

Orphan drugs for sickle vaso-occlusion: dawn of a new era of targeted treatment

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Abstract: While an orphan disease in the USA, sickle cell disease (SCD), a group of genetic disorders of hemoglobin structure and function, is a major public health problem in much of the rest of the world, particularly sub-Saharan Africa. The pathophysiology of SCD stems from the formation of sickle hemoglobin polymers that deform the erythrocyte into a characteristic sickle shape, the rapidity of which is regulated by its intracellular hemoglobin concentration. Subsequent vaso-occlusion is dependent on adhesion of sickled erythrocytes, and perhaps other cellular elements, including leucocytes and platelets, to abnormal vascular endothelium using a number of receptor–ligand pairs. This propensity for vaso-occlusion may be enhanced by altered vascular tone from excessive amounts of vaso-constrictive factors or diminished amounts of vasodilatory factors. Acute pain is the hallmark symptom caused by sickle polymer formation and subsequent vaso-occlusion, and is represented in the endpoints of most previous and current clinical trial designs. Numerous failures of prior investigational agents have frustrated clinicians and patients alike. Hydroxyurea is currently the only US Food and Drug Administration-approved drug for SCD and reduces the frequency of vaso-occlusive complications in many individuals. A considerable therapeutic need remains as hydroxyurea usage is currently not approved for all types of SCD, is not always clinically effective, and requires frequent monitoring. Recent improvements in our understanding of SCD pathophysiology have generated many new therapeutic targets and associated investigational agents. For example, a number of more specific fetal hemoglobin inducers and several therapies to reduce sickle polymer formation are being tested in preclinical and early phase clinical trials. Several agents that target receptor–ligand interactions which mediate cellular adhesion to vascular endothelium have shown considerable promise and are entering Phase III trials, but present some continuing challenges in clinical trial design and conduct. Gene therapy trials are poised to start and offer the potential for curative therapy.

Keywords: sickle cell disease, pain, clinical trials, investigational agents

Introduction

The replacement of glutamine with valine at the sixth amino acid position in the β -globin subunit is the mutation diagnostic for sickle hemoglobin. In sufficient concentrations, molecules of this abnormal hemoglobin polymerize upon deoxygenation into long polymers that physically deform the erythrocyte into the characteristic “sickle” shape and ultimately obstruct blood flow leading to subsequent local tissue ischemia.¹ Such polymer formation can also occur to varying degrees by the copolymerization of sickle hemoglobin with other structural hemoglobin variants, such as hemoglobin C, D, E, or O_{Arab}. When inherited as a compound heterozygote with sickle hemoglobin, mutations in the β -globin gene that cause premature termination of normal β -globin protein production, or that produce structural hemoglobin variants that are ineffectively

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synthesized or are markedly unstable (β -thalassemias), also result in a sickling disorder.^{2,3}

Early clinical trials of potential treatments to prevent sickle hemoglobin polymerization and subsequent vaso-occlusion were hampered by a limited understanding of the complex pathophysiology of sickle cell disease (SCD). The many basic science advances in the last 30 years have provided a number of different drug targets that have been explored in clinical trials (Figure 1). However, an effective pharmacologic strategy remains elusive as most of these agents studied to date have failed in late stage trials. The increasing focus on translational medicine and advances in drug development have brought a number of new highly targeted agents to mid and late stage SCD clinical trials that are the focus of this review, and show promise of finally providing effective therapies for many of the acute complications of SCD in children and adults.

First a brief history lesson: SCD as the first molecular disease

James Herrick provided the very first clinical description of SCD in the USA over 100 years ago, when he reported a 20-year old male of West Indian origin with fever, pain, anemia, pallor, jaundice, leg ulcers, and sickle-shaped red blood cells.⁴ Subsequent case series over the next 40 years provided more in-depth clinical descriptions, and key observations on the role of spleen,⁵ central nervous system lesions,⁶ and infectious complications.⁷ The hypothesis for the role of vaso-occlusion is attributed to Diggs and Ching in 1934.⁸ In 1940, the key effect of deoxygenation on sickle cells was noted by Sherman⁹

and the “vicious” cycle of sickling was proposed by Ham and Castle.¹⁰ The likely ameliorating role of high fetal hemoglobin in newborns with SCD was described by Watson in 1948,¹¹ while in 1956, Ingram demonstrated that a glutamic acid to valine substitution at position 6 of the β -globin chain was the causative mutation in sickle cell anemia (SCA, homozygous SS).¹²

Pauling et al’s 1949 characterization of SCA as a molecular disease¹³ engendered the expectation that a quick cure would be developed now that the genetic abnormality had been identified; yet this dream remains largely unfulfilled. While many individuals are less symptomatic with the widespread introduction of hydroxyurea (HU) therapy (see “HU – A ‘lead-off home run’ for fetal hemoglobin inducers”), and a few are cured by allogeneic hematopoietic stem-cell transplantation,¹⁴ treatment for most SCD complications relies on supportive care that has changed little from the 1950s.

SCD – USA orphan disease but a global health problem

The sickle mutation is believed to have arisen independently many thousands of years ago in at least three areas of Africa, and in the Middle East or Indian subcontinent.^{15,16} The prevalence of the sickle gene was maintained and expanded in these areas by reproductive advantage as the heterozygous condition, sickle trait, provided some degree of protection from severe malaria.¹⁷ The sickle gene was introduced into the Western hemisphere primarily by Africans forcibly taken to the Americas through the transatlantic slave trade, with largest numbers being distributed to the USA, Brazil, and the Caribbean.

