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Developing new pharmacotherapeutic approaches to treating sickle-cell disease

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Abstract

Survival for patients with SCD has been prolonged by improvements in supportive care, including vaccinations, antibiotic prophylaxis, and overall medical management, including transfusion. However, there remains only one approved, partially effective drug for sickle cell disease—hydroxyurea (hydroxycarbamide). The world desperately needs better ways of both treating and preventing the recurrent painful vaso-occlusive episodes pathognomonic of sickle cell disease as well as the end-organ damage that still leads inexorably to severely shortened life expectancies throughout the world.

Based on accumulating knowledge about how the abnormal red blood cells of sickle cell disease cause the double scourge of acute painful episodes and progressive end-organ damage, both pharmaceutical enterprises and individual investigators are now pursuing multiple new avenues for treating sickle cell disease. As a result, many compounds are in active development, both in preclinical models as well as in phase I, II, and III clinical trials. These agents target many pathophysiologic processes thought to be critical in sickle cell disease, including the chemical and physical behavior of haemoglobin S, cell adhesion, coagulation pathways, platelet activation, inflammatory pathways, and upregulation of haemoglobin F expression. In addition, recent explorations of the genetic variations that predispose to certain types of sickle cell disease-related tissue injury, such as stroke or nephropathy, are expected to lead to identification of drugs targeting the pathways uncovered by such work. Thus, the next five to ten years holds a promise of new treatments for sickle cell disease.

Keywords

Red cells; Thrombosis; Cell-cell interactions; Clinical trial; Rheology

Disease mechanisms and druggable targets

The cause of sickle cell disease (SCD) is straightforward and well known: A single base pair change in the gene encoding the haemoglobin (Hb) β chain produces a peptide in which one amino acid (glutamic acid) is replaced by valine. That mutation directly affects only the Hb protein, whose expression is limited to inside the red blood cell. Nonetheless, the abnormalities of sickle (SS) red blood cells (RBCs) are quite wide-ranging and include young mean RBC age, cellular dehydration, HbS polymer formation, surface

phosphatidylserine exposure, upregulated adhesive properties, oxidatively damaged membrane and intracellular proteins, reduced ability to export nitric oxide and ATP, and abnormal cell-cell signaling.¹ As a result, multiple SS RBC characteristics put into motion a cascade of events (Figure 1) that lead to the archetypal manifestation of SCD, painful vaso-occlusion. Cell adhesion, inflammatory pathways involving both mononuclear and polynuclear leucocytes, abnormal activation of coagulation, and oxidative damage are likely among the most important, though not the only, factors contributing to vaso-occlusion and organ damage in sickle cell disease (SCD).^{2,3}

To date, the improvements we have seen in survival in SCD have arisen in large part from advances in supportive care. Prophylactic penicillin and vaccination against the pneumococcus have made major contributions to improvement in childhood mortality;⁴ the discoveries that risk of stroke could be identified by transcranial doppler imaging and that strokes could be largely prevented by regular transfusion decreased both the mortality and morbidity of SCD in childhood.⁵ The use of transfusions in the contexts of stroke, splenic sequestration and acute chest syndrome have also been life-saving advances.

Over the last several decades, we have learned much about the pathophysiologic pathways that contribute to vaso-occlusion (Figure 1). When HbS is the principal haemoglobin in the red cell, we observe that sickle RBCs have shortened lifespans, so that reticulocytes and relatively young mature RBCs predominate in the circulation. These red cells are abnormally adherent to other blood cells as well as to endothelial cells, leading to obstruction of circulation through small blood vessels, particularly post-capillary venules. In addition, young red cells have over-active signaling pathways that allow activation of multiple adhesion receptors on the red cell surface. Patients with SCD also have elevated leucocyte and platelet counts, as well as increased numbers of activated leucocytes and platelets. Leucocyte and platelet activation in turn lead to production and release of pro-inflammatory cytokines, which cause both endothelial cell activation and injury as well as further leucocyte activation. In addition, there is chronic activation of coagulation pathways, which is further stimulated during vaso-occlusion. And vaso-occlusion itself leads to activation of hypoxia/reperfusion injury pathways. Thus, a broad range of biomarkers related to activation of coagulation, leucocyte activation, endothelial cell activation, and adhesion pathways are all elevated in SCD. And it is these many pathways, as well as the abnormalities of the sickle red cell itself, that comprise potential targets of pharmacologic therapies in SCD. (See Table 1.)

