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## Emerging drugs for sickle cell anemia.

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## **Emerging Drugs for Sickle Cell Anemia**

### **Abstract**

**Introduction:** Sickle cell anemia (SCA) is caused by a single point mutation, which results in chronic vasculopathy and end organ damage. Over the past decades, hydroxyurea (HU) has been the standard treatment and therapies aimed at increasing fetal hemoglobin (Hb) F were the focus of research. Our increased understanding of the complex pathophysiology of SCA has led to the development of novel targets involved in vaso-occlusion.

**Areas covered:** In this review, we describe the pathophysiology of sickle cell disease (SCD) and provide an in-depth analysis of the current and new pharmacologic therapies in the field.

**Expert opinion:** SCA is a heterogeneous disease that has caused tremendous global morbidity and early mortality. More effective, individualized and inexpensive therapies are needed. New therapies targeting multiple pathways in its complex pathophysiology are under investigation.

**Keywords:** Hydroxyurea, Sickle cell, Sickle cell anemia, Sickle cell disease, Sickle cell therapy, Sickle cell treatment

## 1. Background

Sickle cell anemia is among the most common inherited hemolytic anemia, and affects an estimated 70,000–100,000 persons in the United States and likely millions worldwide.

The true global incidence of SCD is unknown. The World Health Organization has estimated that each year 220,000 babies are born with SCD in Africa, and that SCD accounts for up to 16% of deaths of children aged <5 years in some African countries [1, 2]. The reported prevalence of the sickle cell trait in African Americans varies from 6.7% to 10.1% and in Africans the range is from 10 to 40% across equatorial Africa and decreases to between 1 and 2% on the North African coast and less than 1% in South Africa [3-5]. The prevalence of the sickle cell trait varies widely worldwide and may be as high as 50% in certain regions [5-7]. The prevalence of SCA is approximately 1 in 600 newborn African American infants and 150,000 to 300,000 newborn Africans [8-10].

Sickle cell anemia is a hereditary disorder of Hb where the sickle gene is inherited, homozygously, from both parents. The sickle mutation is the result of a single base change (GAT→GTT) in the sixth codon of exon 1 of the  $\beta$ -globin gene responsible for the synthesis of the  $\beta$ -globin polypeptide of the Hb molecule ( $\alpha_2\beta_2$ ). This change I, in turn, results in replacement of a normal glutamic acid with valine at position 6 of the  $\beta$ -globin chain and the formation of sickle Hb. Sickle erythrocytes are rigid with decreased deformability and reduced life span resulting in hemolysis, vaso-occlusive disease, vasculopathy and subsequent inflammation and end organ damage [11, 12].

Clinical manifestations of SCD includes pain syndromes, anemia and its sequelae, organ failure including infection/inflammation and co-morbid conditions [13]. Despite having a common genetic basis and similar pathophysiology, individual patients with SCA have a highly variable clinical phenotype. Clinical care for affected individuals has been mostly supportive, including red blood cell transfusions (RBC), iron chelation, intravenous hydration, analgesics and antibiotics. The development of drugs to increase fetal Hb has been the major therapeutic strategy in the treatment of this disorder. Although new fetal Hb–modulating agents are being studied, only HU has shown long-term benefit and remains the only United States Food and Drug Administration (FDA) approved therapy for SCA since 1998 [14]. The major aim of this review is to describe emerging pharmacologic therapies for SCA.

## **2. Medical Need**

The ideal drug for SCD would have analgesic properties, be able to prevent vaso-occlusive pain crises (VOCs) or abort with a rapid onset of action, would decrease the severity and frequency of VOCs, have limited hazardous side effect profile and be effective in all patients, and available globally. Currently HU is the only agent that fits certain of these criteria. It decreases the frequency of VOCs, acute chest syndrome (ACS), and thus improves quality of life and mortality in those with SCD. However HU is not effective in about 25% of those with SCD and may be teratogenic and possibly carcinogenic [15]. Management of patients with SCD in VOCs includes additional supportive measures with intravenous fluids, and analgesia and prophylaxis against thromboembolism when they are hospitalized.

### 3. Existing treatment

Current approaches to management of SCD include supportive therapies such as folic acid, immunization and psychosocial support; symptomatic treatment which involves analgesics and blood transfusion; abortive therapy such as nitric oxide (NO); curative therapies such as bone marrow transplantation and gene therapy [13, 16]. Preventative therapy is another current approach to the management of SCD which involves prophylactic antibiotics for infants and children, immunization, avoidance of known precipitating factors, cellular rehydration and HU for the induction of Hb F [13, 16-21].

