SICKLE CELL DISEASE: CHALLENGES AND PROGRESS

Beyond hydroxyurea: new and old drugs in the pipeline for sickle cell disease

Marilyn J. Telen

Division of Hematology, Department of Medicine, and Duke Comprehensive Sickle Cell Center, Duke University, Durham, NC

Despite Food and Drug Administration (FDA) approval of hydroxyurea to reduce the frequency of vaso-occlusive episodes, sickle cell disease (SCD) has continued to be treated primarily with analgesics for pain relief. However, elucidation of the multiple pathophysiologic mechanisms leading to vaso-occlusion and tissue injury in SCD has now resulted in a burgeoning effort to identify new treatment modalities to prevent or ameliorate the consequences of the disease. Development of new drugs as well as investigation of drugs previously used in other settings have targeted cell adhesion, inflammatory pathways, upregulation of hemoglobin F, hemoglobin polymerization and sickling, coagulation, and platelet activation. Although these efforts have not yet yielded drugs ready for FDA approval, several early studies have been extremely encouraging. Moreover, the marked increase in clinical pharmaceutical research addressing SCD and the new and old drugs in the pipeline make it reasonable to expect that we will soon have new treatments for SCD. (*Blood*. 2016;127(7):810-819)

Introduction

The simplicity of the genetic mutation that causes sickle cell disease (SCD) belies the complexity of the disease's pathophysiology. A single base-pair change (A→T), and the ensuing alteration of one amino acid (glutamic acid replaced by valine) in the β chain of hemoglobin (Hb), a protein only expressed in erythrocytes, nevertheless causes a multiorgan disease with many complex pathophysiologic mechanisms (Figure 1). Thus, therapeutic approaches may target the "root cause" (ie, by replacement of the abnormal hemoglobin), as do stem cell transplantation and gene therapy, or one or more of the many damaging and interwoven pathways responsible for the disease's cardinal manifestations—episodic severely painful vaso-occlusive episodes (VOC), hemolytic anemia, and progressive multiorgan damage.

Red cells that contain primarily HbS or HbS with one of the variants that interacts with it, such as HbC, are abnormal in many respects, including that as a result of hemolysis they are overall much younger than normal erythrocytes.[1] The fundamental defect in sickle red blood cells (SS RBCs) is the insolubility of HbS when it becomes deoxygenated, leading to formation of polymers that aggregate into tubular fibers and, as they enlarge, deform red cells, causing the characteristic sickle shape. In addition, SS RBCs become dehydrated, have abnormally activated intracellular signaling pathways, have decreased nitric oxide[2] and adenosine triphosphate[3] content and antioxidant capacity, demonstrate oxidative damage to many cellular components,[4] and reflect dysregulation of miRNAs and gene expression during erythropoiesis.[5,6] Cellular dehydration contributes to deoxygenated hemoglobin polymer formation and ultimately cell sickling and hemolysis. Signaling pathways downstream of the β2 adrenergic receptor and protein kinase A result in activation of MEK and ERK[7] as well as several cell surface adhesion receptors.[8-10] Oxidative damage of membrane proteins and aggregation of proteins along the inner surface of the plasma membrane led to further intracellular abnormalities.[4,6]

At their surfaces, SS RBCs demonstrate altered lipid sidedness, with markedly increased phosphatidylserine exposure.[4] Along with the formation of microparticles, phosphatidylserine exposure contributes to the procoagulant activity of SS RBCs. SS RBCs also evince abnormal adhesive properties, including activation of known adhesion receptors (including BCAM/Lu, ICAM-4, and CD44) and increased interactions with leukocytes, platelets, endothelial cells, and extracellular matrix proteins. Abnormal SS RBC cell-cell signaling can activate both leukocytes and endothelial cells,[11,12] making both more easily involved in adhesive interactions and also driving endothelial cell expression of procoagulant proteins.

SS RBCs are also stiffer than normal red cells in the circulation. Wide-field digital interferometry (WFDI) examination of normal red cells, normal-appearing SS RBCs, and sickled RBCs has shown that normal-appearing HbSS red cells are 2 to 3 times stiffer than HbAA red cells, and sickled RBCs are about 2 times stiffer than normal-appearing SS RBCs.[13]

Thus, new drug development as well as trials of existing compounds have targeted one or more of these pathophysiologic factors (Figure 1) in an effort to improve the overall prognosis of SCD as well as to reduce or treat its cardinal manifestation, vaso-occlusion. Given the diversity of therapeutic targets and pharmacokinetics of potential drugs, trials of new therapies have focused on a variety of different outcomes, including prevention of SCD events (such as the frequency of both VOC and acute chest syndrome (ACS), both reduced by hydroxyurea) and the ability to shorten the course of acute VOC. However, because resolution of VOC-related pain is a patient-reported outcome generally not accepted as a clinical outcome for SCD therapies, other end points—such as length of stay, time to discharge, or time to readiness for discharge—have been used. Unfortunately, these end points occur at highly variable time points among patients, so that achievement of statistically significant differences has been quite challenging. Partly for that reason, phase 2 studies have often used more easily quantified and sometimes less variable surrogate end points, although not always with great success.

Submitted September 1, 2015; accepted October 21, 2015. Prepublished online as *Blood* First Edition paper, January 12, 2016; DOI 10.1182/blood-2015-09-618553.

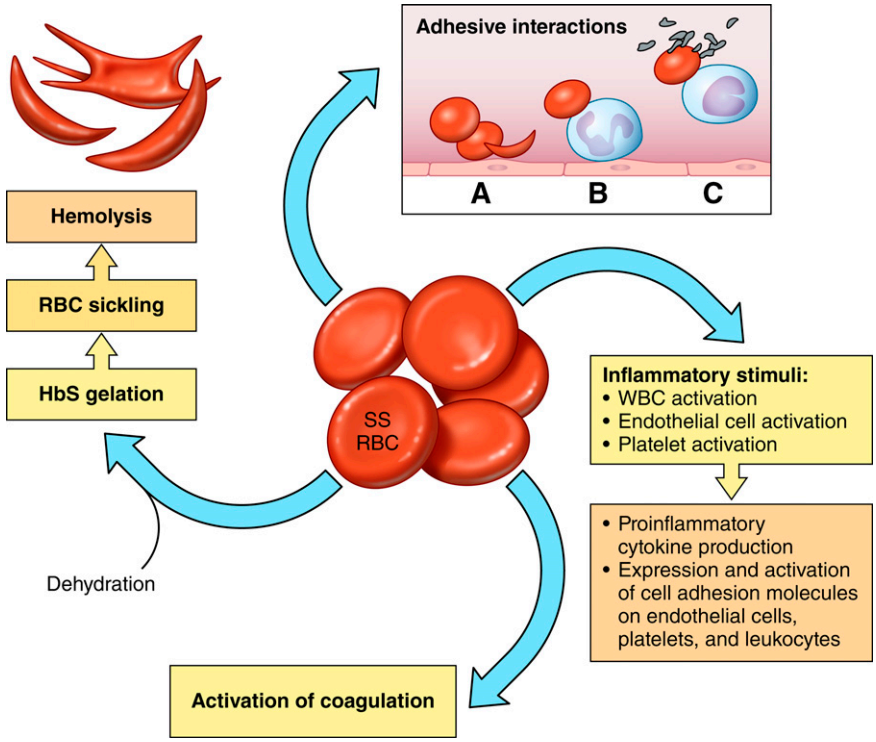

Figure 1. The sickle red blood cell (SS RBC) as source of multiple pathophysiologic pathways. Red cells with predominantly HbS (SS RBCs) become rapidly dehydrated, which increases the propensity of HbS to polymerize when deoxygenated. Pharmacologic reagents that prevent dehydration may therefore also reduce HbS polymerization and hemolysis. Altered lipid sidedness (phosphatidylserine exposure) may play a role in SS RBC adhesion and also promote activation of coagulation. Oxidative damage of red cell membrane proteins likely contributes to altered cell elasticity. Abnormal adhesive properties lead to SS RBC adhesion to endothelial cells (A), SS RBC adhesion to neutrophils (B), and adhesive interactions that result in heterocellular aggregate formation involving SS RBCs, monocytes, and platelets (C). Abnormal intracellular signaling increases the activation state of red cell adhesion molecules, and increased adhesive interactions then lead to abnormally active cell-cell signaling, which leads to activation of both other blood cells and endothelial cells. Both SS RBCs and hypoxia/reperfusion also lead to activation of inflammatory pathways involving both mononuclear and polymorphonuclear leukocytes. Platelet activation also contributes to inflammatory pathways as well as activation of coagulation.