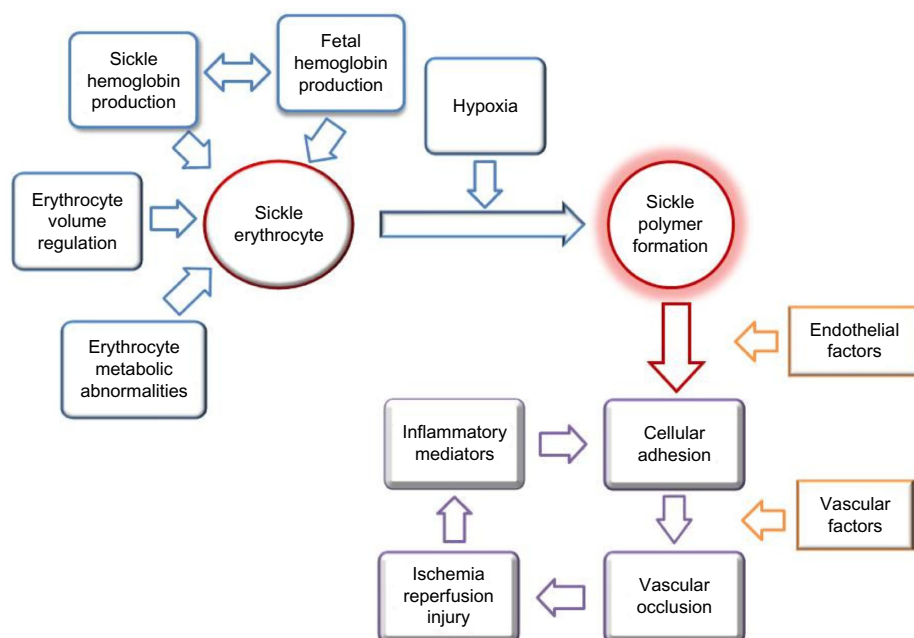


Figure 1 Pathophysiology of sickle vaso-occlusion.

Based on estimates of sickle gene frequencies, it is expected that over 300,000 newborns with SCA were born globally in 2010; an estimated 80% of newborns with SCA occurred in sub-Saharan Africa alone, with three countries (Nigeria, India, and the Democratic Republic of the Congo) representing 57% of the annual number of newborns with SCA globally.¹⁸ Estimates suggest that over 6,000 annual births and 100,000–150,000 Latin Americans are affected by SCD.¹⁹ By comparison, estimates of annual births with SCA in the USA or in the UK are in the range of 1,000–2,000, with estimates of over 100,000 individuals living with SCD in the USA,²⁰ consistent with orphan disease status. SCA represents a large proportion of SCD in the Americas, UK, and certain regions of Africa, while higher proportions of hemoglobin SC are observed in Burkina Faso and hemoglobin S β -thalassemias in Greece and India.²¹ Continued immigration from countries with a high sickle hemoglobin prevalence to high-income countries, such as the USA and UK, will contribute to the increasing health burden of these hemoglobinopathies in the future.²²

While it represents some significant logistical challenges, the availability of this large non-USA sickle cell patient population is a significant advantage for enrolling in large clinical trials and has allowed several current Phase III SCD clinical trials to meet enrollment milestones in a much more timely fashion than with USA sites alone. In contrast, once marketing approval has been obtained, the likely high cost of most new drugs may be a substantial barrier to their use in these low-income countries with the highest prevalence of affected individuals. Lessons learned from the introduction and use of antiretroviral therapies in these low- and middle-income countries may be relevant to future use of anti-sickling therapies.²³

Pathophysiology – a multiplicity of potential targets

The pathophysiology of vaso-occlusion in SCD is quite complex and involves both erythrocyte sickling and subsequent vascular occlusion²⁴ (Figure 1). The former is related to the rapidity and extent of sickle polymer formation, which is regulated by cell volume and hemoglobin concentrations,²⁵ while the latter is dependent on adhesion of sickled erythrocytes, and perhaps other cellular elements, including leucocytes and platelets, to abnormal vascular endothelium using a number of receptor–ligand pairs.^{26,27} This propensity for vaso-occlusion may be enhanced by altered vascular tone from excessive amounts of vaso-constrictive factors, such as ET-1,²⁸ or diminished amounts of vaso-dilatory factors, particularly nitric oxide (NO).²⁹

As part of the “vicious” cycle of vaso-occlusion, the subsequent ischemic tissue injury results in the accumulation of endogenous factors released from cells that reside or infiltrate into the injured area. These factors represent a wide array of signaling molecules, including cytokines, and chemokines, extracellular proteases, peptides, eicosanoids and related lipids, neurotrophins, as well as protons, which damage local nociceptive nerves leading to increased spontaneous firing that is perceived as acute pain,³⁰ the hallmark complication of this disorder. Many factors contribute to a substantial inflammatory response, which may be a key feature of SCD pathobiology that is likely enhanced by the consequences of reperfusion injury.³¹

While the focus for much of the treatment of SCD has been on these acute vaso-occlusive events (Figure 2), much of the morbidity and mortality encountered in children, and particularly in adults, is related to chronic organ damage from vasculopathy in the brain, lungs, kidney, and eyes.³² This injury to vascular endothelium also is a complex process,³³ likely fueled by the inflammation resulting from recurrent sickling/ vaso-occlusion, oxidative stress,³⁴ and hypercoagulability.³⁵ The central pathophysiology that relates these features of a “vascular sub-phenotype” of SCD to vaso-occlusion appears to be the downstream effects of sickling-related intravascular hemolysis and its impact on NO biology (Figure 3).^{36,37}

Protean clinical manifestations provide many potential trial endpoints

The many complications of SCD provide a number of clinically relevant events to consider as potential endpoints for interventional clinical trials (Figure 2). Pain in SCD can begin early in the first year of life as levels of fetal

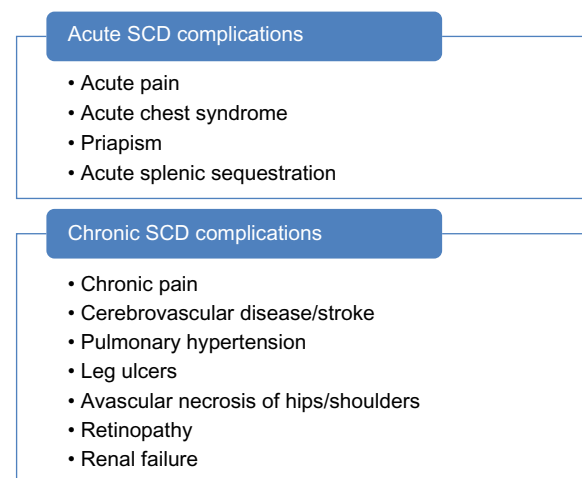


Figure 2 Acute and chronic complications of sickle cell disease (SCD).