Hydroxyurea was identified as a potent Hb F inducer and was subsequently found to be both a feasible and effective treatment option for SCA [12]. The mechanisms by which HU induces Hb F production are not fully clear. It was felt to inhibit ribonucleotide reductase, an intracellular enzyme that converts ribonucleotides to deoxyribonucleotides, necessary for DNA synthesis and repair. More complex effects involve the production of NO, guanylyl cyclase and cGMP-dependent protein kinase pathway important in inducing expression of the  $\gamma$ -globin gene. Additionally HU improves erythrocyte deformability, lowering of circulating leukocytes and reticulocytes, and reduces hemolysis [2, 14, 22]. Since its first clinical application reported in 1984 by Platt and colleagues many trials were performed [18]. The Multicenter Study of Hydroxyurea in SCA (MSH), a placebo controlled randomized phase III trial of 299 adults with severe SCA, which terminated early due to significant reductions in frequency of VOC, ACS, need for blood transfusion and delayed onset of first VOC [23, 24]. This study led to the FDA approval of HU for therapy in severely affected adults with SCA in 1998. Last year

witnessed the culmination of a decade-long Phase III clinical trial (BABY HUG) involving infants with SCA randomized either to HU (fixed dose 20 mg/kg/day) or placebo. BABY HUG showed that HU did not clearly prevent organ damage in a 2-year treatment period, but significantly decreased pain, ACS, hospitalizations, and transfusions in children [25-30].

Additionally, some of the current approaches to the management of SCD could be pharmacologic or non-pharmacologic especially when it comes to pain management. Examples of non-pharmacologic treatments include meditation, therapeutic massage, transcutaneous electrical nerve stimulation, heat and cold packs, distraction, relaxation, music, guided imagery, self-hypnosis, acupuncture and biofeedback [13, 16]. Current examples of pharmacologic therapies include the use of NSAIDs, opioids, adjuvants, steroids, etc [13].

#### **4. Market review**

Sickle cell disease is a global disease affecting millions of people worldwide and hundreds of thousands in the United States. It not only affects those of African descent, but also persons of Middle Eastern, Indian, Latin American, and Mediterranean descent. It has received very little attention and even less research funding. National Institute of Health (NIH) grants for sickle cell research was much less than that for less common inherited diseases. In 1972, the National Sickle Cell Anemia Control Act (NSCACA) was signed, which paved the way for more research funding and established screening and

education programs. The NIH dedicated \$10 million to be spent on SCD research at that time [13].

The economic burden to patients with SCD is significant [31-35]. Many patients are living in poverty with their illness due to chronic pain, and physical disability limiting their ability to work and contribute to society [13]. The economic burden on society was estimated at \$1.1 billion in 2009 [31]. This number is projected to increase as patients with SCD are living longer as we continue to improve supportive care. A solution to this problem is not simple requiring multidisciplinary action with increased funding, legislation, research and supportive services. Simple therapy with HU is still not available to the millions in Africa today. As we continue to push for new therapies for SCD, HU has tremendous potential on the global market.

## **5. Current research goals**

Hydroxyurea has many qualities of the ideal agent for SCD and it is felt to be successful primarily through its actions of increasing fetal Hb. Increased fetal Hb has been associated with a less severe phenotype, which has spurred interest in the identification of pharmacologic agents capable of inducing Hb F expression. The sickle cell population has varied phenotype, which makes targeted therapy more attractive to achieve better outcomes. There are fewer patients with SCD in the United States where most clinical trials are available. There are also challenges with clinical trial enrollment since patients are impoverished and in pain limiting their abilities to effectively participate in a clinical trial. Research focus is changing to incorporate a multi-agent approach to treating patients and in re-engineering clinical trial design.

## 6. Scientific rationale

Since sickle cells were first described in 1910 and mutation causing abnormal Hb S was identified in 1949, the complex mechanism underlying its pathophysiology continues to evolve [36]. A cascade of events driven by endothelial damage and inflammation leads to vasculopathy. The inciting event is injury to the RBC membrane. Hemoglobin S polymerization impairs deformability of the RBC and causes oxidative injury and destruction of the RBC. Red blood cell injury exposes phosphatidyl serine and releases Hb and other intracellular contents. This in turn depletes NO, increases endothelial adherence, releases pro-inflammatory cytokines and activates the coagulation cascade causing ischemia, reperfusion injury and vascular damage [11, 15, 36].

Damaged sickle cells are prone to adhere to the endothelium by adhesion molecules. The RBC membrane receptors VLA-4/ $\alpha_4\beta_1$  bind to endothelial receptors directly to vascular cell adhesion molecule 1 (VCAM-1) and interacts with subendothelial matrix proteins (BCAM/LU,  $\alpha_4\beta_1$  with the laminin and VWF) [37, 38]. Red blood cell interactions with the vascular endothelium also lead to the production of oxygen radicals by activating transcription factor NF- $\kappa$ B. Nuclear factor kappa-light-chain-enhancer of activated B cells up-regulates the production of endothelial adhesion molecules such as E-selectin, VCAM-1, and intracellular adhesion molecule-1 (ICAM-1). P-selectin and E-selectin on endothelial cells have been suggested to participate in VOC [39, 40]. In preclinical studies an anti-P-selectin molecule showed increased microvascular flow and reduced adhesion of leukocytes to the endothelium [39]. Intracellular adhesion molecule-4



(ICAM-4), another RBC membrane protein, participates in adhesion can be activated by epinephrine to adhere to endothelial membrane and exacerbate vasoocclusive disease and also increased leukocyte adhesion to endothelium [40]. When treated with Propranolol (a  $\beta$ -adrenergic receptor antagonist) VOCs were diminished [41, 42]. In addition to adherence to endothelial cells, RBCs in SCA also adhered strongly to leukocytes in veno-occlusive crises via interactions with P-selectin and E-selectin. This interaction is propagated by TNF-alpha. Selectins function in adhesion to the vessel wall by recruiting rolling particles and cells and also contribute to cell activation.

