

Chapter 2

Pathobiology of Sickle Cell Disease

Vaso-occlusion and Targeted Therapies

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Abbreviations

HbS	Sickle hemoglobin
RBC	Red blood cell
SCD	Sickle cell disease
VOC	Vaso-occlusive crisis

Sickle cell disease (SCD) originates from a single base pair change in the β -globin subunit, yet the complex manifestations that result are manifold. The abnormal HbS is insoluble when deoxygenated, leading to polymer formation. These cells are less deformable and are prone to hemolysis. Wide-field digital interferometry demonstrates that sickle red blood cells (RBCs) are stiffer than those with normal adult hemoglobin [1]. Adhesion of low-density sickle RBCs and reticulocytes in postcapillary venules leads to trapping of the older, more dense, and misshapen SS-RBCs and results in reduced blood flow, hence contributing to vaso-occlusive crisis (VOC) [2]. The sickle RBC is only one reason for the systemic multi-organ damage in this disease. Many cells that are not affected by the β -globin mutation play a role in this lifelong debilitating illness. The interactions between the sickle RBCs, endothelium, leukocytes, platelets, cytokines, and inflammatory mediators are all responsible for a chronic inflammatory state and cumulative organ injury [3, 4]. Sickle RBCs easily dehydrate, leading to HbS polymerization and subsequently to altered shape and

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surface cell properties. This leads to hemolysis and triggers activation of coagulation factors, platelets, white blood cells, endothelium, and intracellular signaling pathways [5]. This chapter will highlight the underlying pathophysiology of the ischemia reperfusion injuries, the abnormal interactions between the red cell and its surrounding environment (particularly the endothelium, neutrophils, and platelets), the prothrombotic milieu, and the novel therapies that are being investigated to treat this disease.

Adhesion Pathways

Adhesive Interactions of Red Cells and Leukocytes with the Endothelium

The mutated HbS causes deformation of RBC membranes by polymer formation, RBC membrane damage via iron-mediated generation of oxidants [6], and altered lipid properties [7]. The endothelium in SCD has an activated phenotype, demonstrated by the upregulation of adhesion molecules such as vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), and the selectin family [8, 9]. Selectins regulate leukocyte adhesion to the endothelium, and their expression is enhanced by inflammatory cytokines such as tumor necrosis factor (TNF- α) or interleukin-1 (IL-1) [10]. Two of the selectins (P-selectin and E-selectin) are expressed on the endothelium and can markedly slow down the rolling of the white blood cells with the additional interactions of cytokines and inflammatory markers [8, 11]. Activated $\alpha_M\beta_2$ (macrophage-1 antigen [MAC-1]) on adherent neutrophils captures sickle RBCs leading to decreased blood flow [12]. Sick RBCs can also adhere via numerous adhesive partners to the endothelium directly (e.g., VCAM-1), with or without intervening bridging molecules (thrombospondin, von Willebrand factor [VWF]) or with subendothelial matrix proteins (laminin, VWF) [13, 14]. Activation of NF- κ B upregulates expression of these adhesion molecules (E-selectin, VCAM-1, and ICAM-1) on the surface of the endothelium [2, 15]. Activated circulating endothelial cells and increased levels of plasma sVCAM-1, P-selectin, and E-selectin have all been implicated in participating in VOC [11, 16, 17]. Specific erythrocyte ligands also play a role in adhesion such as Lutheran blood group antigen [18], VLA-4 [19, 20], CD 36, and sulfated glycolipids [21]. Basal cell adhesion molecule/Lutheran blood group (BCAM/LU) and ICAM-4 can both be activated by epinephrine [2, 22]. Activated ICAM-4 by epinephrine leads to VOC and increased leukocyte adhesion to the endothelium via endothelial $\alpha\beta_3$ integrin [23, 24].

Neutrophil-RBC Interactions

Neutrophils participate in the pathogenesis of SCD, and *in vitro* studies demonstrate that sickle RBCs directly bind to neutrophils [25]. This is supported by *in vivo* studies in Berkeley SCD mice where the dynamics of circulating blood cells are

analyzed in the cremasteric microcirculation using intravital microscopy [11, 25]. Clinically, patients with more severe symptoms have higher neutrophil counts than racially matched controls [26, 27]. Patients that have required GCSF or GMCSF for treatment of other comorbidities such as neutropenia or stem cell harvest have had severe or fatal VOC [28–32].

Therapeutic Interventions Targeting Adhesion Molecules

Inhibition of these adhesion molecules, or their downstream targets, has been the focus for novel therapeutic targets. Administration of an anti-P-selectin aptamer in SCD mice resulted in a decreased adhesion of sickle RBCs by 80–90%, increased microvascular flow velocities, and reduced adhesion of the leukocyte to the endothelium [16]. In a recent publication in the *New England Journal of Medicine*, Ataga et al. describe the results of a double-blind, randomized, placebo-controlled, phase 2 trial of crizanlizumab for treating pain crises. Crizanlizumab is a humanized monoclonal antibody against the adhesion molecule P-selectin, and adult sickle cell patients were prophylactically administered with the medication over 52 weeks. In the high-dose crizanlizumab arm, there was a significantly lower rate of sickle cell-related pain crises per year than placebo (1.63 vs. 2.98) and a low incidence of adverse events [17]. Rivapansel (GMI1070) a pan-selectin inhibitor (particularly against E-selectin) has been shown in sickle cell mice to improve sickle RBC-leukocyte interactions leading to improved microcirculatory blood flow and reduced VOCs [33]. The phase 2 clinical studies have shown the drug to be a safe intervention with a markedly reduced use of opioids during hospitalization (83% reduction compared to placebo) and a trend toward a faster resolution of VOC (41 h versus 63 h) [34]. Currently a phase 3 study of rivapansel (NCT02187003) in adults is ongoing.