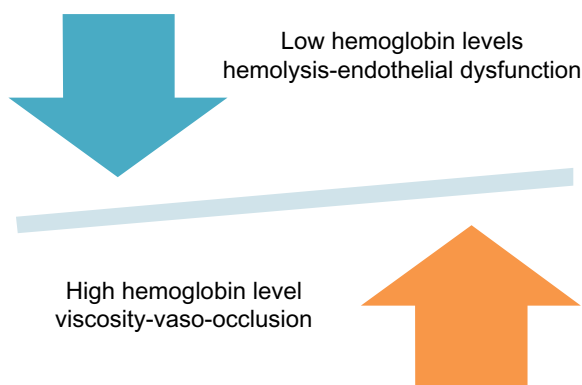


Figure 3 Competing effects of hemoglobin in sickle cell disease pathophysiology.

hemoglobin decline.³⁸ Most infants with pain in the first year of life have pain locations (hands/feet) and signs or symptoms (swelling or tenderness) consistent with dactylitis, or “hand-foot syndrome”, which becomes less common in older children, and rare after 5–7 years of age.^{39,40} Children with SCD often experience increasingly frequent acute pain through childhood into adolescence or young adulthood.⁴¹ While frequent hospitalizations are the major source of health care utilization, the large majority of these painful episodes are managed at home, particularly in children.⁴² Most episodes are relatively brief, typically 2–3 days, with pain of moderate intensity, usually in lower legs, back, or chest wall.⁴³ A small number of children, often in their early adolescent years, change from a pattern of episodic acute pain to frequently recurrent acute pain or chronic pain.^{44,45} SCD pain is an even more complex experience in adults, with features of both acute recurrent pain and chronic pain,^{46,47} and is more often managed in an acute care setting compared to children.⁴⁸ In a large national history study, more frequent episodes of acute pain were associated with higher baseline hematocrit and lower levels of fetal hemoglobin.⁴⁴ A pattern of frequent pain, defined as three or more yearly serious pain episodes, conferred an increased risk for mortality.⁴⁴ Comorbidities such as mental health issues often contribute to more frequent health care utilization in SCD adults with chronic pain.^{49,50}

These episodes of more intense pain require initial evaluation and treatment in acute care settings, usually Emergency Departments, which can be challenging in busy urban hospitals.⁵¹ Some larger SCD programs have developed dedicated acute treatment settings, “day hospitals” with some success,⁵² but despite aggressive analgesic therapy, hospitalization for continued management of these episodes remain frequent.⁵³ Inpatient management usually consists of supportive care with intravenous (IV) fluids and analgesics until the pain subsides.⁵⁴

Change in pain intensity, rather than change in pain frequency, is another obvious pain-related endpoint, but measurement may require different assessment measures if both pediatric and adult participants are included. A significant amount of pain may remain at the end of hospitalization in those patients with coexistent chronic pain, making it difficult to ascertain when acute vaso-occlusive-related pain has resolved. Reduction in standard of care analgesic usage is a frequent pain-related endpoint in analgesic trials, but is complex in acute vaso-occlusive pain as many patients may have substantial degrees of opioid tolerance from prior chronic oral opioid usage, and analgesic therapy often needs to be individualized. Most such acute pain studies ultimately utilize a composite of these measures as a primary endpoint, which continues to be refined based on feasibility and performance in subsequent studies.

Approximately 10%–20% of these pain episodes requiring hospitalization are further complicated by the development of new pulmonary infiltrates associated with fever and hypoxia, often within the first 48–72 hours of admission, referred to as an acute chest syndrome.^{55,56} Some of these pulmonary episodes represent an infectious process, particularly in young children. However, in the setting of continued vaso-occlusion, most infiltrates, particularly in the lung bases,⁵⁷ are caused by pulmonary parenchymal damage from vaso-occlusion occurring in the distal pulmonary circulation. These events typically prolong the length of hospitalization, and are treated with antibiotics, bronchodilators, and blood transfusions. Some patients with unusually severe disease will require mechanical ventilation and other respiratory support, many of whom will also develop neurologic events and acute kidney injury.⁵⁸

Vaso-occlusion also occurs less commonly in other vascular beds, including the spleen, where life-threatening trapping of sickled erythrocytes and platelets can cause acute splenic sequestration with acute left upper quadrant visceral pain from rapid enlargement of the splenic capsule.⁵⁹ Similarly, vaso-occlusion in the penile circulation causes acute episodes of painful priapism that is typically treated with aspiration and alpha-adrenergic agents.⁶⁰

Several other complications likely represent a combination of progressive vascular changes and vaso-occlusion. Progressive endothelial damage occurring in the large vessels of the cerebral vascular circulation, likely related to increased blood flow from chronic anemia, leads to overt strokes in children⁶¹ and adults.⁶² More prevalent neurologically silent infarction from vascular occlusion in smaller vessels also occurs, but is of less certain etiology.⁶³ Routine screening of young SCD children with transcranial Doppler ultrasound has dramatically decreased the incidence of large vessel strokes.⁶⁴

While most children identified with abnormal transcranial ultrasound studies require lifelong transfusion therapy,⁶⁵ some may benefit from daily use of HU instead.⁶⁶

Vascular changes related to hemolysis-related damage also accounts for the development of pulmonary hypertension in adolescents and young adults,⁶⁷ usually diagnosed by an elevated tricuspid regurgitant jet velocity on cardiac echocardiography and confirmed by cardiac catheterization.⁶⁸ An increased risk for mortality has been associated with a tricuspid regurgitant jet velocity ≥ 2.5 m/s, an N-terminal pro-brain natriuretic peptide (NT-pro-BNP) level ≥ 160 pg/mL, or right heart catheterization-confirmed pulmonary hypertension.⁶⁹ Prostacyclin agonist or endothelin receptor antagonist therapies have been successful in selected patients.