Modulating Haemoglobin F and S Expression

Initial attempts to utilize therapeutic agents that addressed SCD mechanisms have targeted production of HbF. Hydroxyurea (hydroxycarbamide) was shown to decrease subjects' median vaso-occlusive crisis rate by approximately 50%, and HbF levels were inversely associated with crisis rate.⁶ In addition, hydroxyurea decreased rates of hospitalization, acute chest syndrome, and transfusion. Clinical response was also associated with reduction in neutrophil and reticulocyte counts. The US Food and Drug Administration approved hydroxyurea for SCD in 1998. However, not all patients respond to hydroxyurea, leading to study of other drugs that might increase the expression of HbF. These have included several

different classes of drugs. Since silencing of the gene encoding HbF is believed to occur through DNA methylation, drugs that inhibit DNA methyl-transferase have been studied for their effect on HbF expression. Two such drugs, the cytosine analogues 5-azacytidine and 5-aza-2'-deoxycytidine (decitabine), have been used to obtain HbF responses in individuals not responsive to hydroxyurea,^{7,8} although neither has gained wide acceptance or approval for use in SCD. Arginine butyrate and HQK-1001 (2,2-dimethylbutyrate) have also been studied. A phase 2 study of HQK-1001, however, was disappointing, as it resulted in only a modest HbF response.⁹ In addition, HQK-1001 led to a paradoxical increase in frequency of vaso-occlusive episodes. Other approaches are being explored, including use of lysine specific demethylase-1 inhibition, which appears effective in animal models.¹⁰

Immunomodulatory drugs such as vorinostat, pomalidomide, and panobinostat have also been studied for their ability to raise HbF levels. Pomalidomide, a drug currently used to treat multiple myeloma, can induce HbF production by reducing the levels of transcriptional repressors of fetal globin gene expression.¹¹ In addition, Dulmovits et al. showed that pomalidomide was effective in inducing HbF expression in erythroid cells from individuals with and without SCD.¹¹ A Phase I study of pomalidomide in SCD showed promising results,¹² although a phase II study has not been started. Early phase studies of vorinostat and panobinostat are either underway or complete but have yet to report results.

Attempts to replace synthesis of HbS with synthesis of either HbA, HbF, or a different mutated nonsickling Hb (such as that encoded by the human beta A-T87Q-globin gene)¹³ are also ongoing. Hematopoietic stem cell transplantation has continued to become safer,¹⁴⁻¹⁷ with less myeloablative regimens leading to adequate donor cell engraftment, reduction in graft-versus-host disease, and fewer adverse effects of the pre- and post-transplant drug regimens.¹⁸ Gene therapy is also being studied. In general, gene therapy attempts to insert a gene encoding HbA or another non-sickling gene into autologous hematopoietic stem cells, in order to produce enough non-HbS globin to interfere with the sickling phenomenon and produce a phenotype closer to that of HbS trait. One ongoing phase I/II study is using a Lentivirus vector and a gene encoding beta A-T87Q-globin (NCT02151526, clinicaltrials.gov) and is sponsored by Bluebird Bio.

Inhibiting Adhesion and Cell-Cell Interactions

Work by many investigators have identified cell adhesion and cell-cell interactions as critical to the process of vaso-occlusion in SCD (Figure 1). Indeed, SCD severity was linked to the degree of red cell adhesion demonstrable *in vitro* nearly more than decades ago.¹⁹ That work led to in-depth studies of the mechanisms of sickle red cell adhesion, which we now know involves multiple red cell receptors and endothelial ligands, as well as several potential bridging molecules.²⁰⁻²⁵ In addition, we have also discovered that sickle red cells contain active signaling pathways that lead to activation of many of the red cell adhesion receptors discovered as contributing to vaso-occlusion.²⁶⁻²⁹ Finally, in addition to interacting with endothelial cells and extracellular matrix molecules such as laminin and thrombospondin, sickle red cells activate circulating leukocytes, which then adhere to endothelium and “capture” circulating red cells, also promoting vaso-occlusion. This process involves selectins as well as CD44.³⁰⁻³² Both leukocytes and red cells may then also aggregate in the

circulation; these aggregates may then also involve platelets.^{20,33} Pharmacologic agents targeting adhesion in SCD may thus target specific receptors, their ligands, or the signaling pathways that cause activation of adhesive interactions.

The first anti-adhesive molecule to be studied clinically was poloxamer-188, a surfactant that acts as a nonspecific inhibitor of cell adhesion by altering the way cells and molecules interact with water.³⁴ The first phase III study of this drug showed statistically significant but quite modest improvement in duration of painful episodes.³⁵ A second phase III study of the drug in vaso-occlusion has recently been conducted, but results are still pending (NCT01737814, [ClinicalTrials.gov](https://clinicaltrials.gov)).

