decreased frequency of vaso-occlusive complications as well as RBC transfusion requirements [26, 28]. However, decreased hemolysis with subsequent increases in hemoglobin is likely to be associated with improvements in fatigue, and may be associated with a decreased risk of such complications as gallstones, leg ulcers, chronic kidney disease and possibly mortality risk [7, 14, 169]. Increase in hemoglobin is the primary endpoint in an ongoing phase 3 trial of voxelotor (NCT03036813), with evaluation of patient-reported outcomes as key secondary endpoints.

Clinical Trials in Sub-Saharan Africa and India

More than 300,000 infants with sickle cell anemia are born worldwide every year, but less than 1% of these births occur in North America and Europe where the vast majority of SCD research is undertaken [170]. As a result of scarce healthcare resources as well as limited awareness by healthcare providers and the general public in resource-poor countries, substantial early mortality rates are observed in SCD populations in these countries [171]. Whilst much needs to be done to decrease the morbidity and mortality due to SCD in low-income nations, it is important that new drugs are tested in these environments, in parallel with such testing in high- and middle-income nations. Hydroxyurea has been shown to be efficacious in resource-rich nations [26, 28, 45]. Until recently, there was substantial concern regarding the safety of hydroxyurea in the setting of a high burden of infectious disease and malnutrition. With demonstration of the safety of hydroxyurea in sub-Saharan Africa [172], multiple studies evaluating its efficacy, including the REACH (NCT01966731) and SPRING (NCT02560935) trials, are ongoing. The present enthusiasm for the development of new drugs for SCD provides an opportunity to involve sites in low- and middle-income nations, particularly sub-Saharan Africa and India, which have the highest burden of disease, in these clinical studies. Not only does this offer patients the opportunity to participate in cutting-edge research to assess the safety and efficacy of novel drugs, it also contributes

substantially to capacity building by improving local infrastructure, strengthening of health systems and training of local providers.

Conclusion

Although a rare disease in the U.S and Europe, SCD is highly prevalent worldwide. There have been significant advances in our understanding of the pathophysiology of SCD over the past several decades. With its high morbidity and mortality, improved treatments are required, not only to prevent the occurrence of complications, but also to shorten the duration of acute complications. Hydroxyurea, and more recently, L-glutamine, are approved for the treatment of sickle cell anemia. An improved understanding of the pathophysiology of SCD, combined with the success of several recently approved drugs for other orphan diseases has increased interest in the development of drugs for SCD. Drugs that are being evaluated include hemoglobin F inducers, anti-sickling agents, anti-adhesive agents, anti-inflammatory agents, antioxidants, anticoagulants and anti-platelet agents. The enthusiasm for development of new drugs offers hope for the development of multiple pharmacotherapies for the treatment of SCD-related complications.

Expert Opinion

Despite the success of allogeneic bone marrow transplantation and the curative potential of gene therapy, these treatments are not widely available, especially in resource-poor countries. The development of drug therapies for SCD that are safe, effective and affordable is highly warranted. The increased number of drugs in testing offers hope for the availability of more pharmacologic therapies for SCD. With its complex pathophysiology, it is unlikely that individual drugs will ameliorate all of the complications of this disease. The availability of multiple drugs offers an opportunity for individualized therapy based on the presence of SCD-related complications, as well as for combination drug therapy based on different mechanisms of action and side effect profiles. It is important that clinical trials of novel drugs for SCD involve individuals in resource-poor countries, where the burden of disease is high. In addition to acute pain episodes, evaluation of the effect of novel agents on other SCD-related clinical complications, PROs, exercise capacity and validated surrogate endpoints is warranted.

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Article Highlights

- While multiple agents have been tested, only two drugs are approved by regulatory agencies for treatment of sickle cell disease (SCD)
- The development of effective drug therapies for SCD is based on an adequate understanding of its pathophysiology
- Multiple drugs, including anti-sickling agents, anti-adhesive agents, antiinflammatory agents, anti-oxidants, anticoagulants and anti-platelet agents are being evaluated as treatments for SCD
- The development of drugs with different mechanisms of action offers opportunities for combination and personalized therapy
- In addition to acute pain crisis, the evaluation of other SCD-related complications, exercise capacity, patient reported outcomes and validated surrogate endpoints are necessary to advance drug development in SCD
- The present enthusiasm for drug development provides an opportunity to involve sites in resource-limited countries, which have the highest burden of SCD, in trials of novel drug therapies.