Development of drugs targeting cell adhesion

Drugs targeting either red cell or leukocyte adhesion (or both) are attractive therapeutic modalities in treating SCD (Table 1), because multiple adhesive interactions have been demonstrated as contributing to vaso-occlusion (Figures 1 and 2) and both ex vivo and in vivo animal studies have suggested the potential efficacy of such an approach.

Selectin inhibitors

Drugs targeting selectin-mediated adhesion are being especially actively investigated. Such drugs inhibit adhesion and/or activation of leukocytes that are otherwise recruited to inflamed vessels. Human studies were preceded by several animal studies showing that inhibition of both P-selectin–mediated and E-selectin–mediated adhesion led to reduction of vaso-occlusion in in vitro and in murine models.[11,14-18] Several selectin inhibitors are now in various stages of investigation.

Table 1. Recently completed and ongoing studies targeting adhesion

Study title	Clinical trials #/Phase	Intervention	Status	Primary sponsor
Selectin inhibitors				
Study of GMI-1070 for the Treatment of Sickle Cell Pain Crisis	NCT01119833 Phase 2	GMI-1070 (rivipansel)	Complete	GlycoMimetics
Efficacy and Safety of Rivipansel (GMI-1070) in the Treatment of Vaso-Occlusive Crisis in Hospitalized Subjects With Sickle Cell Disease	NCT02187003 Phase 3	GMI-1070 (rivipansel)	Ongoing	Pfizer
Study to Assess Safety and Impact of SelG1 With or Without Hydroxyurea Therapy in Sickle Cell Disease Patients With Pain Crises	NCT01895361 Phase 2	SelG1	Ongoing	Selexys
Sevuparin Infusion for the Management of Acute VOC in Subjects With SCD	NCT02515838 Phase 2	Sevuparin	Ongoing	Dilaforette
β blockers				
Study of Propranolol as Anti-Adhesive Therapy in Sickle Cell Disease (SCD)	NCT01077921 Phase 2	Propranolol	Complete	Duke Univ.
Propranolol and Red Cell Adhesion in Non-asthmatic Children with Sickle Cell Disease	NCT02012777 Phase 1	Propranolol	Ongoing	Univ. of Miami
Other inhibitors of adhesion				
Phase III Randomized Study of Poloxamer 188 for Vaso-Occlusive Crisis of Sickle Cell Disease	NCT00004408 Phase 3	Poloxamer 188	Complete	Mast Therapeutics. CytRx
Evaluation of Purified Poloxamer 188 in Vaso-Occlusive Crisis of Sickle Cell Disease (EPIC)	NCT01737814 Phase 3	Poloxamer 188	Ongoing	Mast Therapeutics

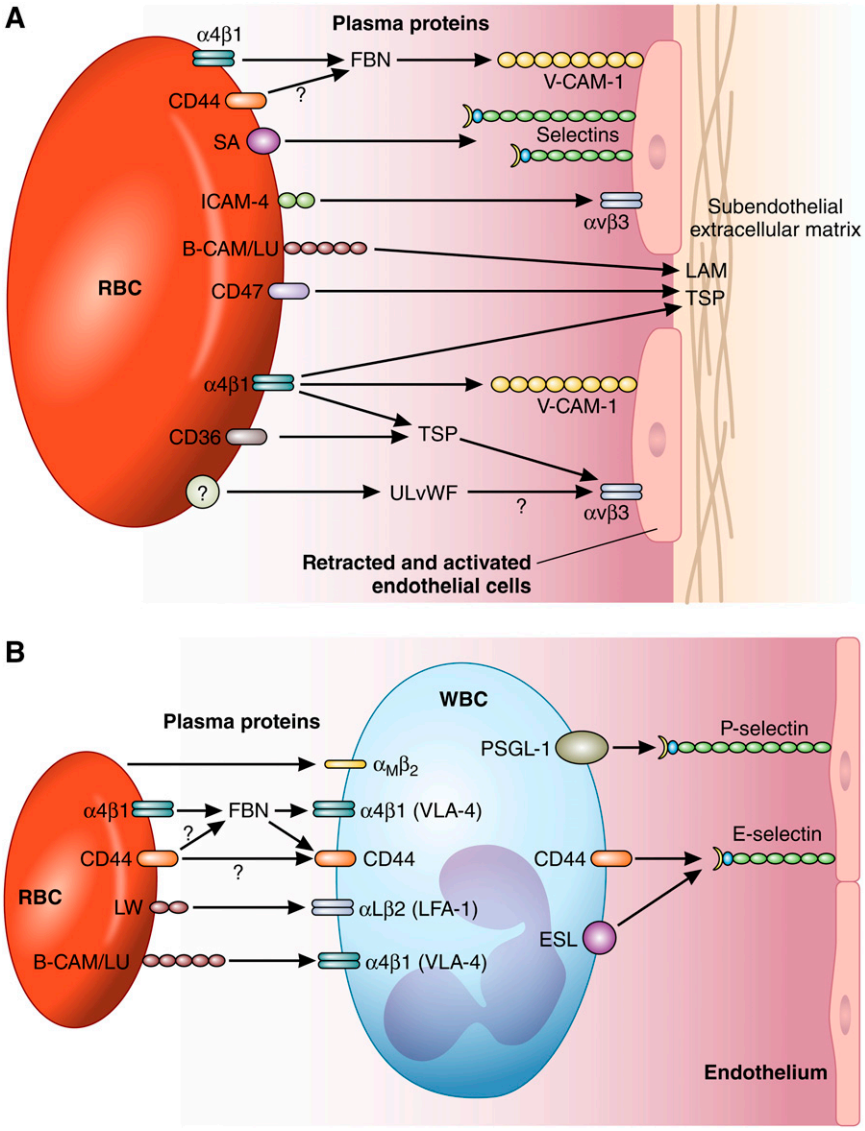

Figure 2. Adhesive interactions involving SS RBCs. (A) Multiple interactions between SS RBCs and endothelial cells, extracellular matrix, and plasma proteins. Red cells express multiple adhesion molecules that recognize ligands on either plasma protein "bridging molecules" or endothelial cell surfaces. Specific adhesion receptors on red cells that contribute to vaso-occlusion include ICAM-4 (the LW blood group protein, a receptor for several integrins), BCAM/Lu (the Lutheran blood group protein, a receptor for laminins (LAM) containing the α5 chain [laminins-10 and -11]), and CD44 (which bears the Indian blood group antigens), which is involved in binding to fibronectin and E-selectin. SA, sialic acid; FBN, fibronectin; TSP, thrombospondin; ULvWF, ultra-large von Willebrand factor. (B) Interactions between SS RBCs and leukocytes adherent to vessel walls. Many stimuli may activate leukocyte adhesion, including cytokines and contact with SS RBCs. Adhesion to endothelial cells initially occurs via selectins expressed by endothelial cells but ultimately likely also involves endothelial integrins. ESL-1, E-selectin ligand-1; PSGL-1, P-selectin glycoprotein ligand-1.

GMI-1070 (now called rivipansel) is currently in a phase 3 study for acute vaso-occlusive episodes. Study of the drug in sickle mice showed that GMI-1070 could both prevent VOC and reduce the severity of ongoing VOC.[15] In phase 1 studies in individuals with SCD in steady state, GMI-1070 was well tolerated, and data suggested that biomarkers of endothelial activation, leukocyte activation, and activation of coagulation were reduced as a result of drug administration.[19] A phase 2 study of the drug during VOC then showed a marked reduction in opioid requirements as well as trends toward shorter duration of VOC and shorter length of hospital stays.[20] The drug was also well tolerated during VOC. The phase 3 study of rivipansel treatment during VOC (NCT02187003) started in June 2015 and is expected to be completed in July 2018. Although selectin inhibitors have the potential to prevent leukocyte migration to sites of infection, clinical infections related to rivipansel have not been observed to date.

SelG1 is a humanized monoclonal antibody against P-selectin. A phase 1 dose-finding study was reported in abstract form in 2013.[21] This single-center, double-blind, placebo-controlled, ascending single and multiple-dose study of IV SelG1 in healthy adult subjects showed the drug to be well tolerated and effective in blocking P-selectin function for approximately one month at well-tolerated dose levels. The phase 2 study (NCT01895361) is designed as a prophylactic study to prevent VOC events during 50 weeks of treatment every 28 days with IV SelG1 and is still actively treating patients.

