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2015 Clinical trials update in sickle cell anemia

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Abstract

Polymerization of HbS and cell sickling are the prime pathophysiological events in sickle cell disease (SCD). Over the last 30 years, a substantial understanding at the molecular level has been acquired on how a single amino acid change in the structure of the beta chain of hemoglobin leads to the explosive growth of the HbS polymer and the associated changes in red cell morphology. O_2 tension and intracellular HbS concentration are the primary molecular drivers of this process, and are obvious targets for developing new therapies. However, polymerization and sickling are driving a complex network of associated cellular changes inside and outside of the erythrocyte, which become essential components of the inflammatory vasculopathy and result in a large range of potential acute and chronic organ damages. In these areas, a multitude of new targets for therapeutic developments have emerged, with several ongoing or planned new therapeutic interventions. This review outlines the key points of SCD pathophysiology as they relate to the development of new therapies, both at the pre-clinical and clinical levels.

Clinical Presentation and Pathophysiology of Sickle Cell Disease

The clinical phenotype of patients with sickle cell disease (SCD) can be exceptionally diverse, despite a finite number of mutations. Clinical manifestations range from almost no symptoms to multiple, potentially fatal, events. Ballas et al. divided the complications secondary to SCD according to three main categories including hematological, pain, and complications affecting major organs, in order to more effectively standardize their definition [1]. While management guidelines are available [2,3], not every SCD patient can be treated exactly the same way given each patients individual manifestations of disease and variable response to therapies.

The most common *acute* manifestations of the disease include vaso-occlusive crisis (VOC), acute chest syndrome, stroke, priapism, sudden deafness, and acute anemia, particularly

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from aplastic crisis and splenic sequestration. Individuals with SCD are also more susceptible to stroke and serious bacterial infections. The spectrum of clinical manifestations is age dependent; women with SCD are particularly at risk during pregnancy. In addition to hemolytic anemia, common *chronic* complications affect major organs, such as brain, kidney, heart, lung, skin, retina, vestibular-cochlear systems, and bone. Some of these complications are seen predominantly in adults. Iatrogenic complications should also be considered, such as delayed hemolytic transfusion reactions and impotence due to inadequate treatment of priapism. Over their lifetime, patients differentially accumulate a wide spectrum of functional defects, which are either passive sequelae of the disease or further reinforce disease pathophysiology and worsen its clinical manifestations, such as renal tubular dysfunctions, which facilitate the development of acidosis.

Acute manifestations of the disease are managed by treating the associated symptoms. In the acute setting, pain crises are treated with hydration, warm packs, and analgesics ranging from nonsteroidal anti-inflammatory drugs (NSAIDs) to opioids. Acute chest syndrome, which typically manifests with respiratory symptoms ranging from an increased respiratory rate to desaturations, is treated with efforts to increase oxygen carrying capacity, i.e. supplemental oxygen, incentive spirometry, and exchange blood transfusion. If pain is associated with acute chest syndrome, analgesics are provided to remove the associated reduction in ventilation. In splenic sequestration, severe, acute anemia is a life-threatening symptom, and is thus treated with top-up blood transfusion. Stroke and serious bacterial infections are treated as they would be in non-SCD but the hemorrhagic risks associated with therapy should be carefully evaluated and glucocorticoids should be used with caution. In addition, SCD patients with stroke receive either simple or exchange transfusions depending on their hemoglobin level and capacity to rapidly achieve a reduction to less than 30% for the remaining SS RBC measured by % HbS after transfusion.

Chronic manifestations sometimes need to be managed symptomatically, but are preferably managed *prophylactically* in an effort to decrease morbidity associated with each condition. This often requires nonspecific treatment, but treatments that aim at decreasing the pathophysiology associated with SCD, i.e. hemolysis and vaso-occlusion, are particularly needed. The complex clinical nature and evolving pathophysiology of SCD emphasizes the need for very well coordinated follow-up and transition from childhood to adult care.

Preventative measures are currently the hallmark of the management of both acute and chronic manifestations of SCD. Even before disease confirmation, after an initiation newborn screen returns positive for SCD, infants are started on penicillin prophylaxis. The meningococcal conjugate vaccine (MCV4) and pneumococcal polysaccharide vaccine (PPV23), in addition to routine childhood immunizations, are given to all children with SCD for added protection against encapsulated bacteria. These interventions are meant to prevent illness and/or death secondary to severe bacterial infections in the setting of functional asplenia. Another example of prophylaxis is the use of chronic transfusion, and now possibly hydroxyurea, for stroke prevention. Up until recently, children with SCD and elevated Transcranial Doppler (TCD) blood velocities were encouraged to begin a chronic transfusion regimen in efforts to reduce the % HbS containing RBC thus lessening vascular complications. In patients with known stroke or silent cerebral infarcts, chronic transfusions

have been shown to result not only in a significant risk reduction of infarct recurrence, but also improved quality of life [4]. However, chronic, monthly blood transfusions are not without potential sequelae, most notably iron overload. The TWiTCH study set out to determine if daily hydroxyurea could lower TCD velocity in children with SCD in a similar way as blood transfusion. This trial was terminated early in November 2014 because hydroxyurea was determined to be noninferior to transfusion therapy in reducing TCD blood velocity. Publication of these data will be an important contribution to the ongoing discussions concerning whether or not hydroxyurea can provide an alternative to chronic blood transfusion for children at risk of developing stroke [5].

The only curative option currently available for SCD is hematopoietic stem cell transplant (HSCT). This approach is mature enough to have resulted in practice guidelines regarding its indications and management [6,7]. However, the protocols currently in use are numerous and the numbers of treated patients in each of the protocols are limited. When one considers the substantial reduction in mortality achieved with noncellular therapies over the last 20 years, long-term mortality for older HSC-based therapies is similar to that of optimal medical care. In addition, long-term complications of HSC therapies are not well documented; the increased long-term risk of solid tumors in transplanted SS patients is an area of particular concern.

Major Therapeutic Strategies for Sickle Cell Disease

HbS polymerization and erythrocyte sickling

Seminal work by the NIH group of Eaton and coworkers has elucidated the interplay of O_2 tension and HbS concentration in the kinetics of HbS polymerization [8–11] (Tables I–IV). The extreme dependence of the delay time for HbS polymerization on HbS concentration is a fundamental characteristic underlying SCD pathophysiology. It explains the very low clinical expression in sickle cell trait, the presence of disease in HbSC compound heterozygosity, the beneficial effects of increasing concentrations of HbF, and the modifications in disease severity when the cellular concentration of HbS is decreased by concomitant alpha or beta thalassemia or iron deficiency [18]. Four major avenues of potential therapies directly or indirectly target HbS polymerization, with the final objective of decreasing polymerization and sickling.

Increasing intracellular concentration of HbF—An increasing fraction of HbF inside the sickle erythrocyte is profoundly relevant not only for the concomitant decrease in HbS, but also for the unique capability of HbF to inhibit HbS polymerization. Large studies like the Cooperative Study of Sickle Cell Disease have shown that HbF is among the most important disease modifiers [19,20]. Similarly, studies on a variety of patient cohorts outside of the U.S. have shown lower disease severity in the presence of higher values of HbF. In a study involving Saudi and African American sickle cell patients, despite similar hemoglobin beta (HBB) gene cluster haplotypes, different clinical phenotypes in the Saudi cohort were attributed to their higher values of HbF [21]. Effects of several HbF modifiers have been described on both HbF values and other hematological parameters [21–25].

Studies by Platt et al., Charache et al., and others have led to the approval of hydroxyurea as a therapeutic agent in adult patients [26,27]. Early studies of 5-azacytidine and hydroxyurea treatment in SCD patients were notable for an increase in F reticulocytes and HbF elevation. This often led to an increase in hemoglobin despite mild bone marrow suppression noted in other cell lines. More than a decade later, clinical benefits were demonstrated including a reduction in pain crises and an associated reduction in mortality with increasing number of cumulative years on hydroxyurea therapy [27,28].

While hydroxyurea has not yet been approved by the FDA for use in children with SCD, several studies and its more aggressive inclusion in the most recent SCD management recommendations speak to its safety and effectiveness in a pediatric population [2,29]. In the phase 1/2 HUG-KIDS trial, 52 children were treated at Maximum Tolerated Dose (MTD) for 1 year, with no significant clinical adverse effects; hematologic toxicities were found to be mild and reversible [30]. In the Baby Hug phase 3 clinical trial, SCD infants 9 to 18 months of age treated with hydroxyurea and compared with a placebo, had reduced pain scores and fewer pain crises and acute chest syndrome [31,32]. Importantly, hydroxyurea was not associated with an increased risk of serious bacterial infections. Subsequent studies have also shown no short-term effect of hydroxyurea on growth and some positive effects on renal function [33–35]. With this supportive evidence, the most recent SCD management guidelines recommend the use of hydroxyurea in both adults with three or more VOCs in any 12-month period and, regardless of symptoms, in children greater than 9 months of age diagnosed with sickle cell anemia [2,29]. Despite the overwhelming evidence supporting the clinical benefits of hydroxyurea, only one out of four adult patients and possibly even fewer are treated with this drug [36]. Extending hydroxyurea therapy to all eligible patients should be a major target for all therapeutic interventions for SCD. Hydroxurea is also being tested in a Phase 2 study in adult and pediatric patients with Hb SC disease (SCYTHE, NCT02336373). Change in quality of life after 6 months at MTD is the primary endpoint of this study. In adults, phlebotomy is allowed if quality of life does not improve after 6 months at MTD.