Two groups have demonstrated the efficacy of RNA aptamers to inhibit P-selectin-mediated RBC adhesion to endothelial cells [16, 35] in preclinical models, lending further support to this adhesive target, but currently there are no open clinical trials for these agents.

In a multicenter phase 3 study, poloxamer, a surfactant that inhibits cell adhesion, did not meet its primary efficacy endpoint of reduction in the mean duration of VOC (82 h in the vepoloxamer group compared to 78 h in the placebo group in the intent-to-treat population ($p = 0.09$). There were also no statistically significant differences between treatment groups in the intent-to-treat population across the two secondary efficacy endpoints, rate of rehospitalization for VOC, and the occurrence of acute chest syndrome [36].

Intravenous γ -globulin (IVIG) inhibits leukocyte activation and adhesion by decreasing leukocyte-erythrocyte interaction and improving microcirculation [37]. Fc γ receptors are on many different hematopoietic cells including neutrophils and macrophages and can be of the activating or inhibitory subtype. Engagement of Fc γ RIII receptors (activating receptor subtype) on neutrophils triggers phagocytosis, reactive oxygen production, and release of inflammatory cytokines [37]. Surprisingly, IVIG binds the Fc γ RIII receptor reducing Mac-1 activity and

mediates these interactions by recruitment of Src homology 2 (SH2)-containing tyrosine phosphatase-1 (SHP-1) that inhibits downstream Src kinase [37]. In SCD mice, IVIG reverses VOC by inhibiting neutrophil adhesion to the endothelium and modulating the interactions between leukocytes and circulating red blood cells [38, 39]. In a phase 1 trial in pediatric and adult SCD patients with acute VOC, IVIG also decreased human neutrophil Mac-1 function and was safe and well tolerated [40]. Currently a phase 2 trial of IVIG is recruiting patients (NCT01757418). While this section highlighted a selected few therapeutic interventions, Table 2.1 summarizes the studies discussed above as well as additional agents targeting cell adhesion.

Table 2.1 Novel agents in clinical trials targeting adhesion

Study title	Intervention	Clinical trials/phase	Status
<i>Selectin inhibitors</i>			
Study of GMI-1070 for the Treatment of Sickle Cell Pain Crisis	GMI-1070 (rivapansel) [34]	NCT01119833 Phase 2	Complete
Efficacy and Safety of Rivipansel (GMI-1070) in the Treatment of Vaso-Occlusive Crisis in Hospitalized Subjects with Sickle Cell Disease	GMI-1070 (rivapansel)	NCT02187003 Phase 3	Ongoing
Study to Assess Safety and Impact of SelG1 with or Without Hydroxyurea Therapy in Sickle Cell Disease Patients with Pain Crises	SelG1 [17]	NCT01895361 Phase 2	Complete
Sevuparin Infusion for the Management of Acute VOC in Subjects With SCD	Sevuparin	NCT02515838 Phase 2	Ongoing
Phase 1–2 Trial of Gamunex (Intravenous Gammaglobulin) for Sickle Cell Acute Pain	IVIG	NCT01757418 Phase 1 [40]/2	Ongoing
<i>β-Blockers</i>			
Study of Propranolol as Anti-adhesive Therapy in Sickle Cell Disease (SCD)	Propranolol [41]	NCT01077921 Phase 2	Complete
Propranolol and Red Cell Adhesion in Non-asthmatic Children with Sickle Cell Disease	Propranolol	NCT02012777 Phase 1	Ongoing
<i>Other inhibitors of adhesion</i>			
Phase III Randomized Study of Poloxamer 188 for Vaso-Occlusive Crisis of Sickle Cell Disease	Poloxamer [36]	NCT00004408 Phase 3	Complete
Evaluation of Purified Poloxamer 188 in Vaso-Occlusive Crisis of Sickle Cell Disease (EPIC)	Poloxamer	NCT01737814 Phase 3	Complete
A Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics Study of PF-04447943, Coadministered With and Without Hydroxyurea, In Subjects with Stable Sickle Cell Disease	PDE9 inhibitor 1	NCT02114203 Phase 1	Complete

Inflammatory Pathways

Adenosine and Invariant Natural Killer T (iNKT) Cells

Invariant natural killer T (iNKT) cells are increased in number and activity in SCD [42–44] and promote the inflammatory cascade. Adenosine A2A receptors (A2AR) are expressed on iNKT cells, and activation of these receptors downregulates the activity of iNKT cells [43, 45, 46]. Regadenoson is a selective A2AR agonist that is approved for radionuclide myocardial imaging. A side effect of this drug can be decreased blood pressure and reflexive tachycardia. Low-dose infused regadenoson was postulated to have the selective binding of A2AR receptor and to have less cardiac toxicity. Animal models showed that an A2AR agonist led to reversal of pulmonary dysfunction in mice [43], and a phase 1 study in adults with SCD demonstrated that low-dose regadenoson infusion decreased activation of iNKT cells during a VOC without significant toxicity [47]. A phase 2 trial during VOC in pediatric and adult patients is currently ongoing (NCT01788631).

