



Review Article

New Therapeutic Options for the Treatment of Sickle Cell Disease

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Competing interests: The authors have declared that no competing interests exist.

Abstract. Sickle cell disease (SCD; ORPHA232; OMIM # 603903) is a chronic and invalidating disorder distributed worldwide, with high morbidity and mortality. Given the disease complexity and the multiplicity of pathophysiological targets, development of new therapeutic options is critical, despite the positive effects of hydroxyurea (HU), for many years the only approved drug for SCD.

New therapeutic strategies might be divided into (1) pathophysiology-related novel therapies and (2) innovations in curative therapeutic options such as hematopoietic stem cell transplantation and gene therapy. The pathophysiology related novel therapies are: a) Agents which reduce sickling or prevent sickle red cell dehydration; b) Agents targeting SCD vasculopathy and sickle cell-endothelial adhesive events; c) Anti-oxidant agents.

This review highlights new therapeutic strategies in SCD and discusses future developments, research implications, and possible innovative clinical trials.

Keywords: Sickle cell disease; Hemoglobinopathy; Vaso-occlusive events; Hydroxyurea; Selectin inhibitors.

Citation: Matte A., Zorzi F., Mazzi F., Federti E., Olivieri O., De Franceschi L. New therapeutic options for the treatment of sickle cell disease. *Mediterr J Hematol Infect Dis* 2019, 11(1): e2019002, DOI: <http://dx.doi.org/10.4084/MJHID.2019.002>

Published: January 1, 2019

Received: October 1, 2018

Accepted: November 11, 2018

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Introduction. Sickle cell disease (SCD) is a hemoglobinopathy which affects approximately 100,000 individuals in the United States and almost 20,000-25,000 subjects in Europe, mainly immigrants from endemic areas such as Sub-Saharan Africa to European countries.¹⁻³ Estimates of the number of affected newborn in 2010 are of approximately 312,302 subjects with 75.5% being born in Africa.⁴ The invalidating impact of SCD on patient survival, quality of life and cost for health systems,² requires the development of new therapeutic options to treat sickle cell related acute and chronic complications.

SCD is caused by a point mutation in the β -globin gene resulting in the synthesis of pathological hemoglobin S (HbS). HbS displays peculiar biochemical characteristics, polymerizing when deoxygenated with associated reduction in cell ion and water content (cell dehydration), increased red cell density and further acceleration of HbS polymerization (**Figure 1**).⁵⁻⁷

Pathophysiological studies have shown that dense, dehydrated red cells play a central role in acute and chronic clinical manifestations of SCD, in which intravascular sickling in capillaries and small vessels leads to vaso-occlusion and impaired blood flow with ischemic/reperfusion injury.^{5,8-10} In microcirculation, vaso-occlusive events (VOC) result from a complex and still partially known scenario, involving the interactions between different cell types, including dense red cells, reticulocytes, abnormally activated endothelial cells, leukocytes, platelets and plasma factors (**Figure 1**).^{5,9-13} Acute VOCs have been associated with increased expression of pro-adhesion molecules such as vascular adhesion molecule-1 (VCAM-1), intracellular adhesion molecule-1 (ICAM-1) or selectins (**Figure 1**).^{5,9,11,12,14,15} These molecules are important in recruitment and adhesion of both neutrophils and sickle red cells to the abnormally activated vascular endothelial surface.^{11,16} In addition, the presence of free Hb and free heme

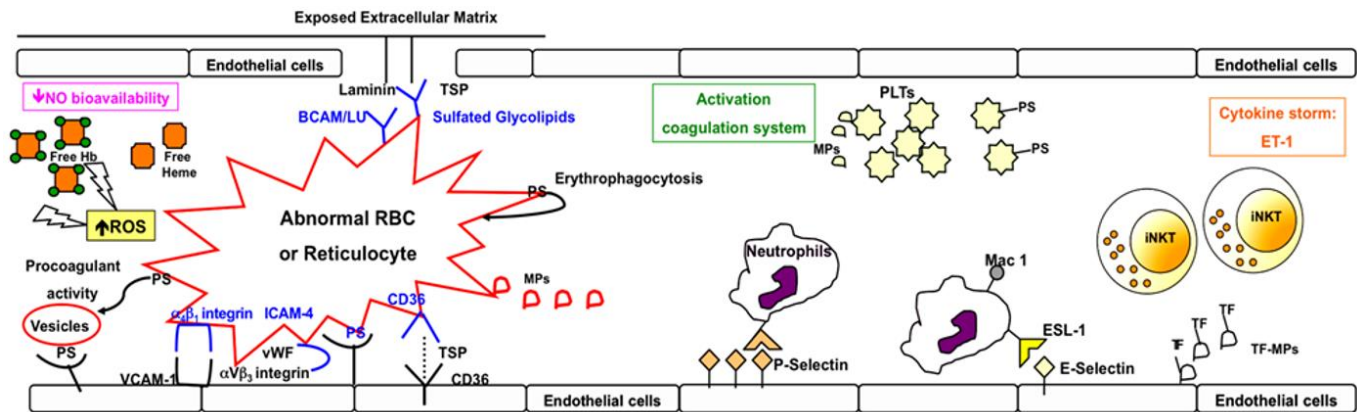


Figure 1. Schematic diagram of the mechanisms involved in the pathogenesis of acute sickle cell related vaso-occlusive events. These involve the adherence of sickle red blood cells (RBCs) or reticulocytes and neutrophils to the abnormally activated endothelial cells, with the participation of activated and phosphatidyl-Serine (PS)-rich platelets (PLTs), activation of the coagulation system, and activation of a cytokine storm. PS: Phosphatidyl-Serine; TSP: thrombospondine; vWF: von Willebrand factor; BCAM/LU: Lutheran blood group protein; ICAM-4: Landstein-Weiner (LW) blood group glycoprotein; MPs: microparticles; Mac1: $\beta 2$ integrins ($\alpha_M\beta_2$ or CD11b/CD18); ESL-1: neutrophil E-selectin ligand -1; Hb: hemoglobin; ROS: reactive oxygen species; iNKT: invariant natural killer T cells; ET-1: endothelin-1; NO: nitric oxide (modified from De Franceschi L *et al.* Seminars in Thrombosis, 37: 266; 2011).

contribute to the local reduction of nitric oxide (NO) bioavailability, establishing an endovascular high pro-oxidant and pro-inflammatory environment. This is associated with modulation of innate immunity and increased iNKT lymphocytes, increase levels of vascular active cytokines such as endothelin 1, combined with the final contribution of platelets (**Figure 1**).^{5,9,14,17-20}

Hydroxyurea is the Gold- Standard Treatment for Sickle Cell Disease. Hydroxyurea or hydroxycarbamide (HU) is the key therapeutic tool for SCD approved by Food and Drug Administration (FDA) and European Medical Agency (EMA). US and European guidelines highlighted that HU should be available for all SCD patients from pediatric to adult populations.^{21,22}

Studies in SCD show a multimodal action of HU, which (i) increases HbF production, resulting in delayed HbS polymerization; (ii) reduces hemolysis and increase NO availability targeting cGMP production; (iii) modulates endothelial activation and reduces neutrophil counts, contributing to the reduction of chronic inflammation (**Figure 2**).²³⁻²⁷ Long-term use of HU has been shown to be safe and well-tolerated in large cohorts of children and adults with SCD, reducing mortality and morbidity of both children and adult patients.^{21,28-31} Indeed, HU reduces (i) the frequency of VOC and the rate of hospitalization; (ii) the incidence of ACS; (iii) the transfusion requirements; and (iv) the severity of dactylitis in SCD pediatric population.^{21,32-36} HU might also be used in combination with transfusion regimen in selected SCD population such as SCD children with progressive cerebrovascular disease in the absence of antigen- matched sibling donor.³⁷ Furthermore, recent reports propose HU as acceptable alternative to chronic transfusion regimen in SCD patients with history of abnormalities at the transcranial doppler scan (TCD),

used to screen for cerebrovascular disease in pediatric patients.³⁸⁻⁴⁰ This requires a close follow-up by TCD scan every 3 months, with the possibility to switch-back to chronic transfusion regimen if abnormal transcranial velocities are again documented.³⁸⁻⁴⁰ Noteworthy, increase reticulocyte count before HU treatment and high leukocyte count after HU have been identified as risk factor for reversion to abnormal TCD velocities in SCD pediatric patients. Thus, again chronic inflammation and vasculopathy seems to be key determinants of severe chronic complications in SCD.³⁸⁻⁴⁰