Selectins are adhesion receptors that mediate rapid on-off interactions and are theorized to provide the earliest cell-cell interactions in the process of vaso-occlusion. Therefore they have attracted many investigators and pharmaceutical efforts aimed at developing inhibitors of selectin-mediated interactions.

P-selectin is expressed by both endothelial cells and platelets and its expression at the cell surface is upregulated by a variety of stimuli. P selectin expression is higher in sickle mice than in normal mice, and knock out of P selectin expression in sickle mice abrogated the ability of pro-inflammatory cytokines to cause vaso-occlusion.²² One of the classical inhibitors of P-selectin-mediated adhesion is heparin. Matsui et al. showed that heparin efficiently inhibited sickle red cell adhesion to immobilized P selectin at concentrations similar to those routinely achieved during heparin therapy *in vivo*.³⁶ In addition, heparinoid molecules with low anticoagulant activity are also able to inhibit P-selectin mediated adhesion.³⁷ Two preclinical studies also demonstrated that inhibition of P selectin could interfere with sickle red cell adhesion and vaso-occlusion.^{38,39} Thus, several early phase studies have been conducted to investigate the use of heparinoids for vaso-occlusion. Qari et al performed a randomized, double-blind study of tinzaparin in over 250 SCD patients with acute vaso-occlusion and found that tinzaparin was associated with a significant reduction in duration of pain and hospitalization, although the drug was stopped for bleeding in a small number of patients.⁴⁰ The non-anticoagulant heparinoid sevuparin is currently in phase II study for vaso-occlusion, having been shown in *in vitro* and animal studies to be able to inhibit sickle red cell adhesion to endothelial cells and prevent vaso-occlusion in an animal model.⁴¹ Recently, another drug—SelG1 (Selexys Pharmaceuticals)—has been used in a clinical trial to determine if monthly use could prevent vaso-occlusive episodes (NCT01895361, [ClinicalTrials.gov](https://clinicaltrials.gov)); the study has completed accrual but results are still pending.

E selectin is also a target of new therapeutics. A small carbohydrate molecule, GMI-1070, was first shown to be able to relieve vaso-occlusion in sickle mice.³⁰ Now named rivipansel, it was then successfully carried forward into phase I and phase II studies.^{42,43} Rivipansel was well tolerated, decreased biomarkers associated with vaso-occlusion—including sE-selectin, sP-selectin, and sICAM-1—and reduced time to resolution of pain and amount of opioids required when used to treat patients with vaso-occlusion. A phase III study of rivipansel for vaso-occlusive episodes is currently underway (NCT02187003, [ClinicalTrials.gov](https://clinicaltrials.gov)).

The anti-inflammatory effects of intravenous gamma globulin have also been investigated in the context of SCD. IVIg reverses acute vaso-occlusion in sickle mice through reduction in neutrophil adhesion.^{44,45} A study (NCT01757418, clinicaltrials.gov) of a single dose of IVIg in pain episodes also showed that IVIg can stabilize neutrophil Mac-1 activation, although the small study did not allow detection of differences in clinical outcomes between placebo and study drug.⁴⁶

Cell adhesion might also be potentially reduced by interfering with the red cell signaling pathways that activate adhesion receptors. β 2 adrenergic signaling pathways activate several adhesion receptors, including the BCAM/Lu laminin receptor and the ICAM-4 (LW) receptor for β 3 integrins.^{25,28,47} De Castro et al. showed that propranolol could prevent vaso-occlusion *in vivo* in an animal model and that a single oral dose of propranolol could prevent activation of sickle red cell adhesion by epinephrine *in vitro*.⁴⁸ Results from a phase II study are pending. In addition, the MEK/ERK pathway is activated downstream of the β 2 adrenergic receptor and also regulates red cell adhesion.²⁹ Recently, Zennadi has also shown that MEK inhibition effectively blocks sickle cell adhesion and vaso-occlusion *in vivo*.^{49,50} Since several MEK inhibitors are now either FDA-approved or in clinical trials for various cancers, it is reasonable to expect that these drugs will also be investigated for their effects on vaso-occlusion in SCD.