The overall success of hydroxyurea has led to efforts exploring its potential synergy with other drugs such as magnesium [37]. A study investigating adjuvant magnesium pidolate in patients with HbSC disease treated with hydroxyurea was unfortunately closed early due to slow enrollment; no difference in the primary outcome of hyperdense cells or the secondary outcome of clinical events were observed across the four treatments arms (HU + Mg, HU + placebo, Mg + placebo and placebo + placebo) [38]. In vitro studies have provided evidence that hydroxyurea reduces the endothelial expression of adhesive ligands like Vascular Cell Adhesion Molecule-1 (VCAM-1) and the activation of Lutheran/basal cell-adhesion molecule (Lu/BCAM) [39,40]. Montelukast is a leukotriene receptor antagonist currently approved for prophylaxis and chronic treatment of asthma. In vitro data suggest that it inhibits eosinophil adhesion to VCAM-1 [41]. An ongoing trial is investigating whether the addition of montelukast versus placebo to hydroxyurea leads to a measurable reduction in soluble VCAM-1 (Vanderbilt University, NCT01960413).

NHLBI is currently conducting a trial in 24 patients, age 15 and older, to test a new dosing algorithm to maximize response and reduce side effects of hydroxyurea (NCT02225132,

Primary outcome: Maximal HbF values). Baylor College of Medicine is testing a new approach to shorten the time required to achieve MTD for hydroxyurea (NCT02042222, up to 105 patients, age 1–16 years.). It is however not clear how adherence to treatment can be eventually improved by the optimization of MTD for hydroxyurea. While HbF is a widely used endpoint to assess both MTD and therapeutic effectiveness, other endpoints such as the % of dense red blood cells are relevant for disease pathophysiology, have shown value and could be used to guide treatment [42,43].

Other compounds known to increase HbF are currently being investigated clinically. Decitabine (5-aza-2′-deoxycytidine), a drug commonly used to treat myelodysplastic syndrome, inhibits DNA methyltransferase. It is currently 4 years into a phase 2 clinical trial, testing the safety and effectiveness of weekly to biweekly injections administered over 1 year in SCD patients older than 18 years in whom hydroxyurea was not deemed effective or tolerable (NCT01375608, Table II). Oral Decitabine plus Tetrahydrouridine, a cytidine deaminase inhibitor, is also being investigated in a similar patient population and is currently in year 2 of its phase 1 clinical trial. MTD, safety, and effect on HbF induction are being determined for Pomalidomide, a derivative of thalidomide that also inhibits angiogenesis, in SCD patients greater than 18 years of age and who are also hydroxyurea refractory or intolerant (Table I). A phase 2 clinical trial for 2,2-dimethylbutyrate's (HQK-1001), an oral HbF inducer, was recently terminated early for lack of effects [15,44]. Inhibition of histone deacetylase (HDAC) is being investigated as a mechanism to increase HbF; Vorinostat's phase 2 clinical trial was recently closed due to the lack of measurable effects, while panobinostat (LBH589) is still in phase 1.

Still in development are a handful of compounds that have some promising preclinical data (Table IV). *BCL11A* is a transcriptional suppressor of HbF production; a substantial component of HbF variability is associated with genetic polymorphisms in *BCL11A* [45,46]. Acetylon's ACY-957, also a selective HDAC ½ inhibitor, is thought to downregulate *BCL11A*, and upregulate *GATA 2*, which conversely induces HbF (ASH 2014 #335). Pracinostat (SB939), another HDAC inhibitor, and PB-04 also induced HbF production in erythroid precursors in SCD and beta thalassemic patients (ASH 2014, # 2687). Ferritin heavy chain (FtH), a protein that stimulates gamma globin, is activated by EdX-17, augmenting HbF levels in betaYac mice to greater than 25% (ASH 2014, # 1357). Lysine Specific Demethylase-1 (LSD-1) inhibitor, RN-1, which has previously demonstrated a two to threefold increase in F cells while inducing γ-globin mRNA levels five to eightfold in mice (ASH 2014, # 561) has also induced high levels of HbF, F reticulocytes, and F cells in baboons (ASH 2014, #336). Erythroid Kruppel like factor (*KLF1*), involved in β-globin induction and γ-globin suppression, has also been targeted using antisense oligonucleotides (ASOs) in both murine and human cell lines. Subcutaneous administration of KLF1 ASOs in mice and rats has been shown to reduce β-globin production (ASH 2014, #4038). Valproic acid and trichostatin have been shown to increase γ-globin gene expression in human liquid erythroid cultures [47]. Trichostatin A (Errant Gene Therapeutics) is a histone deacetylase inhibitor, which may induce HbF expression, but no data have been published or presented so far. For many of the preclinical leads described above, it should be noted that ability of inducing HbF production in vitro in cell lines does not necessarily translate into in vivo

efficacy in humans. The limited availability of nonhuman primate models also poses a significant challenge to the pre-clinical development of these compounds.

Reducing intracellular concentration of HbS—This avenue comprises either modification of ion transport across the erythrocyte membrane with the intent of preventing sickle cell dehydration or reduction of HbS concentration by iron deficient erythropoiesis. Given the unique dependence of the kinetics of HbS polymerization on HbS concentration, the presence of erythrocyte dehydration in SCD has important implications for pathophysiology and was originally and unsuccessfully targeted in 1980 in a study aimed to induce red cell swelling with hypo-osmolarity [48]. Over the last 30 years, the major pathways which are responsible for erythrocyte dehydration have been characterized in substantial detail [49]. They include the K-Cl cotransport (KCC1,3,4) [50–54], the Ca-activated Gardos channel (KCCN4) [55–59], and P_{sickle} (most probably mediated by the mechano-sensitive ion channel Piezo 1) [60–62]. Pharmacological inhibition of these pathways has been demonstrated in vitro and in vivo in transgenic mouse models. Clinical trials with dietary magnesium supplementation (Mg-pidolate) in patients with SCD have shown increases in red cell magnesium content, inhibition of K-Cl cotransport, and improvements in erythrocyte hydration, but there have been no completed controlled trials testing clinical efficacy of this approach [63,64]. A phase 1 study of oral Mg-pidolate supplementation established the MTD for children with SCD concomitantly treated with hydroxyurea [36]. As discussed above, a study on oral Mg pidolate supplementation in patients with SC disease was terminated early due to poor enrollment, but the limited data collected showed no changes in erythrocyte Mg or cell dehydration [37]. Clinical studies on the inhibition of the Gardos channel were first conducted in the acute settings with IV cetiedil [65] and subsequently with oral clotrimazole and senicapoc as prophylactic agents. These latter studies resulted in reduction of cell dehydration, improvement of anemia, but no measurable improvements in the relevant clinical endpoints, and an actual increase in painful crises in the subgroup of patients not receiving hydroxyurea [66–68]. Since Hb increased in all senicapoc-treated patients, it is possible that the associated increase in blood viscosity negated the benefit of reducing cellular HbS concentration, and resulted in increased vaso-occlusion. However, there was no correlation between Hb values or changes in Hb values and pain rates in patients treated with senicapoc. In vitro and in vivo data have shown a role for endothelin-1 (ET-1) receptor blockade in reducing erythrocyte dehydration, most likely due to a functional connection between activation of this receptor and activation of the Gardos channel [69]. It remains to be determined if targeting ET-1 can achieve a greater reduction in cell dehydration than targeting the Gardos channel itself.

Isolated reports of improvement in SCD due to concomitant hypochromia induced by iron deficiency have so far not translated into viable therapeutic strategies and have not considered potentially associated reduction in HbF [70,241160071]].

Direct inhibition of HbS polymerization—In vivo chemical modification of HbS resulting in inhibition of polymerization has been an elusive therapeutic goal. Obvious challenges to this approach are the high concentration of Hb in the erythrocyte requiring a substantial amount of modifying compound to be absorbed by the GI tract and to cross the

erythrocyte membrane without affecting other crucial cellular functions. Several compounds have been tested, all with disappointing results so far. Na cyanate mediates an irreversible carbamylation of the aminoterminal valine of HbS which results in reduced polymerization and sickling [72]. In vivo studies with extracorporeal carbamylation showed measureable changes in several hematological parameters [73], however, significant toxicities were observed most likely due to carbamylation of other targets, resulting in peripheral neuropathy, CNS toxicity, weight loss, and cataract formation. BW12C, a substituted benzaldehyde, showed significant in vivo modification of HbS when administered parenterally to patients with SCD [74,75], but it was not further developed due to significant toxicity observed with a related compound.

The food additive vanillin binds to HbS and reduces polymerization and sickling by both an allosteric shifting of oxygen affinity and a stereospecific inhibition of polymer assembly [76]. Reduced polymerization and sickling were reported following a double-blind, placebo-controlled in vivo study using 1 g vanillin/day for 40 days in 30 patients with SCD in Cuba [77]. However, no additional studies were carried out with this compound. Recent evidence suggests that vanillin may adversely affect red cell ion transport and produce K loss and dehydration of sickle cells [78]. INN-270 and TD-7, two derivatives of vanillin, demonstrate high rates of HbS binding and modification resulting in a shift to a higher oxygen affinity hemoglobin state (ASH 2014, #218) [79]. Sickle mouse studies using these two compounds are ongoing.

Indirect inhibition of HbS polymerization by increasing oxygen affinity—Several compounds have been identified that indirectly inhibit HbS polymerization by shifting the partial pressure of oxygen at which 50% of hemoglobin is saturated with oxygen (P_{50}). For some of the compounds described in the preceding section, such as BW12C, it is not completely understood how much of the antisickling effect is due to inhibition of polymerization and how much to a left-shift in P_{50}. A general concern for this approach is that a reduction in tissue oxygen delivery may produce a compensatory increase in hemoglobin, with concomitant increased viscosity and vaso-occlusion [80]. Sudden interruption of this kind of treatment may also expose patients to substantial complications due to the increased overall mass of circulating sickle cells.

Even including some of the drugs discussed in the preceding section, a limited number of clinical trials have been conducted so far. Oral administration of Tucaresol, a substituted benzaldehyde, resulted in measureable changes in oxygen affinity, but also in significant toxicity [81].