Although HU should be available for all SCD subjects, the major limitation is the poor adherence of adults SCD patients to HU therapy. Different studies have identified multiple factors to be involved in reduced adherence of SCD patients to HU such as (i) chronicity of the treatment; (ii) socio-economic reasons; and (iii) adhesion barriers related to the transition from pediatric to adult care system.⁴¹⁻⁴⁴

The dissemination of the use of HU is particularly important in underdeveloped countries with high incidence of SCD such as in the sub-Saharan African areas.⁴⁵ Recently, Opoka *et al.* reported safety of use for HU at the dosage of ~20 mg/Kg/d in African children from Uganda, a malaria endemic area (NOHARM study, NCT01976416).⁴⁶ This study further supports the importance of HU as a front-line medical treatment for SCD patients all over the world. Noteworthy, in geographical context where frequent hematologic monitoring is not available, Toya *et al.* have recently reported the beneficial effects of low dose HU (10 mg/Kg/d) on SCD acute clinical manifestations in Nigerian patients.⁴⁷

Novel Therapeutic Approaches to Treat Sickle Cell Disease. In the last two decades, the availability of

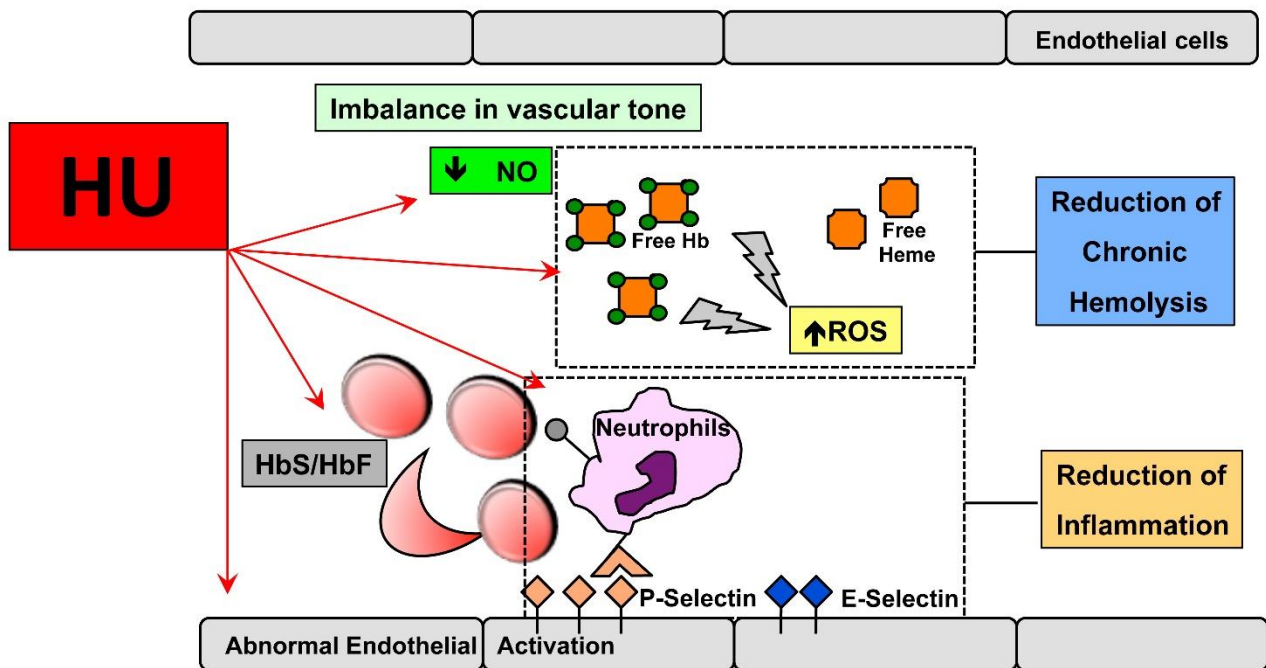


Figure 2. Schematic diagram of multimodal therapeutic action of hydroxyurea (HU) in sickle cell disease. ROS: reactive oxygen species; Hb: hemoglobin; NO: nitric oxide; HbS: sickle hemoglobin; HbF: fetal hemoglobin.

mouse models for SCD has allowed both characterization of the pathogenesis of sickle cell related organ damage(s) and identification of pathophysiology-based new therapeutic options in addition to HU.^{5,7,11,12,48-50}

As shown in **Table 1**, pathophysiology related novel therapies for SCD can be divided into:

- Agents which reduce/prevent sickle red cell dehydration or red cell sickling or HbF inducers;
- Agents targeting SCD vasculopathy and sickle cell-endothelial adhesive events;
- Anti-oxidant agents.

Agents Which Reduce/Prevent Sickle Red Cell Dehydration and Sickling. Different agents targeting sickle red cells have been developed to prevent or limit HbS polymerization or to block the mechanism(s) involved in red cell dehydration.^{14,18,19,48,51-55} Targeting the reduction of circulating dense red cells and/or sickled red cells is very important, since these cells are easily trapped in microcirculation and participate to the pathogenesis of acute VOC.

Recent reports indicate GBT440, an oral direct anti-sickling agent, to be beneficial in SCD. GBT440 (or voxelotor) blocks HbS intermolecular contacts, preventing the generation of HbS fibers and red cell sickling.⁵⁶⁻⁶⁰ GBT440 has been shown (i) to ameliorate *in vitro* SCD red cell features such as red cell deformability or viscosity and (ii) to improve sickle red cell survival with decrease reticulocyte count.⁵⁶⁻⁶⁰ Preliminary data on phase I/II double blind placebo study with GBT440 in healthy volunteers and few SCD

patients show safety and tolerability of GBT440 associated with an amelioration of hemolytic indices and a reduction in reticulocyte count (#NCT02285088).^{55,61,62} Blyden et al. have reported the compassionate use of voxelotor, at the dosage of 900 mg/d up to 1500 mg/d for 24 weeks in a small group of subjects with severe untreatable SCD. Voxelotor beneficially impacts SCD patient well-being with a reduction in number of hospitalization for severe VOC compared to patient's clinical history.⁶³ These data further support the on-going phase III clinical trial on voxelotor in a larger population of SCD patients (HOPE; #NCT03036813).

Agents Targeting SCD Vasculopathy and Sickle Cell-Endothelial Adhesive Events. SCD is not only a hemolytic anemia but also a chronic inflammatory disorder characterized by abnormally activated vascular endothelial cells, amplified inflammatory response, and the release of soluble factors, which promote abnormal adhesive interactions between erythrocytes, endothelial cells, and neutrophils.^{5,7,10,12,64,65} An increased number of circulating, abnormally activated endothelial cells has been identified in SCD patients during acute VOCs, indicating the presence of chronic vasculopathy, worsened by acute events.⁶⁶ Thus, SCD is characterized by a chronic inflammatory vasculopathy that favors the recruitment of leukocytes and the entrapment of dense red cells with the generation of heterotypic aggregates (thrombi) with ischemic/reperfusion local damage.

In this context, the major objectives of therapeutic strategies targeting sickle cell vasculopathy are to

reduce or prevent vascular endothelial activation and damage. The end-point of anti-adherence therapy, alternatively, is to interfere with the initialization and/or amplification of adhesive events.

In SCD, agents targeting SCD vasculopathy and sickle cell-endothelial adhesive events (**Figure 3**) can be divided into:

- i. Molecules targeting hemolysis-induced vasculopathy;
- ii. Agents that modulate the abnormal vascular tone;
- iii. Agents interfering with red cell vascular adhesion events.

i. *Molecules targeting hemolysis-induced vasculopathy.* The chronic hemolytic anemia of SCD is for one-third intravascular and for two-third extravascular, via the reticulo-endothelial systems. Free Hb is present in the peripheral circulation of SCD patients, reacting with plasma nitric oxide (NO) with production of reactive oxygen species (ROS) and generation of MetHb. This is a key step for the release of free heme.^{9,67,68}

The physiological systems binding free Hb or free heme are haptoglobin (Hp) and hemopexin (Hx), respectively.

Table 1. Novel Therapeutic Targets in SCD and Experimental Treatments.