Patients with SCD have chronic elevation of proinflammatory cytokines at baseline, including C-reactive protein, TNF, IL-1, IL-8. Damaged RBCs, activated endothelial cells, leukocytes and platelets contribute to a proinflammatory environment. Sickled RBCs stimulate endothelial cells to release TNF-alpha and IL1-beta. There is increased production of placental growth factor, which activates monocytes to release reactive oxygen species which enhances inflammation. Additionally invariant natural killer T (iNKT) cells are activated in patients with SCD suggesting that iNKT cells may play a critical role in mediating inflammation. Intravascular hemolysis results in release of cell free Hb in plasma, and heme release that contribute to the inflammation [38, 43].

Nitric oxide is produced by the endothelium from arginine and causes vasodilation by binding to endothelin-1, a vasoconstrictor. Intravascular hemolysis releases Hb, which scavenges NO in the plasma and subendothelial spaces. Depletion of NO leads to vasoconstriction and formation of reactive oxygen species. Nitric oxide also

downregulates adhesion molecules, (VCAM-1), ICAM-1 and E-selectin. Erythrocyte arginase released during hemolysis decreases arginine levels and decreases NO production. The byproducts of these reactions: urea, proline, polyamines and free radicals cause vascular remodeling and vasculopathy. Patients with SCD have elevated asymmetric dimethylarginine, which inhibits arginine transport, promotes endothelial dysfunction [15, 44, 45].

These inflammatory processes activate the coagulation cascade. Phosphatidylserine expression on RBC surface and microparticles activates tissue factor and in turn, the extrinsic coagulation cascade. Tissue factor also promotes inflammation and endothelial damage. In preclinical studies in transgenic sickle mice, lowering tissue factor levels resulted in lower plasma levels of IL-6 and soluble VCAM-1 [46].

Sickle cell disease is a chronic inflammatory state and reactive oxygen species (ROS) are increased at baseline compared with normal controls. Hemolysis releases Hb, and iron products, which increase ROS that generate superoxide ( $O_2^-$ ) and peroxynitrate ( $ONOO^-$ ), which promotes an inflammatory response and causes cell death. Patients with SCD have impaired buffer system with decreased glutathione, and other antioxidants [47-49].

## **7. Competitive environment**

### **7.1 Fetal Hemoglobin Inducing agents**

Two novel Hb F-inducing agents, pomalidomide and sodium dimethylbutyrate , are

promising targets to increase fetal Hb. Pomalidomide is an orally active thalidomide analogue. Preclinical studies showed that it induced Hb F production in a SCD model with similar efficacy as HU. Surprisingly, pomalidomide improved erythropoiesis in comparison to myelosuppression seen with HU. However, when given in combination with HU, this effect was lost and fetal Hb levels were suppressed [50]. A phase I study of pomalidomide in SCD was completed. Twelve patients enrolled and data has not been published (clinicaltrials.gov, trial identification NCT01522547).

HQK-1001, sodium dimethylbutyrate is an orally-active short chain fatty acid derivative, under development by HemaQuest™ for the treatment of SCA and beta-thalassemia. It was found to increase fetal Hb and Hb levels in Phase I trials [51]. A randomized, placebo-controlled Phase II trial enrolled 77 patients with SCD in Canada, Egypt, Jamaica, Lebanon and the US and has been terminated. The primary objective was to evaluate for increase in fetal Hb levels. Interim analysis was expected in late 2013 and this data has not been yet been published. A phase III trial is expected in 2015 [51].

Additionally L-arginine, a substrate for NO, was evaluated in combination with HU in a small randomized trial of 21 adult patients with SCD. There was a greater response in fetal Hb levels and reticulocyte count in the group receiving combination therapy versus HU alone. This study suggests that fetal Hb synthesis depends on NO effect on erythroid progenitors [52].

Decitabine is an intravenous cytosine analog 5-aza-2'-deoxycytidine which

hypomethylates DNA by inhibiting DNA methyltransferase. It is approved for treatment of myelodysplastic syndrome. It increases fetal Hb by reactivating the silenced  $\gamma$ -globin through hypomethylation at its promoter site. In a small study of 8 patients refractory or intolerant to HU, it increased Hb F and Hb levels when administered subcutaneously [53]. Ongoing trials will further clarify its efficacy and tolerability. A phase II study with planned enrollment of 40 patients with high risk SCD is recruiting (NCT01375608). A phase I combination study of oral decitabine with tetrahydrouridine, a competitive inhibitor of cytidine deaminase, is also recruiting and its aim is to evaluate oral bioavailability of decitabine in combination therapy [54] (NCT 01685515).