Down-regulating Inflammation

As noted above, many pro-inflammatory pathways are believed to contribute to the pathophysiology of SCD vaso-occlusion. Sickle red cells themselves are able to activate leukocytes and endothelial cells.^{32,51} Relatively large numbers of circulating leukocytes are activated in SCD,⁵²⁻⁵⁴ and levels of pro-inflammatory cytokines are also higher than normal.⁵⁵⁻⁵⁷ The high frequency of asthma and airway hyperresponsiveness in children with SCD are both believed to arise from inflammatory processes and to be associated with earlier mortality.^{58,59} Lymphocytes, and particularly iNKT cells are believed to also contribute to the inflammatory pathways exacerbating vaso-occlusion.⁶⁰⁻⁶² Finally, once vaso-occlusion occurs, hypoxia/reperfusion tissue injury follows, restarting the cycle of inflammation and leading to progressive organ damage.⁶³ Therefore, many investigators are targeting inflammatory pathways as a means to reduce the pathophysiology of vaso-occlusion.

Corticosteroids have been used in SCD but have a mixed record in the setting of vaso-occlusive events. The most attractive and best documented indication for their use is in acute chest syndrome. However, no adequately controlled randomized prospective trials of steroids in acute chest syndrome have been conducted. A recent review of the potential benefits and hazards of corticosteroid use points to risks ranging from increased risk of readmission due rebound pain crisis to more serious events, such as stroke, renal infarction, and even death.⁶⁴

Several newer therapies are being developed to target invariant natural killer T(iNKT) cells, which are more numerous and more often activated in SCD than in normal subjects.⁶⁵ iNKT cells are known to play an important role in ischemia/reperfusion injury. In sickle mice, the adenosine A2A receptor agonist regadenoson, which inhibits iNKT cell activity, showed

ability to reduce lung inflammation.⁶⁵ An early phase study in SCD patients also showed that a single infusion of regadenoson was able to reduce iNKT cell activation, as measured by phospho-NF- κ B p65 expression in iNKT cells.⁶⁰ A phase II study of regadenoson for vaso-occlusive events is underway (NCT01788631, clinicaltrials.gov) and anticipated to be completed in the fall of 2016. In addition, a second drug targeting iNKT cells, NKTT 120, a humanized monoclonal antibody (NKT Therapeutics) against iNKT cells, is also being evaluated in SCD. A pharmacodynamic study has been completed.(NCT01783691, clinicaltrials.gov)

Other potentially anti-inflammatory drugs are also being studied at this time. In SCD mice, the 5-lipoxygenase inhibitor zileuton was able to reduce airway hyper-responsiveness and decrease leukotriene levels.⁵⁹ A phase I study using zileuton in both adults and children with SCD (NCT01136941, clinicaltrials.gov) has been completed and reported in abstract form, although no efficacy data are available.⁶⁶ Cysteinyl leukotriene E4 is a biomarker of increased pain rate in children and adults with SCD.⁶⁷ Montelukast, which blocks leukotrienes, is currently being used in a phase II trial in SCD, with the object of reducing another key biomarker of endothelial injury, soluble vascular cell adhesion molecule-1 (sVCAM-1)(NCT01960413, clinicaltrials.gov).

Statins, such as simvastatin, have also garnered interest. In a pilot study, simvastatin improved many markers of endothelial injury in SCD.⁶⁸

Anticoagulants and Anti-platelet Agents

The activation of coagulation pathways in SCD has been well characterized, and the source of activation of coagulation well described.⁶⁹⁻⁷⁷ Thus, it is not surprising that the use of anticoagulants in SCD has also been of interest for several decades. Use of the oral anticoagulant acecoumerol was able to normalize biomarkers of coagulant activation (F1.2),⁷⁸ although a second study failed to show improvement in endothelial markers of activation.⁷⁹ As mentioned above, the low molecular weight heparin tinzaparin used at full anticoagulant dose was judged effective in treating acute vaso-occlusive symptoms in one single institution study,⁴⁰ but additional studies of low molecular weight heparins have not been reported. In another study of prophylactic dose low molecular weight heparin that did not meet its accrual target, dalteparin was considered possibly effective, since dalteparin was associated with a more pronounced decrease in pain scores at day 3 than was the placebo.⁸⁰(NCT01419977, ClinicalTrials.gov) Recently, investigators have turned their attention to new oral anticoagulants, such as apixaban and rivaroxaban (NCT02179177 and NCT02072668, respectively, clinicaltrials.gov).

Anti-platelet agents have also generated interest among SCD investigators, since platelets contribute to both the activation of coagulation as well as stimulation of inflammatory pathways. Among the studies concluded to date, however, results have been disappointing. Eptifibatide, which inhibits the α IIB β III platelet integrin and reduces release of CD40 ligand from platelets, nonetheless did not improve the clinical course of vaso-occlusive episodes in a small study.⁸¹ In a much larger multicenter and international study, prasugrel failed to reduce the frequency of vaso-occlusive episodes in individuals age 2-17 years, despite the

fact that the drug reduced the levels of P selectin expressed on platelet surfaces as well as in soluble form in plasma.⁸² Ticagrelor is currently in Phase II study to determine if it can reduce the number of days with pain in adults with SCD (NCT02482298).