Leukotrienes

Leukotrienes are proinflammatory lipid molecules produced by all leukocytes in response to various stimuli. Leukotrienes LTC₄, LTD₄, and LTE₄ are produced by mast cells and macrophages and as a group are classified as cysteinyl LTs (CysLTs). In the lung, CysLTs cause airway edema, smooth muscle proliferation, and fibrotic tissue formation [48]. In the endothelium they cause vasoconstriction, upregulation of adhesion molecules, and recruitment of inflammatory cells such as eosinophils, monocytes, and T cells [49–53]. Secretory phospholipase A2 (sPLA2) which releases arachidonic acid, the precursor of leukotrienes, is increased in individuals with acute chest syndrome [54]. LTE₄ is elevated in adults and children with SCD at baseline and increases during pain crisis [55–58]. Montelukast is a CysLT inhibitor and an FDA-approved drug for asthma. Currently an 8-week phase 2 study of the addition of montelukast is being conducted (NCT01960413) in individuals on hydroxyurea with outcomes looking at tissue injury, lung function, and microvascular blood flow. Another FDA-approved drug for asthma, zileuton, is being examined in a phase 1 trial (NCT01136941) in children and adults. Zileuton inhibits 5-lipoxygenase a key leukotriene synthetic enzyme. In a mouse model, zileuton attenuated the amount of activated neutrophils and decreased sickle RBC adherence in the lung [59]. Corticosteroids are potent antileukotrienes by inhibiting the release of arachidonic acid. The Inhaled Mometasone to Reduce Painful Episodes in Patients With Sickle Cell Disease (IMPROVE) trial (NCT02061202) is a phase 2 trial ongoing currently to investigate if individuals without asthma could have decreased VOCs with inhaled corticosteroids [60].

Oxidative Stress and Impaired Nitric Oxide Biology

Plasma hemoglobin released from hemolyzed sickle erythrocytes consumes nitric oxide (NO) [61] 1000-fold faster than intraerythrocytic hemoglobin [62, 63]. NO has multiple vascular effects including vasodilation, anti-adhesive, antithrombotic, and antioxidant [64]. Reduced endothelial NO bioavailability in SCD impairs downstream vascular functions of NO, like vasodilation. Decreased NO also results in increased expression of cell adhesion molecules, VCAM-1, ICAM-1, P-selectin, and E-selectin [64]. Decreased NO bioavailability occurs in SCD at baseline and is associated with VOCs and acute chest syndrome [65, 66]. Statins modulate NO production through upregulation of endothelial nitric oxide synthase and hence are protective against endothelial injury [67, 68]. Children with SCD were treated with simvastatin for 21 days and had decreased IL-6 levels and CRP with increased NO metabolites (NOx) [69].

L-arginine is an obligate substrate for NO and is relatively deficient in SCD due to high levels of plasma arginase released from hemolyzed erythrocytes. L-arginine supplementation improves erythrocyte integrity [70], and inhibition of arginase in sickle cell mice reverses endothelial dysfunction and vascular stiffness [71]. Exogenous supplementation of L-arginine (100 mg/kg three times a day) was administered during VOC for 5 days in a double-blind, randomized controlled trial in children. The treatment was well tolerated and had significant reduction in opioid use and lower pain scores [72].

Omega-3 fatty acids have been demonstrated in preclinical models to also mitigate vasculopathy. This is achieved by various mechanisms such as favorable changes in the red cell membrane lipid composition, modulation of inflammation and coagulation, and production of nitric oxide [73]. In two single-center studies, omega-3 fatty acids have shown to decrease the frequency and severity of VOC episodes in adults and children [74, 75]; multicenter studies are ongoing NCT02973360. Table 2.2 highlights other treatments that are being investigated in modulating oxidative stress and decreasing inflammatory markers to improve outcomes in SCD.

Table 2.2 Interventions targeting inflammation

Study title	Intervention	Clinical trials/phase	Status
<i>Adenosine and invariant NKT cells</i>			
Adenosine 2A Agonist Lexiscan in Children and Adults With Sickle Cell Disease	Regadenoson [76]	NCT01085201 Phase 1	Complete
A Phase II Trial of Regadenoson in Sickle Cell Anemia	Regadenoson	NCT01788631 Phase 2	Ongoing
Safety, Pharmacokinetic, and Pharmacodynamic Study of NKTT120 in Adult Patients with Stable Sickle Cell Disease (SCD)	NKTT120 [76]	NCT01783691 Phase 1	Complete

Table 2.2 (continued)