Vorinostat, also known as suberanilohydroxamic acid (SAHA) is inhibitor of histone deacetylases (HDAC). Vorinostat is marketed under the name Zolinza™ for the treatment of cutaneous T cell lymphoma (CTCL). In preclinical studies, HDAC inhibitor trichostatin A (TSA) inhibited pulmonary vascular endothelial VCAM-1 and tissue factor expression, prevented vascular stasis in sickle mice, served as an iron chelator, and increased Hb F. Vorinostat, a TSA analogue exhibits the same effects [55]. Vorinostat is currently being evaluated in a Phase II clinical trial with target enrollment of 15 patients with SCD (NCT01000155).

## **7.2 Targeting adhesion**

Rivipansel sodium, GMI-1070, is a small molecule pan-selectin inhibitor that binds to E, P and L selectin that was developed by GlycoMimetics to target inflammation in sickle VOCs. It improves blood flow by inhibiting E-selectin and neutrophil activation. A randomized, double-blind, adaptive Phase II trial of rivipansel in pediatric SCD patients

with VOC of age 12-18yr is complete. There was >50% reduction in time to transition to oral pain medications, as well as a reduction in time to hospital discharge, in each case compared to standard treatment for pain was observed. Statistical significance was achieved for time to transition to oral pain medication, however results for time to discharge and time to resolution of VOC, the primary endpoint, were not statistically significant. Safety results for the pediatric patients were consistent with prior findings in treatment of adult patients [56].

A randomized, double-blind, placebo-controlled Phase II trial in 76 subjects hospitalized for sickle cell VOC assessing GMI-1070 is complete. Data showed that the patients treated with rivipansel sodium experienced reduction in duration of VOC, length of hospital stay and reduction in the use of opioids for pain relief. Both adult and pediatric patients demonstrated improvement and adverse event rates were comparable between rivipansel sodium and placebo (NCT01119833). Results of these studies have not yet been published in a peer-reviewed journal [57].

Intravenous immunoglobulin (IVIG) also inhibits leukocyte adhesion and activation by binding to Fc $\gamma$ RIII expressed on neutrophils [58]. A Phase I/II trial is currently recruiting to evaluate Gamunex (Intravenous Gammaglobulin) versus normal saline in sickle cell acute pain (NCT 01757418).

Endothelial P selectin is involved in microvascular blood flow. Heparin, in addition to its anticoagulant effects, decreases adhesion of sickled erythrocytes by inhibiting P-selectin. Two clinical trials are evaluating the effect of prophylactic dose low-molecular-weight heparin (LMWH) to ameliorate the vaso-occlusive process and unfractionated heparin in

ACS (NCT 01419977, NCT02098993).

In a randomized clinical trial of 253 patients, Tinzaparin, a low-molecular-weight heparin, showed reduced duration of VOC and no severe bleeding complications [59]. These results need to be validated in a multicenter study. A recent phase II trial of an oral P-selectin inhibitor (pentosan polysulfate sodium) similar to heparin but with greater P-selectin blocking ability than heparin showed improved microvascular flow in SCD patients in a phase 1 study [60]. The efficacy of SelG1, a humanized anti-P-selectin monoclonal antibody, in preventing VOC will be evaluated in an upcoming phase 2 trial in combination with HU (NCT01895361).

Studies suggest stress related adrenergic stimuli like epinephrine may contribute to vaso-occlusion. De Castro and colleagues conducted preclinical and phase I studies to evaluate the role of propranolol, a beta adrenergic blocker, in SCD.

Propranolol significantly reduced RBC adhesion in a dose-dependent manner. Adverse events were not severe, did not vary with the dose administered and no elevation in heart rate was noted. These results imply that  $\beta$ -blockers have a potential role in inhibiting RBC adhesion [61]. A phase 2 study of propranolol in SCD has been completed and no data has been reported at the time that this manuscript was written (NCT 01077921).

The importance of activated platelets on the vasculopathy of SCD have led to clinical trials with antiplatelet therapy [62]. Eptifibatide, a synthetic GPIIb/IIIa peptide

antagonist which inhibits platelet aggregation, was initially developed to treat acute arterial thrombosis. It was launched in the US in 1998 as Integrillin to treat angina, acute coronary syndromes and for use in percutaneous coronary angioplasty and it is approved in Europe. It has decreased platelet activation and increased vasodilation in the sickle cell population in clinical trials [63]. A placebo controlled phase 2 trial was completed. Thirteen patients with SCD were randomized to receive either eptifibatide or placebo. There were no major bleeding episodes in either arms and one minor bleeding episode in the eptifibatide arm. There were no differences in the median times to discharge, median times to crisis resolution or the median total opioid use. In this small study, eptifibatide appeared to be safe, but did not improve the times to crisis resolution or hospital discharge. Adequately powered studies are required to evaluate the safety and efficacy of eptifibatide in SCD [64].