Two groups have also investigated the ability of RNA aptamers to inhibit P-selectin–mediated RBC adhesion to endothelial cells.[14,22] However, these reagents have not entered clinical trials.

Heparins as inhibitors of adhesion

Heparins have a well-known ability to inhibit adhesive interactions via P-selectin.[16,23] Sevuparin, a derivative of low-molecular-weight heparin (LMWH), is being developed as a P-selectin blocker. Sevuparin retains the P-selectin–binding domain of heparin but largely lacks anticoagulant properties. It has been reported in abstract to inhibit SS RBC adhesion to endothelial cells in vitro and to reduce tumor necrosis factor–induced vaso-occlusion in vivo.[24] After in vitro studies showing effect-inhibiting *Plasmodium falciparum* rosettes,[25] sevuparin was first used in a phase 1/2 study as adjunctive therapy for malaria (NCT01442168). Sevuparin is now in an international phase 2 trial (NCT02515838) for the management of acute VOC.

In a single-center, randomized double-blind study of tinzaparin for acute VOC episodes, therapeutic-dose tinzaparin anticoagulation was associated with significantly shorter periods of severe pain, overall

Table 2. Recently completed and ongoing studies targeting inflammation

Study title	Clinical trials #/Phase	Intervention	Status	Primary sponsor
Adenosine and invariant NK T (iNKT) cells				
Adenosine 2A Agonist Lexiscan in Children and Adults With Sickle Cell Disease	NCT01085201 Phase 1	Regadenoson	Complete	Dana-Farber Cancer Institute
A Phase II Trial of Regadenoson in Sickle Cell Anemia	NCT01788631 Phase 2	Regadenoson	Ongoing	Dana-Farber Cancer Institute
Safety, Pharmacokinetic, and Pharmacodynamic Study of NKTT120 in Adult Patients With Stable Sickle Cell Disease (SCD)	NCT01783691 Phase 1	NKTT120	Complete	NKT Therapeutics
Leukotrienes				
Phase 2 Study of Montelukast for the Treatment of Sickle Cell Anemia	NCT01960413 Phase 2	Montelukast	Ongoing	Vanderbilt Univ.
Trial of Zileuton CR in Children and Adults With Sickle Cell Disease	NCT01136941 Phase 1	Zileuton	Complete	Children's Hospital Medical Center, Cincinnati
Neutrophil adhesion and non-specific anti-inflammatory reagents				
Intravenous Gammaglobulin for Sickle Cell Pain Crises	NCT01757418 Phase 1/2	IVIg	Ongoing	A. Einstein College of Medicine, Yeshiva Univ.
Effect of Simvastatin Treatment on Vaso-occlusive Pain in Sickle Cell Disease	NCT01702246 Phase 2	Simvastatin	Ongoing	Children's Hospital & Research Center Oakland

duration of pain episode, and overall duration of hospitalization.[26] However, issues regarding potential study bias and standard of care make the study difficult to interpret.[27] In addition, it is difficult to attribute the effects of tinzaparin to either its anticoagulant or antiadhesive properties.

Targeting signaling pathways that activate adhesion molecules

The role of the β_2-adrenergic signaling pathway in activation of both the BCAM/Lu and ICAM-4 (LW) red cell adhesion receptors has suggested the possibility that beta-blockers might reduce RBC adhesion and thus have a salutary effect on SCD. A proof-of-principle phase 1 study in humans showed that oral propranolol was associated with a reduction in the ability of epinephrine to stimulate SS RBC adhesion in vitro.[28] A phase 2 double-blind crossover study (NCT01077921) in 25 subjects showed that propranolol treatment was associated with decreased levels of all measured biomarkers (soluble E-selectin, P-selectin, ICAM-1, vascular cell adhesion molecule-1 [VCAM-1]) compared both with baseline values and with placebo, but these differences were not statistically significant.[29] A second phase 1 study of propranolol in children without asthma (NCT02012777) is currently enrolling. Given the high prevalence of asthma in children with SCD, concerns remain that beta-blockers may not be tolerated by many patients with SCD.

A second signaling pathway of interest involves the kinases MEK and ERK, which in red cells appear to become activated downstream of the β_2-adrenergic receptor and protein kinase A.[7] Several MEK inhibitors have been developed as treatment of oncological diseases, as in cancer cells MEK and ERK act downstream of EGFR. In a murine model of vaso-occlusion involving infusion of human SS RBCs, exposure of SS RBCs to a MEK inhibitor reduced red cell adhesion to tumor necrosis factor–stimulated endothelial vessel walls and diminished trapping of SS RBCs in organs, concomitantly improving the circulatory half-life of SS RBCs.[30] Thus, although clinical trials of MEK inhibitors in SCD have not yet been undertaken, this remains an interesting pathway for future drug development. Although reportedly well tolerated by most patients, MEK inhibitors have been associated with anemia and thus may exacerbate anemia in SCD.

Nonspecific inhibitors of adhesion

Poloxamer 188 is a surfactant that alters the way cells and molecules interact with water. Intravenous administration of poloxamer 188 acts by multiple mechanisms to improve microvascular blood flow, most notably in ischemic vascular beds, by inhibiting cell adhesion, lowering blood viscosity, and decreasing friction along the vessel wall.[31] After successful early-phase clinical studies,[32,33] poloxamer 188 was tested for efficacy during acute VOC in a phase 3 clinical trial (NCT00004408), resulting in a modest reduction in duration of painful episodes.[34,35] It is now being studied in a second phase 3 trial (NCT01737814) for its ability to reduce the time to resolution of VOC.

Development of drugs targeting inflammatory pathways

Vaso-occlusion not only directly injures tissue via local hypoxemia but also engenders an inflammatory response typical of hypoxia/reperfusion injury,[18,36] involving both mononuclear as well as polymorphonuclear leukocytes, along with production of a large array of proinflammatory cytokines. As described elsewhere in this issue, leukocytes, platelets, and multiple proinflammatory pathways contribute to the pathophysiology of SCD. Therefore, several approaches are being studied to determine whether downregulation of inflammatory pathways will ameliorate aspects of SCD (Table 2).

Adenosine and invariant NK T cells

Invariant NKT (iNKT) cells are implicated in the pathogenesis of ischemia/reperfusion injury.[37,38] iNKT cell activation can be downregulated by activation of the adenosine A2A receptor (A2AR), and adenosine can reduce activation of and cytokine production by iNKT cells through this receptor.[39-41] Expression of the A2AR on pulmonary iNKT cells is markedly increased in sickle mice, and recent studies demonstrate that blockade of iNKT cell activation in mice with SCD reduces pulmonary inflammation and injury.[39] Adenosine also normally enhances 2,3-bisphosphoglycerate production via activation of the adenosine A2B receptor on RBCs, thus promoting O_2 release. However, this same action, by promoting hemoglobin deoxygenation, might also promote polymerization of HbS and subsequent sickling.[42]

Regadenoson is a partially selective adenosine A_{2A} receptor agonist. This drug is being studied for usefulness during VOC, which is theorized to involve iNKT cells as important contributors to the

inflammatory components of VOC pathophysiology. Regadenoson is currently Food and Drug Administration (FDA)-approved for use in radionuclide myocardial perfusion imaging, similar to injectable formulations of adenosine and dipyridamole. However, regadenoson is more selective than these other agents for the adenosine A_{2A} receptor. Furthermore, animal studies have shown that ATL146e, another adenosine A_{2A} receptor agonist, caused a dose-dependent reversal of pulmonary dysfunction in sickle mice.[39] Moreover, a phase 1 human study (NCT01085201) of regadenoson in individuals with SCD found that regadenoson could be safely administered to both children and adults in both steady state and during VOC, despite its demonstrated association with both cardiac and respiratory side effects outside the SCD context. The investigators found that iNKT cells demonstrated increased activation in individuals with SCD in steady state compared with controls; this activation was more marked during VOC. After a 24-hour infusion of regadenoson during VOC, phospho-NF-κB p65 activation in iNKT cells decreased significantly, and administration during VOC was not associated with toxicity.[43] A phase 2 study of regadenoson in children and adults experiencing VOC (NCT01788631) is now underway to determine whether regadenoson infusion reduces iNKT cell activation among individuals with SCD VOC or ACS, compared with placebo. This study is also designed to look at length of hospital stay for VOC or ACS with regadenoson or placebo to determine whether regadenoson improves respiratory symptoms and reduces opioid use, and to study the effect of regadenoson on inflammatory biomarkers. The investigators hope to complete accrual by mid to late 2016.