Anti-oxidants

Degradation of HbS leads to generation of reactive oxygen species and oxidative damage of red cell proteins.⁸³ The degree to which sickle red cells have deficient defense against oxidative damage has been associated with degree of anaemia.⁸⁴ Therefore, studies have been conducted and others are underway to explore the potential utility of anti-oxidant compounds, including arginine, alpha lipoic acid, omega 3 fatty acids, N-acetylcysteine, glutamine, Nrf2 activators,⁸⁵⁻⁸⁸ haptoglobin, hemopexin⁸⁹ and others. Although studies in murine models have looked promising,^{90,91} clinical studies have not yet shown definitive benefit to date, although some remain ongoing.

Summary

Our expanded understanding of the complex pathophysiology of SCD has created multiple opportunities for targeted drug development aimed at reducing the frequency of vaso-occlusive episodes, ameliorating the clinical course of vaso-occlusive episodes when they do occur, and forestalling end-organ damage. To date, some studies have looked promising, while others have failed to produce encouraging results. While this circumstance should likely be expected due to our inability to judge which pathophysiological processes are most critical, optimism remains that at least some of the agents in development will be proven efficacious.

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Figure 1. Pathophysiologic processes contributing to vaso-occlusion and constituting potentially druggable targets.

Table 1**Druggable Targets in Sickle Cell Disease**

Therapeutic Target	Rationale	Examples of Potentially Therapeutic Agents
Synthesis of HbF	Increased HbF is associated with fewer vaso-occlusive episodes and a milder clinical course	<ul style="list-style-type: none"> • Hydroxyurea • Decitabine • Vorinostat, Panibostat • Pomalidomide • HQK-1001
Synthesis of HbS	Replace synthesis of HbS with either HbF or Hb A, to reduce HbS-related cellular damage	<ul style="list-style-type: none"> • Hematopoietic stem cell transplantation • Gene therapy
Cell adhesion	Both sickle red cells and leukocytes adhere to each other and to endothelial cells, leading to vaso-occlusion	<ul style="list-style-type: none"> • SelGI • PF-04447943 • Rivipansel (GMI-1070) • MST-188 (Poloxamer-188) • Sevuparin • Propranolol • IVIg
Inflammation	Sickle red cell interaction with both leukocytes and endothelial cells leads to activation of those cells. In addition, vaso-occlusion results in hypoxia/reperfusion injury and inflammation.	<ul style="list-style-type: none"> • Regadenoson • NKTT120 • Zileuton • Montelukast • IVIg • Simvastatin
Activation of coagulation	Coagulation is chronically activated in SCD and is believed to contribute to vaso-occlusion and organ damage.	<ul style="list-style-type: none"> • Tinzaparin • Apixaban • Enoxaparin • Unfractionated heparin • N-acetyl cysteine
Platelet activation	Platelet activation promotes thrombosis as well as inflammation.	<ul style="list-style-type: none"> • Ticagrelor • Prasugrel • Eptifibatide • Aspirin
Red cell "sickling"	Many agents seek to bind CO to Hb or otherwise increase O ₂ affinity, in order to reduce HbS gelation	<ul style="list-style-type: none"> • MP4CO • SCD-101 • Sanguinate (PEG-bHb-CO) • AES-103
Red cell dehydration and hemolysis	Red cell dehydration is a contributor to HbS gelation, cell deformation and hemolysis. Some drugs can inhibit the Gardos channel and thus increase cell hydration.	<ul style="list-style-type: none"> • Clotrimazole • ICA-17043 (senicapoc)
Oxidant damage	Anti-oxidant compounds may improve red cell survival and reduce tissue injury.	<ul style="list-style-type: none"> • L-glutamine • Alpha-lipoic acid • Arginine • omega3 fatty acids • N-acetylcysteine • Nrf2 activators (mono- and di-methylfumarate, sulforaphane) • Haptoglobin • Hemopexin
Endothelial damage and vascular biology	Vasculature in SCD shows signs of chronic activation, damage, and remodeling. Abnormal NO availability is thought to contribute.	<ul style="list-style-type: none"> • Inhaled and intravenous NO • 6R-BH4 (sapropterin dihydrochloride) • Statins • Bosentan • Losartan • Varespladib • Mg sulfate