While uncommon in the pediatric age group, leg ulcers, typically over the medial malleolus of either or both ankles, can be a source of considerable pain and disability and are difficult to manage.⁷⁰ The role of venous stasis or other vascular factors remains controversial.⁷¹ Avascular necrosis in the vertebral column, shoulder, or hips, from vascular compromise of the cortical bone and/or bone marrow circulations can be a source of acute pain as well as chronic pain in adolescent and young adult patients. Some patients progress to joint collapse, particularly at the head of the femur, which ultimately requires joint replacement for relief of chronic pain or to improve physical functioning.⁷²

Severe anemia, characteristic of SCA and S β 0 thalassemia, also is a potential therapeutic target. Lower

hemoglobin levels are associated with a higher frequency of stroke, renal disease, leg ulcers, and pulmonary hypertension secondary to hemolysis-related endothelial dysfunction,³⁷ but a lower frequency of vaso-occlusive pain,⁷³ likely as a result of lower blood viscosity. This typifies the delicate balance in treating sickle cell complications with investigational agents where a therapeutic improvement in one feature may conversely have a negative impact on the frequency of other complications (Figure 3).

Hemostatic abnormalities related to sickling and hemolysis-related changes are also common.⁷⁴ Changes include thrombin activation, altered levels of anticoagulant proteins, impaired fibrinolysis, and platelet activation. Platelet activation may be particularly problematic as it may contribute to cell-cell interactions that may impact sickle erythrocyte-endothelial cell adhesion and subsequent vaso-occlusion. Antithrombotic therapies have been clinically useful to treat thrombotic complications of SCD, and have shown some preliminary benefit as a potential vaso-occlusive therapy.

Therapeutic trials

Design considerations for vaso-occlusion therapy trials

The design of new therapeutics for sickle vaso-occlusion presents many challenges. Numerous agents have been developed to intervene in the many facets of SCD pathophysiology (Figure 4). There remains limited understanding of the potential efficacy that could be obtained from intervening

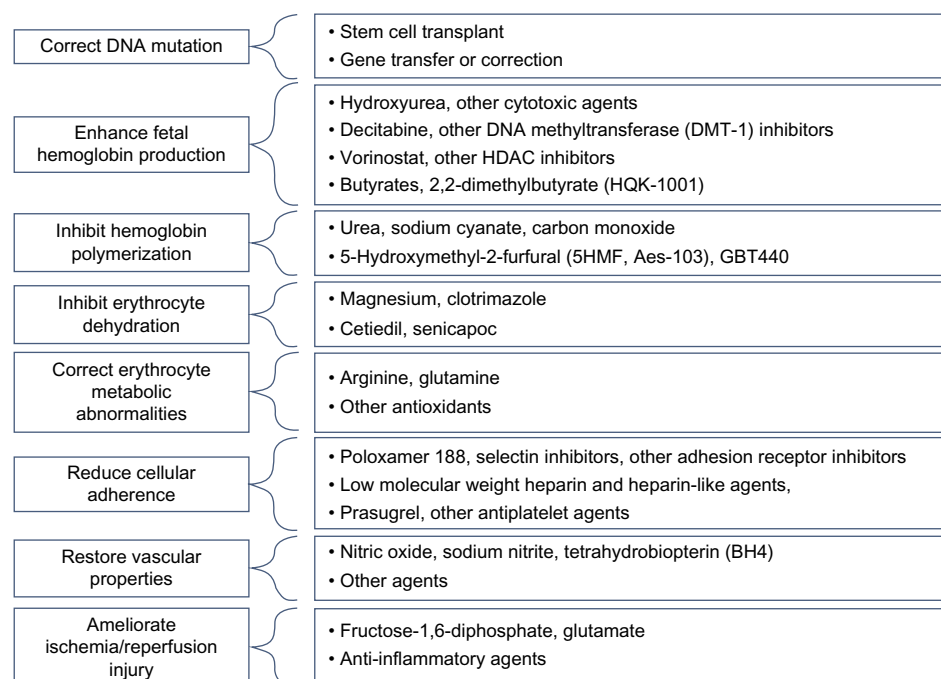


Figure 4 Therapeutic targets and corresponding investigation agents in sickle cell disease.

at any one particular point in the process leading to vaso-occlusion and subsequent tissue injury (Figure 1), but the most successful therapies to date have directly impacted sickle polymer formation rather than any of its “downstream” consequences.

Comparison of test article to placebo in the context of best standard care is the most ethically appropriate design, particularly for children, but the clinical practice for analgesics, fluids, oxygen, and transfusions all vary considerably across clinical sites.⁵⁴ This site-to-site variability may need to be considered in statistical analysis strategies. In the absence of an effective surrogate marker or ability to measure vaso-occlusion in humans, studies of therapies for vaso-occlusion have had to rely on clinical outcomes of “crisis resolution” as endpoints. These too have been challenging as the most obvious clinical endpoint, duration of hospitalization, can be impacted by social considerations, transportation issues, and treatment of other comorbidities such as fever or acute chest syndrome.