Targets	Agents and Mechanism of Action		References
Sickle red cell dehydration or red cell sickling	Anti-sickling agent	GBT440 is an oral direct anti-sickling agent, to be beneficial in SCD. GBT440 (or voxelotor) blocks HbS intermolecular contacts, preventing the generation of HbS fibers and red cell sickling (#NCT02285088). on-going phase III clinical trial on voxelotor in a larger population of SCD patients (HOPE; #NCT03036813).	56-60
SCD vasculopathy and sickle cell-endothelial adhesive events	Molecules targeting hemolysis-induced vasculopathy	Haptoglobin (Hp) and hemopexin (Hx) respectively binding free Hb or free heme	67-76
	Agents that modulate the abnormal vascular tone	- NO donors such as nitrate or NCX1443 or L-Arginine - Bosentan: Endothelin-1 (ET-1) receptors' blocker	18, 77-81, 84, 85, 89, 90, 91
	Agents interfering with red cell vascular adhesion events	- <i>Molecules interfering with the physical properties of the red cell-endothelial adhesion process.</i> RheothRx (Poloxamer 188), a non-ionic surfactant copolymer was shown to improve microvascular blood. Mast Therapeutics announced in 2016 negative results for a new phase III trial with Vepoloxamer (MST-188).	96-99
		- <i>Molecules specifically interfering with sickle cell-endothelial adhesive mechanisms. Selectins blockers:</i> (i) pan-Selectin antagonist (GMI-1070, rivipansel; #NCT01119833); (ii) humanized anti-P-Selectin antibody (SelG1, crinalizumab; SUSTAIN, #NCT0185361); (iii) P-selectin-aptamer; and (iv) sevuparin.	11, 12, 15, 50, 65, 104, 107-112
		- <i>Molecules modulating inflammatory pathways involved in sickle cell endothelial adhesion.</i> (i) Regadenoson, a selective A2A adenosine receptor agonist, reduces iNKT activation but it fails in interfering with the severity of the acute clinical manifestations of SCD patients enrolled in randomized phase II clinical trial (#NCT01788631) (ii) Antibodies against iNKT cells (NKTT120) (#NCT01783691). (iii) omega-3 fatty acids supplementation. A phase II multicenter randomized double-blind placebo-controlled study in SCD patients reported that ω-3 fatty acid supplementation reduced pain episode in SCD subjects (SCOT, #NCT02973360).	14, 15, 99, 115-128
- <i>Molecules affecting platelet function.</i> Ticagrelor, a direct anti-platelet agent (HESTIA1, #NCT02214121). A phase III clinical trial with ticagrelor in adults with SCD is on-going (#NCT02482298)	11, 131		
Oxidative stress	Anti-oxidant agents	- <u>N-Acetyl-Cysteine (NAC), an exogenous thiol donor.</u> A clinical trial with high dose of NAC during acute VOCs related to SCD is ongoing (#NCT 01800526). - <u>L-Glutamine.</u> Glutamine is involved in GSH metabolism. A multicenter, randomize, placebo-controlled double-blind phase III clinical trial with L-glutamine (0.3 g/Kg twice a day) supplementation reduced the mean number and length of hospitalization, associated with increased median time to the first crisis.	134-140

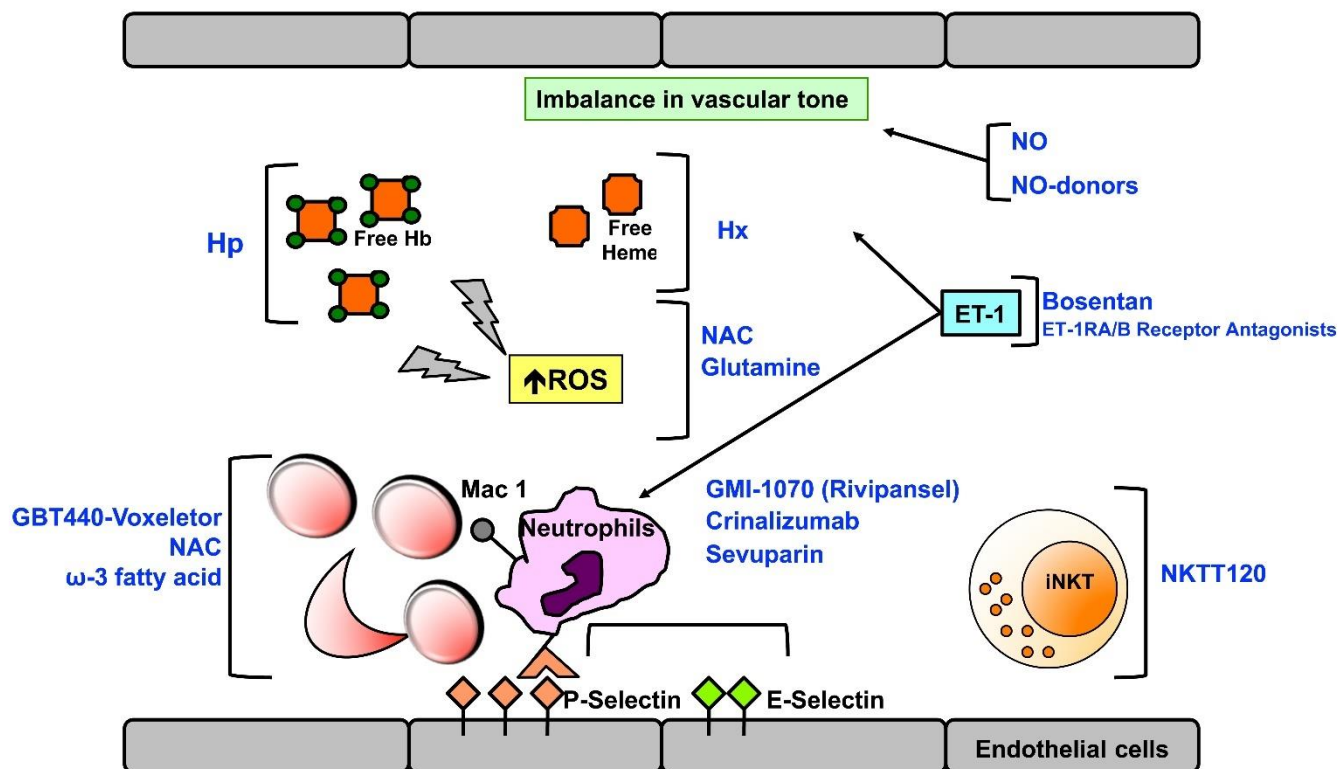


Figure 3. Schematic diagram of the mechanisms of action of pathophysiology based new therapeutic options for treatment of sickle cell disease and sickle cell vasculopathy. Hp: haptoglobin; Hx: hemopexin; NAC: N-Acetyl-cysteine; Ab: antibody; ROS: reactive oxygen species; iNKT: invariant natural killer T cells; NKTT120: humanized monoclonal antibody specifically depleting iNKT; NO: nitric oxide; ET-1: endothelin-1; ET-R: endothelin-1 receptor.

In SCD patients, both Hp and Hx levels are significantly reduced in steady state compared to healthy controls; they further decrease during acute VOCs.^{67,69} The highly pro-oxidant environment with the presence of free heme and free Hb promotes inflammation and abnormal vascular activation with increased expression of adhesion vascular molecules such as VCAM-1, ICAM-1 or E-selectin.^{67,69} Studies in mouse models for SCD have shown that free heme induces vascular stasis and leukocyte extravasation with the trapping of dense red cells and neutrophils in microcirculation.⁷⁰⁻⁷²

In human SCD patients, free Hb and free heme increase during acute VOCs with further reduction in Hp and Hx levels (Figure 1).^{72,73} Noteworthy, Hp levels correlate with pulmonary hypertension,⁶⁷ suggesting that the blockage of free-Hb by Hp might possibly affect SCD related organ damage. In mouse models for SCD, the infusion of Hp has been shown to prevent vascular stasis. Encouraging data from small, *in vivo* human studies with infused Hp show that Hp protects the kidneys from free Hb-related tubular damage in patients who have undergone cardiopulmonary surgery or endoscopic sclerotherapy.⁶⁷ Few case reports are present in the literature on the use of Hp in patients with hemolytic crisis and inherited red cell disorders.^{67,74} Thus, Hp might be as a possible new therapeutic tool to be further explored in SCD.