Investigational therapies targeting multiple pathways are being studied. Rivaroxaban, a new orally active direct Factor-Xa inhibitor and serine protease inhibitor was FDA approved in the US as an anticoagulant for prophylaxis and treatment in acute coronary syndromes, cerebral ischemia, pulmonary embolism and venous thrombosis. It is currently being evaluated in a Phase II clinical trial in SCD to reduce inflammation, coagulation, endothelial cell activation, and improve microvascular blood flow in patients during the non-VOC, steady state (NCT02072668).

### **7.3 Targeting inflammation**

In SCA patients there is increase in the number of activated invariant natural killer T

(iNKT) cells. Regadenoson is an  $A_{2A}$  receptor ( $A_{2A}R$ ) agonist reduces iNKT activation and thus decreases inflammation. A phase 1 study in 27 adults with SCD showed a 48% decrease in activation of iNKT cells compared to baseline after regadenoson was administered with no toxicities identified [65]. NKTT-120 is a humanized monoclonal antibody designed to target invariant Natural Killer T cells. Preclinical studies showed depletion of iNKT cells and no effect on other natural killer cells. The T cell antibody response was not impaired in response to a KLH challenge [66]. A phase I trial planned to enroll 21 patients with SCD is currently recruiting (NCT 01783691).

Carbon monoxide inhibits inflammation and hypoxia-induced vasoocclusion in transgenic mice [67]. MP4-CO is a human Hb conjugated to carbon monoxide via an MP4 molecule. It decreased mortality in sickle cell mice from hypoxia reperfusion injury and has anti-inflammatory properties [68]. A phase I study that enrolled 32 patients with SCD is complete. Data is not yet available (NCT01356485). A phase II study in patients with SCD has been withdrawn prior to enrollment.

The vascular injury seen in SCD has been described to share similarities with that of atherosclerosis. Statins decrease inflammation and improve endothelial function in cardiovascular disease and is under study in SCD. A pilot study of 26 patients treated with simvastatin showed a dose related decrease in inflammatory biomarkers (C-reactive protein and IL-6 levels) and increased NO metabolite levels [69]. A phase II trial of atorvastatin is enrolling by invitation (NCT01732718).



Omega-3 fatty acids, EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid), are noted for their antiadhesive, anti-inflammatory, and vasodilatory effects. These are decreased in patients with SCD. A single institution study in Sudan was designed to evaluate its therapeutic effects in patients with SCD in a randomized, placebo-controlled, trial. One hundred and forty patients received omega-3 capsules for 1 year and omega-3 treatment reduced the rate of VOC events, red blood cell transfusions, and white blood cell count. The therapy was well tolerated and warrants further study [70].

Sickle cell disease patients have elevated levels of 5-lipoxygenase, a potent inflammatory leukotriene. Zileuton, a specific inhibitor of 5-lipoxygenase, is FDA approved for asthma. Beneficial effects in the SCD animal model have led to a phase 1 trial in SCD (NCT01136941).

*N*-acetylcysteine (NAC), is the rate limiting substrate for glutathione generation, an important antioxidant with pleiotropic effects on inflammation [71]. NAC inhibits dense cell formation and restores glutathione levels toward normal. In pilot studies the administration of *N*-Acetylcysteine resulted in a reduction of oxidative stress. A phase 2 double-blind, randomized clinical trial was completed to determine the efficacy of NAC in decreasing dense cell and irreversible sickle cell formation and VOC episodes in SCD. NAC inhibited dense cell formation, restored glutathione levels toward normal, and decreased VOC episodes [72]. A phase III trial is underway (NCT01849016).

Prasugrel (Effient <sup>TM</sup>) is a member of the thienopyridine class of ADP receptor antagonist, like clopidogrel (Plavix <sup>TM</sup>), which inhibits ADP induced platelet activation and aggregation by irreversibly binding to P2Y<sub>12</sub> receptors. It is FDA approved for use in acute coronary syndromes. In randomized trials in SCD, platelet activation was prevented and preliminary results suggest a decrease in pain rate [73, 74]. A phase 2 randomized, double-blind, placebo-controlled study examined safety of prasugrel measured by hemorrhagic events. Patients were randomized to prasugrel 5 mg daily or placebo for 30 days. There were no hemorrhagic events requiring medical intervention in either study arm. Platelet surface P-selectin and plasma soluble P-selectin, both biomarkers of platelet activation, were significantly reduced in SCD patients receiving prasugrel compared with placebo. This study showed a decrease in platelet activation biomarkers and a trend toward decreased pain [75]. A phase II trial to determine dosing was completed in children and results are pending (NCT01476696). A phase 3 study in children is currently recruiting (NCT01794000).

Dysregulation of NO metabolism is a common denominator in the pathogenesis of vasculopathy. Inhaled NO in SCD has resulted in variable clinical responses. A recent phase 2 trial for adult SCD patients treated with NO for painful VOCs showed no benefit [76]. A phase II trial to evaluate NO to treat acute VOCs was completed. Results showed a longer time to resolution of VOCs with NO, however statistical analysis is not yet available (NCT00094887). Two trials in adults with SCA were completed and results have not been reported (NCT0009581, NCT00352430). Another study evaluating NO therapy for VOCs and ACS in children with SCD is recruiting (NCT01089439,

NCT00142051).