Most agents, including HU, have been tested only in individuals with the more severe sickling genotypes of SS and S β^0 -thalassemia, so their effectiveness in other sickling genotypes is poorly understood. While this knowledge and treatment gap may be less of an issue in the pediatric population where these other sickling phenotypes are often less symptomatic, it significantly impacts the availability of therapies for the adult sickle cell population where most individuals are symptomatic regardless of sickle genotype. Inclusion of all sickling disorders in late stage trials would address this critical problem,⁷⁵ but requires careful attention to inclusion/exclusion criteria and analysis of safety assessments as baseline hematologic and metabolic parameters will vary by sickle genotype. If endpoints are symptom-based, the enrollment may need to be stratified by sickle genotype to balance across study arms, which may impact sample size considerations.

Participant age is another important design consideration. The frequency of vaso-occlusive pain and other SCD complications are age-dependent,⁴⁴ so designs that require a minimum pain frequency may have difficulty recruiting adequate numbers of young children unless a large number of clinical sites are utilized. Similarly, some complications such as leg ulcers, priapism, or hip avascular necrosis are uncommon in children. Age-related changes in drug distribution, metabolism, and clearance are well-understood pharmacologic issues in children,⁷⁶ but SCD children also have substantially increased renal blood flow and resultant increases in glomerular filtration rates.⁷⁷ These changes may impact the therapeutic levels of agents or their metabolites

that have substantial degrees of renal clearance, which may require further pharmacokinetic testing and alternative dosing strategies when expanding clinical trials into younger SCD populations.

“Painful lessons” – past treatment failures

In contrast to the success of a number of multisite clinical trials exploring the role of transfusion therapy or HU for central nervous system complications of SCD,^{65,78,79} the therapeutic arena in sickle cell vaso-occlusion therapy has been dominated by a number of multisite studies with negative results. Early studies by the first SCD clinical trial group, the Cooperative Urea Trials Group, evaluated the relative effectiveness of anti-sickling therapy with alkali or urea, compared to invert sugar, administered intravenously in the treatment of the painful episodes of SCA over a 48-hour period using a double-blind protocol in the early 1970s.⁸⁰ While the clinical trial design was remarkably good by today’s standards, neither urea nor alkali administration was found to be superior to invert sugar alone in shortening the duration of crisis episodes.

The pioneering studies by Hebbel et al⁸¹ and Kaul et al,⁸² demonstrating the tendency for sickle erythrocytes to adhere to vascular endothelium, in vitro and in vivo, suggested anti-adhesion therapy could stop and/or reverse sickle vaso-occlusion. In an initial pilot study, a nonionic block copolymer surfactant with hemorheologic and antithrombotic properties, poloxamer 188, reduced total analgesic use and pain intensity, and showed trends for shorter duration of painful episodes and total days of hospitalization.⁸³ However, a subsequent larger randomized, placebo-controlled Phase III trial showed a statistically significant but clinically disappointing small (9-hour) decrease in the duration of painful episodes compared with the patients receiving placebo.⁸⁴ In subgroup analysis, children and patients who were receiving concomitant HU had a more significant reduction in crisis duration (16–21 hours). Whether these findings represented more efficacy of this agent in these subgroups, or a more effective statistical analysis strategy, was uncertain. After a hiatus of almost 15 years, this agent is being studied again in an ongoing pediatric and young adult Phase III trial (MAST Therapeutics, NCT01737814) using a different endpoint, analgesic usage, and a different statistical analysis plan.

The strong dependence of the rate of sickle polymerization on the intracellular hemoglobin concentration suggested that modulation of erythrocyte volume might be an effective therapeutic strategy. An early study of induced hyponatremia

with fluids and desmopressin proved too difficult to control,⁸⁵ but encouraging pilot studies with magnesium⁸⁶ and with clotrimazole⁸⁷ suggested that inhibition of the calcium activated potassium channel (Gardos channel) could be effective in increasing erythrocyte volume and reducing sickling. Senicapoc, a novel Gardos channel inhibitor, increased hemoglobin concentration with a concomitant decrease in total reticulocyte number and various markers of erythrocyte destruction in a Phase II study,⁸⁸ but a subsequent Phase III study was stopped early by its data safety monitoring board (DSMB) due to a lack of efficacy when it determined that despite improvements in anemia and hemolysis, there was no significant improvement in the rate of sickle cell painful episodes.⁸⁹ Many have speculated that the increase in hematocrit and resulting blood viscosity offset the beneficial anti-sickling effect of an increased erythrocyte volume reflecting the complex nature of sickle polymer formation and vaso-occlusion.

Similarly, IV magnesium also has been studied in two randomized, double-blind, placebo-controlled trials in children requiring admission to hospital for treatment of severe pain with IV analgesics.^{90,91} Magnesium was well tolerated, but had no effect on hospital duration, pain scores, or cumulative analgesia use in either study. However, a number of important future feasibility issues were successfully addressed in these studies, including recruitment and initial administration of study drug in the Emergency Department setting.