The active ingredient for AES-103 (Baxter International Inc.) is 5-hydroxymethyl-2-furfural (5-HMF), which has been shown to form a high-affinity Schiff-base adduct with HbS and indirectly inhibits sickling via a leftward oxygen curve equilibrium shift (ASH 2014 # 2699) [82]. In vitro 5-HMF reduces sickling-induced dehydration (Gardos and P_{sickle}) while it increases K loss and dehydration mediated by the K-Cl cotransport [83]. A phase 1 clinical trial tested oral doses of AES-103 up to 4,000 mg in 18 patients with SCD, some treated with hydroxyurea, with no significant side effects [84]. AES-103 is currently being tested in a phase 2 clinical trial (Table II).

GBT440 (formerly GTx011, Global Blood Therapeutics, South San Francisco, CA), increases HbS oxygen affinity and diminishes cell sickling in vitro, while it prolongs red cell survival in vivo in sickle (Townes) mice (ASH 2014 #217, # 1370). A phase 1/2 clinical trial is ongoing to assess safety, pharmacokinetics and pharmacodynamics of oral GBT440 (single and multiple doses, administered once daily) in healthy subjects and in patients with SCD (Table I).

While reducing oxygen carrying capabilities, CO has multiple potentially beneficial effects in SCD, which include inhibition of polymerization, increase in oxygen affinity, reduced inflammation, and increase antioxidant responses. Delivery of CO via a pegylated hemoglobin saturated with CO has improved vaso-occlusion in a mouse model of SCD [85]. It remains to be determined if this can be achieved in humans without significant side effects.

The inflammatory vasculopathy of sickle cell disease

Adhesion of sickle cells to endothelium and vaso-occlusion—Adhesion of sickle cells to endothelium plays a major role in SCD pathophysiology, especially as it relates to sickle vaso-occlusion. It is generally believed that vaso-occlusion develops from adhesion of sticky sickle RBCs to endothelium, followed by trapping and polymerization of rigid, less deformable cells [86,87].

The mechanisms supporting the adhesion of sticky sickle erythrocytes to endothelium as well as additional, cooperative adhesive interactions involving activated leukocytes, monocytes, and platelets are of great interests, since interfering with these mechanisms has the potential to greatly impact clinical severity. As we learn more about the specific receptors involved in the adhesion process, new therapeutic targets are coming into play to prevent and/or reverse vaso-occlusion.

Selectins (P, E, and L) play a crucial role in adhesion of leukocytes to endothelium [88]. In sickle erythrocytes, P-selection and E-selectin have therefore been explored as potential targets. Considerable debate has been generated regarding the single selective (P or E) versus pan-selectin blockade. There are also concerns about the fact that selectins mediate adhesion mostly in the presence of shear stress [89,90], and it is not clear how much this mechanism is actively engaged when the blood flow is reduced or stopped as presumably happens in VOC. Matsui et al. demonstrated that the mechanism of decreased sickle cell adhesion via unfractionated heparin is through P-selectin inhibition [91]. Unfractionated heparin was shown to bind to P- and L-selectins and inhibit their function, while low molecular weight heparins did not [92]. Promising results in a small cohort of patients with SCD were obtained with Pentosan Polysulfate Sodium (PPS), an agent used for treatment of pain associated with interstitial cystitis (Elmiron, Janssen Pharmaceuticals). PPS was shown to block P-selection adhesive processes in vitro, to normalize microcirculatory blood flow, and to reduce markers of vascular injury in vivo [93]. Availability of an orally-absorbed selectin inhibitor would be a major advantage compared with those requiring SC injections. In 2007, a randomized, double-blind trial in 253 patients with acute VOC showed positive results on duration of crises, hospitalization and pain intensity with the daily SC

administration of 175 IU/Kg of Tinzaparin, a LMWH [94]. US marketing of Tinzaparin, (Innohep, LEO Pharma, Ballerup, Denmark) was discontinued in February 2011.

Sevuparin, a chemically modified heparin, currently being studied as a antimalarial agent, has also been shown to reduce sickle red cell and leukocyte adhesion in vitro, to stimulated endothelial cells [95]. Given promising in vivo studies in nude mice demonstrating decreased vaso-occlusion following TNF-α exposure, Dilaforette AB (Sweden) and Ergomed plc (UK) are planning a phase 2 study using sevuparin as an acute therapy for VOCs (Table II). Hemostatic balance is of critical pathophysiological importance in SCD: hemorrhagic (retinal, renal, brain) disease as well as thrombotic events (pulmonary embolism, phlebitis) are not uncommon. Thus contrary to Tinzaparin, LMWH agents like Sevuparin and pentosan that have limited anticoagulation properties may provide a safer alternative.

GMI-1070 (Rivipansel, GlycoMimetics Inc.) is a E-selectin inhibitor which was shown to be safe and to produce some positive biomarker changes in a phase 1 study when injected IV in 15 SCD patients [96]. GMI-1070 was studied in phase 2 study with the primary outcome measure being a reduction in time to resolution of VOC. No statistically significant effects were observed on the primary outcome measure, while plasma E-selectin (ASH # 2704), opioid and overall pain medication usages were significantly reduced [97–100]. Despite having failed its primary outcome, GMI-1070 will be tested in a phase 3 trial, on SCD patients 6 years of age and older hospitalized for pain (Table III). The primary outcome of this study is time to readiness to discharge, while secondary outcomes include amount and duration of opioids and readmission rates. The initiation of this phase 3 trial has been delayed due to manufacturing issues.

The SUSTAIN trial is a phase 2 multicenter trial currently assessing whether SelG1 (Selexys Pharmaceuticals, Oklahoma City, OK and Novartis Pharmaceuticals), a humanized monoclonal antibody to P-selectin, is safe and effective when given IV to patients on or off hydroxyurea (Table II). ARC5690, an anti-P-selectin aptamer, was considered for preclinical development by Archemix, but this company is now in liquidation (Table IV) [101].

Almost 15 years ago, IV Poloxamer 188 (Flocor), purified surfactant, was tested in a large phase 3 clinical trial to assess its efficacy in reducing the duration of painful crises. A limited effect was seen in children and subjects concomitantly treated with hydroxyurea [102]. A smaller study subsequently showed measurable improvement in microcirculatory parameters during VOC [103]. A second phase 3 trial with this compound, now renamed MST-188 is currently under way (Mast Therapeutics, San Diego, CA) to test its effectiveness in shortening VOC in children and adults (Table III).

Very late antigen 4 (VLA-4) or α4β1 is a cell surface integrin that mediates reticulocyte interactions with the endothelium, vascular adhesion molecule 1 (VCAM1), and plasma fibrinogen. Natalizumab, a recombinant humanized antibody currently approved for relapsing multiple sclerosis (MS) and Crohn’s disease, binds to the α4 subunit of VLA-4. Sickle cell reticulocyte and leukocyte adhesion to VCAM-1 was blocked in whole blood

samples obtained from subjects with SCD and saturated with Natalizumab at plasma trough concentrations measured in MS and Crohn's disease patients, (ASH 2014, #221).

RBC have beta adrenergic receptors that may, under adrenergic stress, activate some adhesion molecules like RBC LU-BCAM, favoring initiation of VOC as well as generating vasospasm. Propranolol, a frequently used beta-blocker in children and adults, has been shown to inhibit epinephrine upregulation of sickle RBC endothelial adhesion in animal studies and in a phase 1 clinical study [104].

PF04447943 (PDE9i, Pfizer Inc.), an inhibitor of phosphodiesterase-9A enzyme, was unsuccessfully tested in a Phase 2 trial in Alzheimer's disease [105]. This compound decreased adhesion in a SCD mouse model (ASH 2014, # 2694). A phase 1 clinical trial is planned for patients with SCD on or off hydroxyurea (Table I).

A phase 1 study in 15 patients with Hb SS or Hb Sβthal experiencing an acute painful crisis showed changes in neutrophil activation markers following a single infusion of intravenous immunoglobulin (IVIG, from 100 to 800 mg/kg). Although not significant, a trend toward increased re-admissions with high dose IVIG was noted in this trial [106].

Vasculopathy of sickle cells and coagulation—Virchow's triad describes the three broad categories of factors leading to thrombosis. In SCD, endothelial damage (caused by direct vascular damage from ischemic injury and free hemoglobin), stasis (consequence of decreased blood flow secondary to erythrocyte adhesion and increased viscosity), and hypercoagulability (due to externalization of phosphatidlserine on erythrocytes promoting subsequent thrombin generation, platelet adhesion and WBC activation) are potential contributors to thrombus formation. Lastly, platelet elevation and activation in SCD accelerates the hemostasis cascade.

Eptifibatide, an inhibitor of the platelet αIIbβ3 receptor pathway was studied in a small group of patients with SCD during acute VOC but showed no measurable benefits [16]. Abciximab (ReoPro, a platelet glycoprotein IIb/IIIa receptor antagonist), is currently being studied as IV infusion in the treatment of acute VOC in SCD patients 5 to 25 years of age. Ticagrelor and Prasugrel are oral platelet aggregation inhibitors that act via inhibition of the adenosine diphosphate receptor P2Y12. A phase 2 study tested oral administration of Prasugrel (5 mg/day for 30 days, $n = 41$) vs. placebo ($n = 21$) in patients with SCD, demonstrating its safety and some changes in markers of platelet activation [107]. Prasugrel is currently in a phase 3, double-blind, placebo-controlled study involving up to 220 pediatric patients, which will test its efficacy in reducing VOC, as a composite point of either painful crisis or acute chest syndrome. This study will use the VerifyNow® P2Y12 test, which quantifies the extent of platelet function inhibition via the P2Y12 pathway, to titrate Prasugrel to a maintenance dose for a treatment period between 9 and 24 months.