In the complex scenario of the pathogenesis of SCD vasculopathy, Hx, a high affinity heme binding protein,

represents another interesting molecule that might be explored as a novel therapeutic option (Figure 1). The supplementation of Hx in mouse models for SCD has been shown to reduce heme induced oxidative stress, vascular endothelial injury, inflammation, and vascular stasis.⁹ Recently, a link between increased free heme and complement activation has been reported in cell- and animal-based model for SCD.⁷⁵ Hx significantly reduces complement deposition in kidney from humanized SCD mice, highlighting the importance of controlling free heme plasma level as additional tool to limit inflammatory vasculopathy and related severe organ damage in SCD. The importance of optimal levels of Hp and Hx is also supported by a recent report on the use of therapeutic plasma exchange in SCD with severe VOC, resistant to red cell exchange.⁷⁶

Further studies need be carried out to develop and understand the potential clinical use of Hp and/or Hx in management of severe complication related to excess of free heme in SCD patients.

i. Agents that modulate the abnormal vascular tone. Vascular tone results from the balance between vaso-dilatory factors such as nitric oxide (NO) and vaso-constrictor factors such as the endothelin-1 (ET-1) system (Figure 1).^{18,77-81} In SCD, reduced NO local bioavailability, a consequence of the presence of free Hb, contributes to chronic vaso-constriction and amplifies the expression of vascular adhesion molecules.^{77,82,83} In addition, the release of arginase in

peripheral circulation by sickle red cells during chronic hemolysis, subtracts arginine from the urea cycle in endothelial cells, and further contributes to NO deficiency.^{77,82-85} Plasma NO metabolites are decreased in SCD patients during acute VOCs and decreased exhaled NO has also been reported. Thus, therapeutic strategies to supplement or modulate NO might beneficially interfere with the pathogenesis of acute SCD related clinical manifestations such as VOCs. Initial trials showed some positive, encouraging effects of inhaled NO on acute VOCs.^{82,86,87} However, a subsequent multicentric, double-blind, randomized placebo-controlled study in SCD with VOCs using inhaled NO showed no clinically significant effects.⁸² New NO donors such as nitrate or NCX1443 need to be further evaluated in humanized animal-based pre-clinical studies.^{83,88} Another possible strategy to increase NO production in SCD is the supplementation of L-Arginine. Oral L-Arginine (i) decreases artery pulmonary pressure in SCD; (ii) improves leg ulcers; and (iii) contributes in pain control in SCD.^{84,85,89} The co-administration of L-Arginine with HU has been reported to increase levels of nitrate, suggesting L-Arginine as an adjuvant molecule in treatment of SCD.^{84,85,89}

Endothelin-1 is a potent vasoconstrictor and bronchoconstrictor, whose plasma and urinary values are increased in SCD subjects in steady state and during acute VOCs.^{18,90,91} In a mouse model for SCD, the ET-1 receptors' blocker, bosentan, prevented hypoxia induced organ damage and affect neutrophil mediated inflammatory response, suggesting the modulation of the ET-1 system as an additional therapeutic option to interfere with the pathogenesis of SCD related clinical manifestation(s).^{18,92,93} It is of interest to note that increased ET-1 and high ET-1 levels have been shown to positively correlate with pain rating in children with SCD.⁹⁴ This has been recently investigated in humanized mouse model for SCD, showing that endothelin receptor-type A might be involved in inflammatory mediated pain component throughout the modulation of Nav1.8 channel in primary sensing neurons.⁹⁵

ii. *Agents interfering with red cell vascular adhesion events.* In SCD, anti-adherence therapeutic strategies might represent an interesting, novel therapeutic strategy to prevent the generation of acute VOCs and to lessen SCD related organ damage (**Figure 1 and 3**). The anti-adherence therapeutic options might be divided into three groups based on their mechanism of action:

- a) *Molecules interfering with the physical properties of the red cell-endothelial adhesion process;*
- b) *Molecules specifically interfering with sickle cell-endothelial adhesive mechanisms;*

c) *Molecules modulating inflammatory pathways involved in sickle cell endothelial adhesion;*

d) *Molecules affecting platelet function.*

a) *Molecules interfering with the physical properties of the red cell-endothelial adhesion process.*

RheothRx (Poloxamer 188), a non-ionic surfactant copolymer was shown to improve microvascular blood flow by lowering viscosity and frictional forces. RheothRx was shown some beneficial effects on pain intensity and duration of hospitalization in a pilot study on SCD patients experiencing moderate to severe vaso-occlusive crisis.⁹⁶ RheothRx was tested in a phase III clinical study for treatment of VOCs in SCD. Although P188 has been shown to shorten the duration of pain crisis, its effects on acute events were limited.^{97,98} Mast Therapeutics announced in 2016 negative results for a new phase III trial with Vepoloxamer (MST-188), a IV agent tested to assess its effect on reducing the duration of vaso-occlusive crises.⁹⁹

b) *Molecules interfering with sickle cell-endothelial adhesive mechanisms.*

Recent studies in SCD have identified different mechanisms involved in sickle cell-endothelium adhesive events, which may be of therapeutic relevance (**Figure 1**): (i) the integrin $\alpha 4\beta 1$ receptor of fibronectin and the vascular adhesion molecule-1 (VCAM-1), E-selectin and P-selectin; (ii) the thrombospondin and/or collagen and receptor CD36, present on the surface of endothelial cells, platelets and reticulocyte-rich subpopulations of normal and sickle red cells; (iii) the sulfate glycolipids, which bind thrombospondin, von-Willebrand factor multimer and laminin; (iv) the Lutheran blood group proteins (BCAM/LU), whose expression is increased in red cells from SCD patients; (v) the ICAM-4 (Landstein-Weiner blood group glycoprotein-LW), which binds $\alpha V\beta 3$ integrin receptors; and (vi) the exposure of PS detectable in a subpopulation of sickle red cells, which participates both in cell-cell adhesion to activated vascular endothelium surface and in the activation of a coagulation system. Monoclonal antibodies against the adhesion molecules or short synthetic peptides interfering with ICAM-4 or $\alpha V\beta 3$ integrin have been shown to reduce adhesion events in SCD mouse models (**Figure 1**). It is of interest to note that antibodies against adhesion molecules block the heme induced vascular stasis, supporting again the connection between heme, vasculopathy, and adhesion events in SCD.^{68,100,101} Among the agents interfering with red cell vascular adhesion events, the blockade of adhesion mechanisms through interference with Selectins seems to be a novel powerful therapeutic option for clinical management of SCD. Selectins are a family of molecules mediating adhesion of blood cells with activated vascular endothelial cells. and play a key role in leukocyte recruitment as well as in sickle red cell adhesion to

inflammatory activated vascular endothelium. In addition, studies have shown that P-selectin are increased in plasma of SCD patients.^{65,102-106} Different therapeutic strategies have been developed, to block selectins: (i) pan-Selectin antagonist (GMI-1070, rivipansel); (ii) humanized anti-P-Selectin antibody (SelG1, crinalizumab); (iii) P-selectin-aptamer; and (iv) sevuparin.^{11,12,15,50,65,104,107-112} Rivipansel is a glycomimetic pan-selectin antagonist, which was tested in phase-I and -II studies in SCD. Rivipansel showed a safe profile, reducing the levels of E-Selectin in SCD patients during acute VOCs.^{107,113} In phase II study, rivipansel beneficially affected the number of pain crisis in a small number of SCD subjects (#NCT01119833). However, these data were obtained including some SC patients, which generates some difficulties in their interpretation. An on-going phase II study is focused on SCD children.

Crinalizumab is a humanized P-Selectin antibody, which has been tested in a multinational double-blind placebo-controlled trial (SUSTAIN, #NCT0185361).^{15,111} SCD subjects (SS, SC, S β ⁺ and S β ⁰ genotype) were treated with crinalizumab either 2.5 or 5 mg/Kg every 4 weeks. Crinalizumab at the dosage of 5 mg/Kg every 4 weeks reduced the number of pain crisis and increased the time between VOCs in SCD independently from possible preceding HU treatment.^{15,111,112}

An additional strategy targeting P-Selectins is represented by the use of low molecular weight heparins, such as tinzaparin, which has been shown to block the P-Selectin system and to reduce the duration and the severity of VOCs in few cases of SCD patients.^{12,50} Sevuparin is a derivative of low-molecular weight heparin, lacking anticoagulant activity and it has been evaluated in SCD.^{109,114} Sevuparin acts on multiple targets: (i) P and L-selectins; (ii) thrombospondin-Fibronectin-Von Willebrand factor; and (iii) sickle-leukocyte-endothelial cells interaction. A phase II multicenter international trial on sevuparin in acute VOCs is ongoing.

c) Molecules modulating inflammatory pathways involved in sickle cell endothelial adhesion. Another set of novel therapeutic option is represented by agents modulating the inflammatory pathways that participate to adhesion events in SCD.