Study title	Intervention	Clinical trials/phase	Status
<i>Leukotrienes</i>			
Phase 2 Study of Montelukast for the Treatment of Sickle Cell Anemia	Montelukast	NCT01960413 Phase 2	Ongoing
Trial of Zileuton CR in Children and Adults with Sickle Cell Disease	Zileuton	NCT01136941 Phase 1	Complete
Inhaled Mometasone to Reduce Painful Episodes in Patients with Sickle Cell Disease (IMPROVE)	Mometasone	NCT02061202 Phase 2	Ongoing
<i>Other anti-inflammatory reagents</i>			
Effect of Simvastatin Treatment on Vaso-occlusive Pain in Sickle Cell Disease	Simvastatin [69]	NCT01702246 Phase 2	Complete
Atorvastatin Therapy to Improve Endothelial Function in Sickle Cell Disease	Atorvastatin	NCT00072826 Phase 1	Complete
The Effect of Factor Xa Inhibition, with Rivaroxaban, on the Pathology of Sickle Cell Disease	Rivaroxaban	NCT02072668 Phase 2	Ongoing
<i>Antioxidants</i>			
A Phase III, Prospective, Randomized, Double-Blind Placebo-Controlled, Parallel-Group, Multicenter Study of L-Glutamine for Sickle Cell Anemia and S β 0-Thalassemia	L-glutamine [77]	NCT01179217 Phase 3	Complete
A Phase 2, Randomized, Double-Blind, Placebo-Controlled, Parallel-Group, Dose-Finding Study of SC411 in Children with Sickle Cell Disease	Omega-3 fatty acids [78]	NCT02973360 Phase 2	Ongoing
A Phase 1 Study of Continuous Intravenous L-Citrulline During Sickle Cell Pain Crisis or Acute Chest Syndrome	L-citrulline	NCT02697240 Phase 1	Ongoing
N-Acetylcysteine in Patients with Sickle Cell Disease: Reducing the Incidence of Daily Life Pain	N-acetyl cysteine	NCT01849016 Phase 3	Complete
A Pilot Study of N-Acetylcysteine in Patients with Sickle Cell Disease	N-acetyl [79] cysteine	NCT01800526 Phase 1/2	Ongoing
Physiological Effect of Sulforaphane Obtained from Broccoli Sprouts Homogenates (BSH) on the HbF and Anti-oxidative Capacity of Human Sickle RBC	Broccoli sprouts [80]	NCT01715480 Phase 1	Complete
Arginine Supplementation in Sickle Cell Anemia: Physiological and Prophylactic Effects	L-arginine [72]	NCT00513617 Phase 2	Complete

NRF2 nuclear factor (erythroid-derived 2)-like 2, *NAD* nicotinamide adenosine dinucleotide, *NO* nitric oxide, *RBC* red blood cell

Role of Fetal Hemoglobin

Hemoglobin F interferes with polymerization of HbS [81]. Individuals with hereditary persistent fetal hemoglobin are clinically very different since the elevated fetal hemoglobin ameliorates SCD severity by preventing polymerization of HbS. Hydroxyurea is currently the only FDA-approved drug for SCD that has been shown to elevate fetal hemoglobin production [81–83], but many other medications are currently being investigated in clinical trials. Intravenous sodium butyrate infusion was effective in increasing fetal hemoglobin from 7% to 22% in a small adult study [84]. A randomized placebo-controlled trial of HQK-1001 had to be halted after a planned interim analysis showed no significant increase in fetal hemoglobin in the HQK-1001 group [85]. Gene therapy rather than pharmacologic therapy may be the answer to solving this dilemma. The locus control region (LCR) is the major transcriptional enhancer of the β -globin gene. Blobel and colleagues successfully redirected globin synthesis from the adult β -globin promoter to the fetal γ -globin promoter by custom-designed zinc finger-Ldb1 fusion proteins (ZF-Ldb1) that redirected binding of the long-range enhancer [86, 87]. ZF-Ldb1 sickle-treated hematopoietic cells from individuals with SCD showed more than twice the increase of HbF (45%) and a concomitant decrease in HbS (50%) compared to various pharmacologic treatments [86]. *In vivo* work needs to be performed to further determine the feasibility of this in clinical practice.

Anti-sickling Agents

Common themes of agents that target sickling focus on ways to prevent polymerization of the HbS. By shifting the oxyhemoglobin dissociation curve to the left, improved oxygenation of hemoglobin will decrease sickling. AES-103 (5-hydroxymethyl furfural) is a compound made of a five-carbon-ring aromatic aldehyde that exists naturally in coffee, honey, and dried fruits. *In vitro* assays and sickle mice data both show decreased sickling and polymer formation with improved red cell survival [88]. Compounds that shift the oxyhemoglobin dissociation curve to the left (AES-103 and GBT440) [89, 90] have been well tolerated in adult patients with SCD in early phase 1 studies [91]. AES-103 was renamed Bax 555 when Baxalta was acquired by Shire and the phase 2 trial was terminated. GBT440 is a small molecule that increases HbS affinity for oxygen, delays *in vitro* HbS polymerization, and prevents sickling of RBCs. In a mouse model, GBT440 extends the half-life of RBCs, reduces reticulocyte counts, and prevents ex vivo RBC sickling [89]. A phase 3 trial for GBT440 is registered and recruiting (NCT03036813). Carbon monoxide (CO) attaches to hemoglobin and acts as an anti-sickling agent by preventing HbS polymerization. Sanguinate is a pegylated hemoglobin product that delivers CO to HbS and has been shown to be safe in a phase 1 trial [92, 93]. It also