Arginine is depleted in hemolysis due to the release of arginase and leads to decreased NO formation. In SCD patients with pulmonary hypertension, arginine supplementation increases plasma NO and rapidly decreases pulmonary artery pressure by 15% [77]. A recent randomized, double-blind, placebo-controlled study of high-dose arginine supplementation in hospitalized SCD patients with VOC was completed and found a > 56% reduction in narcotic use in patients receiving arginine compared with controls [78]. A phase II randomized trial in 38 children showed a significant reduction in opioid use and lower pain scores at discharge in those treated with arginine in comparison to the placebo arm. There was no significant difference in hospital length of stay and no toxicity was noted [79]. A study was just completed in children with SCD to evaluate the effectiveness of arginine at increasing NO levels, improving RBC function, and reducing hospitalizations and pain medication use. This was done by measuring gardos channel activity, mean corpuscular Hb concentration (MCHC) and NO levels. There was only statistically significant difference in low dose arginine with decreased MCHC versus placebo. Data is available but has not been published (NCT00513617). Other studies have been completed and awaiting analysis and 2 are currently recruiting (NCT01142219, NCT00056433, NCT00029731, NCT00004412).

#### **7.4 Targeting oxidative injury**

The primary buffer for reactive oxygen species is reduced glutathione. Glutamine is metabolized to glutamate, the glutathione precursor, and preserves intracellular

nicotinamide adenine dinucleotide (NAD), which is necessary for glutathione recycling. Oral supplementation of glutamine in SCD increases the NAD redox potential and may improve sickle erythrocyte adhesiveness [80, 81]. Oral glutamine is also used as a dietary supplement and it decreases the resting energy expenditure in children with SCD. A multicenter phase III trial of glutamine supplementation in 230 children to prevent VOC is completed and results are not available (NCT01179217). Two phase II trials are also complete and results have not been posted (NCT00125788, NCT00131508).

Alpha-lipoic acid augments cellular stress response by increasing the transcription of antioxidant genes, decreasing NF- $\kappa$ B, and increasing glutathione synthesis. Acetyl-L-carnitine is an essential nutrient that facilitates the entry of long-chain fatty acids into the mitochondria and decreases lipid peroxidation in tissue. Alpha-lipoic acid and acetyl-L-carnitine have a synergistic antioxidant effect [82]. A recent Phase II trial combining antioxidants enrolled 42 patients to determine whether alpha-lipoic acid and acetyl-L-carnitine will lower systemic inflammation in patients with SCD. This study is complete, however data is not available for review (NCT01054768).

Aes-103, 5-hydroxymethyl-2-furfural (5HMF), is a naturally occurring oral aldehyde that interacts with Hb S in red blood cells increasing oxygen affinity and decreasing Hb S polymerization. A preclinical study showed that 5-HMF decreased RBC dehydration in a deoxygenated state and inhibited activation of the Gardos channel and phosphatidylserine. We are awaiting results of two completed phase I trials and a phase II trial is currently recruiting [83-85] (NCT01597401, NCT01871142, NCT01987908).

## **8. Potential development issues**

The ideal drug to decrease the global disease burden would be an oral agent with no special handling and storage requirements. Many new drugs currently in clinical trial would mainly provide benefit for patients in the developed world. Additionally certain drugs like the hypomethylating agents may only be ideal in certain patient populations, more specifically in adults. Studies in the pediatric population are lacking and will warrant further study. It has been studied in children with acute myelogenous leukemia, however further long term data to evaluate long term side effects are needed. Its potential teratogenic and carcinogenic potential may limit applicability in women of childbearing age and in children. When looking at combining therapies, potential for hazardous side effects can be preventative. For example, bleeding risk is increased with antiplatelet therapy such as prasugrel, in combination with anticoagulation, such as heparin [13, 60, 75].

## **9. Conclusion**

Hydroxyurea has proven to be effective in SCD and has decreased morbidity and mortality in randomized phase III trials in adults and children. It is available both in the US and the rest of the world. Access in Africa still remains a challenge. Our improved understanding of the pathophysiology and its protean pathways led to many new drug targets. We reviewed 27 new drugs in development for the treatment of patients with SCD. Table 1 includes all the drugs discussed in this paper as well as others.

The agents that primarily increase fetal Hb include pomalidomide, sodium dimethylbutyrate, decitabine and vorinostat. Pomalidomide, a thalidomide analog, is in a phase I trial and the hypomethylating agent, decitabine, and the HDAC inhibitor, vorinostat, are in phase II trials. Sodium dimethylbutyrate, a short chain fatty acid, has completed phase II studies and a phase III trial is expected.