A second drug targeting iNKT cells has also been studied in phase 1 (NCT01783691) and has been granted fast-track status by the FDA. This humanized monoclonal antibody against iNKT cells depletes iNKT cells in vivo in animals. A completed nonrandomized, nonplacebo-controlled phase 1b, dose-escalation single-dose study of the safety, pharmacokinetics, pharmacodynamics, and biological activity of IV NKTT120 in adults with SCD in steady state has been reported in abstract form.[44] However, a phase 2 study of this drug is not yet listed as active at ClinicalTrials.gov.

Downregulation of leukotrienes

Given the association of SCD and asthma, along with the evidence that inflammatory pathways are important to both initial vaso-occlusion and tissue injury, targeting inflammatory mediators such as leukotrienes also appears to be a promising approach for the development of novel therapies for SCD.[45] Cysteinyl leukotriene (CysLT), leukotriene E4 (LTE4), is elevated in steady-state SCD, and its level is correlated with pain event rate.[46] Montelukast, already FDA-approved for treatment of asthma, is a CysLT inhibitor that acts by binding to CysLT receptors. Therefore, to test that LTE4 has a pathogenic role in sickle vasculopathy and vaso-occlusion, an 8-week phase 2 study (NCT01960413) of montelukast is being conducted in patients already on stable doses of hydroxyurea, with the surrogate end point of a reduction in sVCAM-1 levels. In this trial, investigators are looking at whether montelukast has additive effects in patients already taking hydroxyurea, compared with study participants treated with hydroxyurea only. Outcomes to be measured also include markers of tissue injury, lung function, and forearm microvascular blood flow.

Another approach is to target a key leukotriene synthetic enzyme, 5-lipoxygenase (5-LO). Zileuton, also FDA-approved for asthma, acts as a leukotriene inhibitor by inhibiting 5-LO, thus interfering with leukotriene formation. In SCD, placenta growth factor is elevated due to hyperplastic erythropoiesis and leads to activation of both monocytes and endothelial cells by inducing 5-LO,[47] which leads to increased production of leukotrienes, including LTB4, a very potent chemoattractant and neutrophil and endothelial cell activator, and LTE4, which is associated with lung inflammation. In addition, interleukin-13 (IL-13) is known to increase expression of VCAM-1, which is elevated in SCD and known to be associated with mortality.[48,49] In the context of SCD, zileuton reduces adhesion of PMN and sickle RBC to rat pulmonary vasculature[50]; it has also been shown to reduce IL-13 production by murine splenic lymphocytes, and thus it has been postulated to be potentially beneficial in SCD.[51] Zileuton has been examined in a phase 1 study (NCT01136941) in children and adults with SCD, to determine whether it can safely inhibit 5-LO activity in individuals with SCD and if it will significantly reduce leukotrienes and biomarkers of inflammation. Zileuton has also been noted to be associated with increased HbF,[52] and its effect on HbF levels in patients with SCD is also being examined.

Neutrophil adhesion and nonspecific antiinflammatory reagents

Intravenous γ globulin (IVIg) reduces inflammation via inhibition of neutrophil adhesion. In sickle mice exposed to an inflammatory challenge as a stimulus for VOC,[53,54] IVIg infusion caused a rapid reduction in adherent leukocytes, markedly decreased leukocyte-erythrocyte interactions, and increased microcirculatory blood flow as well as survival. This effect occurs via IVIg's ability to block the FcγRIII, leading to inhibition of neutrophil adhesion, reduction of RBC capture by leukocytes, and reduced Mac-1 activity as a result of recruitment of Src homology 2–containing tyrosine phosphatase-1.[55] Recently reported results of a phase 1/2 study of IVIg for vaso-occlusion (NCT01757418) confirmed that IVIg decreased human neutrophil Mac-1 function. IVIg in this small study was not associated with reduced duration of pain crisis, total opioid use, or time to hospital discharge, although the study was likely not powered to detect such differences.[56]

Statins decrease endothelial inflammation in cardiovascular disease, so they have also been studied in SCD. A pilot study of 21 days of simvastatin in children with SCD looked at both safety issues as well as the effect of simvastatin on biomarkers of vascular dysfunction in SCD. Simvastatin resulted in dose-dependent statistically increased NOx levels and decreased C-reactive protein and IL-6 levels, while having no effect on vascular endothelial growth factor, sVCAM-1, or tissue factor expression.[57] A phase 2 study of the ability of simvastatin to reduce the frequency of acute pain episodes is currently ongoing (NCT01702246).

Induction of HbF and antisickling agents

Irreversible sickling does not appear to be the driving factor for red cell adhesion and subsequent vaso-occlusion that occurs typically in postcapillary venules, because morphologically normal sickle red cells adhere as avidly to the endothelium as do sickled cells. Nevertheless, sickling leads to entrapment, or at least slow passage of cells through capillaries, as well as to hemolysis.

Induction of HbF

Drugs that increase HbF levels are the archetypal antisickling agents, because HbF interferes with polymerization of HbS, thereby lessening hemolytic rate and resulting in the increase in total Hb levels seen with hydroxyurea therapy.[58] A number of drugs in addition to hydroxyurea are known to result in elevated HbF levels, at least temporarily (Table 3). For example, histone deacetylase inhibitors, including butyrates, generally increase HbF levels.[59-63] Decitabine demethylates and reactivates

Table 3. Recently completed and ongoing studies of HbF induction and antisickling agents

Study title	Clinical trials #/Phase	Intervention	Status	Primary sponsor
HbF Induction				
Study of Decitabine and Tetrahydrouridine (THU) in Patients With Sickle Cell Disease	NCT01685515 Phase 1	Decitabine and Tetrahydrouridine	Ongoing	Cleveland Clinic
Decitabine for High-Risk Sickle Cell Disease	NCT01375608 Phase 2	Decitabine	Suspended	NIH Clinical Center, NHLBI
Phase II Randomized Trial:Arginine Butyrate Plus Standard Local Therapy in Patients With Refractory Sickle Cell Ulcers	NCT00004412 Phase 2	Arginine Butyrate	Complete	Boston Med Ctr
Phase 1 Placebo Controlled Study of the Safety, Activity and Pharmacokinetics of HQK-1001 in Healthy Subjects	NCT00717262 Phase 1	HQK-1001	Complete	HemaQuest
Phase 1/2 Study to Evaluate the Safety, Tolerability and Pharmacokinetics of HQK-1001 Administered Daily in Patients With Sickle Cell Disease	NCT00842088 Phase 1/2	HQK-1001	Complete	HemaQuest
A Study of HQK-1001 in Patients With Sickle Cell Disease	NCT01322269 Phase 2	HQK-1001	Complete	HemaQuest
Effects of HQK-1001 in Patients With Sickle Cell Disease	NCT01601340 Phase 2	HQK-1001	Terminated	HemaQuest
Study to Determine the Maximum Tolerated Dose, Safety and Effectiveness of Pomalidomide for Patients With Sickle Cell Disease	NCT01522547 Phase 1	Pomalidomide	Complete	Celgene
Hemoglobin-modifying and anti-sickling agents				
Dose-Escalation Study of SCD-101 in Sickle Cell Disease	NCT02380079 Phase 1	SCD-101	Ongoing	Invenux; SUNY-Downstate Med Ctr
Safety Study of MP4CO in Adult Sickle Cell Patients	NCT01356485 Phase 1	MP4CO	Complete	Sangart
Study of SANGUINATE™ Versus Hydroxyurea in Sickle Cell Disease (SCD) Patients	NCT01848925 Phase 1	Sanguinate	Complete	Prolong Pharmaceuticals
Study of SANGUINATE™ In the Treatment of Sickle Cell Disease Patients With Vaso-Occlusive Crisis	NCT02411708 Phase 2	Sanguinate	Ongoing	Prolong Pharmaceuticals
A Study of the Efficacy and Safety of ICA-17043 (With or Without Hydroxyurea) in Patients With Sickle Cell Anemia.	NCT00040677 Phase 2	Senicapoc (ICA-17043)	Complete	Icagen
A Stratified Sickle Event Randomized Trial (ASSERT)	NCT00102791 Phase 3	Senicapoc (ICA-17043)	Terminated (lack of efficacy)	Icagen
A Study Evaluating the Long-Term Safety of ICA-17043 in Sickle Cell Disease Patients With or Without Hydroxyurea Therapy	NCT00294541 Phase 3	Senicapoc (ICA-17043)	Terminated	Icagen
A Single Dose Study of the Safety, Blood Levels and Biological Effects of Aes-103 Compared with Placebo in Subjects With Stable Sickle Cell Disease	NCT01597401 Phase 1	Aes-103	Complete	Baxalta US
Evaluation of Different Dose Regimens of Aes-103 Given for 28 Days to Subjects With Stable Sickle Cell Disease	NCT01987908 Phase 2	Aes-103	Terminated	Baxalta US

expression of the methylated γ globin gene. Other drugs, such as thalidomide and related compounds, act by different mechanisms.