acts as an oxygen transfer agent and has anti-inflammatory properties [94]. A phase 2 study of sanguinate for VOC in adults (NCT02411708) is currently recruiting patients in an adult ambulatory setting. SCD-101 a botanical-derived drug is currently in a phase 1 trial (NCT02380079) in adults with SCD. While *in vivo* and *in vitro* studies show anti-sickling activity, the underlying mechanism is unknown, but has been well tolerated in an adult cohort [95]. Table 2.3 highlights the various treatments that are being investigated to target fetal hemoglobin induction and enhance anti-sickling.

Table 2.3 Therapeutics targeting hemoglobin F induction and anti-sickling

Study title	Intervention	Clinical trials/phase	Status
<i>Hemoglobin F induction</i>			
Phase 1 Placebo-Controlled Study of the Safety, Activity and Pharmacokinetics of HQK-1001 in Healthy Subjects	Oral sodium butyrate (HQK-101)	NCT00717262 Phase 1	Complete
Phase 1/2 Study to Evaluate the Safety, Tolerability and Pharmacokinetics of HQK-1001 Administered Daily in Patients with Sickle Cell Disease	Oral sodium butyrate [96] (HQK-101)	NCT00842088 Phase 1/2	Complete
A Study of HQK-1001 in Patients with Sickle Cell Disease	Oral sodium butyrate [97] (HQK-101)	NCT01322269 Phase 2	Complete
A Study of HQK-1001 in Patients with Sickle Cell Disease	Oral sodium butyrate [85] (HQK-101)	NCT01601340 Phase 2	Terminated
Study of Panobinostat (LBH589) in Patients with Sickle Cell Disease (LBH589)	Panobinostat	NCT01245179 Phase 1	Ongoing
Study of Decitabine and Tetrahydrouridine (THU) in Patients With Sickle Cell Disease	Decitabine-THU	NCT01685515 Phase 1	Ongoing
Study to Determine the Maximum Tolerated Dose, Safety and Effectiveness of Pomalidomide for Patients with Sickle Cell Disease	Pomalidomide [98]	NCT01522547 Phase 1	Complete
Use of Metformin as a Fetal Hemoglobin Inducer in Patients with Hemoglobinopathies	Metformin [99]	NCT02981329 Phase 1	Ongoing
<i>Anti-sickling agents</i>			
Dose-Escalation Study of SCD-101 in Sickle Cell Disease	SCD-101 [95]	NCT02380079 Phase 1	Ongoing
Study of SANGUINATE™ Versus Hydroxyurea in Sickle Cell Disease (SCD) Patients	Sanguinate [92]	NCT01848925 Phase 1	Complete

(continued)

Table 2.3 (continued)

Study title	Intervention	Clinical trials/phase	Status
Study of SANGUINATE™ In the Treatment of Sickle Cell Disease Patients with Vaso-Occlusive Crisis	Sanguinate	NCT02411708 Phase 2	Ongoing
Safety Study of MP4CO in Adult Sickle Cell Patients	MP4CO [100]	NCT01356485 Phase 1	Complete
A Study of the Efficacy and Safety of ICA-17043 (With or Without Hydroxyurea) in Patients with Sickle Cell Anemia	Senicapoc (ICA-17043) [101, 102]	NCT00040677 Phase 2	Complete
A Stratified Sickle Event Randomized Trial (ASSERT)	Senicapoc (ICA-17043) [101, 102]	NCT00102791 Phase 3	Terminated
A Study Evaluating the Long-Term Safety of ICA-17043 in Sickle Cell Disease Patients With or Without Hydroxyurea Therapy	Senicapoc (ICA-17043) [101, 102]	NCT00294541 Phase 3	Terminated
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	Aes-103 (Bax 55)	NCT01597401 Phase 1	Complete
Evaluation of Different Dose Regimens of Aes-103 Given for 28 Days to Subjects with Stable Sickle Cell Disease	Aes-103 (Bax 55)	NCT01987908 Phase 2	Terminated
A Study of the Safety, Blood Levels and Biological Effects of GBT440 in Healthy Subjects and Subjects with Sickle Cell Disease	GBT440	NCT02285088 Phase 1	Ongoing
A Study of the Safety, Blood Levels and Biological Effects of GBT440 in Healthy Subjects and Subjects with Sickle Cell Disease	GBT440 [91]	NCT02285088 Phase 1	Complete
Study to Evaluate the Effect of GBT440 Administered Orally to Patients with Sickle Cell Disease (GBT_HOPE)	GBT440	NCT03036813 Phase 3	Ongoing

Chronic Pain in Sickle Cell Disease

Frequent and persistent pain is common in SCD, particularly in the adult population. According to a recent study, patients report sickle cell pain characteristics that are consistent with both nociceptive and neuropathic pain, contrary to prior belief that all SCD pain is nociceptive [103]. Trifluoperazine, a potent Ca/calmodulin protein kinase IIa inhibitor commonly used to treat neuropathic pain, has been recently studied in adult SCD patients. Half of the patients in this phase 1 trial reported a