The compelling role for NO in the pathobiology of vaso-occlusion prompted a number of attempts to manipulate this pathway as a therapeutic target. A large randomized placebo-controlled Phase III study of NO inhalation therapy was subsequently conducted, using a primary endpoint, which has been adapted for many subsequent clinical trials, of time to resolution of painful crisis, defined by: 1) freedom from parenteral opioid use for 5 hours; 2) pain relief as assessed by visual analog pain scale scores of 6 cm or lower (on 0–10 cm scale); 3) ability to walk; and 4) patient's and family's decision, with physician consensus, that the remaining pain could be managed at home. Unfortunately, there was no significant change in the primary endpoint between the inhaled NO and placebo groups, nor were there significant differences in secondary outcome measures, including length of hospitalization, visual analog pain scale scores, or cumulative opioid usage.⁹²

A number of other clinical trials have explored alternative targets to normalized NO biology. The use of sodium nitrite as alternative source of NO has also been explored,

largely by investigators at the National Institutes of Health Clinical Center.⁹³ The proposed mechanism involves a novel physiological function of human hemoglobin as an oxygen- and pH-dependent nitrite reductase potentially converting nitrite to NO along the physiological oxygen gradient in arterial blood, accentuating vasodilation in hypoxic tissue. Sodium nitrite infusions were well tolerated without hypotension, clinically significant methemoglobinemia or other untoward events in Phase I/II clinical trials,⁹⁴ and demonstrated dose-dependent increases in forearm blood flow. Subsequent pilot studies of sodium nitrite during vaso-occlusive crises have not been promising, but the drug is being explored as a topical agent for treatment of sickle leg ulcers (NCT01316796).⁹⁵

NO production could also be restored and endothelial dysfunction reduced or reversed by restoring production of NO. Since tetrahydrobiopterin (BH4) is an essential cofactor required for the activity of eNOS, supplementation with exogenous BH4 or therapeutic approaches that increase endogenous amounts of BH4 could be possible therapeutic strategies. To explore this concept, BioMarin conducted a Phase IIa dose-escalation trial in SCD of oral sapropterin, a synthetic BH4 approved for the treatment of phenylketonuria.⁹⁶ While well tolerated, the effects on physiological and biochemical markers of endothelial function in SCD were unimpressive and further studies have not been pursued.

Ischemia-related tissue damage, with its own complex pathophysiology,³¹ is the likely downstream consequence of sickle vaso-occlusion. Numerous drug development programs have attempted to identify cytoprotective agents for tissues ranging from the heart to the brain, including the development of fructose-1,6-diphosphate by Cypros Pharmaceuticals, which was intended to restore adenosine triphosphate (ATP) levels in ischemic tissues.⁹⁷ An initial dose-escalation Phase IIa trial of 47 SCD patients evaluated a single dose at three dose levels of Fructose di-phosphate (FDP) (50, 100 and 250 mg/kg). The primary endpoint of the study was the level of pain intensity reported during the first 24 hours following drug or placebo administration. All of the drug-treated patients demonstrated reductions in pain levels, which reached statistical significance at the two lower dose groups. Unfortunately, a larger Phase III study in hospitalized vaso-occlusive pain was stopped early for futility, perhaps reflecting a lack of efficacy in more established pain, which may be a potential issue for similar Phase III trial designs of agents intended to reverse rather than prevent sickle vaso-occlusion.

Newer agents – the next generation of targeted therapies

Enhancement of fetal hemoglobin production

Fetal hemoglobin, as its name implies, is the dominant hemoglobin produced later in human fetal development. It begins to be replaced by adult hemoglobin shortly after birth in a process called “hemoglobin switching”, the biochemical and genetic basis of which has been extensively studied.⁹⁸ Recovery from a period of anemia or hypoxia in response to increased erythropoietin production, so-called “stress erythropoiesis”,⁹⁹ is another clinical situation that reverses the fetal-to-adult hemoglobin switch. This process was studied in animals with anemia induced with a myelosuppressive chemotherapy agent, 5-azacytidine.¹⁰⁰ Subsequent studies in humans, including those with SCD, documented similar increases in fetal hemoglobin, but this agent was thought to be too toxic for long-term use. HU was subsequently studied as a fetal hemoglobin inducer in SCD as it was a less toxic myelosuppressive agent with some long-term experience in the treatment of polycythemia vera.¹⁰¹

HU – A “leadoff home run” for fetal hemoglobin inducers

HU remains the only US Food and Drug Administration-approved disease-modifying therapy for SCD, although repeated scheduled transfusions of red blood cells can also decrease disease severity.¹⁰² The approval was based on a single randomized clinical trial, the MSH (Multicenter Study of Hydroxyurea for Sickle Cell Anemia), that enrolled 299 adults (mean age of 30.5 years), and almost all had SCA.¹⁰³ The primary endpoint was a reduction in the frequency of painful crises over a 2-year period, but the trial was closed earlier than expected, after demonstration that the median pain rate was reduced by almost 50% (2.5 vs 4.5 events per year) in patients assigned to HU therapy. Hospitalizations, episodes of chest syndrome, and numbers of transfusions were also lower in patients treated with HU. A subsequent follow-up study of these trial participants demonstrated a continued clinical improvement in morbidity and mortality.¹⁰⁴ A similar Phase III trial of HU in SCA infants and young children did not meet its primary endpoint of preventing a decline in organ function, but showed similar reductions in the frequency of acute pain, chest syndromes, and transfusions.^{105,106}

The large Phase III MSH trial was preceded by a number of pilot studies,¹⁰⁷ and was supported by previous studies of cytotoxic chemotherapeutic agents in animals¹⁰⁸

and humans,¹⁰⁹ and a wealth of clinical and laboratory data on the role of fetal hemoglobin in ameliorating sickle polymer formation. The clinical benefit of higher levels of fetal hemoglobin was also supported by the relatively mild clinical manifestations among Bedouin Arabs from the Saudi Arabian peninsula and certain tribes from central India with individuals who had homozygous sickle hemoglobin and relatively high amounts of fetal hemoglobin,¹¹⁰ and the beneficial effect of high fetal hemoglobin in newborns and young infants with SCD.³⁸ This “perfect storm” of substantial clinical benefit, impressive supportive preclinical and laboratory data, and strong correlative clinical models, allowed regulatory approval with a single pivotal Phase III trial, but has set a high standard for comparison in future regulatory deliberations for new therapies.