Butyrates were the first histone deacetylase inhibitors to be extensively studied in SCD.[64-66] Sodium butyrate infusion was effective in a small study if given as pulse rather than continuous therapy.[65] However, the orally available sodium dimethyl butyrate (HQK-1001) has produced generally unimpressive results in several studies,[60-62] and in one study pain episodes were more frequent in patients receiving sodium dimethyl butyrate.

Pomalidomide has demonstrated the ability to upregulate HbF production in vitro and in sickle mice.[67,68] A dose-finding study in individuals with SCD (NCT01522547) has been completed, but results reported only in abstract[69] found increases in HbF and total Hb only at the highest dose or with >6 months of exposure.

Overall, optimally efficient induction of HbF may require combined use of drugs with different molecular and epigenetic mechanisms.[70]

Hemoglobin modification and antisickling agents

Carbon monoxide (CO) is a potent antisickling agent, because it attaches to Hb and, while attached, can prevent or reverse HbS polymerization. At very low levels, CO may also have antiinflammatory effects. Sickling may also be ameliorated by shifting the oxyhemoglobin dissociation curve to the left (Aes-103) or preventing cell dehydration through Gardos channel inhibition (ICA-17043, senicapoc).[71-75] Despite its ability to reduce sickling and increase Hb levels, however, senicapoc did not reduce the frequency of VOC in a phase 3 clinical trial,[72] perhaps because it did not reduce the sickling-independent adhesivity of the red cells themselves. This problem may also turn out to plague other agents designed to reduce sickling.

Sanguinate is a bovine pegylated Hb product designed to reduce sickling by delivering CO to HbS and then carrying O_2. It was given safely to normal healthy subjects in a phase 1 trial,[76] as well as to SCD

Table 4. Recently completed and ongoing studies involving anticoagulants

Study title	Clinical trials #/Phase	Intervention	Status	Primary sponsor
An Exploratory Study of Anticoagulation For Pulmonary Hypertension in Sickle Cell Disease	NCT01036802 Phase 2	Warfarin	Terminated	Univ. of North Carolina-Chapel Hill
Treatment of Sickle Cell Patients Hospitalized in Pain Crisis With Prophylactic Dose Low-molecular-weight Heparin (LMWH) Versus Placebo	NCT01419977 Phase 2	Dalteparin	Completed	Duke Univ.
Unfractionated Heparin in Acute Chest Syndrome: A Pilot Feasibility Randomized Controlled Trial of Unfractionated Heparin vs Standard of Care in Acute Chest Syndrome	NCT02098993 Phase 2	Unfractionated heparin	Recruiting	Univ. of Pittsburgh
The Effect of Factor Xa Inhibition, With Rivaroxaban, on the Pathology of Sickle Cell Disease	NCT02072668 Phase 2	Rivaroxiban	Recruiting	Univ. of North Carolina-Chapel Hill
Impact of Daily Prophylaxis Dose Anticoagulation With a Factor Xa Inhibitor (Apixaban) in Patients With Sickle Cell Disease	NCT02179177 Phase 2	Apixiban	Recruiting	Duke Univ.
A Pilot Study of *N*-acetylcysteine in Patients With Sickle Cell Disease	NCT01800526 Phase 0	N-acetyl cysteine	Recruiting	Puget Sound Blood Center

subjects (NCT01848925). It has been granted orphan drug status and is now in a randomized, double-blind placebo-controlled phase 2 study of individuals with SCD experiencing VOC (NCT02411708), examining both safety and time to discharge.

MP4CO, or pegylated carboxyhemoglobin, markedly induced hepatic heme oxygenase-1 activity and inhibited NF-κB activation and microvascular stasis in sickle mice.[77] However, although a phase 1 safety study has been completed (NCT01356485), a planned phase 2 study was withdrawn from ClinicalTrials.gov before enrollment, and the sponsor has ceased operations.

SCD-101 is a botanical-derived drug currently in a phase 1 study (NCT02380079) to determine dose and ability to inhibit sickling in vivo. Finally, a phase 1 study of Aes-103 (NCT01597401) (active ingredient 5-hydroxymethyl-2-furfural) has been completed in subjects with stable SCD.[78] A phase 2 study in stable SCD (NCT01987908) has also just been completed, but results are not yet available.

Anticoagulants and antiplatelet agents

Although coagulation is chronically activated in SCD,[79-83] no specific clinical complication of SCD has yet been clearly associated with a polymorphism of the coagulation pathway, and anticoagulation has an uncertain role in SCD in the absence of clinically apparent thromboembolic disease. Nevertheless, activation of coagulation and platelets is felt to contribute to vascular blockade, endothelial damage, and stimulation of inflammatory pathways. Thus, both anticoagulants

Table 5. Recently completed and ongoing studies involving antiplatelet agents

Study title	Clinical trials #/Phase	Intervention	Status	Primary sponsor
Abciximab (ReoPro) as a Therapeutic Intervention for Sickle Cell Vaso-Occlusive Pain Crisis	NCT01932554 Phase 2	Abciximab	Withdrawn	St. Louis Univ.
Dipyridamole/Magnesium To Improve Sickle Cell Hydration	NCT00276146 Phase 2	Dipyridamole, Magnesium	Withdrawn	Children's Hospital Medical Center, Cincinnati
A Phase I/II Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Safety of Eptifibatide as Treatment of Acute Pain Episodes in Sickle Cell Disease	NCT00834899 Phase 1, 2	Eptifibatide	Terminated	Univ. of North Carolina-Chapel Hill
An Assessment of Prasugrel on Healthy Adults and Sickle Cell Adults	NCT01178099 Phase 1	Prasugrel	Completed	Ely Lilly
A Study of Prasugrel in Pediatric Participants With Sickle Cell Disease	NCT01476696 Phase 2	Prasugrel	Completed	Ely Lilly
Prasugrel Versus Placebo in Adult Sickle Cell Disease	NCT01167023 Phase 2	Prasugrel	Completed	Ely Lilly
A Phase 3, Double-Blind, Randomized, Efficacy and Safety Comparison of Prasugrel and Placebo in Pediatric Patients With Sickle Cell Disease.	NCT01794000 Phase 3	Prasugrel	Active, not recruiting	Eli Lilly
Aspirin Prophylaxis in Sickle Cell Disease	NCT00178464 Phase 1, Phase 2	Aspirin	Completed	Univ. of Rochester
A Pharmacokinetic (PK) and Pharmacodynamic (PD) Dose-ranging Phase II Study of Ticagrelor Followed by a 4 Weeks Extension Phase in Pediatric Patients With Sickle Cell Disease	NCT02214121 Phase 2	Ticagrelor	Recruiting	AstraZeneca
A Study to Evaluate the Effect of Ticagrelor in Reducing the Number of Days With Pain in Patients With Sickle Cell Disease (Hestia2)	NCT02482298 Phase 2	Ticagrelor	Recruiting	AstraZeneca

and antiplatelet agents have attracted champions who have led or are leading clinical investigations of these agents (Tables 4 and 5). However, concerns remain regarding bleeding risk, especially in association with Moya Moya vascular disease of the central nervous system.

Vitamin K antagonists

In the 1960s, a small study of warfarin anticoagulation in 12 patients, with duration of treatment ranging from 12 to 34 months, reported a slight decrease in VOC rate but a significant number of bleeding episodes.[84] Warfarin was subsequently reported to be associated with lower D-dimer levels in individuals with SCD with VOC.[85] More recently, a study of anticoagulation with warfarin for pulmonary hypertension (NCT01036802) was terminated because of difficulty accruing patients.

Heparins

Heparins may potentially exert both antiadhesive effects (discussed above) and anticoagulation effects. However, well-designed placebo-controlled studies with various LMWHs are needed to confirm or dismiss the results of the single study of full anticoagulation doses of tinzaparin reported thus far.[26] A somewhat more recent study of the LMWH dalteparin (NCT01419977) looked at the effect of prophylactic dosing on change in D-dimer, change in pain score, and change in the thrombin generation assay during VOC. Results, provided in abstract form,[86] report that prophylactic dose dalteparin did not significantly affect markers of activation of coagulation, although pain scores at days 1 and 3 decreased more markedly in patients treated with dalteparin than with placebo.