Apixaban, a factor Xa inhibitor, is being investigated as a prophylactic agent in a phase 3 study (Table III). The main objective of its study is to reduce mean daily pain scores in patients greater than 18 years of age with SCD. Rivaroxaban, also a factor Xa inhibitor, is

under evaluation in a phase 2 trial, to examine its effects on vascular cell adhesion molecule-1 (VCAM-1) and interleukin-6 (IL-6) levels after 4 weeks of therapy (Table II).

White cells and other cellular and soluble mediators of inflammation—Several classic studies have shown that the baseline WBC is a strong predictor of ACS [108], silent strokes [109], and early mortality in patients with SCD [20]. It is well known that inflammation is a key component of the pathophysiology of SCD. Several anti-inflammatory drugs have been and are used in the treatment of acute and chronic events in SCD, including NSAID and steroids. Hydroxyurea therapy significantly decreases WBC counts, but how much this change contributes to the beneficial effects of hydroxyurea remains undetermined. Ischemia-reperfusion injury with release of inflammatory cytokines has also been invoked as a potential mechanism leading to acute and chronic tissue damage. More recent studies have identified regulatory steps leading to the inflammatory state of SCD, which are also potential candidates for therapeutic intervention [110–112].

Invariant Natural Killer T (iNKT) cells are currently being investigated as a potential mediator of the inflammatory state of SCD, and activation of these cells has been demonstrated during VOC [13]. The humanized monoclonal antibody NKTT120 has been shown to safely deplete iNKT cells in adult sickle cell patients up to a dose of 0.3 mg/kg (ASH 2014, # 2178). iNKT cells are laden with adenosine receptors. Regadenoson, an adenosine receptor 2A agonist, currently used to increase coronary blood flow during cardiac nuclear stress scan, (Lexiscan, Astellas USLLC) is being studied for the acute treatment of VOC, with the intent to down-regulate the inflammatory cascade that is initiated by iNKT activation. In a phase 1 study in patients with SCD (21 at baseline and 6 during VOC), a 24-h infusion of regadenoson was shown to result in decrease iNKT activation [13]. A double-blind, placebo controlled, phase 2 study is ongoing to determine the effects of a 48-h IV infusion of regadenoson on iNKT cell activation in 96 patients with sickle cell anemia admitted for acute VOC or mild to moderate ACS (Table II). Other adenosine receptor ligands are being considered for potential development in SCD, such as PNQ103 (Advinus Therapeutics, Pune, India), and A2a PAM (Addex Therapeutics, Geneve Switzerland).

Zileuton (ZYFLO CR®, Chiesi) is a structural analog of hydroxyurea, which decreases leukotriene production by inhibiting 5-Lipoxygenase, and is currently marketed for prevention of asthma in children and adults. Zileuton also induces HbF in erythroid progenitors through a mechanism that involves l-arginine/nitric oxide/cyclic GMP [113]. A phase 1 study in 11 patients with SCD showed safety of higher doses than those approved for asthma [12], supporting the feasibility of a future phase 2 study.

Dimethyl fumarate (Tecfidera, Biogen Idec) currently approved in the USA for the treatment of relapsing multiple sclerosis, is being considered for SCD based on both its general anti-inflammatory properties as well as the ability to activate NRF2 signaling which is involved in drug-induced HbF expression [114,115].

The use of the inhaled corticosteroid, Mometasone, in sickle cell patients with cough or wheeze but no diagnosis of asthma, is also currently being investigated to assess if it can

decrease general pulmonary inflammation. ReveraGen Biopharma (Silver Spring, MD) is currently developing VBP15, a “dissociative” steroid, which retains steroidal efficacy with reduced side effects, for Duchenne Muscular dystrophy, and has received NIH funding to begin its development for SCD. This is relevant for SCD because there are concerns about oral glucocorticoids possibly promoting VOC and accelerating the development of osteonecrotic complications.

Atorvastatin and Simvastatin, two frequently used lipid lowering medications, have been considered for SCD. However, Atorvastatin treatment (10–20 mg/day) did not produce measurable improvements in vasodilatory responses in a small cohort of SCD patients [116]. Simvastatin increased nitric oxide metabolites while decreasing C-reactive protein (CRP) and interleukin-6 (IL-6) in patients with SCD [117]. Lovaza, an omega-3 fatty acid ethyl ester, is also being investigated for its role in inflammation in pediatric SCD patients (Table II). In sickle mice, a diet supplemented with omega-3 fatty acid (fish oil) reduced several systemic inflammatory biomarkers and improved hypoxia-reoxygenation associated organ damage [118]. Preclinical data show a possible role for inhibition of the mitogen-activated protein kinase ERK1/2 in reducing adhesion and vaso-occlusion (Table IV) [119].

NO, arginine, and hemolysis as a key mediators of vasculopathy—Nitric oxide is a potent vasodilator produced from the metabolism of L-arginine by NO synthase, and plays a key role in vascular physiology and pathophysiology. In SCD, the transfer of NO from HbS to the membrane is impaired, resulting in an impaired capability of red cells to mediate vasodilation [120]. The chronic hemolytic state of SCD increases plasma Hb values and substantially limits NO bio-availability, resulting in a NO-deficient state [121]. This NO-deficient state is believed to be the main determinant for the development of pulmonary hypertension [122–124], although there have been different estimates about the true prevalence of this complication [125], and some spirited academic debates about the hemolysis hypothesis [125–127]. Initial studies showed some promise for inhaled NO in the treatment of acute VOC [128], but a large randomized, placebo-controlled, double-blind study, showed no effect on the time of crisis resolution [129]. Another study on inhaled NO for the treatment of acute crises in pediatric patients was terminated early due to slow enrollment, but no data are yet available about this trial (Table II). Some case reports have suggested a possible therapeutic role for inhaled NO in ACS [130,131]. A case-controlled trial of inhaled NO in adult patients with ACS has recently been completed in Creteil, France and results have been submitted for publication. Patients with SCD exhibit a dysfunctional regulation of arterial tone, which may impair response to variation in blood flow or shear stress [132,133]. Despite abundant scientific evidence and strong rationale, studies targeting NO and associated regulatory pathways have been largely disappointing.

A trial of sildenafil in SCD disease patients with pulmonary hypertension (TRV ≥2.7 m/s and 6 min walk distance between 150 and 500 m) was terminated early due to serious adverse events in the sildenafil arm (mostly increased hospitalization for pain) [134]. A small study on prevention of recurrent ischemic priapism with sildenafil had inconclusive results [135]. Similarly, studies assessing endothelin receptor blockade (ASSET-1 and -2, with Bosentan) as a potential therapy for pulmonary hypertension in SCD were inconclusive due to poor enrollment [136]. Safety and efficacy of Macitentan (Actelion, Switzerland) will

be tested in a single center open label trial for precapillary pulmonary hypertension in SCD at Boston University (Table II).

Low levels of plasma arginine have been demonstrated in steady state SCD, with further decreases due to acute events and chronic vascular damage. Human studies have been performed with arginine, based on the notion that administration of this compound may improve the relative NO deficiency in SCD, with conflicting results [137]. Questions have been raised about what should be the optimal oral arginine dose, suggesting that not enough arginine was used in a negative NIH-sponsored, phase 2 trial, whose results have never been formally published. A more recent study compared parenteral L-arginine (100 mg/kg tid for 5 days) and placebo administration in 38 children with SCD. Although length of stay was not affected, parenteral opioid use (~50%) and pain scores were significantly diminished with L-arginine [138]. It has also been suggested that administration of arginine and hydroxyurea may be superior to arginine monotherapy.

Some key mechanisms associated with hemolysis have been identified as toxic to the body and contributory to the pathophysiology seen in SCD. Hemolysis leads to the release of free hemoglobin into the extravascular space, the depletion of nitric oxide, and the creation of toxic free radicals and hemin [139]. Haptoglobin itself is protective and by binding to hemoglobin prevents movement of hemoglobin across endothelial layers. It also limits NO depletion and thus the release of free radicals and hemin. However, in SCD, haptoglobin is readily depleted and free hemoglobin must be cleared by heme oxygenase-1 (HO-1), as the presence of free hemoglobin can cause multiorgan damage. Preclinical studies show that exogenous haptoglobin decreases the production of HO-1, likely due to a decrease in the presence of free hemoglobin [140]. Bio Products Laboratory has received EU Orphan Drug designation for a preparation of haptoglobin to be administered IV to patients with SCD. Preclinical studies in dogs have shown that this animal model can be used to estimate clearance of haptoglobin-bound Hb [141].

Vasodilation-increased blood flow-perfusion-oxygenation—$MgSO_4$, known for his vasodilatory properties, has also been studied in SCD. However, as detailed above, two randomized clinical trials showed no effect of IV $MgSO_4$ on the resolution of acute painful crises (ASH 2014 # 88) [17].

A hemoglobin based blood substitute was tested in a clinical trial in 1997, under the assumption that this oxygen carrier could potentially reverse VOC [142]. While no toxicity was observed in this first study, subsequent studies for other indications not related to SCD have shown an association with death and myocardial infarction [143], resulting in diminished enthusiasm about these compounds as therapeutic agents.

MP4CO was a human hemoglobin, obtained from blood donors, conjugated with polyethylene glycol (PEG) and saturated with CO. It was produced with the idea that CO delivery to ischemic areas would improve perfusion and reduce microvascular stasis and with the assumption that 5 to 10% HbCO would be nontoxic and could limit or reverse HbS polymerization as well as producing a vasodilatory effect. Preclinical studies in sickle mice showed positive changes in inflammatory parameters and mortality [85], but no data are

available on a phase Ib study funded by Sangart, a company which is no longer active (Table I). Sanguinate, a pegylated bovine carboxyhemoglobin was tested in a phase 1 trial (Table I). According to a press release on April 14, 2015 by Prolong Pharmaceuticals (South Plainfield, NJ; http://www.prolongpharma.com/press-14APR2015) phase 2 trials are planned for VOCs and leg ulcers. A topical sodium nitrate 2% cream was shown to be safe and well tolerated in a phase 1 study in 18 patients with SCD [14]. Increases in per-wound cutaneous blood flow and decreased ulcer size were also observed in this trial.