Table 2.4 Therapeutic targets investigating chronic pain

Study title	Intervention	Clinical trials/phase	Status
Clinical Trial to Study the Safety and Tolerability of Memantin Mepha® in Sickle Cell Disease Patients	Memantine hydrochloride	NCT02615847 Phase 2	Ongoing
Pain Management of Vaso-Occlusive Crisis in Children and Young Adults with Sickle Cell Disease	Gabapentin	NCT01954927 Phase 2	Ongoing
Cannabinoid-Based Therapy and Approaches to Quantify Pain in Sickle Cell Disease	Vaporized cannabis	NCT01771731 Phase 1	Ongoing

50% reduction in their chronic pain, suggesting a role for neuropathy in the pathogenesis of SCD pain [104]. SCD mice also exhibit altered sensitivity to pain as demonstrated by musculoskeletal and cutaneous hyperalgesia [105]. Nociceptive neurons in the spinal cord of BERK sickle mice have increased phosphorylation of mitogen-activated protein kinases (MAPKs) that are known to contribute to neuronal hyperexcitability, including c-Jun N-terminal kinase (JNK), p44/p42 extracellular signal-regulated kinase (ERK), and p38, which suggests that central sensitization contributes to the pain phenotype [106]. In SCD mice, activators of neuropathic and inflammatory pain (p38 mitogen-activated protein kinase, STAT3, and mitogen-activated protein kinase/extracellular signal-regulated kinase) are increased in the spinal cord in addition to neurochemical changes in the peripheral nerves [107]. Mast cells in murine models promote neurogenic inflammation and nociceptor activation through the release of substance P in the skin and dorsal root ganglion [108]. Targeting mast cells in sickle cell mice by small molecule inhibitors or by stabilizing mast cell degranulation ameliorates hyperalgesia [108, 109]. Although treatments for neuropathic pain appear to be promising as novel therapeutics for chronic pain in SCD, further investigations are urgently needed. Table 2.4 summarizes the treatments that are being investigated in chronic pain.

Role of Activated Coagulation in SCD

Venous thromboembolism (VTE) has been an underappreciated complication of HbSS, although the increased incidence and recurrence of thrombosis in SCD patients suggest a chronic hypercoagulable state [110]. It has been reported that up to 25% of adults with SCD have developed VTE, with the median age of first VTE being considerably younger than in the general population [111] and comparable to the age observed in families with high-risk thrombophilia [112]. The risk of VTE in SCD is heightened by recurrent hospitalizations, prolonged episodes of immobility, frequent use of central venous catheters, and infection [113]. Increased mortality was observed in adults with SCD and thrombosis [111, 114]. There is an increased prevalence of pulmonary embolism found in SCD patients at autopsy, especially

those with sudden death [115, 116], and it has been suggested that pulmonary embolism may underlie development of some cases of pulmonary hypertension. Additionally, both retrospective and prospective analyses of patients with acute chest syndrome report increased pulmonary embolism [115–118]. Use of administrative discharge databases further corroborates the increased incidence of pulmonary embolism in adults with SCD when compared to age- and race-matched controls [118, 119]. In the pediatric SCD population, central venous catheter placement increases the risk of DVT [120, 121]. Pregnancy-related VTE is also increased in women with SCD [122, 123].

Nearly every component of coagulation, including platelets, is affected by SCD. Tissue factor (TF) is an essential component of the factor VIIA-TF complex enzyme, the initiator of blood coagulation *in vivo*. TF is expressed by endothelial cells and monocytes, and increased levels are reported in SCD [124–127]. The number of circulating TF-laden cells and microparticles increases during painful crises, as compared to steady state [124, 127, 128]. In general, increased numbers of TF-expressing endothelial cells, monocytes, red blood cells, and their associated microparticles influence the coagulation cascade [129]. In accordance with this, there is an association between increased markers of hemolysis in SCD and whole blood TF procoagulant activity [130].

An overall increased state of thrombin generation in SCD is evidenced by chronic elevation of procoagulant proteins such as thrombin-antithrombin (TAT) complexes, prothrombin fragments (F1.2) and D-dimers, and other markers of thrombin generation [131]. Moderately decreased levels of the anticoagulant proteins C and S are observed in patients with SCD in steady state, and these may be further decreased during acute pain episodes [132–135]. Decreased levels of factor V have also been reported, suggesting chronic consumption of procoagulant factors due to the increase in tissue factor expression and thrombin generation [110, 136].

Von Willebrand factor (VWF) has also been implicated in the thrombophilic state of SCD [137]. Extracellular hemoglobin binds with high affinity to VWF, thus preventing VWF from being cleaved by ADAMTS-13. This could be considered a form of acquired ADAMTS-13 deficiency [138]. The inability to proteolyze VWF leads to accumulation of ultra-large, extremely adhesive VWF multimers in circulation and on the endothelium [138]. Plasma free heme also induces exocytosis of VWF from Weibel-Palade bodies [139], and total activity of VWF has been shown to directly correlate with hemolysis [140]. This pathophysiology is demonstrated clinically by the description of a thrombotic thrombocytopenic purpura-like syndrome in SCD patients [141, 142].