There is also an ongoing feasibility study of unfractionated heparin in ACS (NCT02098993) in which the primary outcome is time to discharge. At this time, however, it is unclear whether this will lead to a larger multicenter phase 3 trial of unfractionated heparin for ACS.

Direct thrombin and factor X inhibitors

With the advent of orally available direct thrombin and factor X inhibitors, investigators interested in the role of coagulation in SCD have begun investigating these newer agents. Current studies are looking at the potential benefit of rivaroxiban and apixiban in outpatients with SCD. The rivaroxaban study (NCT02072668) is looking at the effect on levels of sVCAM-1 and IL-6, whereas the apixaban study is looking at daily pain scores.

Antiplatelet agents

Platelets and platelet activation are thought to contribute to SCD pathophysiology by multiple mechanisms, including participation in formation of heterocellular blood cell aggregates,[87,88] release of proinflammatory cytokines,[89] and activation of coagulation pathways. Therefore, a number of platelet antagonists have been examined in SCD. In a small study, eptifibatide failed to reduce time to VOC resolution (NCT00834899),[90] and no further studies of this drug are currently ongoing. Prasugrel has shown promising results in sickle mice,[91] in which it partially attenuated both basal and agonist-stimulated platelet activation. A multicenter phase 2 study of prasugrel in adults with SCD showed that biomarkers of in vivo platelet activation were significantly reduced in individuals with SCD receiving prasugrel compared with placebo; however, only nonsignificant decreases in pain were observed. A phase 3 study of prasugrel (NCT01794000) to determine whether it affects the number of VOC events per year is ongoing. A phase 2 study of ticagrelor (NCT02482298) is also ongoing to determine whether the drug reduces the number of days of pain, pain intensity, and analgesic use.

Outlook for the future

New pharmacotherapeutic approaches to SCD are being explored more actively than ever, and both clinical investigators and pharmaceutical companies are engaged in developing better treatment to prevent VOC as well as to abrogate the process once it occurs. With our expanded knowledge of the pathophysiologic mechanisms of vaso-occlusion, and indeed of how SS RBCs engender the widespread effects we observe in patients, drug therapy targeted to specific pathophysiologic mechanisms is becoming a realistic approach.

However, given the panoply of effects SS RBCs have, it is likely that, ultimately, optimal therapy will only be achieved with a multitargeted approach, such as that now used for acute coronary syndromes. Unfortunately, the limited number of individuals with SCD in developed countries and the dearth of resources available where most of the world's patients live will likely make definitive studies of many pharmacologic agents difficult to accomplish. Although early phase studies may find biomarkers of inflammation, endothelial activation, or activation of coagulation to be helpful, there is insufficient evidence that any biomarkers are predictive of meaningful clinical response, although some clearly correlate with survival.[49,92]

Thus, real progress in treating SCD will be critically dependent on participation of clinical sites throughout the world, as well as on the availability of funds to support the arduous research approaches needed to confirm safety and efficacy in this highly variable disease.

Acknowledgments

Because of limitations to length of the paper, many significant studies and contributions to this field have not been included in this review, and the author apologizes to investigators whose work was not included in this report.

This work was supported in part by the National Institutes of Health, National Heart, Lung, and Blood Institute (R01 HL107608) and by the Doris Duke Charitable Foundation.

Authorship

Contribution: M.J.T. wrote the article.

Conflict-of-interest disclosure: M.J.T. has received research funding from Dilaforette, the maker of sevuparin, and Glyco-Mimetics, the maker of GMI-1070 (rivipansel), and has consulted for Pfizer, Inc regarding development of the phase 3 clinical trial of rivipansel.

Off-label drug use: Drugs described in this report of research studies are not being recommended for clinical off-label use.

Correspondence: Marilyn J. Telen, Box 2615, Duke University Medical Center, Durham, NC 27710; e-mail: marilyn.telen@duke.edu.