Studies in different models of hypoxia/reoxygenation stress have shown that adenosine is released from cells and interacts with A (1-3) receptors (AR), which are present on endothelial cells, leukocytes and iNKT cells. This promotes the activation of the transcriptional factor NF- κ B, which orchestrates the inflammatory response. iNKT are a subgroup of T lymphocytes that affects both innate and adaptive immunity, participating to inflammatory cascade.¹¹⁵⁻¹¹⁷ Increased iNKT circulating cells have

been observed in SCD subjects on both steady state and during acute VOCs. Antibodies against iNKT cells (NKTT120) have been developed, based on the key role that adhesion and inflammation are involved in the pathogenesis of severe acute complication of SCD (#NCT01783691).^{15,99,115} Field *et al.* recently reported the failure of regadenoson in reducing iNKT activation and in interfering with the severity of the acute clinical manifestations of SCD patients enrolled in randomized phase II clinical trial (#NCT01788631).¹¹⁸

An attempt to target inflammatory vasculopathy and to modulate inflammatory response has been made based on the evidences in other diseases such as in cardiovascular disease looking to dietary manipulation with omega-3 fatty acids (ω -3 PUFAs). Supplementation with omega-3 fatty acids has been reported to (i) beneficially affect red cell membrane lipid composition; (ii) modulate soluble and cellular inflammatory response and coagulation cascade; and (iii) to favor NO production.¹¹⁹⁻¹²² In SCD, the fatty acid profile of sickle erythrocytes is altered compared to healthy controls, with a relative increase in the ratio of ω -6 to ω -3 PUFAs, in agreement with sustained chronic inflammation.^{123,124} In humanized mouse model for SCD, PUFA supplementation protects against acute sickle cell-related lung and liver damages during hypoxia/reoxygenation induced VOCs.¹⁴ A phase II multicenter randomized double-blind placebo-controlled study in SCD patients reported that ω -3 fatty acid supplementation reduced pain episode in SCD subjects (SCOT, #NCT02973360).¹²⁵⁻¹²⁸

d) Molecules affecting platelet function. The role of platelets in clinical manifestations of SCD on both steady state and acute events has been only partially characterized and much still remains to be investigated.^{5,11,50} Early evidence on the beneficial effects of ticlopidine on reducing the rate of pain crisis highlighted the potential role of platelet activation and aggregation during acute events in SCD.¹²⁹ However, a multicentric phase 2 study on prasugrel, a third-generation anti-platelet agent, in adult with SCD showed a reduction of platelet activation without change in pain rate.¹³⁰ Recently, ticagrelor, a direct anti-platelet agent with some effects on vascular tone and inflammatory response has been evaluated in a dose-finding study on SCD children (HESTIA1, #NCT02214121).^{11,131} Ticagrelor was well tolerated without significant drug related adverse events, in particular no hemorrhagic events were reported. Noteworthy, in SCD children ticagrelor induced platelet inhibition similar to that reported in adults with acute coronary disease.¹³¹ A phase III clinical trial with ticagrelor in adults with SCD is on-going (#NCT02482298).^{11,131}

Anti-Oxidant Agents and Sickle Cell Disease. SCD is also characterized by a highly pro-oxidant environment

due to the elevated production of reactive oxygen species (ROS) generated by increased levels of pathological free heme and iron and a reduction in anti-oxidant systems such as GSH (**Figure 1**).^{5,7,12,132,133} N-Acetyl-Cysteine (NAC), an exogenous thiol donor, has been studied both *in vitro* and *in vivo* in SCD patients. NAC supplementation (1,200-2,400 mg/day) was shown to reduce the formation of dense red cells and the rate of hemolysis and to increase GSH levels in SCD subjects. However, Sins et al. have recently reported a randomized, placebo-, double-blind trial (#NCT01849016) on NAC in SCD. Although the study shows a failure of NAC in affecting acute clinical manifestations of SCD, the Authors point out that the low adherence of SCD to NAC treatment might be responsible for the reduced biological effect of NAC in SCD. A clinical trial with high dose of NAC during acute VOCs related to SCD is ongoing (#NCT 01800526).¹³⁴⁻¹³⁶

L-Glutamine is a likely anti-oxidant agent in SCD. Glutamine is involved in GSH metabolism since it preserves NADPH levels required for GSH recycling, and it is the precursor for nicotinamide adenine dinucleotide (NAD) and arginine.¹³⁷⁻¹³⁹ A first randomized, double blind, placebo-controlled parallel group trial with L-glutamine supplementation in SCD patients showed reduction in number of hospitalization compared to historic patients data.¹³⁸ Recently, a multicenter, randomize, placebo-controlled double-blind phase III clinical trial with L-glutamine (0.3 g/Kg twice a day) involving 230 SS/Sbeta⁰ patients with ≥ 2 pain crisis showed that L-glutamine supplementation reduced the mean number and length of hospitalization, associated with increased median time to the first crisis.¹³⁷ Both studies have several limitations such as (i) the high rate of patient drop-out; (ii) the presence of fatal events due to multiorgan failure in L-glutamine arm; (iii) the lack of effects on hematologic parameters and hemolytic indices; and (iv) the absence of clear data on L-glutamine mechanism of action.^{137,140} Since no information are available on long-term use of L-glutamine supplementation as well on the systemic effects of L-glutamine, the sickle cell scientific community should use caution in prescribing L-glutamine supplement for both adult and pediatric SCD patients.¹⁴⁰ Future studies are required to further define

the role of anti-oxidant treatments in the clinical management of SCD subjects.

Curative Options in Sickle Cell Disease. In the last two decades, progresses on hematopoietic stem cell transplantation (HSCT) strategies have allowed to offer a new curative option to patients with SCD. The major limitation in diffusion of HSCT is (i) the availability of leukocyte antigen (HLA)-matched sibling donor; (ii) the toxicities associated with myeloablative conditioning; and (iii) inflammatory vasculopathy.¹⁴¹⁻¹⁴⁵ Recently, lentiviral gene therapy has been reported to be safe and to positively impact hematologic phenotype in a child with SCD.¹⁴⁶ Different clinical trials on gene therapy in SCD are on-going in various countries.¹⁴¹⁻¹⁴⁴

Finally, the development of CRISPR/Cas9 genome editing (GE) strategy has been reported to represent a new potential therapeutic tool for genetic correction of SCD.¹⁴⁷⁻¹⁴⁹ However, in SCD GE is still limited to cell- and/or animal-based studies.

Conclusions. In conclusion, the emerging picture for new treatment of SCD is that formation of dense red cells, vasculopathy, adhesion events and inflammation as well as oxidative stress might constitute new pharmacological targets (**Figure 3**).

Promising data have been reported on new therapeutic tools interfering with P-selectin and modulating inflammatory vasculopathy. However, some concerns have been expressed about possible reductions of appropriate inflammatory responses to pathogens, although the initial trials did not show any signal in this direction. A new field of combinatorial therapy for SCD will require a holistic approach, considering the improvement of patient quality of life as an important outcome in designing new clinical studies.

Acknowledgments. We would like to thank Dr Carlo Brugnara (Boston Children's Hospital, Harvard Medical School, Boston, MA; USA) for fruitful discussion and manuscript revision.

Competing Interests and Funding. The Authors declare that they have no conflict of interest. This work was supported by FUR-UNIVR (LDF).

References:

1. Modell B, Darlison M. Global epidemiology of haemoglobin disorders and derived service indicators. *Bulletin of the World Health Organization* 2008;86:480-487. <https://doi.org/10.2471/BLT.06.036673> PMID:18568278 PMCID:PMC2647473
2. Murray CJ, Vos T, Lozano R, et al. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 2012;380:2197-2223. [https://doi.org/10.1016/S0140-6736\(12\)61689-4](https://doi.org/10.1016/S0140-6736(12)61689-4)
3. Weatherall DJ, Clegg JB. Inherited haemoglobin disorders: an increasing global health problem. *Bulletin of the World Health Organization* 2001;79:704-712. PMID:11545326 PMCID:PMC2566499
4. Piel FB, Patil AP, Howes RE, et al. Global epidemiology of sickle haemoglobin in neonates: a contemporary geostatistical model-based map and population estimates. *Lancet* 2013;381:142-151. [https://doi.org/10.1016/S0140-6736\(12\)61229-X](https://doi.org/10.1016/S0140-6736(12)61229-X)
5. De Franceschi L, Cappellini MD, Olivieri O. Thrombosis and sickle cell disease. *Semin Thromb Hemost* 2011;37:226-236. <https://doi.org/10.1055/s-0031-1273087> PMID:21455857
6. Eaton WA, Hofrichter J. Sickle cell hemoglobin polymerization.