Mechanisms of action of pharmacologic agents that have been evaluated and/or are being tested in sickle cell disease.

RBCs - Red Blood cells; HbF – Hemoglobin F; NO – Nitric Oxide; Invariant NKT cells – Invariant Natural Killer T cells. Modified with permission from [173]

Table 1:

Ongoing Clinical Trials of Anti-Sickling Agents in Sickle Cell Disease

Drug	Study Title (Sponsor)	Mechanism of Action	Clinical Phase	NCT Number	Sponsor
Sanguinate	A Study of SANGUINATE for the Treatment of Vaso-occlusive Crisis (VOC) in Adult Sickle Cell Disease Patients	Oxygen transfer agent	Phase 2	NCT02672540	Prolong Pharmaceuticals
Voxelotor	Study to Evaluate the Effect of GBT440 Administered Orally to Patients With Sickle Cell Disease (GBT_HOPE) (GBT_HOPE)	Increase hemoglobin oxygen affinity (left shift of oxyhemoglobin dissociation curve)		NCT03036813	Global Blood Therapeutics
Panobinostat	Study of Panobinostat (LBH589) in Patients With Sickle Cell Disease (LBH589)	Increase hemoglobin F (histone deacetylase inhibitor)	Phase 1	NCT01245179	Novartis
INCB059872	A Study to Evaluate Safety, Pharmacokinetic, and Biological Activity of INCB059872 in Subjects With Sickle Cell Disease	Increase hemoglobin F by inhibition of lysine-specific demethylase 1 (LSD1)	Phase 1	NCT03132324	Incyte Corporation
Decitabine and Tetrahydropyridine	Study of Decitabine and Tetrahydropyridine (THU) in Patients With Sickle Cell Disease	Increase hemoglobin F (hypomethylating agent)	Phase 1	NCT01685515	The Cleveland Clinic
Metformin	Fetal Hemoglobin Induction Treatment Metformin (FITMet)	Increase hemoglobin F	Phase 1	NCT02981329	Baylor College of Medicine

Table 2:

Ongoing Clinical Trials of Anti-Adhesion Agents in Sickle Cell Disease

Drug	Study Title	Mechanism of Action	Clinical Phase	NCT Number	Sponsor
Crizanlizumab	Pharmacokinetics and Pharmacodynamics Study of SEG101 (Crizanlizumab) in Adult Sickle Cell Disease (SCD) Patients With Vaso-Occlusive Crisis (VOC)	Anti-adhesive (anti-P-selectin agent)	Phase 2	NCT03264989	Novartis
Rivipansel	Efficacy and Safety of Rivipansel (GMI-1070) in the Treatment of Vaso-Occlusive Crisis in Hospitalized Subjects With Sickle Cell Disease	Anti-adhesive (pan-selectin inhibitor)	Phase 3	NCT02187003	Pfizer
Sevuparin	Sevuparin Infusion for the Management of Acute VOC in Subjects With SCD	Anti-adhesive (binds to P- and L-selectins, fibronectin, thrombospondin and von Willebrand factor)	Phase 2	NCT02515838	Modus Therapeutics AB

Table 3:
Ongoing Clinical Trials of Anti-Oxidants and Anti-Inflammatory Agents in Sickle Cell Disease

Drug	Study Title	Mechanism of Action	Clinical Phase	NCT Number	Sponsor
N-acetyl cysteine	A Pilot Study of N-acetyl/cysteine in Patients With Sickle Cell Disease (NACinSCD)	Anti-oxidant (increased glutathione)	Phase 1/2	NCT01800526	Bloodworks; University of Washington
Omega-3 fatty Acids (Docosahexaenoic Acid (DHA) and Eicosapentaenoic Acid (EPA))	Omega-3 Fatty Acids in Sickle Cell Disease	Anti-inflammatory	Phase 1/2 /	NCT02947100;	NIH/GMS; Thomas Jefferson University; Solutex GC, S.L.
Azithromycin	Macrolide Therapy to Improve Forced Expiratory Volume in 1 Second in Adults With Sickle Cell Disease; A Pilot Study of Azithromycin Prophylaxis for Acute Chest Syndrome in Sickle Cell Disease	Anti-inflammatory agent (antibiotic)	Phase 1; Phase 1	NCT02960503; NCT02630394	Vanderbilt University; University of Mississippi Medical Center
Canakinumab	Study of Efficacy, Safety and Tolerability of ACZ885 (Canakinumab) in Pediatric and Young Adult Patients With Sickle Cell Anemia	Anti-inflammatory agent (anti-IL-1 β)	Phase 2	NCT02961218	Novartis Pharmaceuticals
Omega-3 fatty acid (DHA)	Sickle Cell Omega-3 Treatment Trial (SCOT Trial) (SCOT)	Anti-inflammatory	Phase 3	NCT02604368	Sancilio and Company, Inc
Atorvastatin	Effect of Atorvastatin on Endothelial Dysfunction and Albuminuria in Sickle Cell Disease (ENDO)	Anti-inflammatory; pleiotropic (statin)	Phase 2	NCT01732718	University of North Carolina, Chapel Hill
Mometasone Furoate	Inhaled Mometasone to Reduce Painful Episodes in Patients With Sickle Cell Disease (IMPROVE)	Anti-inflammatory (steroid)	Phase 2	NCT02061202	Icahn School of Medicine at Mount Sinai
Montelukast	Phase 2 Study of Montelukast for the Treatment of Sickle Cell Anemia	Anti-inflammatory (leukotriene receptor antagonist)	Phase 2	NCT01960413	Vanderbilt University Medical Center