The efficacy of HU for the treatment of vaso-occlusion in SCD is generally attributed to its ability to increase fetal hemoglobin. However, the cellular mechanisms by which this occurs are not completely understood, and other mechanisms may account for the clinical benefit of this agent. For example, HU reduces the expression of adhesion molecules on sickle erythrocytes,¹¹¹ and cyto-reduction of neutrophils, monocytes, and reticulocytes may also have a beneficial effect on blood viscosity or cell–cell interactions.¹¹²

Other fetal hemoglobin inducers

A number of additional fetal hemoglobin inducers have been developed based on the evolving research on the importance of epigenetic changes, such as DNA methylation and histone modification, in regulating fetal hemoglobin production.¹¹³ These agents will likely be initially tested as alternatives to HU, recognizing that some individuals taking this drug may not achieve a clinically effective response, particularly in those with low initial fetal hemoglobin levels.^{114,115} Other patients may benefit from a drug that requires less frequent monitoring, or that has less reproductive risk. Trial designs that test fetal hemoglobin inducers with different mechanisms of action separately and in combination may ultimately be necessary to achieve clinically useful increases in broad populations of SCD adults and children.

DNA hypomethylation agents that act by inhibiting DNA methyltransferase initially built on the success of 5-azacytidine using a derivative, 5-aza-2'-deoxycytidine (decitabine). Substantial elevations of fetal hemoglobin have been demonstrated in a number of Phase I and II trials of decitabine in SCD adults,¹¹⁶ but further development has been hampered by the absence of an oral dosing form, and occurrence of dose-related neutropenia and thrombocytosis.

HDAC inhibitors have been another promising class of agents to induce fetal hemoglobin production in SCD.¹¹⁷ Short chain fatty acids such as butyrate cause significant increases in fetal hemoglobin levels, but the practical use of IV butyrate analogs has been complicated by their relatively low potency and short half-life. Similarly, a Phase II clinical trial of a recently developed oral dosing form, 2,2-dimethylbutyrate (HKK-1001),¹¹⁸ was recently terminated early for lack of effects. With their clinical use in a variety of hematologic malignancies and solid tumor malignancies, newer HDAC inhibitors have been explored in Phase I and II trials in SCD, including vorinostat and panobinostat.¹¹⁷ Unfortunately, vorinostat's Phase II clinical trial was recently closed due to the lack of measurable effects. A number of newer more selective HDAC inhibitors are in preclinical development. Thalidomide, a drug used for multiple myeloma treatment with immunomodulatory and anti-angiogenic properties, upregulates γ -globin gene expression in vitro.¹¹³ Pomalidomide, a thalidomide derivative, has been used in a phase I study in SCD adults (NCT01522547).

Polymerization inhibitors

While initially envisioned once the mechanism of sickle polymerization was characterized, this therapeutic area has made little headway until recently. A naturally occurring aromatic aldehyde, 5-hydroxymethyl-2-furfural (5-HMF), binds to the valine residue of hemoglobin producing a left shift in the oxygen equilibrium curve, which reduces erythrocyte sickling in animal models.¹¹⁹ Reduction in P_{sickle} -mediated Ca^{2+} by 5-HMF following erythrocyte sickling has also been observed suggesting that this agent may also have an additional anti-sickling effect by inhibiting the deleterious sequelae of Gardos channel activation.¹²⁰ Single doses of 5-HMF were well tolerated in a Phase I study, and showed changes in blood oxygen levels during a hypoxia challenge test consistent with the effects of a left-shifting allosteric hemoglobin modifier.¹²¹ This agent is completing Phase II trials in SCD sponsored by AesRx, LLC, and is expected to enter Phase III trials late in 2015 with sponsorship by Baxter.

Another novel orally bioavailable small molecule, GTx011 (GBT440) (Global Blood Therapeutics, South San Francisco, CA, USA), that binds to the N-terminal α -globin chain of hemoglobin via a reversible Schiff base has been evaluated in preclinical studies. Inhibition of sickle hemoglobin polymerization in vitro improved erythrocyte deformability and blood viscosity,¹²² decreased the p50 value of human blood, and in a murine model of SCD (Townes SS mice),

GTx011 prevented ex vivo sickling of RBCs and prolonged erythrocyte half-life.¹²³ This agent has entered Phase I testing in SCD patients (NCT 02285088).

Anti-adhesion therapies/anti-hemostatic agents

Much of the current enthusiasm and activity in SCD clinical trials rests in this therapeutic area, especially for agents targeting selectins,¹²⁴ although agents targeting integrin-mediated interactions are also being developed or repurposed for SCD. For example, a humanized anti-P-selectin monoclonal antibody (SelG1) is being developed by Selexys Pharmaceuticals as a treatment for SCD. A Phase I clinical study has evaluated the safety, pharmacokinetics, pharmacodynamics, and immunogenicity of SelG1 in a single-center, double-blind, placebo-controlled, ascending single dose and multiple dose study of IV-administered SelG,¹²⁵ and a Phase II study in adults with SCD is underway.

An alternative approach, a small molecule pan-selectin inhibitor (GMI-1070, rivipansel) is being developed by Glyco-Mimetics, Inc., and now Pfizer, to inhibit blood cell adhesion in sickle vaso-occlusive episodes. After a successful initial Phase I/II study in SCD,¹²⁶ a subsequent Phase II study of GMI-1070 was conducted in a prospective multicenter, randomized, placebo-controlled, double-blind, study of 76 SCD children and adults hospitalized for vaso-occlusive pain.¹²⁷ Comparing active treatment group to placebo, clinically meaningful reductions in mean and median times to crisis resolution of 41 and 63 hours were demonstrated. Also in secondary analyses, mean cumulative IV opioid analgesic use was reduced by 83% with GMI-1070 vs placebo ($P=0.010$). A randomized placebo-controlled Phase III trial sponsored by Pfizer using crisis resolution as its primary endpoint has recently been opened. This study is currently unique in its inclusion of all sickle genotypes, in both children and adults.