- Advances in protein chemistry 1990;40:63-279.
[https://doi.org/10.1016/S0065-3233\(08\)60287-9](https://doi.org/10.1016/S0065-3233(08)60287-9)
7. De Franceschi L, Corrocher R. Established and experimental treatments for sickle cell disease. *Haematologica* 2004;89:348-356. PMID:15020275
 8. Ballas SK, Smith ED. Red blood cell changes during the evolution of the sickle cell painful crisis. *Blood* 1992;79:2154-2163. PMID:1562742
 9. Vinchi F, De Franceschi L, Ghigo A, et al. Hemopexin therapy improves cardiovascular function by preventing heme-induced endothelial toxicity in mouse models of hemolytic diseases. *Circulation* 2013;127:1317-1329.
<https://doi.org/10.1161/CIRCULATIONAHA.112.130179>
 PMID:23446829
 10. Hebbel RP, Vercellotti G, Nath KA. A systems biology consideration of the vasculopathy of sickle cell anemia: the need for multi-modality chemo-prophylaxis. *Cardiovasc Hematol Disord Drug Targets* 2009;9:271-292. <https://doi.org/10.2174/1871529X10909040271>
 PMID:19751187 PMCID:PMC2914570
 11. Telen MJ. Beyond hydroxyurea: new and old drugs in the pipeline for sickle cell disease. *Blood* 2016;127:810-819.
<https://doi.org/10.1182/blood-2015-09-618553>
 PMID:26758919 PMCID:PMC4760087
 12. Manwani D, Frenette PS. Vaso-occlusion in sickle cell disease: pathophysiology and novel targeted therapies. *Blood* 2013;122:3892-3898. <https://doi.org/10.1182/blood-2013-05-498311>
 PMID:24052549 PMCID:PMC3854110
 13. Hebbel RP. Adhesion of sickle red cells to endothelium: myths and future directions. *Transfus Clin Biol* 2008;15:14-18. <https://doi.org/10.1016/j.tacl.2008.03.011> PMID:18501652
 14. Kalish BT, Matte A, Andolfo I, et al. Dietary omega-3 fatty acids protect against vasculopathy in a transgenic mouse model of sickle cell disease. *Haematologica* 2015;100:870-880.
<https://doi.org/10.3324/haematol.2015.124586>
 PMID:25934765 PMCID:PMC4486221
 15. Ataga KI, Kutlar A, Kanter J, et al. Crizanlizumab for the Prevention of Pain Crises in Sickle Cell Disease. *N Engl J Med* 2017;376:429-439.
<https://doi.org/10.1056/NEJMoa1611770>
 PMID:27959701 PMCID:PMC5481200
 16. Hidalgo A, Chang J, Jang JE, et al. Heterotypic interactions enabled by polarized neutrophil microdomains mediate thromboinflammatory injury. *Nature medicine* 2009;15:384-391.
<https://doi.org/10.1038/nm.1939>
 PMID:19305412 PMCID:PMC2772164
 17. Dalle Carbonare L, Matte A, Valenti MT, et al. Hypoxia-reperfusion affects osteogenic lineage and promotes sickle cell bone disease. *Blood* 2015;126:2320-2328.
<https://doi.org/10.1182/blood-2015-04-641969> PMID:26330244
 18. Sabaa N, de Franceschi L, Bonnin P, et al. Endothelin receptor antagonism prevents hypoxia-induced mortality and morbidity in a mouse model of sickle-cell disease. *J Clin Invest* 2008;118:1924-1933.
<https://doi.org/10.1172/JCI33308>
 PMID:18382768 PMCID:PMC2276396
 19. Wieschhaus A, Khan A, Zaidi A, et al. Calpain-1 knockout reveals broad effects on erythrocyte deformability and physiology. *Biochem J* 2012;448:141-152. <https://doi.org/10.1042/BJ20121008>
 PMID:22870887 PMCID:PMC3955119
 20. Siciliano A, Turrini F, Bertoldi M, et al. Deoxygenation affects tyrosine phosphoproteome of red cell membrane from patients with sickle cell disease. *Blood Cells Mol Dis* 2010;44:233-242.
<https://doi.org/10.1016/j.bcmd.2010.02.007> PMID:20206558
 21. Yawn BP, Buchanan GR, Afenyi-Annan AN, et al. Management of sickle cell disease: summary of the 2014 evidence-based report by expert panel members. *JAMA* 2014;312:1033-1048.
<https://doi.org/10.1001/jama.2014.10517> PMID:25203083
 22. Engert A, Balduini C, Brand A, et al. The European Hematology Association Roadmap for European Hematology Research: a consensus document. *Haematologica* 2016;101:115-208.
<https://doi.org/10.3324/haematol.2015.136739>
 PMID:26819058 PMCID:PMC4938336
 23. Platt OS. Hydroxyurea for the treatment of sickle cell anemia. *N Engl J Med* 2008;358:1362-1369. <https://doi.org/10.1056/NEJMct0708272>
 PMID:18367739
 24. Yarbro JW. Mechanism of action of hydroxyurea. *Semin Oncol* 1992;19:1-10. PMID:1641648
 25. Charache S. Mechanism of action of hydroxyurea in the management of sickle cell anemia in adults. *Semin Hematol* 1997;34:15-21. PMID:9317197
 26. Charache S, Terrin ML, Moore RD, et al. Effect of hydroxyurea on the frequency of painful crises in sickle cell anemia. Investigators of the Multicenter Study of Hydroxyurea in Sickle Cell Anemia [see comments]. *New England Journal of Medicine* 1995;332:1317-1322.
<https://doi.org/10.1056/NEJM199505183322001> PMID:7715639
 27. Saleh AW, Hillen HF, Duits AJ. Levels of endothelial, neutrophil and platelet-specific factors in sickle cell anemia patients during hydroxyurea therapy. *Acta Haematol* 1999;102:31-37.
<https://doi.org/10.1159/000040964> PMID:10473885
 28. Ware RE, de Montalembert M, Tshililo L, et al. Sickle cell disease. *Lancet* 2017;390:311-323.
[https://doi.org/10.1016/S0140-6736\(17\)30193-9](https://doi.org/10.1016/S0140-6736(17)30193-9)
 29. Rigano P, De Franceschi L, Sainati L, et al. Real-life experience with hydroxyurea in sickle cell disease: A multicenter study in a cohort of patients with heterogeneous descent. *Blood Cells Mol Dis* 2018;69:82-89. <https://doi.org/10.1016/j.bcmd.2017.08.017>
 PMID:29107441
 30. Pule GD, Mowla S, Novitzky N, et al. A systematic review of known mechanisms of hydroxyurea-induced fetal hemoglobin for treatment of sickle cell disease. *Expert Rev Hematol* 2015;8:669-679.
<https://doi.org/10.1586/17474086.2015.1078235>
 PMID:26327494 PMCID:PMC4829639
 31. Jison ML, Munson PJ, Barb JJ, et al. Blood mononuclear cell gene expression profiles characterize the oxidant, hemolytic, and inflammatory stress of sickle cell disease. *Blood* 2004;104:270-280.
<https://doi.org/10.1182/blood-2003-08-2760>
 PMID:15031206 PMCID:PMC5560446
 32. Stettler N, McKiernan CM, Melin CQ, et al. Proportion of adults with sickle cell anemia and pain crises receiving hydroxyurea. *JAMA* 2015;313:1671-1672.
<https://doi.org/10.1001/jama.2015.3075> PMID:25919532
 33. Wong TE, Brandow AM, Lim W, et al. Update on the use of hydroxyurea therapy in sickle cell disease. *Blood* 2014;124:3850-3857.
<https://doi.org/10.1182/blood-2014-08-435768>
 PMID:25287707 PMCID:PMC4271176
 34. Crosby WH, Dameshek W. The significance of hemoglobinemia and associated hemosiderinuria, with particular references to various types of hemolytic anemia. *J Lab Clin Med* 1951;38:829. PMID:14889070
 35. Voskaridou E, Christoulas D, Bilalis A, et al. The effect of prolonged administration of hydroxyurea on morbidity and mortality in adult patients with sickle cell syndromes: results of a 17-year, single-center trial (LaSHS). *Blood* 2010;115:2354-2363.
<https://doi.org/10.1182/blood-2009-05-221333> PMID:19903897
 36. Wang WC, Ware RE, Miller ST, et al. Hydroxycarbamide in very young children with sickle-cell anaemia: a multicentre, randomised, controlled trial (BABY HUG). *Lancet* 2011;377:1663-1672.
[https://doi.org/10.1016/S0140-6736\(11\)60355-3](https://doi.org/10.1016/S0140-6736(11)60355-3)
 37. Brousse V, Gandhi S, de Montalembert M, et al. Combined blood transfusion and hydroxycarbamide in children with sickle cell anaemia. *Br J Haematol* 2013;160:259-261.
<https://doi.org/10.1111/bjh.12104> PMID:23116405
 38. Benaudin F, Verlhac S, Arnaud C, et al. Long-term treatment follow-up of children with sickle cell disease monitored with abnormal transcranial Doppler velocities. *Blood* 2016;127:1814-1822.
<https://doi.org/10.1182/blood-2015-10-675231> PMID:26851292
 39. Ware RE, Davis BR, Schultz WH, et al. Hydroxycarbamide versus chronic transfusion for maintenance of transcranial doppler flow velocities in children with sickle cell anaemia-TCD With Transfusions Changing to Hydroxyurea (TWITCH): a multicentre, open-label, phase 3, non-inferiority trial. *Lancet* 2016;387:661-670.
[https://doi.org/10.1016/S0140-6736\(15\)01041-7](https://doi.org/10.1016/S0140-6736(15)01041-7)
 40. Helton KJ, Adams RJ, Kesler KL, et al. Magnetic resonance imaging/angiography and transcranial Doppler velocities in sickle cell anemia: results from the SWITCH trial. *Blood* 2014;124:891-898.
<https://doi.org/10.1182/blood-2013-12-545186>
 PMID:24914136 PMCID:PMC4126329
 41. Inoue S, Kodjebacheva G, Scherrer T, et al. Adherence to hydroxyurea medication by children with sickle cell disease (SCD) using an electronic device: a feasibility study. *Int J Hematol* 2016;104:200-207.
<https://doi.org/10.1007/s12185-016-2027-x> PMID:27225236
 42. Han J, Bhat S, Gowhari M, et al. Impact of a Clinical Pharmacy Service on the Management of Patients in a Sickle Cell Disease Outpatient Center. *Pharmacotherapy* 2016;36:1166-1172.
<https://doi.org/10.1002/phar.1834>
 PMID:27639254 PMCID:PMC5373798
 43. Green NS, Manwani D, Qureshi M, et al. Decreased fetal hemoglobin over time among youth with sickle cell disease on hydroxyurea is associated with higher urgent hospital use. *Pediatr Blood Cancer* 2016;63:2146-2153. <https://doi.org/10.1002/psc.26161>