Oxidative damage

Oxidative stress is thought to play a role in the pathophysiology of SCD, both at the erythrocyte level, as well as in the development of VOC. Several commonly used supplements are currently being investigated based on their anti-oxidant properties. Sorghum bicolor extract, Jobelyn is often used as a dietary supplement in Nigeria. Its antioxidant and anti-inflammatory properties have led to its investigation as a potential SCD agent, with an ongoing trial in Lagos, Nigeria comparing the effect of daily Jobelyn at different daily doses (500 mg, 250 mg, and 2 mg) on quality of life (Table II).

It has been hypothesized that *N*-acetylcysteine (NAC) may help reduce oxidative damage associated with SCD, based on its capability of increasing antioxidant systems like the one based on glutathione. This is currently being tested in a phase 3 trial (Table III).

The amino acid L-glutamine has been shown to increase erythrocyte nicotinamide adenine dinucleotide redox potential (NADH). A randomized, placebo controlled trial evaluated the efficacy and safety in SCD patients >5 years of age and with a history of at least two VOC in the previous 12 months. In the 2014 ASH abstract (ASH 2014, # 86) clinical benefit was reported in the treatment arm with patients receiving L-glutamine having significantly fewer days to first VOC, number of painful crises, and hospital days. However, Form 10-K filed by Emmaus Life Sciences Inc. with the U.S. Securities and Exchange Commission, have disclosed significant concerns raised by the FDA, namely that the primary endpoint of the trial did not reach statistical significance, that the results were inconsistent among regions, and that the reduction in painful crises, was not clinically meaningful and was inconsistent across regions. These concerns resulted in the recommendation that a second trial be conducted in patients with higher baseline levels of painful crises [144].

Cell-based therapies

Cell based therapies currently provide the only curative options for SCD, and they comprise both gene therapies and stem cell transplantation (Table V).

Gene therapies—Several companies have developed groundbreaking gene therapies for SCD that, using a lentiviral vector, insert a functional human beta or gamma globin gene into a patient's own hematopoietic stem cells. These are then infused into a patient during an autologous stem cell transplant.

Bluebird Bio is currently assessing the safety and efficacy of the LentiGlobin BB305 in children and adults with SCD (trials NCT 02140554 and NCT02151526). The use of this vector, which contains a B-globin gene (BA-T87Q) and produces hemoglobin BA-T87Q has

already been proven successful in patients with Beta-thalassemia major (ASH 2014, # 549). The therapeutic Hb was designed with a unique mutation which, while preserving normal O_2 carrying properties, strongly inhibits HbS polymerization. In addition, the lack of a functional LTR, the use of erythroid specific promoters, and chromatin insulator element make the chance of leukemia unlikely. Preliminary results have been presented on the first SCD patient treated under this protocol, showing that after 6 months Hb A^{T87Q} comprised 45% of Hb production with associated improvement in transfusion requirements and hemolytic parameters. One additional SCD patient has been treated under a similar protocol at the NIH.

The University of California, Los Angeles is currently investigating the use of the recombinant beta globin gene (HBBAS3) in a lentiviral vector [145]. It too has the T87Q blocking site. In vitro data have been recently published showing that therapeutic levels can be achieved for the production of an antisickling Hb using insulated, self-inactivating and erythroid-specific lentiviral vectors in cultured CD34+ cells from SCD patients [146].

The Children's Hospital Medical Center at Cincinnati has developed a novel human gamma-globin gene vector. It is currently recruiting adult SCD patients for its phase 1 trial. Boston Children's Hospital is set to open a phase 1/2 trial that unlike the approaches highlighted above, involves the insertion of a beta or gamma globin gene, seeks to knockout BCL11A thus increasing fetal hemoglobin and bypassing the mutated sickle globin gene.

Preclinical evidence shows promise for the use of zinc-finger nucleases (ZFNs) in site-specific correction of the HbS mutation in mouse and human sickle CD34+ precursors [147]. However, new powerful genome editing techniques like CRISPR are likely to revolutionize gene therapy for hemoglobinopathies [148].

Several companies, including Errant Gene therapeutics and Editas Medicine are currently working with CRISPR-Cas9 systems to develop strategies to modify the mutated sickle cell gene, while Sangamo Biosciences is attempting to develop Zinc Finger Nuclease (ZFN) mediated gene correction. OrphageniX has tried a different approach and is developing therapies based on targeted gene alteration (TGA) which involves the delivery of strategically mismatched oligonucleotides to the nucleus of the cell, feeding off of the body's natural DNA repair processes.

Stem cell transplantation—Hematopoietic stem cell transplant (HSCT) is currently the only approved cure for SCD. In centers with considerable experience with this approach, and when patients are treated before transplant with hydroxyurea, or if the transplant took place after January 2000, event-free survival rates of 97.4% and 95.3%, respectively, have been reported [149,150]. Overall survival is similar for either bone marrow transplant or cord blood transplant [151].

However, the potential associated morbidity and mortality often make HSCT a less considered treatment option [7]. For many providers, the difficulty in recommending transplant lies in our inability to predict clinical severity before the advent of significant morbidity, which in some cases can greatly affect transplant outcomes. Guidelines

recommending stem cell transplant as a therapy for severe disease as opposed to a cure for all patients with the mutation, has also led to underuse.

In reality, overall survival and disease free survival in select populations has been shown to be equal to that of patients treated symptomatically without transplant [152]. Therefore, experts recommend that symptomatic patients with an HLA-matched sibling undergo transplantation preferably when they are pre-school age [6]. However, a major problem within the sickle cell community is the lack of healthy, HLA-matched siblings. In those without a sibling match, transplantation is only recommended in the setting of a controlled clinical trial.

At present, there are several ongoing clinical trials that are studying the success of diverse myeloablative, nonmyeloablative, and reduced intensity regimens in both matched sibling and unrelated sibling donor transplants. A reduced intensity regimen is meant to decrease morbidity and mortality associated with transplant, especially in those with preexisting co-morbidities. It also increases the likelihood of mixed donor chimerism, which, if high enough in SCD, can result in predominance of donor cells in the circulation [153]. Mixed donor chimerism and the addition of antibodies against mature lymphocytes, namely anti-thymocyte globulin or alemtuzumab, provide additional graft versus host disease prophylaxis. Case Comprehensive Cancer Center is planning a trial that uses solely fludarabine for conditioning (NCT0206559). A preliminary study has provided encouraging evidence that fludarabine can be used to reduce exposure to busulfan and cyclophosphamide in children with SCD undergoing HLA-matched HSCT [154].

A nonmyeloablative regimen composed of low dose radiation, alemtuzumab, and sirolimus is also being studied in older patients by University of Texas Southwestern Medical Center, University of Illinois at Chicago (UIC), and the National Heart Lung and Blood Institute (NHLBI). UT Southwestern is using peripheral stem cells while NHLBI and UIC are using bone marrow as their stem cell source. In another study, NHLBI has proposed using the same conditioning regimen backbone with additional pentostatin and oral cyclophosphamide in patients considered at high risk for transplant failure (i.e. female recipients of male donors those with preexisting allo-antibodies and those receiving peripheral stem cells).

As not all patients have a fully matched HLA sibling for transplant or an unrelated match, haploidentical transplantation has been proposed as an alternative. New York Medical College, Vanderbuilt-Ingram Cancer Center, UIC, and NHLBI all have ongoing trials.

Treatment of pain in sickle cell disease

Ballas et al. described the hallmark of SCD as acute continuous pain as it is the most common reason for inpatient hospitalization and treatment in the emergency department (Table VI) [155]. At present the mainstay of treatment for pain in VOC is opioids. Several interventional trials are ongoing to assess alternative modalities to decrease either pain scores during and between VOC. Nationwide Children’s Hospital recently completed a trial investigating whether warmed saline given to children who present with VOC may decrease hospital admission, IV analgesic usage, and pain scores. The safety and efficacy of lidocaine 5% plasters in pediatric patients with SCD experiencing neuropathic pain is being

investigated at the Centre Léon Bérard (Lyon, France). Results are not yet available for either of these trials. Still recruiting are trials comparing different opioids such as morphine, hydromorphone, gabapentin, and intranasal fentanyl to those investigating the benefits of music therapy, cannabis, inhaled steroids and nitrous oxide. All studies are welcomed by the SCD community as opioids alone only address one component of the pathophysiology behind VOC i.e. nociception, but fail to address both vaso-occlusion and inflammation. In addition, trials should focus on early treatment during the prodromal stage of VOC as well as on how to minimize the hyperalgesia and fear that often accompanies recurrent painful episodes.

Iron overload in sickle cell disease

Chronic transfusion therapy is still the main therapy for primary and secondary stroke prophylaxis (Table VII). Transfusion therapy is also widely used for acute chest syndrome. Thus, transfusional iron overload is a significant problem for patients with severe SCD. Chelation is typically started 2 to 3 years after chronic transfusion therapy is initiated or once ferritin exceeds 1,000 ng/mL. Unfortunately, current therapies are often not well tolerated, especially in the pediatric setting. Current therapies include oral deferasirox, subcutaneous or IV deferoxamine, and oral deferasirox in combination with deferiprone [156]. Previous studies have demonstrated the safety and efficacy of deferasirox compared with deferoxamine in patients with iron overload and SCD [157]. Apopharma is currently recruiting patients to assess the safety and efficacy of deferiprone compared with deferoxamine. The primary endpoint is change in liver iron concentration. A similar study, yet to open, will take place in Italy. It will include patients as young as 1 month of age and will assess successful chelation based on ferritin and cardiac MRI T2*. Shire recently terminated all clinical trials for deferitazole (SHD602) in the treatment of iron overload.

Additional therapeutic modalities

Orthopedic complications such as osteonecrosis of femur and humeral heads represent an important clinical feature of SCD [158,159]. In recent years, the outcome for procedures like total hip arthroplasty has improved substantially and is not different from patients with osteonecrosis not due to SCD [160].