Eli Lilly is conducting a therapeutic development program in SCD for prasugrel, a thienopyridine $P2Y_{12}$ adenosine diphosphate receptor antagonist that inhibits adenosine diphosphate-mediated platelet activation and aggregation. Drug exposure in Phase I studies was well tolerated, with no bleeding-related events in patients with SCD.¹²⁸ Mean drug concentration–time profiles and platelet reactivity were comparable between healthy subjects and patients with SCD. Subsequent Phase II studies in SCD adults observed a modest decrease in mean pain rate and intensity in the prasugrel arm compared with placebo.¹²⁹ Platelet surface P-selectin and plasma soluble P-selectin, biomarkers of in vivo platelet activation, were significantly reduced in SCD

patients receiving prasugrel compared with placebo suggesting potential effects on sickle erythrocyte adhesion. A Phase III randomized placebo-controlled pediatric trial is ongoing using reduction in pain frequency as the primary study endpoint (NCT01794000). Additional novel features of this trial design include the counting of home-managed pain using electronic diaries, and the extensive use of non-US clinical sites.

Vascular agents

Small pilot studies to enhance NO levels by providing increased amounts of its substrate, arginine, have had some success and are advancing to Phase II studies.^{130,131} A number of preclinical studies have evaluated the utility of carbon monoxide form of polyethylene glycol (PEG)ylated hemoglobin (SANGUINATE™) in ischemia/reperfusion injury settings, including myocardial infarction,¹³² and is now in development for SCD by Prolong Pharmaceuticals.

Cytoprotective agents

Therapies explored to date have been disappointing. However, recent progress has been made with the development of oral glutamine to provide increased defense against oxidant stress in sickle erythrocytes. Glutamine supplementation was expected to increase NAD and NADH levels by increased transport and utilization of glutamine in sickle erythrocytes, which was confirmed in an initial pilot study.¹³³ A subsequent Phase II randomized, double-blind, placebo-controlled, parallel-group, multicenter study evaluated the safety and efficacy of L-glutamine therapy. Eighty-one patients aged 5 years or older diagnosed with SCA or S β 0-thalassemia were randomized to oral L-glutamine at 0.3 g/kg or placebo twice daily for 48 weeks. At 6 months of therapy, L-glutamine treatment reduced the frequency of hospitalization (nearly 40% reduction) and there was a major trend for the decrease in frequency of painful crises (over 50% reduction) favoring the L-glutamine treatment arm.¹³⁴ These results have been replicated in a larger Phase III clinical trial, but the results have not yet been published in a peer-reviewed publication, and further studies will likely be needed for regulatory approval.

Advent of curative therapies

Stem cell transplant

HLA-identical sibling bone marrow transplantation in children with severe SCD currently provides a curative outcome in more than 90% of the recipients.¹⁴ Health-related quality of life largely returns to that of the normal population by 1 year

post-transplant, and acute and some chronic sickle complications also resolve in this time period.¹³⁵ While some progress is being made in using unrelated and alternative donor sources in the absence of suitable HLA-matched siblings,¹³⁶ this remains a fundamental barrier to more widespread use of this therapeutic strategy. Similarly, the current risk of loss of fertility, and the risk of post-transplant complications remain unacceptable to many potentially eligible families.¹³⁷

Gene therapy

Correction of the genetic defect in the patient's own hematopoietic stem cells would obviate the need for alternative sources of bone marrow to produce normal red blood cells, prevent therapy-related immunological side effects (graft vs host disease/graft rejection), and obviate the need for prophylactic therapies such as HU.¹³⁸ Gene insertion therapies have been developed to insert γ -globin-based or modified β -globin-based vectors for SCD gene therapy, largely using lentiviruses. Both approaches have shown proof of concept in mouse models of SCD.^{139,140} Bluebird Bio, Inc. has initiated a Phase I trial in adults with severe SCD to evaluate the safety of transplantation of autologous CD34+ stem cells transduced ex vivo with the LentiGlobin BB305 lentiviral vector inserting a bioengineered recombinant β -globin that prevents axial and lateral contacts in the sickle polymer originally developed by Levasseur et al.¹⁴¹ A similar Phase I study using autologous CD34+ stem cells transduced by a vector which substitutes the exons of γ -globin gene for β -globin in the β -globin vector has been opened at Cincinnati Children's Hospital. Myeloablative chemotherapy is used to provide space for the transduced stem cell to grow, so this procedure will share some of the risks of bone marrow transplantation, as well as yet to be established risk of the gene therapy. However, the survival advantage of erythrocytes protected from sickle hemoglobin polymerization by introduction of novel genes provides an opportunity for phenotypic correction of SCD even with modest levels of transduced stem cells similar to that observed with donor chimerism of 10%–30% in patients after traditional bone marrow transplant.¹⁴²

Final thoughts

A number of clinical trial design-related issues remain to be resolved. Since patients typically manage the initial portion of their vaso-occlusive pain at home, studies of those agents intended to accelerate crisis resolution will invariably contain individuals with a heterogeneous distribution of crisis duration before being exposed to the

test article. Whether the duration of prior vaso-occlusion impacts the potential efficacy of these agents is largely unknown. Given the complexity of sickle vaso-occlusion pathophysiology, it is likely that combination therapy may provide optimum inhibition of sickle polymerization and its consequences to maximize clinical benefit.¹⁴³ However, few studies have attempted such multiple combination comparisons so feasible and regulatory acceptable study designs remain largely unexplored. The regulatory considerations for investigational agents that might provide only modest incremental benefit in ameliorating sickle cell-related complications have yet to be clarified. Finally if effective, short-term intervention studies for acute complications, such as pain, will need to be followed by much larger and more expensive studies to examine their impact on less frequent vaso-occlusion complications, such as acute chest syndrome, or priapism. Longer term multi-year studies will also be needed to examine their impact on preventing chronic organ damage in spleen, kidneys, and the lung.

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