The drugs targeting adhesion primarily inhibit selectins and include rivipansel sodium, heparin, tinzaparin, pentosan polysulfate and SelG1. These are all in phase I and II trials. IVIG, propranolol, eptifibatid, and rivaroxaban also prevent adhesion to the vascular endothelium and are in phase II trials in the sickle cell population. We have long term experience and data with use of heparin, IVIG, tinzaparin, propranolol, eptifibatid and rivaroxaban for other hematologic illnesses and in the field of cardiology where they were studied in phase III trials and approved for many other indications.

Targets of the inflammatory process include regadenoson, carbon monoxide, simvastatin, atorvastatin, omega-3 fatty acids, zileuton, N-acetylcysteine, prasugrel, NO and arginine. Prasugrel and N-acetylcysteine have reached phase III trials. Nitric oxide and arginine also function indirectly as fetal Hb induction agents. Arginine, a natural amino acid, is being evaluated in adults and children and has reached phase III studies.

Drugs under study to prevent oxidative injury include glutamine, alpha-lipoic acid, acetyl-L-carnitine and Aes-103. Glutamine, a dietary supplement, had reached phase III studies in children. Alpha-lipoic acid and acetyl-L-carnitine are studied as combination

therapy in phase II studies.

## **10. Expert Opinion**

There has been tremendous advance in our knowledge of the pathophysiology of sickle cell vascular injury over the past decade resulting in new therapeutic targets. The field is witnessing promising translational studies hoping to replace or use with HU as the primary pharmacologic therapy for patients with SCD. We reviewed therapies targeting increases in fetal Hb and the complex pathways in adhesion and inflammation.

Hydroxyurea is an oral agent that has decreased morbidity and mortality in adults and children with SCA. It decreases recurrent VOCs, ACS, blood transfusion requirements, and improves quality of life mainly through increasing fetal Hb production. It is inexpensive and potentially available worldwide. It is cytotoxic, which may cause myelosuppression and it is contraindicated in pregnancy due to potential teratogenicity. Its carcinogenic effects are unknown and long term studies have failed to document this.

Decitabine is an attractive agent as it induced fetal Hb with similar disadvantageous risk profile like HU with potential myelosuppression, teratogenicity and carcinogenicity. It is already approved therapy for myelodysplastic syndrome, and acute myeloid leukemia; conditions more prevalent in the elderly. It is being evaluated in oral form and in combination therapy currently and further testing is warranted in the pediatric population.

The oral HDAC inhibitor, vorinostat, is appealing for its potential multiple effects. In preclinical studies, its analogue TSA, not only induced Hb F, but reduced endothelial activation, prevented vascular stasis and exhibited iron chelation properties. Both of these agents are in phase II trials.

Glutamine, prasugrel, and N-acetylcysteine have reached phase III trials. These work differently, targeting oxidative injury and inflammation and do not share the same side effect profile. A combination of any of these agents together or in combination with a fetal Hb inducing agent such as HU or vorinostat is a potential strategy to combat this disease.

Studies involving NO so far have been disappointing in the sickle cell population. It is surprising that arginine therapy was more promising than NO since its role is to increase NO. Nevertheless this natural amino acid is an ideal agent for a combination regimen. Similarly, omega-3-fatty acids has multiple effects, is effective in other illnesses, has a good side effect profile making it an ideal agent to combine with other therapies.

In the sickle cell population, there are challenges with clinical trial enrollment since it is a relatively rare and clinically heterogeneous disease. A paradigm shift in clinical trial design would improve outcome. Due to the complex pathophysiology of the disease, clinical trials targeting a multi-agent approach may be more successful as in oncology where combination chemotherapy regimens have been more efficacious. Trial design in SCD over the past 3 decades has historically incorporated all patients with SCA. Vichinsky proposed reassessing endpoints to see a benefit in a targeted phenotype and



including quality of life measures. A modification of this concept is to target the different phenotypes upfront as in recent successful lung cancer drug trials, which incorporated biomarkers in patient selection.

In summary, our greater understanding of the pathophysiology of SCD has led to many new targets for drug therapy and with a paradigm shift in clinical trial design, we are in an exciting position to improve care for the millions who suffer with SCD.

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**Table 1. Emerging Drug Therapies for the Treatment of Sickle Cell Disease**