Renal disease occurs early in SCD and may ultimately lead to chronic renal failure. The pathophysiology of the SCD nephropathy is complex, and the clinical manifestations are variable [161,162]. Hyperfiltration is a common finding in children with SCD, but hypofiltration can also be present in a smaller subset [163]. Hydroxyurea therapy can play a significant role in retarding the progression of renal disease in SCD [164]. The angiotensin II receptor losartan is currently being studied for the prevention of sickle nephropathy in children and young adults with SCD (Table II, CT01479439 and NCT02373241).

Impaired rheology plays a key role in the pathogenesis of the complications of HbSC disease, and is believe to result in the 70% incidence of retinopathy and 29% incidence of sensorineural ontological disease, substantially higher than in non-SCD populations [165,166]. Limited evidence suggests a beneficial effect of phlebotomy in preventing recurrence of acute complications [165].

Despite overwhelming evidence for the benefit of vaccination in children with SCD, pneumococcal vaccination rates are still suboptimal in the US and the incidence of invasive pneumococcal disease is still greater than in the general population [167,168]. Similarly disappointing data have been reported for adherence to penicillin prophylaxis and for influenza vaccination [169].

Relevant endpoints for SCD clinical trials

The clinical trial which resulted in the approval of hydroxyurea for adults with SCD remains so far the only prototype for current trials aiming at pharmacologic reductions of clinical complications of SCD [27]. To the best of our knowledge, the FDA has not allowed phase 3 trials based on biomarkers or other disease indicators outside of transfusion and acute events (acute chest and/or VOCs resulting in patient admission). Alternative assessment of pain and disease severity (pain diaries) have been proposed but not fully validated. Validation of novel endpoints for SCD, especially related to pain, remains an important target for future studies.

Methods

Information on clinical trials was obtained from several sources. (a) Clinical-Trials.gov (https://clinicaltrials.gov) was searched for studies including the term sickle; studies were not included if the status of the study was listed as completed (with a completion date before 2013), terminated, unknown, or withdrawn. Observation trials were not included. (b) The WHO International Clinical Trials registry platform http://apps.who.int/trialsearch/ and the European registry (https://www.clinicaltrialsregister.eu/) were searched for additional studies not listed in the ClinicalTrials.gov database. Studies were then organized based on clinical phase (1, 2, 3), with separate tables for studies on cell-based therapies, pain treatment and iron overload. Information for preclinical studies was obtained from literature searches and review of abstracts presented at the 2013, 2014 American Society of Hematology and 2014 and 2015 European Hematology Association meetings.

Conclusions

A substantial number of clinical trials are ongoing or planned to test new treatments for SCD. The majority of these trials is supported by private companies, which represents a profound and positive change compared with the prior decade. This is most likely the result of the significant investments in fundamental research on SCD pathophysiology that NIH supported in the past 30 years and the realization for many biotechnology and pharmaceutical companies that the orphan status of SCD provides unique advantages for drug development and marketing. We hope that these studies will result in the approval of new treatment for SCD, either as a single agent or in combination with hydroxyurea.

Treatment or prevention of VOC in SCD is the central aim of new therapies. They will permit a better quality of life and facilitate more normal educational or occupational activities and ultimately translate into a significantly longer life expectancy. The development of these novel therapies poses a significant challenge for the so-called curative therapeutic approaches, which will have to demonstrate substantial advantages in cost and short-term and long-term toxicities to be considered as a serious, realistic option. These

improved, noncurative therapeutic options also provide unexpected ethical and moral challenges in considering interruption of pregnancy following prenatal diagnosis of SCD.

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TABLE I

Planned, ongoing or recently completed Phase 1 trials

Company	Drug	Mechanism	Indication	Route	Clintrials.gov	Status
HB F-Anti-sickling						
The Cleveland Clinic	Decitabine (Dacogen) and tetrahydrouridine	HbF induction	Prophylaxis HbSS, HbSβ^0, HbSβ$^+$, HbSC ≥18 years	Oral	NCT01685515	Open ASH 2014 # 90
Celgene	Pomalidomide (Pomalyst)	HbF induction	Prophylaxis HbSS, HbSβ^0 18 years > 60 years	Oral	NCT01522547	Completed
Novartis Pharmaceuticals	Panobinostat (Farydak)	HbF induction (HDAC inhibition)	Prophylaxis HbSS, HbSβ^0 ≥18 years	Oral	NCT01245179	Open
AesRx (Baxter International)	5-hydromethyl-2-furfural (AES-103)	Increased O_2 affinity	Prophylaxis HbSS 18 years ≥ 65 years	Oral	NCT01597401	Completed
Prolong Pharmaceuticals	PEGylated carboxyhemoglobin bovine (Sanguinate)	O_2 and CO delivery (oxygenation and anti-inflammation)	Prophylaxis Healthy 18 years ≥ 45 years	IV	NCT01847222	Completed; ASH 2014 #1372
Prolong Pharmaceuticals	PEGylated carboxyhemoglobin bovine (Sanguinate)	O_2 and CO delivery (oxygenation and anti-inflammation)	Prophylaxis HbSS, HbSβ^0, HbSβ$^+$, HbSC 18 years ≥ 65 years	IV	NCT01374165	Recruitment suspended
Prolong Pharmaceuticals	PEGylated carboxyhemoglobin bovine (Sanguinate)	O_2 and CO delivery (oxygenation and anti-inflammation)	Prophylaxis HbSS ≥18 years	IV	NCT01848925	Completed
Invenux, LLC	SCD-101	Inhibition of sickling	Prophylaxis HbSS, HbSβ^0 18 years ≥ 55 years	Oral	NCT02380079	Open
Global Blood Therapeutics	GBT440 (formerly GTx011)	Increased O_2 affinity	Prophylaxis Healthy, 18 years ≥ 55 years HbSS, 18 years ≥ 60 years	Oral	NCT02285088	Open; ASH 2014 # 217 (mouse) and #1370 (in vitro)
Anti-inflammatory						
NKT Therapeutics	NKTT120	Anti-inflammation	Prophylaxis HbSS, HbSβ^0 18 years ≥ 60 years	IV	NCT01783691	Completed; ASH 2014 #2718
Children's Hospital Medical Center	Zileuton (ZYFLO)	Anti-inflammation	Prophylaxis HbSS, HbSβ^0, HbSβ$^+$, HbSC ≥12 years	Oral	NCT01136941	Completed; [12]

Company	Drug	Mechanism	Indication	Route	Clintrials.gov	Status
NHLBI	Regadenoson (Lexiscan)	Anti-inflammation	VOC diagnosis HbSS, HbSβ^0 18 years ≥ 70 years	IV	NCT01566890	Completed; [13]
Pfizer	PF-04447943	Phosphodiesterase 9 inhibitor	Prophylaxis HbSS, HbSβ^0 18 years ≥ 70 years	Oral	NCT02114203	Open
Vanderbilt University	Budenoside	Anti-inflammation	Post-ACS prophylaxis HbSS, HbSβ^0 1 years > 4 years	Inhaled	NCT02187445	Open
Anti-adhesion						
University of Miami and Duke University	Propranolol	Anti-adhesion	Prophylaxis HbSS, HbSβ^0 7 years ≥ 17 years	Oral	NCT02012777	Open
University of Miami and Duke University	Propranolol	Anti-adhesion	Prophylaxis HbSS, HbSβ^0 ≥18 years	Oral	NCT01077921	Completed
Albert Einstein College of Medicine	Immune Globulin (Gamunex-C)	Anti-adhesion	Acute VOC treatment HbSS, HbSβ^0, HbSβ$^+$ 12 years ≥ 65 years	IV	NCT01757418	Completed; [106]
University Hospitals Case Medical Center	Sodium bicarbonate	Alkali therapy	Low serum bicarbonate levels treatment HbSS ≥18 years	Oral	NCT01894594	Open
Other						
Sangart	MP4CO (pegylated carboxyhemoglobin, > 90% CO-Hb saturation)	Anti-sickling and vascular stasis	Prophylaxis HbSS, HbSβ^0 ≥18 years	IV	NCT01356485	Completed Phase Ib Discontinued
NHLBI	Topical sodium nitrite cream	Increased blood flow	Leg ulcers treatment HbSS, HbSβ^0, HbSβ$^+$, HbSC ≥18 years	Topical	NCT01316796	Completed; [14]

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TABLE II

Planned, ongoing or recently completed Phase 2 trials

Funding	Drug/Company	Mechanism	Indication	Route	Clintrials.gov	Status
HB F-Anti-sickling						
NHLBI	Decitabine	HbF induction	Prophylaxis HbSS, HbSβ^0, HbSC ≥18 years	IV	NCT01375608	Open
Hema-Quest Pharmaceuticals	2,2-dimethylbutyrate (HQK-1001)	HbF induction	Prophylaxis HbSS, HbSβ^0 12 years ≥ 60 years	Oral	NCT01601340	Completed; [15]
Baxter International Inc.	5-hydromethyl-2-furfural (AES-103)	Increased O_2 affinity	Prophylaxis HbSS 18 years ≥ 60 years	Oral	NCT01987908	Open
Dana Farber Cancer institute	Vorinostat (Zolinza)	HbF induction	Prophylaxis HbSS, HbSβ^0 ≥18 years	Oral	NCT01000155	Discontinued
Prolong Pharmaceuticals	PEGylated carboxyhemoglobin bovine (Sanguinate)	O_2 and CO delivery (oxygenation and anti-inflammation)	Acute VOC treatment HbSS, HbSβ^0, HbSβ$^+$, HbSC ≥12 years	IV	NCT02411708	Not yet open
Baylor College of Medicine	Hydroxyurea	HbF induction; Rheological improvement	Prophylaxis HbSC 5 years ≥ 21 years	Oral	NCT02336373	Open (SCYTHE)
Baylor College of Medicine	Hydroxyurea ± Phlebotomy	HbF induction; Rheological improvement	Prophylaxis HbSC 18 years ≥ 69 years	Oral	N/A	Not yet open
Anti-adhesion, hemolysis						
Novartis (Selexys)	SelG1	Anti-P-selectin	Prophylaxis HbSS, HbSβ^0, HbSβ$^+$, HbSC 16 years ≥ 65 years	IV	NCT01895361	Open (SUSTAIN)
Vanderbilt University	Montelukast (Singulair)	Anti-adhesion	Prophylaxis HbSS, HbSβ^0 16 years ≥ 60 years	Oral	NCT01960413	Open
St. Louis University	Abciximab (ReoPro)	Anti-adhesion	Acute VOC treatment HbSS, HbSβ^0, HbSC 5 years ≥ 25 years	IV	NCT01932554	Discontinued
Astra Zeneca	Ticagrelor (Brilinta)	PLT aggregation inhibition	Prophylaxis HbSS, HbSβ^0 2 years ≥ 18 years	Oral	NCT02214121	Open
University of North Carolina	Rivaroxaban (Xarelto)	Anticoagulant	Prophylaxis HbSS, HbSβ^0 18 years ≥ 55 years	Oral	NCT02072668	Open by invitation only