Table 4:
Ongoing Clinical Trials of Antiplatelet Agents and Anticoagulants in Sickle Cell Disease

Drug	Study Title	Mechanism of Action	Clinical Phase	NCT Number	Sponsor
Ticagrelor	A Study to Assess the Effect of Ticagrelor in Reducing the Number of Days With Pain in Patients With Sickle Cell Disease (Hestia2)	Antiplatelet agent	Phase 2	NCT02482298	AstraZeneca
Rivaroxaban	The Effect of Rivaroxaban in Sickle Cell Disease	Anticoagulant	Phase 2	NCT02072668	University of North Carolina, Chapel Hill
Apixaban	Apixaban in Patients With Sickle Cell Disease	Anticoagulant	Phase 3	NCT02179177	Duke University Medical Center
Unfractionated heparin	Feasibility Study of Unfractionated Heparin in Acute Chest Syndrome	Anticoagulant; antiadhesive agent	Phase 2	NCT02098993	University of Pittsburgh
Tinzaparin	Therapeutic Anticoagulation Strategy for Acute Chest Syndrome (TASC)	Anticoagulant; antiadhesive agent	Phase 3	NCT02580773	Assistance Publique–Hopitaux de Paris

Table 5:

Ongoing Clinical Trials of Nitric Oxide-Related Drugs and Vasodilators in Sickle Cell Disease

Drug	Study Title	Mechanism of Action	Clinical Phase	NCT Number	Sponsor
Ambrisentan	The Role of Endothelin-1 in Sickle Cell Disease	Vasodilator (endothelin receptor A antagonist)	Phase 1	NCT02712346	Augusta University; Gilead Sciences; NHLBI
Citrulline	A Phase 1 Study of Continuous Intravenous L-citrulline During Sickle Cell Pain Crisis or Acute Chest Syndrome	Increased nitric oxide synthesis	Phase 1	NCT02697240	University of Mississippi Medical Center
Riociguat	A Multi-Center Study of Riociguat in Patients With Sickle Cell Diseases	Vasodilator (soluble guanylate cyclase stimulator)	Phase 2	NCT02633397	University of Pittsburgh
Sodium Nitrite	Topical Sodium Nitrite in Sickle Cell Disease and Leg Ulcers	Vasodilator (local nitric oxide donor)	Phase 2	NCT02863068	Montefiore Medical Center; Food and Drug Administration
Arginine	Arginine Therapy for the Treatment of Pain in Children With Sickle Cell Disease (R34 pK/PD); Arginine Therapy for Sickle Cell Disease Pain	Increased nitric oxide synthesis	Phase 1/2; Phase 2	NCT02447874; NCT02536170	Emory University
Macitentan	Macitentan in Pulmonary Hypertension of Sickle Cell Disease (MENSCH)	Vasodilator (endothelin receptor antagonist)	Phase 1	NCT02651272	Boston University
IMR-687	A Study of IMR-687 in Adult Patients With Sickle Cell Anaemia (Homozygous HbSS or Sickle- β^0 Thalassemia)	Phosphodiesterase-9 inhibitor	Phase 2	NCT03401112	Imara, Inc
Losartan	Preventing Sickle Cell Kidney Disease	Vasodilator (angiotensin receptor blocker)	Phase 2	NCT02373241	University of Alabama at Birmingham