References

1. Telen MJ. It really IS the red cell. *Blood.* 2008; 112(3):459-460.
2. Pawloski JR, Hess DT, Stamler JS. Impaired vasodilation by red blood cells in sickle cell disease. *Proc Natl Acad Sci USA.* 2005;102(7): 2531-2536.
3. Sabina RL, Wandersee NJ, Hillery CA. Ca^{2+}-CaM activation of AMP deaminase contributes to adenine nucleotide dysregulation and phosphatidylserine externalization in human sickle erythrocytes. *Br J Haematol.* 2009;144(3):434-445.
4. Kuypers FA. Hemoglobin s polymerization and red cell membrane changes. *Hematol Oncol Clin North Am.* 2014;28(2):155-179.
5. Chen SY, Wang Y, Telen MJ, Chi JT. The genomic analysis of erythrocyte microRNA expression in sickle cell diseases. *PLoS One.* 2008;3(6):e2360.
6. Sangokoya C, Telen MJ, Chi JT. microRNA miR-144 modulates oxidative stress tolerance and associates with anemia severity in sickle cell disease. *Blood.* 2010;116(20):4338-4348.
7. Zennadi R, Whalen EJ, Soderblom EJ, et al. Erythrocyte plasma membrane-bound ERK1/2 activation promotes ICAM-4-mediated sickle red cell adhesion to endothelium. *Blood.* 2012;119(5): 1217-1227.
8. Zennadi R, Hines PC, De Castro LM, Cartron JP, Parise LV, Telen MJ. Epinephrine acts through erythroid signaling pathways to activate sickle cell adhesion to endothelium via LW-alphavbeta3 interactions. *Blood.* 2004;104(12):3774-3781.
9. Gauthier E, Rahuel C, Wautier MP, et al. Protein kinase A-dependent phosphorylation of Lutheran/ basal cell adhesion molecule glycoprotein regulates cell adhesion to laminin alpha5. *J Biol Chem.* 2005;280(34):30055-30062.
10. Hines PC, Zen Q, Burney SN, et al. Novel epinephrine and cyclic AMP-mediated activation of BCAM/Lu-dependent sickle (SS) RBC adhesion. *Blood.* 2003;101(8):3281-3287.
11. Zennadi R, Chien A, Xu K, Batchvarova M, Telen MJ. Sickle red cells induce adhesion of lymphocytes and monocytes to endothelium. *Blood.* 2008;112(8):3474-3483.
12. Shiu YT, Udden MM, McIntire LV. Perfusion with sickle erythrocytes up-regulates ICAM-1 and VCAM-1 gene expression in cultured human endothelial cells. *Blood.* 2000;95(10):3232-3241.
13. Shaked NT, Satterwhite LL, Telen MJ, Truskey GA, Wax A. Quantitative microscopy and nanoscopy of sickle red blood cells performed by wide field digital interferometry. *J Biomed Opt.* 2011;16(3):030506.
14. Burnette AD, Nimjee SM, Batchvarova M, et al. RNA aptamer therapy for vaso-occlusion in sickle cell disease. *Nucleic Acid Ther.* 2011;21(4): 275-283.
15. Chang J, Patton JT, Sarkar A, Ernst B, Magnani JL, Frenette PS. GMI-1070, a novel pan-selectin antagonist, reverses acute vascular occlusions in sickle cell mice. *Blood.* 2010;116(10):1779-1786.
16. Embury SH, Matsui NM, Ramanujam S, et al. The contribution of endothelial cell P-selectin to the microvascular flow of mouse sickle erythrocytes in vivo. *Blood.* 2004;104(10):3378-3385.
17. Matsui NM, Borsig L, Rosen SD, Yaghmai M, Varki A, Embury SH. P-selectin mediates the adhesion of sickle erythrocytes to the endothelium. *Blood.* 2001;98(6):1955-1962.
18. Kaul DK, Hebbel RP. Hypoxia/reoxygenation causes inflammatory response in transgenic sickle mice but not in normal mice. *J Clin Invest.* 2000;106(3):411-420.
19. Wun T, Styles L, DeCastro L, et al. Phase 1 study of the E-selectin inhibitor GMI 1070 in patients with sickle cell anemia. *PLoS One.* 2014;9(7): e101301.
20. Telen MJ, Wun T, McCavit TL, et al. Randomized phase 2 study of GMI-1070 in SCD: reduction in time to resolution of vaso-occlusive events and decreased opioid use. *Blood.* 2015;125(17): 2656-2664.
21. Mandarino D, Kawar Z, Alvarez R, Falconer D, Rollins SA, Rother RP. Placebo-controlled, double-blind, first-in-human, ascending single dose and multiple dose, healthy subject study of intravenous-administered SelG1, a humanized anti-P-selectin antibody in development for sickle cell disease [abstract]. *Blood.* 2013;122(21). Abstract 970.
22. Gutsaeva DR, Parkerson JB, Yerigenahally SD, et al. Inhibition of cell adhesion by anti-P-selectin aptamer: a new potential therapeutic agent for sickle cell disease. *Blood.* 2011;117(2):727-735.
23. Matsui NM, Varki A, Embury SH. Heparin inhibits the flow adhesion of sickle red blood cells to P-selectin. *Blood.* 2002;100(10):3790-3796.
24. Batchvarova M, Shan S, Zennadi R, et al. Sevuparin reduces adhesion of both sickle red cells and leukocytes to endothelial cells In vitro and inhibits vaso-occlusion in vivo [abstract]. *Blood.* 2013;122(21). Abstract 182.
25. Leitgeb AM, Blomqvist K, Cho-Ngwa F, et al. Low anticoagulant heparin disrupts Plasmodium falciparum rosettes in fresh clinical isolates. *Am J Trop Med Hyg.* 2011;84(3):390-396.
26. Qari MH, Aljaouni SK, Alardawi MS, et al. Reduction of painful vaso-occlusive crisis of sickle cell anaemia by tinzaparin in a double-blind randomized trial. *Thromb Haemost.* 2007;98(2): 392-396.
27. van Zuuren EJ, Fedorowicz Z. Low-molecular-weight heparins for managing vaso-occlusive crises in people with sickle cell disease. *Cochrane Database Syst Rev.* 2013;6:CD010155.
28. De Castro LM, Zennadi R, Jonassaint JC, Batchvarova M, Telen MJ. Effect of propranolol as antiadhesive therapy in sickle cell disease. *Clin Transl Sci.* 2012;5(6):437-444.
29. De Castro LM. Propranolol: Anti-adhesive SCD treatment. (A phase II study of propranolol as anti-adhesive therapy for sickle cell disease). 9th Annual Sickle Cell Disease Research and Educational Symposium and 38th National Sickle Cell Disease Scientific Meeting. Hollywood, FL; 2015.
30. Zennadi R. MEK inhibitors, novel anti-adhesive molecules, reduce sickle red blood cell adhesion in vitro and in vivo, and vasoocclusion in vivo. *PLoS One.* 2014;9(10):e110306.
31. Emanuele RM. FLOCOR: a new anti-adhesive, rheologic agent. *Expert Opin Investig Drugs.* 1998;7(7):1193-1200.
32. Ballas SK, Files B, Luchtman-Jones L, et al. Safety of purified poloxamer 188 in sickle cell disease: phase I study of a non-ionic surfactant in the management of acute chest syndrome. *Hemoglobin.* 2004;28(2):85-102.
33. Adams-Graves P, Kedar A, Koshy M, et al. RheothRx (poloxamer 188) injection for the acute painful episode of sickle cell disease: a pilot study. *Blood.* 1997;90(5):2041-2046.
34. Orringer EP, Casella JF, Ataga KI, et al. Purified poloxamer 188 for treatment of acute vaso-occlusive crisis of sickle cell disease: A randomized controlled trial. *JAMA.* 2001;286(17): 2099-2106.
35. Cheung AT, Chan MS, Ramanujam S, et al. Effects of poloxamer 188 treatment on sickle cell vaso-occlusive crisis: computer-assisted intravital microscopy study. *J Investig Med.* 2004;52(6): 402-406.
36. Hebbel RP. Ischemia-reperfusion injury in sickle cell anemia: relationship to acute chest syndrome, endothelial dysfunction, arterial vasculopathy, and inflammatory pain. *Hematol Oncol Clin North Am.* 2014;28(2):181-198.
37. Wallace KL, Marshall MA, Ramos SI, et al. NKT cells mediate pulmonary inflammation and dysfunction in murine sickle cell disease through production of IFN-γ and CXCR3 chemokines. *Blood.* 2009;114(3):667-676.
38. Field JJ, Nathan DG, Linden J. Targeting iNKT cells for the treatment of sickle cell disease. *Clin Immunol.* 2011;140(2):177-183.
39. Wallace KL, Linden J. Adenosine A2A receptors induced on iNKT and NK cells reduce pulmonary inflammation and injury in mice with sickle cell disease. *Blood.* 2010;116(23):5010-5020.
40. Nowak M, Lynch L, Yue S, et al. The A2aR adenosine receptor controls cytokine production in iNKT cells. *Eur J Immunol.* 2010;40(3):682-687.
41. Linden J. Regulation of leukocyte function by adenosine receptors. *Adv Pharmacol.* 2011;61: 95-114.
42. Zhang Y, Xia Y. Adenosine signaling in normal and sickle erythrocytes and beyond. *Microbes Infect.* 2012;14(10):863-873.
43. Field JJ, Lin G, Okam MM, et al. Sickle cell vaso-occlusion causes activation of iNKT cells that is decreased by the adenosine A2A receptor agonist regadenoson. *Blood.* 2013;121(17):3329-3334.
44. Field JJ, Ataga KI, Majerus E, Eaton CA, Mashal R, Nathan DG. A Phase I single ascending dose study of NKTT120 in stable adult sickle cell patients [abstract]. *Blood.* 2013;122(21). Abstract 977.
45. Knight-Perry J, DeBaun MR, Strunk RC, Field JJ. Leukotriene pathway in sickle cell disease: a potential target for directed therapy. *Expert Rev Hematol.* 2009;2(1):57-68.
46. Field JJ, Strunk RC, Knight-Perry JE, Blinder MA, Townsend RR, DeBaun MR. Urinary cysteinyl leukotriene E4 significantly increases during pain in children and adults with sickle cell disease. *Am J Hematol.* 2009;84(4):231-233.
47. Patel N, Gonsalves CS, Yang M, Malik P, Kalra VK. Placenta growth factor induces 5-lipoxygenase-activating protein to increase leukotriene formation in sickle cell disease. *Blood.* 2009;113(5):1129-1138.
48. Elmariah H, Garrett ME, De Castro LM, et al. Factors associated with survival in a contemporary adult sickle cell disease cohort. *Am J Hematol.* 2014;89(5):530-535.
49. Kato GJ, Martyr S, Blackwelder WC, et al. Levels of soluble endothelium-derived adhesion molecules in patients with sickle cell disease are associated with pulmonary hypertension, organ dysfunction, and mortality. *Br J Haematol.* 2005; 130(6):943-953.
50. Haynes J Jr, Obiako B, King JA, Hester RB, Ofori-Acquah S. Activated neutrophil-mediated sickle red blood cell adhesion to lung vascular endothelium: role of phosphatidylserine-exposed sickle red blood cells. *Am J Physiol Heart Circ Physiol.* 2006;291(4):H1679-H1685.
51. Kuvibidila S, Baliga BS, Gardner R, et al. Differential effects of hydroxyurea and zileuton on interleukin-13 secretion by activated murine spleen cells: implication on the expression of vascular cell adhesion molecule-1 and vasoocclusion in sickle cell anemia. *Cytokine.* 2005;30(5):213-218.