KEYWORDS	SYNONYMS	ABBREV.	CONTEXT
Decitabine	5-aza-2'-deoxycytidine	5-aza-dCyd; DAC; Dacogen; Dacogen (IV); Dacogen (SC); deoxyazacytidine; dezocitidine; E7373; E7373 (IV); E7373 (oral); E7373 (SC); JNJ-30979754 (IV); NSC-127716	Hemoglobin F induction
Sodium butyrate	Butyric acid sodium salt	SB	Hemoglobin F induction
Vorinostat	Suberanolohydroxamic acid	rINN; L-001079038; MK-0683; MSK-390; SAHA (IV); suberoylanilide hydroxamic acid; VOR; vorinostat; Zolinza	Hemoglobin F induction
Panobinostat	(2E)-N-Hydroxy-3-[4-[[[2-(2-methyl-1H-indol-3-yl)ethyl]amino]methyl]phenyl]-2-propenamamide	Faridak; LBH-589; panobinostat lactate;	Hemoglobin F induction
Rivipansel Sodium	GMI-1070	GMI-1010; GMI-1011; GMI-1043; GMI-1070; PF-06460031; PF06460031; selectin inhibitors, GlycoMimetics	Inhibits cellular adhesion
Intravenous immunoglobulin	Intravenous immunoglobulin	IVIG	Inhibits cellular adhesion
Low molecular weight heparin	Low molecular weight heparin	LMWH	Inhibits cellular adhesion
Dalteparin	Dalteparin sodium	Fragim	Inhibits cellular adhesion
SelG1	PselhmAb	PselhmAb; SelG1	Inhibits cellular adhesion
Propranolol	1-(isopropylamino)-3-(1-naphthoxy)propan-2-ol	INN; Verex; Problok; Yissum	Inhibits cellular adhesion
Adenosine 2A receptor antagonist	ADORA2A	CVT-3146	Anti-inflammatory agent
ADP receptor antagonist	Ticlopidine, Tagren; ticlopidine; anagrelal;	KRKA; DE-4160; CS-747; LY-640315; HL-006	Anti-inflammatory agent
Omega-3-acid ethyl esters	Omega-3-acid ethyl esters	Lovaza	Anti-inflammatory agent
N-acetyl cysteine	Acetyl Cysteine	EN-3285; RK-0202	Anti-inflammatory agent
Alpha lipoic acid	Acetate Replacing Factor	Thioctic acid, Meda	Anti-inflammatory agent
Nonionic polyoxyethylene-polyoxypropylene	Poloxamer 188	ANX-188; CRL-5861; Flocor; MST-188; MST188; P-188; P188; poloxamer 188, purified-1, CytRx; purified poloxamer 188, CytRx; vepoloxamer	Surfactant
Purified Poloxamer 188	MST-188	CytRX; RheothRx	Reduces viscosity
Niacin	Pyridine-3-carboxylic acid	Vitamin B <sub>3</sub>	Increases levels of HDL
Rivaroxaban	BAY 59-7939	JNJ-39039039; Xarelto	Antiplatelet agent
Abciximab	c7E3 Fab	7E3; anti-GPIIb/IIIa MAb; CentoRx; ReoPro	Platelet aggregation inhibitor
Eptifibatide	Integrilin	Velofibatide	Antiplatelet agent
Acetylsalicylic acid	Aspirin; Benzon; Acetard; Eurand; Levius	BAN; ASA	Antiplatelet agent
Dipyridamole	Dipyridamine	Elan	Red blood cell hydration
Magnesium	Magnesium citrate, magnesium sulfate, magnesium carbonate	Mg	Red blood cell hydration
Nitric oxide	Nitrogen oxide	NO	Vasodilator
Sodium Nitrate	Caliche	NaNO <sub>3</sub>	Vasodilator
Cholecalciferol	25-hydroxyvitamin D <sub>3</sub>	Vitamin D <sub>3</sub>	Supplementary vitamin
Saproterin dihydrochloride	(6R)-2-Amino-6-[(1R,2S)-1,2-dihydroxypropyl]-5,6,7,8-tetrahydro-4(1H)-pteridinone dihydrochloride	Kuvan	Phenylalanine Hydroxylase activator
Senicapoc	ICA 17043	ICA-17043; KCa3.1 programme, Icagen; senicapoc	Gardos channel inhibitor
Arginine	2-Amino-5-guanidinopentanoic acid	Arg	Vasodilator
HQK 1001	2,2-Dimethylbutyrate	Sodium ST20	Gamma globin gene promoter
MP4CO	Pegylated hemoglobin saturated with carbon monoxide	Haemoglobin, Sangart-3; MP-4, Sangart; MP4; MP4-CO; MP4CO; PEG carboxyhemoglobin, Sangart; Pegylated carboxyhemoglobin, Sangart	Prevents microvascular stasis
L-citrulline	2-amino-5-(carbamoylamino)pentanoic acid	Asklepion; citrupress	Vasodilator
NKTT120	NKTT-120	NKTT-120	Reduce chronic inflammation associated with sickle cell disease
AES-103	5-hydroxymethyl-2-furfural	5-HMF; Xechem	Anti-sickling agent
Varespladib sodium	A-001	A-001; LY-315920; LY-315920Na;	Inhibitor of secretory phospholipases A2 (sPLA2)



		LY315920-Na; LY315920/S-5920 ; S-5920; varepladib sodium;	
TRF-1101	Pentosan polysulfate sodium	TRF-1101	Anti-sickling agent
Glutamine	Glutamine	Gin, Q	Anti-sickling agent
PF 04447943	6-[(3S,4S)-4-methyl-1-(pyrimidin-2-ylmethyl)pyrrolidin-3-yl]-1-(tetrahydro-2H-pyran-4-yl)-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one	PDE-IX; PDE9i; phosphodiesterase 9A inhibitor; PF-4447943	Selective PDE 9 inhibitor