Overall the balance of the coagulation system in SCD is tipped toward thrombosis (Fig. 2.1). This system is a potential target for disease-modifying interventions with anticoagulants. For example, the use of low-dose warfarin was shown to significantly decrease D-dimer during crisis in a small group of patients with SCD [143]. Multiple studies targeting coagulation in SCD are ongoing (Table 2.5).

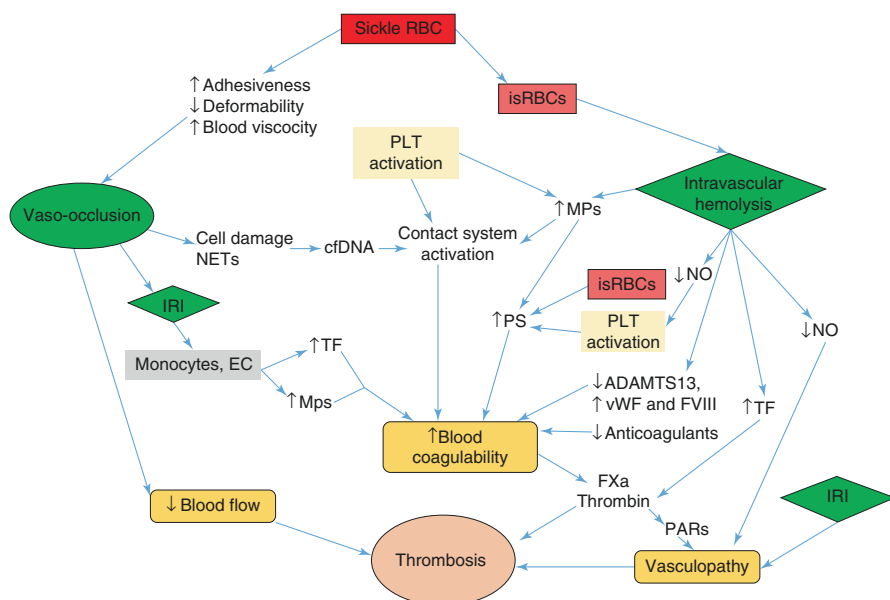


Fig. 2.1 Pathogenesis of thrombosis in sickle cell disease. *RBC* red blood cells, *isRBC* irreversibly sickled red blood cells, *PLT* platelets, *MP* microparticles, *cfDNA* cell-free DNA, *NETs* neutrophil extracellular traps, *NO* nitric oxide, *IRI* ischemic reperfusion injury, *TF* tissue factor, *PARs* protease-activated receptors, *PS* phosphatidylserine, *EC* endothelial cell, *VWF* von Willebrand factor, *FVIII* factor VIII, *FXa* activated factor X. Adapted from Ref. [124]

Table 2.5 Studies involving anticoagulants

Study title	Intervention	Clinical trials/phase	Status
An Exploratory Study of Anticoagulation for Pulmonary Hypertension in Sickle Cell Disease	Warfarin	NCT01036802 Phase 2	Terminated
Apixaban in Patients with Sickle cell Disease	Apixaban	NCT02179177 Phase 3	Ongoing
Treatment of Sickle Cell Patients Hospitalized in Pain Crisis with Prophylactic Dose Low-Molecular-Weight Heparin (LMWH) vs. Placebo	Dalteparin [144]	NCT01419977 Phase 2	Complete
The Effect of Rivaroxaban in Sickle Cell Disease	Rivaroxaban	NCT02072668 Phase 2	Ongoing
Feasibility Study or Unfractionated Heparin in Acute Chest Syndrome	Unfractionated heparin	NCT02098993Phase 2	Ongoing

Targeting Coagulation in SCD

Heparin

Trials of heparins have shown efficacy in treating painful crises. The anti-adhesive effect of heparins mediated via blockade of P-selectin is an additional mechanism of these agents. NCT01419977 studied prophylactic dosing of dalteparin on change in D-dimer, change in pain score, and change in the thrombin generation assay during VOC [144]. Results showed that prophylactic dosing did not significantly affect markers of coagulation; however there was a greater decrease in pain scores at days 1 and 3 in patients treated with dalteparin. A single-center, randomized, double-blind clinical trial showed reduction in the severity and duration of acute VOC when using tinzaparin vs. placebo [145]. A study of the effects of unfractionated heparin in acute chest syndrome in SCD (NCT02098993) is ongoing, with the primary outcome being time to hospital discharge.

Direct Thrombin and Factor X Inhibitors

Current studies of new oral anticoagulants and their potential role in SCD are ongoing. NCT02179177 studies the effect of prophylactic dosing of apixaban on daily pain scores and NCT02072668 the effect of rivaroxaban on sVCAM and IL-6.

Vitamin K Antagonists

NCT01036802 studied anticoagulation with warfarin for pulmonary hypertension, but was terminated due to poor accrual.