- PMid:27573582 PMCID:PMC5072999
44. Smaldone A, Findley S, Manwani D, et al. HABIT, a Randomized Feasibility Trial to Increase Hydroxyurea Adherence, Suggests Improved Health-Related Quality of Life in Youths with Sickle Cell Disease. *J Pediatr* 2018;197:177-185 e172.
 45. Ansong D, Akoto AO, Ocloo D, et al. Sickle cell disease: management options and challenges in developing countries. *Mediterr J Hematol Infect Dis* 2013;5:e2013062. <https://doi.org/10.4084/mjihid.2013.062> PMid:24363877 PMCID:PMC3867228
 46. Opoka RO, Ndugwana CM, Latham TS, et al. Novel use Of Hydroxyurea in an African Region with Malaria (NOHARM): a trial for children with sickle cell anemia. *Blood* 2017;130:2585-2593. <https://doi.org/10.1182/blood-2017-06-788935> PMid:29051184
 47. Tayo BO, Akingbola TS, Saraf SL, et al. Fixed Low-Dose Hydroxyurea for the Treatment of Adults with Sickle Cell Anemia in Nigeria. *Am J Hematol* 2018.
 48. De Franceschi L. Pathophysiology of sickle cell disease and new drugs for the treatment. *Mediterr J Hematol Infect Dis* 2009;1:e2009024.
 49. Stocker JW, De Franceschi L, McNaughton-Smith GA, et al. ICA-17043, a novel Gardos channel blocker, prevents sickled red blood cell dehydration in vitro and in vivo in SAD mice. *Blood* 2003;101:2412-2418. <https://doi.org/10.1182/blood-2002-05-1433> PMid:12433690
 50. De Franceschi L, Saadane N, Trudel M, et al. Treatment with oral clotrimazole blocks Ca(2+)-activated K+ transport and reverses erythrocyte dehydration in transgenic SAD mice. A model for therapy of sickle cell disease. *J Clin Invest* 1994;93:1670-1676. <https://doi.org/10.1172/JCI117149> PMid:7512989 PMCID:PMC294212
 51. Telen MJ. Developing new pharmacotherapeutic approaches to treating sickle-cell disease. *ISBT Sci Ser* 2017;12:239-247. <https://doi.org/10.1111/voxs.12305> PMid:28484512 PMCID:PMC5418585
 52. De Franceschi L, Franco RS, Bertoldi M, et al. Pharmacological inhibition of calpain-1 prevents red cell dehydration and reduces Gardos channel activity in a mouse model of sickle cell disease. *FASEB J* 2013;27:750-759. <https://doi.org/10.1096/fj.12-217836> PMid:23085996 PMCID:PMC3545531
 53. McNaughton-Smith GA, Burns JF, Stocker JW, et al. Novel inhibitors of the Gardos channel for the treatment of sickle cell disease. *J Med Chem* 2008;51:976-982. <https://doi.org/10.1021/jm070663s> PMid:18232633
 54. De Franceschi L, Brugnara C, Rouyer-Fessard P, et al. Formation of dense erythrocytes in SAD mice exposed to chronic hypoxia: evaluation of different therapeutic regimens and of a combination of oral clotrimazole and magnesium therapies. *Blood* 1999;94:4307-4313. PMid:10590075
 55. Li Q, Henry ER, Hofrichter J, et al. Kinetic assay shows that increasing red cell volume could be a treatment for sickle cell disease. *Proc Natl Acad Sci U S A* 2017;114:E689-E696. <https://doi.org/10.1073/pnas.1619054114> PMid:28096387 PMCID:PMC5293101
 56. Dufu K, Oksenberg D. GBT440 reverses sickling of sickled red blood cells under hypoxic conditions in vitro. *Hematol Rep* 2018;10:7419. <https://doi.org/10.4081/hr.2018.7419> PMid:30046411 PMCID:PMC6036981
 57. Metcalf B, Chuang C, Dufu K, et al. Discovery of GBT440, an Orally Bioavailable R-State Stabilizer of Sickle Cell Hemoglobin. *ACS Med Chem Lett* 2017;8:321-326. <https://doi.org/10.1021/acsmchemlett.6b00491> PMid:28337324 PMCID:PMC5346980
 58. Oksenberg D, Dufu K, Patel MP, et al. GBT440 increases haemoglobin oxygen affinity, reduces sickling and prolongs RBC half-life in a murine model of sickle cell disease. *Br J Haematol* 2016;175:141-153. <https://doi.org/10.1111/bjh.14214> PMid:27378309
 59. Dufu K OD, Zhou C, Hutchaleelaha A, Archer DR. GTx011, a potent allosteric modifier of hemoglobin oxygen affinity, prevents RBC sickling in whole blood and prolongs RNC half-life in vivo in a murine model of sickle cell disease. In: *Blood*, editor. American Society of Hematology; 2014. p a217.
 60. Patel M CP, Dufu K, Metcalf B, Sinha U. GTx011, an anti-sickling compound, improves SS blood rheology by reduction of HbS polymerization via allosteric modulation of O2 affinity. In: *Blood*, editor. American Society of Hematology 2014. p a1370.
 61. Blyden G, Bridges KR, Bronte L. Case series of patients with severe sickle cell disease treated with voxelotor (GBT440) by compassionate access. *Am J Hematol* 2018.
 62. Telfer P, Agodoa I, Fox KM, et al. Impact of voxelotor (GBT440) on unconjugated bilirubin and jaundice in sickle cell disease. *Hematol Rep* 2018;10:7643. <https://doi.org/10.4081/hr.2018.7643> PMid:30046415 PMCID:PMC6036983
 63. Estep JH. Voxelotor (GBT440), a first-in-class hemoglobin oxygen-affinity modulator, has promising and reassuring preclinical and clinical data. *Am J Hematol* 2018;93:326-329. <https://doi.org/10.1002/ajh.25042> PMid:29352729
 64. Kato GJ, Heibel RP, Steinberg MH, et al. Vasculopathy in sickle cell disease: Biology, pathophysiology, genetics, translational medicine, and new research directions. *Am J Hematol* 2009;84:618-625. <https://doi.org/10.1002/ajh.21475> PMid:19610078 PMCID:PMC3209715
 65. Kato GJ, Piel FB, Reid CD, et al. Sickle cell disease. *Nat Rev Dis Primers* 2018;4:18010. <https://doi.org/10.1038/nrdp.2018.10> PMid:29542687
 66. Solovey A, Gui L, Ramakrishnan S, et al. Sickle cell anemia as a possible state of enhanced anti-apoptotic tone: survival effect of vascular endothelial growth factor on circulating and unanchored endothelial cells. *Blood* 1999;93:3824-3830. PMid:10339489
 67. Schaer DJ, Buehler PW, Alayash AI, et al. Hemolysis and free hemoglobin revisited: exploring hemoglobin and heme scavengers as a novel class of therapeutic proteins. *Blood* 2013;121:1276-1284. <https://doi.org/10.1182/blood-2012-11-451229> PMid:23264591 PMCID:PMC3578950
 68. Belcher JD, Chen C, Nguyen J, et al. Heme triggers TLR4 signaling leading to endothelial cell activation and vaso-occlusion in murine sickle cell disease. *Blood* 2014;123:377-390. <https://doi.org/10.1182/blood-2013-04-495887> PMid:24277079 PMCID:PMC3894494
 69. Reiter CD, Wang X, Tanus-Santos JE, et al. Cell-free hemoglobin limits nitric oxide bioavailability in sickle-cell disease. *Nat Med* 2002;8:1383-1389. <https://doi.org/10.1038/nm1202-799> PMid:12426562
 70. Belcher JD, Vineyard JV, Bruzzone CM, et al. Heme oxygenase-1 gene delivery by Sleeping Beauty inhibits vascular stasis in a murine model of sickle cell disease. *J Mol Med (Berl)* 2010;88:665-675. <https://doi.org/10.1007/s00109-010-0613-6> PMid:20306336 PMCID:PMC2877767
 71. Belcher JD, Beckman JD, Balla G, et al. Heme degradation and vascular injury. *Antioxid Redox Signal* 2010;12:233-248. <https://doi.org/10.1089/ars.2009.2822> PMid:19697995 PMCID:PMC2821146
 72. Muller-Eberhard U, Javid J, Liem HH, et al. Plasma concentrations of hemopexin, haptoglobin and heme in patients with various hemolytic diseases. *Blood* 1968;32:811-815. PMid:5687939
 73. Ballas SK, Marcolina MJ. Hyperhemolysis during the evolution of uncomplicated acute painful episodes in patients with sickle cell anemia. *Transfusion* 2006;46:105-110. <https://doi.org/10.1111/j.1537-2995.2006.00679.x> PMid:16398738
 74. Ohga S, Higashi E, Nomura A, et al. Haptoglobin therapy for acute favism: a Japanese boy with glucose-6-phosphate dehydrogenase Guadalajara. *Br J Haematol* 1995;89:421-423. <https://doi.org/10.1111/j.1365-2141.1995.tb03322.x> PMid:7873396
 75. Merle NS, Grunenwald A, Rajaratnam H, et al. Intravascular hemolysis activates complement via cell-free heme and heme-loaded microvesicles. *JCI Insight* 2018;3.
 76. de Franceschi L, Malpeli G, Scarpa A, et al. Protective effects of S-nitrosoalbumin on lung injury induced by hypoxia-reoxygenation in mouse model of sickle cell disease. *Am J Physiol Lung Cell Mol Physiol* 2006;291:L457-465. <https://doi.org/10.1152/ajplung.00462.2005> PMid:16603592
 77. Louie JE, Anderson CJ, Fayaz MFK, et al. Case series supporting heme detoxification via therapeutic plasma exchange in acute multiorgan failure syndrome resistant to red blood cell exchange in sickle cell disease. *Transfusion* 2018;58:470-479. <https://doi.org/10.1111/trf.14407> PMid:29193101
 78. Kato GJ, Gladwin MT. Evolution of novel small-molecule therapeutics targeting sickle cell vasculopathy. *JAMA* 2008;300:2638-2646. <https://doi.org/10.1001/jama.2008.598> PMid:19066384 PMCID:PMC2756016
 79. 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;122:2757-2764. <https://doi.org/10.1182/blood-2013-02-486282> PMid:23908468 PMCID:PMC4067504
 80. de Franceschi L, Baron A, Scarpa A, et al. Inhaled nitric oxide protects transgenic SAD mice from sickle cell disease-specific lung injury induced by hypoxia/reoxygenation. *Blood* 2003;102:1087-1096.