Funding	Drug/Company	Mechanism	Indication	Route	Clintrials.gov	Status
Dilaforette AB (Sweden) Ergomed plc (UH)	Sevuparin	Anticoagulant	Acute VOC treatment	IV	N/A	Study design phase
University of Pittsburgh	Unfractionated heparin	Anticoagulant	Acute Chest Syndrome treatment HbSS, HbSβ^0, HbSC 18 years ≥ 20 years	IV	NCT02098993	Open
Boston Children's Hospital	Inhaled NO	NO repletion	Acute VOC treatment HbSS, HbSβ^0, HbSC 9 years ≥ 22 years	Inhaled	NCT00142051	Discontinued
Wake Forest School of Medicine	Beet Juice (Unbeetable)	NO repletion	Prophylaxis HbSS, HbSβ^0 19 years ≥ 65 years	Oral	NCT02162225	Open
Bio Products Laboratory (UK)	BPL Haptoglobin	Free Hb depletion	Prophylaxis	IV	N/A	EU Orphan drug designation 2011
University of North Carolina	Eptifibatide (Integrilin)	PLT aggregation inhibition	Acute VOC treatment HbSS, HbSβ^0, HbSC 18 years ≥ 55 years	IV	NCT00834899	Discontinued; [16]
Anti-inflammatory						
NHLBI	Regadenoson (Lexiscan)	Anti-inflammation	Acute VOC treatment HbSS, HbSβ^0 10 years ≥ 70 years	IV	NCT01788631	Open
Children's Hospital & Research Center	Simvastatin (Zocor)	Anti-inflammation	Prophylaxis HbSS, HbSβ^0 ≥10 years	Oral	NCT01702246	Open
University of North Carolina	Atorvastatin (Lipitor)	Anti-inflammation	Prophylaxis HbSS, HbSβ^0 18 years ≥ 60 years	Oral	NCT01732718	Open by invitation only
	Fish oil (Lovaza)	Anti-inflammation	Prophylaxis	Oral	NCT01202812	Unknown
Thomas Jefferson University, Mount Sinai School of Medicine	Inhaled Mometasone Furoate	Anti-inflammation	HbSS, HbSβ^0 10 years >19 years Acute VOC treatment HbSS, HbSβ^0 ≥15 years	Inhaled	NCT02061202	Open
NKT Therapeutics	NKTT120	Anti-inflammation	Prophylaxis	IV	N/A	Study design phase
Boston University Medical Center	Macitentan (Opsumit)	Endothelin blocker	Pulmonary arterial hypertension treatment	Oral	NCT02126943	Open
Children's Hospital Medical Center	Losartan (Cozaar)	Reduction of intra-glomerular pressure	Sickle nephropathy prophylaxis and/or treatment HbSS, HbSβ^0 ≥6 years	Oral	NCT01479439	Open
University of Alabama at Birmingham	Losartan (Cozaar)	Reduction of intra-glomerular pressure	Sickle nephropathy prophylaxis HbSS, HbSβ^0 6 years ≥ 19 years	Oral	NCT02373241	Open

Funding	Drug/Company	Mechanism	Indication	Route	Clintrials.gov	Status
Lagos State University	Sorghum bicolor (Jobelyn)	Anti-oxidant	Prophylaxis HbSS 14 years ≥ 40 years	Oral	NCT01704794	Open

TABLE III

Currently planned, ongoing, or recently completed Phase 3 trials

Company	Drug	Mechanism	Indication	Route	Enrollment	Clintrials.gov	Status
Eli Lilly & Daiichi Sankyo	Prasugrel (Effient)	Platelet inhibition	Prophylaxis	Oral	240, HbSS, HbSβ^0 2 years ≥ 17 years	NCT01794000	Open
Emmaus Medical	L-glutamine vs placebo	Anti-oxidant	Prophylaxis	Oral	230, HbSS, HbSβ^0 ≥5 years	NCT01179217	Completed; ASH 2014 # 86
MAST Therapeutics	MST-188	Anti-adhesion	Acute VOC treatment	IV	388, HbSS, HbSβ^0, HbSβ$^+$, HbSC 4 years ≥ 65 years	NCT01737814	Open (EPIC)
GlycoMimetics	GM-1070 (Rivipansel)	Anti-P-selectin	Acute VOC treatment	IV	350, HbSS, HbSβ^0, HbSβ$^+$, HbSC ≥6 years	NCT02187003	Phase 2 completed; [100]
BC Children's Hospital and University of BC, Vancouver	$MgSO_4$	Vasodilation? Pain reduction?	Acute VOC treatment	IV	120 (98 enrolled), HbSS, HbSβ^0, HbSβ$^+$, HbSC 4 years ≥ 18 years	NCT00313963	Completed (MAST); [17]
PECARN (Pediatric Emergency Care Applied Research Network)	MgSO4	Vasodilation? Pain reduction?	Acute VOC treatment	IV	208 HbSS, HbSβ^0, HbSβ$^+$, HbSC 4 years ≥ 21 years	NCT01197417	Completed (MAGIC); ASH 2014 # 88
Academisch Medisch Centrum - Universiteit van Amsterdam	N-Acetylcysteine	Anti-oxidant	Prophylaxis	Oral	140, HbSS, HbSβ^0, HbSβ$^+$, HbSC ≥12 years	NCT01849016	Open
Duke University Medical Center	Apixaban (Eliquis)	Anticoagulant	Prophylaxis	Oral	60, HbSS, HbSβ^0, HbSC 18 years ≥ 80 years	NCT02179177	Not yet open

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TABLE IV

Compounds in preclinical development

Company	Drug	Mechanism	Indication/Route	Other	Status
Acetylon Pharmaceuticals	HDAC ½ inhibition	HbF induction		ASH 2014 #335	
EpimedX	EdX-17	HbF induction (ferritin heavy chain activation)		ASH 2014 #1357	
Errant Gene Therapeutics	Trichosic	HbF induction			Early stage of development
Phoenicia Biosciences & NCATSTRND	PB-04	HbF induction	Prophylaxis	ASH 2014 #2687 for other targets	
Isis Pharmaceuticals	Antisense nucleotides	HbF induction via KLF1 reduction		ASH 2014 # 4038	
NIH (academic, U Chicago	RN-1	Inhibition of LSD1, a repressor of gamma-globin expression	SC	ASH 2014 #336	Baboon-study shows HbF induction
Global Blood Therapeutics	GTx011	Increase O_2 affinity	ORAL	ASH 2014 #1370	Sickle mouse study ASH 2014 # 217
Virginia Common- wealth And CHOP	INN-270 and TD-7	Increase O_2 affinity			Sickle mouse study open
Erytech Pharma	Enhoxy	O_2 delivery			EU Orphan drug designation 2012
Biogen Idec	Tysabri (Natalizumab)	Anti-adhesion	IV	ASH 2014 #221	Approved for relapsing MS
Archemix	ARC5690	Aptamer anti-adhesion	IV		In liquidation
Biogen Idec	TYSABRI, Natalizumab	VLA4 blocker, anti-adhesion	Treatment IV		Approved for relapsing MS & Crohn's disease
Acceleron Pharma	Luspatercept (ACE-536)	Ligand trap TGFbeta superfamily	SC	ASH 2014 #4055	Phase 2 in MDS and Beta-thalassemia intermedia
ReveraGen Biopharma	VBP15	Anti-inflammation (like prednisolone, NF-kB inhibition, fewer side effects)	ORAL		Studies in Duchenne muscular dystrophy
Biogen Idec	Tecfidera ® (Dimethyl fumarate)	Anti-inflammation	Prophylaxis ORAL		Approved for relapsing MS
Addex Therapeutics	TFNR1 NAM A2a PAM	A2AR, Anti-inflammation	ORAL		Studies in Parkinson's disease
Advinus Therapeutics	PNQ103	AQ2BAR antagonist	ORAL		Ex-vivo studies on SCD RBCs
Pfizer	PF-04447943	Phosphodiesterase 9 inhibitor	ORAL	ASH 2014 #2694	Unsuccessful in Alzheimer's trial. Reduced vaso-occlusion in SS mouse
Duke University	TBD	MEK1/2 and ERK1/2 inhibition			[170]
Alnylam Pharmaceuticals	ALN-TMP	RNAi *Tmprss6*, to reduce Fe load	Iron overload SC		Studies in Beta-thalassemia