Role of Platelets in SCD

Platelets have been shown to circulate in SCD patients in an activated state in both “steady state” and during painful crisis. This is evidenced by elevated platelet expression of CD62, CD63, PAC, P-selectin, activated glycoprotein IIb/IIIa, plasma soluble factor 4, and β -thromboglobulin [124, 131, 146–150]. It is also proposed that platelets contribute to the inflammatory milieu of SCD via manufacture and release of pro- and anti-inflammatory molecules upon activation [151, 152]. Increasing cytokine levels are associated with increased platelet number in SCD [153]. Platelets are well known to form aggregates in SCD by binding erythrocytes, monocytes, and neutrophils [3, 148, 150, 154]. At the molecular level, the

neutrophil serine/threonine kinase isoform AKT2 plays a critical role in both neutrophil recruitment and neutrophil-platelet interactions resulting in vascular inflammation and lung damage [12]. Inhibition of AKT2 diminishes neutrophil adhesion and neutrophil-platelet interactions, leading to improved blood flow [155] and prolonged survival when coadministered with hydroxyurea [156] in SCD mice. Overall, evidence suggests that the chronic activation of platelets in SCD contributes to the vasculopathy and thrombo-inflammatory state described in SCD. Accordingly, these alterations have been targeted by antiplatelet therapies with the goal of ameliorating the SCD phenotype (Table 2.6).

Table 2.6 Studies involving antiplatelet agents

Study title	Intervention	Clinical trial #/phase	Status
A Phase I/II Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Safety of Eptifibatide as Treatment for Acute Pain Episodes in Sickle Cell Disease	Eptifibatide [157]	NCT00834899 Phase 1, 2	Terminated
An Open-Label, Dose-Ranging Study of Prasugrel in Pediatric Patients with Sickle Cell Disease	Prasugrel [158]	NCT01476696 Phase 2	Complete
A Phase 3, Double-Blind, Randomized, Efficacy and Safety Comparison of Prasugrel and Placebo in Pediatric Patients with Sickle Cell Disease	Prasugrel [159]	NCT01794000 Phase 3	Terminated
A Pharmacokinetic and Pharmacodynamic Assessment of Prasugrel in Healthy Adults and Adults with Sickle Cell Disease	Prasugrel	NCT01178099 Phase 1,2	Complete
Prasugrel Versus Placebo in Adult Sickle Cell Disease	Prasugrel [160]	NCT01167023 Phase 2	Complete
Aspirin Prophylaxis in Sickle Cell Disease	Aspirin	NCT00178464 Phase 1, 2	Complete
Abciximab (ReoPro) as a Therapeutic Intervention for Sickle cell Vaso-Occlusive Pain Crisis	Abciximab	NCT01932554 Phase 2	Withdrawn
Dipyridamole/Magnesium to Improve Sickle Cell Hydration	Dipyridamole and magnesium	NCT00276146 Phase 2	Withdrawn
A Pharmacokinetic (PK) and Pharmacodynamic (PD) Dose-Ranging Phase II Study of Ticagrelor Followed by a 4-Week Extension Phase in Pediatric Patients With Sickle Cell Disease	Ticagrelor	NCT02214121 Phase 2	Complete
A Study to Evaluate the Effect of Ticagrelor in Reducing the Number of Days with Pain in Patients with Sickle Cell Disease (Hestia2)	Ticagrelor	NCT02482298 Phase 2	Complete

Antiplatelet Agents

The effect of aspirin on hemoglobin level and frequency of painful crises have been evaluated in several clinical trials [161–164]. While one study showed an effect on hemoglobin level [163], none showed a significant effect on frequency of painful crises. In a single-site randomized trial, the glycoprotein IIb/IIIa inhibitor, eptifibatide (NCT00834899), was shown to be safe in SCD. However treatment with eptifibatide did not improve the time to crisis resolution or hospital discharge [157]. A multicenter phase 2 trial of the P2Y12 inhibitor, prasugrel (NCT01167023), showed a decrease in platelet activation biomarkers and a trend toward decreased pain that was nonsignificant [160]. A phase 3 randomized, double-blind, placebo-controlled study of prasugrel (NCT01794000) for prevention of VOC also demonstrated a nonsignificant trend toward fewer painful crises in the treatment versus placebo arm [165]. A phase 2 study using ticagrelor (NCT02482298) to determine whether the P2Y12 inhibitor can reduce the number of days of pain, pain intensity, and analgesic use has recently been completed, and results are not yet available. Thus, while platelets have been implicated in the pathophysiology of SCD vaso-occlusion and painful crises, antiplatelet agents have not proven to be effective in targeting that specific clinical outcome. However, given the correlation between hemolysis and activation of the hemostatic system, and the cross talk between coagulation and inflammation, it is possible that different aspects of SCD pathophysiology may be positively affected by antiplatelet therapy.

Summary

Improved understanding of the pathophysiology of sickle cell VOC has led to new targeted therapeutics as well as emerging gene therapies. Given the complexity of the sickle RBC interactions with the endothelium, platelets, and neutrophils, it is likely a multimodal approach will be necessary for optimal results. It is crucial that clinicians, scientists, and patients continue to collaborate together and participate in multi-institutional and international trials for investigating novel treatments in this highly variable disease.

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