52. Haynes J Jr, Baliga BS, Obiako B, Ofori-Acquah S, Pace B. Zileuton induces hemoglobin F synthesis in erythroid progenitors: role of the L-arginine-nitric oxide signaling pathway. *Blood*. 2004;103(10):3945-3950.
53. Chang J, Shi PA, Chiang EY, Frenette PS. Intravenous immunoglobulins reverse acute vaso-occlusive crises in sickle cell mice through rapid inhibition of neutrophil adhesion. *Blood.* 2008; 111(2):915-923.
54. Turhan A, Jenab P, Bruhns P, Ravetch JV, Coller BS, Frenette PS. Intravenous immune globulin prevents venular vaso-occlusion in sickle cell mice by inhibiting leukocyte adhesion and the interactions between sickle erythrocytes and adherent leukocytes. *Blood.* 2004;103(6): 2397-2400.
55. Jang JE, Hidalgo A, Frenette PS. Intravenous immunoglobulins modulate neutrophil activation and vascular injury through FcγRIII and SHP-1. *Circ Res.* 2012;110(8):1057-1066.
56. Manwani D, Chen G, Carullo V, et al. Single-dose intravenous gammaglobulin can stabilize neutrophil Mac-1 activation in sickle cell pain crisis. *Am J Hematol.* 2015;90(5):381-385.
57. Hoppe C, Kuypers F, Larkin S, Hagar W, Vichinsky E, Styles L. A pilot study of the short-term use of simvastatin in sickle cell disease: effects on markers of vascular dysfunction. *Br J Haematol.* 2011;153(5):655-663.
58. Charache S, Barton FB, Moore RD, et al; The Multicenter Study of Hydroxyurea in Sickle Cell Anemia. Hydroxyurea and sickle cell anemia. Clinical utility of a myelosuppressive "switching" agent. *Medicine (Baltimore).* 1996;75(6):300-326.
59. Hebbel RP, Vercellotti GM, Pace BS, et al. The HDAC inhibitors trichostatin A and suberoylanilide hydroxamic acid exhibit multiple modalities of benefit for the vascular pathobiology of sickle transgenic mice. *Blood.* 2010;115(12):2483-2490.
60. Reid ME, El Beshlawy A, Inati A, et al. A double-blind, placebo-controlled phase II study of the efficacy and safety of 2,2-dimethylbutyrate (HQK-1001), an oral fetal globin inducer, in sickle cell disease. *Am J Hematol.* 2014;89(7):709-713.
61. Kutlar A, Reid ME, Inati A, et al. A dose-escalation phase IIa study of 2,2-dimethylbutyrate (HQK-1001), an oral fetal globin inducer, in sickle cell disease. *Am J Hematol.* 2013;88(11):E255-E260.
62. Kutlar A, Ataga K, Reid M, et al. A phase 1/2 trial of HQK-1001, an oral fetal globin inducer, in sickle cell disease. *Am J Hematol.* 2012;87(11): 1017-1021.
63. Okam MM, Esrick EB, Mandell E, Campigotto F, Neuberg DS, Ebert BL. Phase 1/2 trial of vorinostat in patients with sickle cell disease who have not benefitted from hydroxyurea. *Blood.* 2015;125(23):3668-3669.
64. Ikuta T, Atweh G, Boosalis V, et al. Cellular and molecular effects of a pulse butyrate regimen and new inducers of globin gene expression and hematopoiesis. *Ann N Y Acad Sci.* 1998;850: 87-99.
65. Atweh GF, Sutton M, Nassif I, et al. Sustained induction of fetal hemoglobin by pulse butyrate therapy in sickle cell disease. *Blood.* 1999;93(6): 1790-1797.
66. Atweh GF, Schechter AN. Pharmacologic induction of fetal hemoglobin: raising the therapeutic bar in sickle cell disease. *Curr Opin Hematol.* 2001;8(2):123-130.
67. Meiler SE, Wade M, Kutlar F, et al. Pomalidomide augments fetal hemoglobin production without the myelosuppressive effects of hydroxyurea in transgenic sickle cell mice. *Blood.* 2011;118(4): 1109-1112.
68. Moutouh-de Parseval LA, Verhelle D, Glezer E, et al. Pomalidomide and lenalidomide regulate erythropoiesis and fetal hemoglobin production in human $CD34^+$ cells. *J Clin Invest.* 2008;118(1): 248-258.
69. Kutlar A, Swerdlow PS, Meiler SE, et al. Pomalidomide in sickle cell disease: Phase I study of a novel anti-switching agent [abstract]. *Blood.* 2013;122(21). Abstract 777.
70. Fard AD, Hosseini SA, Shahjahani M, Salari F, Jaseb K. Evaluation of novel fetal hemoglobin inducer drugs in treatment of β-hemoglobinopathy disorders. *Int J Hematol Oncol Stem Cell Res.* 2013;7(3):47-54.
71. Ataga KI, Orringer EP, Styles L, et al. Dose-escalation study of ICA-17043 in patients with sickle cell disease. *Pharmacotherapy.* 2006; 26(11):1557-1564.
72. Ataga KI, Reid M, Ballas SK, et al; ICA-17043-10 Study Investigators. Improvements in haemolysis and indicators of erythrocyte survival do not correlate with acute vaso-occlusive crises in patients with sickle cell disease: a phase III randomized, placebo-controlled, double-blind study of the Gardos channel blocker senicapoc (ICA-17043). *Br J Haematol.* 2011;153(1):92-104.
73. Ataga KI, Smith WR, De Castro LM, et al; ICA-17043-05 Investigators. Efficacy and safety of the Gardos channel blocker, senicapoc (ICA-17043), in patients with sickle cell anemia. *Blood.* 2008; 111(8):3991-3997.
74. Ataga KI, Stocker J. Senicapoc (ICA-17043): a potential therapy for the prevention and treatment of hemolysis-associated complications in sickle cell anemia. *Expert Opin Investig Drugs.* 2009; 18(2):231-239.
75. Stocker JW, De Franceschi L, McNaughton-Smith GA, Corrocher R, Beuzard Y, Brugnara C. ICA-17043, a novel Gardos channel blocker, prevents sickled red blood cell dehydration in vitro and in vivo in SAD mice. *Blood.* 2003;101(6):2412-2418.
76. Misra H, Lickliter J, Kazo F, Abuchowski A. PEGylated carboxyhemoglobin bovine (SANGUINATE): results of a phase I clinical trial. *Artif Organs.* 2014;38(8):702-707.
77. Belcher JD, Young M, Chen C, et al. MP4CO, a pegylated hemoglobin saturated with carbon monoxide, is a modulator of HO-1, inflammation, and vaso-occlusion in transgenic sickle mice. *Blood.* 2013;122(15):2757-2764.
78. Abdulmalik O, Safo MK, Chen Q, et al. 5-hydroxymethyl-2-furfural modifies intracellular sickle haemoglobin and inhibits sickling of red blood cells. *Br J Haematol.* 2005;128(4):552-561.
79. Ataga KI, Key NS. Hypercoagulability in sickle cell disease: new approaches to an old problem. *Hematology Am Soc Hematol Educ Program.* 2007;2007:91-96.
80. Ataga KI, Orringer EP. Hypercoagulability in sickle cell disease: a curious paradox. *Am J Med.* 2003; 115(9):721-728.
81. Babiker MA, Ashong EF, Bahakim H, Gader AM. Coagulation changes in sickle cell disease in early childhood. *Acta Haematol.* 1987;77(3):156-160.
82. Francis RB Jr. Platelets, coagulation, and fibrinolysis in sickle cell disease: their possible role in vascular occlusion. *Blood Coagul Fibrinolysis.* 1991;2(2):341-353.
83. Shah N, Thornburg C, Telen MJ, Ortel TL. Characterization of the hypercoagulable state in patients with sickle cell disease. *Thromb Res.* 2012;130(5):e241-e245.
84. Salvaggio JE, Arnold CA, Banov CH. Long-term anti-coagulation in sickle-cell disease. A clinical study. *N Engl J Med.* 1963;269:182-186.
85. Ahmed S, Siddiqui AK, Iqbal U, et al. Effect of low-dose warfarin on D-dimer levels during sickle cell vaso-occlusive crisis: a brief report. *Eur J Haematol.* 2004;72(3):213-216.
86. Shah N, Willen S, Telen MJ, Ortel TL. Prophylactic dose low molecular weight heparin (dalteparin) for treatment of vaso-occlusive pain crisis in patients with sickle cell disease [abstract]. *Blood.* 2013;122(21). Abstract 2241.
87. Wun T, Paglieroni T, Field CL, et al. Platelet-erythrocyte adhesion in sickle cell disease. *J Investig Med.* 1999;47(3):121-127.
88. Brittain JE, Knoll CM, Ataga KI, Orringer EP, Parise LV. Fibronectin bridges monocytes and reticulocytes via integrin α4β1. *Br J Haematol.* 2008;141(6):872-881.
89. Lee SP, Ataga KI, Zayed M, et al. Phase I study of eptifibatide in patients with sickle cell anaemia. *Br J Haematol.* 2007;139(4):612-620.
90. Desai PC, Brittain JE, Jones SK, et al. A pilot study of eptifibatide for treatment of acute pain episodes in sickle cell disease. *Thromb Res.* 2013;132(3):341-345.
91. Ohno K, Tanaka H, Samata N, et al. Platelet activation biomarkers in Berkeley sickle cell mice and the response to prasugrel. *Thromb Res.* 2014;134(4):889-894.
92. Telen MJ. Biomarkers and recent advances in the management and therapy of sickle cell disease. F1000 Research Vol. 4 (F1000 Faculty Rev); 2015: 1050 doi:1010.12688/f11000research. 16615.12681.

2016 127: 810-819
doi:10.1182/blood-2015-09-618553 originally published online January 12, 2016

Beyond hydroxyurea: new and old drugs in the pipeline for sickle cell disease

Marilyn J. Telen

Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.