TABLE V

Cell-based therapies: applications to sickle cell disease

Company	Protocol/agent	Mechanism	Clintrials. gov	Enrollment target	Status
		Gene Therapy			
Blue Bird Bio	LentiGlobin BB305 Drug Product	Gene therapy	NCT02140554	8 HbSS, HbSβ^0 ≥ 18 years	Open
Blue Bird Bio	LentiGlobin BB305 Drug Product	Gene therapy	NCT02151526	7 HbSS, HbSβ^0 Beta-thalassemia major 5 years ≥ 35 years	ASH 2014 # 549 & 4797
Children's Hospital LA	Lenti/βAS3-FB lentiviral vector Transduction to express an antisickling (βAS3) gene	Gene therapy	NCT02247843	6 HbSS, HbSβ^0 ≥ 18 years	Open
Children's Hospital Medical Center, Cincinnati	Gamma Globin Lentivirus Vector-mediated gene transfer	Gene therapy	NCT02186418	10 Severe SCD 18 years ≥ 35 years	Open
Errant Gene therapeutics	Lentiviral vector, TNS 9.55.3	Gene therapy	N/A	N/A	Open Phase 1 in Beta-thalassemia (NCT01639690)
OrphageniX	Targeted Gene Alteration (TGA)	Sickle gene repair	N/A	N/A	Preclinical
Sangamo Biosciences (and Biogen Idec?)	ZFN-mediated gene correction	Sickle gene repair	N/A	N/A	Preclinical
Editas Medicine	CRISPR-Cas9 & TALENs	Sickle gene repair-orincrease HbF	N/A	N/A	Preclinical
Boston Children's Hospital	BCL11A knockdown	HbF induction	N/A	N/A	Not yet open
		HSC Transplant			
Cellerant Therapeutics	CLT-001	Purify stem cells for HSCT	N/A	N/A	Discontinued
Gamida Cell Ltd	Nicotinamide (NiCord®)	Expand stem cells, HSCT	NCT01590628	10 HbSS, HbSβ^0, HbSC2 years ≥ 21 years	Open
Morphogenesis	SCPF	Expand stem cells	N/A	N/A	Discontinued
Assistance Publique -Hôpitaux de Paris	Plerixafor	Mobilization of stem cells	NCT02212535	5 HbSS, HbSβ^0 ≥ 18 years	Not yet open
University of Louisville, KY	Alemtuzumab based conditioning	Stable Mixed chimerism induction	NCT01419704	30 hemoglobinopathies, bone marrow failure syndromes ≤ 45 years	DIscontinued
Masonic Cancer Center, University of Minnesota		Stable Mixed chimerism induction	NCT00176852	30HbSS, HbSβ^0, Beta-thalassemia major	Open but not recruiting

Company	Protocol/agent	Mechanism	Clintrials. gov	Enrollment target	Status
				≤ 50 years	
Hackensack University Medical Center	Alemtuzumab, fludarabine, and melphalan	Reduced intensity HSCT	NCT01877837	40 HbSS, HbSβ^0, HbSβ$^+$, HbSC 2 years ≥ 30 years	Open
University of Illinois at Chicago	Alemtuzumab, 300 cGy TBI, and sirolimus	Non-Myeloablative HSCT	NCT01499888	15 HbSS, HbSβ^0, HbSC 18 years ≥ 60 years	Open
University of Illinois at Chicago	Alemtuzumab, 300 cGy TBI, post-SCT cyclophosphamide and sirolimus	HLA-Haploidentical HSCT	NCT02013375	110 Severe SCD 16 years ≥ 60 years	Open
Sidney Kimmel Comprehensive	Fludarabine, cyclophosphamide, and, post-SCT sirolimus	Partially HLA-Mismatched and	NCT00489281	50 Severe SCD 2 years ≥ 70 years	Open
Emory University & NHLBI	Busulfan, fludarabine, anti-thymocyte globulin	Reduced intensity	NCT01565616	15 Severe SCD 16 years ≥ 40 years	Open (STRIDE)
University of Texas Southwestern Medical Center	Low dose irradiation, Alemtuzumab and sirolimus.	Non-Myeloablative HSCT, Mobilized peripheral stem cells	NCT02038478	50 Severe SCD 18 years ≥ 45 years	Open
University of Pittsburgh		Reduced intensity	NCT01962415	30 2 months ≥ 35 years	Open
Fred Hutchinson Cancer Research Center	Treosulfan and fludarabine, ± low dose irradiation	HSCT	NCT00919503	68 ≤ 54 years	Open
New York Medical College	Hydroxyurea, azathioprine, fludarabine, busulfan, thiotepa, cyclophosphamide, and rabbit ATG	Haploidentical T cell- depleted HSCT	NCT01461837	35 Severe SCD 2 years ≥ 21 years	Open
NHLBI	Low dose irradiation, alemtuzumab, sirolimus, and cyclophosphamide	Non-Myeloablative Haploidentical Mobilized peripheral stem cells	NCT00977691	192 Sever SCD and Beta-Thalassemia ≥ 2 years	Open
NHLBI	Low dose radiation (300 rads), oral cyclophosphamide, pentostatin, and sirolimus	Non-Myeloablative Mobilized peripheral stem cells	NCT02105766	142 HbSS, HbSβ^0, 16 years ≥ 80 years Up to 142	Open
NHLBI	Low dose radiation, Alemtuzumab and Sirolimus	HSCT	NCT00061568	124 Severe SCD 2 years ≥ 65 years	Open
Case Comprehensive Cancer Center	Fludarabine	HSCT	NCT02065596	25 subjects HbSS, HbSβ^0, HbSβ$^+$, HbSC ≥ 18 years	Not yet open
Vanderbilt-Ingram Cancer Center	Thymoglobulin, fludarabine cyclophosphamide sirolimus, 200 cGy TBI	Non-myeloablative partially HLA matched and fully HLA matched	NCT01850108	10 Severe SCD 2 years ≥ 70 years	Open

TABLE VI

Currently planned, ongoing, or recently completed clinical trials on treatment of pain in sickle cell disease

Company	Drug	Design	Indication	Phase	Enrollment	Endpoints	Clintrials.gov	Status
Duke University (NHLBI)	Morphine Sulfate or Hydromorphone	Randomized Patient-specific vs. standard dosing	Acute VOC treatment	4	77 HbSS, HbSβ^0, HbSβ$^+$, HbSC ≥21 years	Pain scores	NCT02222246	Not yet open
Children's Healthcare of Atlanta	Morphine or Nubain	Randomized double blind	Acute VOC treatment	3	40 HbSS, HbSβ^0, HbSβ$^+$, HbSC 6 years > 19 years	Pain control acute chest syndrome	NCT01380197	Open but not recruiting; (COMPARE)
University Hospital Case Medical Center	Music therapy vs. Music Listening vs. no intervention	Randomized	Prophylaxis		120 HbSS, HbSβ^0, HbSβ$^+$, HbSC ≥18 years	Pain intensity, relief and mood	NCT02270060	Open
Centre Leon Berard	Lidocaine 5% plaster (versatic 5%)	Open-label	Acute VOC treatment	2	39 6 years > 21 years	Pain scores	NCT01314300	Completed
St. Jude Children's Research Hospital	Gabapentin vs. placebo	Randomized Double blind	Acute VOC treatment	2	190 1 years > 20 years	Pain scales	NCT01954927	Open
University of California, San Francisco (NHLBI)	Vaporized cannabis (4.7% THC/5.1% CBD) vs. placebo	Randomized Crossover Double blind	Chronic pain treatment	1–2	35 HbSS, HbSβ^0, HbSβ$^+$, HbSC ≥18 years	Pain scores	NCT01771731	Open
Montefiore Medical Center	Intranasal fentanyl citrate	Randomized Double blind	Acute VOC treatment	4	200 HbSS, HbSβ^0, HbSβ$^+$, HbSC 3 years > 21 years	Pain scores	NCT01482091	Open
Columbia University	Nitrous oxide 50%	Non-randomized, open label	Acute VOC treatment	2	12 HbSS, HbSβ^0, HbSβ$^+$, HbSC 8 years > 18 years	Pain scores	NCT01891812	Open
Nationwide Children's Hospital, Columbus, OH	Warmed saline	Randomized, open label	Acute VOC treatment		80 HbSS, HbSβ^0, HbSβ$^+$, HbSC 4 years > 21 years	Rate of hospital admission	NCT02316366	Completed
Brooklyn Hospital Center	IV Ketamine	Double blind, placebo-controlled	Acute VOC treatment	2/3	106 HbSS, HbSβ^0, HbSβ$^+$, HbSC	Pain Scores and	NCT02417298	Open

Company	Drug	Design	Indication	Phase	Enrollment	Endpoints	Clintrials.gov	Status
					≥18 years	admission rates		
Makerere University, Kampala Uganda	Low-dose Ketamine vs morphine	Double blind	Acute VOC treatment	4	240 HbSS, HbSβ^0, HbSβ$^+$, HbSC 7 years > 18 years	Pain scores	NCT02434939	Open

TABLE VII

Studies on pharmacological treatment of iron overload in sickle cell disease

Company	Drug	Design	Criteria	Phase	Enrollment	Endpoints	Clintrials.gov	Status
ApoPharma	Deferiprone (Ferriprox) vs deferoxamine (Desferal)	Randomized open label	≥6 years; Baseline LIC > 7 mg/g dw	4	300	Liver iron concentration	NCT02041299 UCTR2013-002181-39-GB	Open (FIRST)
Consorzio per Valutazioni Biologiche e Farmacologiche (EC)	Deferiprone (Ferriprox) vs Deferasirox (Exjade)	Randomized open label	1 month< 18 years	3	344	Ferritin and cardiac iron concentration	NCT01825512	Not yet open
Shire plc (previously Ferrokin Biosciences)	Deferitazole (SPD602 or SSP-004184)	Non-randomized open label (NCT01927913 randomized open label)	Variable	1 and 2	N/A	Liver iron concentration	NCT01363908 NCT01604941 NCT01671111 Withdrawn: NCT02065401 NCT01927913	All trials discontinued July 2014

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