- <https://doi.org/10.1182/blood-2002-07-2135> PMID:12689931
81. De Franceschi L, Platt OS, Malpeli G, et al. Protective effects of phosphodiesterase-4 (PDE-4) inhibition in the early phase of pulmonary arterial hypertension in transgenic sickle cell mice. *FASEB J* 2008;22:1849-1860. <https://doi.org/10.1096/fj.07-098921> PMID:18245171
 82. Gladwin MT, Kato GJ, Weiner D, et al. Nitric oxide for inhalation in the acute treatment of sickle cell pain crisis: a randomized controlled trial. *JAMA* 2011;305:893-902. <https://doi.org/10.1001/jama.2011.235> PMID:21364138 PMCID:PMC3403835
 83. Kim-Shapiro DB, Gladwin MT. Nitric oxide pathology and therapeutics in sickle cell disease. *Clin Hemorheol Microcirc* 2018;68:223-237. <https://doi.org/10.3233/CH-189009> PMID:29614634
 84. Bakshi N, Morris CR. The role of the arginine metabolome in pain: implications for sickle cell disease. *Journal of pain research* 2016;9:167-175. PMID:27099528 PMCID:PMC4821376
 85. Morris CR. Alterations of the arginine metabolome in sickle cell disease: a growing rationale for arginine therapy. *Hematology/Oncology Clinics of North America* 2014;28:301-321. <https://doi.org/10.1016/j.hoc.2013.11.008> PMID:24589268
 86. Weiner DL, Hibberd PL, Betit P, et al. Preliminary assessment of inhaled nitric oxide for acute vaso-occlusive crisis in pediatric patients with sickle cell disease. *JAMA* 2003;289:1136-1142. <https://doi.org/10.1001/jama.289.9.1136> PMID:12622584
 87. Head CA, Swerdlow P, McDade WA, et al. Beneficial effects of nitric oxide breathing in adult patients with sickle cell crisis. *Am J Hematol* 2010;85:800-802. <https://doi.org/10.1002/ajh.21832> PMID:20799359
 88. Abid S, Kebe K, Houssaini A, et al. New Nitric Oxide Donor NCX 1443: Therapeutic Effects on Pulmonary Hypertension in the SAD Mouse Model of Sickle Cell Disease. *J Cardiovasc Pharmacol* 2018;71:283-292. <https://doi.org/10.1097/FJC.0000000000000570> PMID:29438213
 89. Morris CR, Kato GJ, Poljakovic M, et al. Dysregulated arginine metabolism, hemolysis-associated pulmonary hypertension, and mortality in sickle cell disease. *JAMA* 2005;294:81-90. <https://doi.org/10.1001/jama.294.1.81> PMID:15998894 PMCID:PMC2065861
 90. Tharoux PL, Hagege I, Placier S, et al. Urinary endothelin-1 as a marker of renal damage in sickle cell disease. *Nephrol Dial Transplant* 2005;20:2408-2413. <https://doi.org/10.1093/ndt/gfi111> PMID:16144850
 91. Hammerman SI, Kourembanas S, Conca TJ, et al. Endothelin-1 production during the acute chest syndrome in sickle cell disease. *American journal of respiratory and critical care medicine* 1997;156:280-285. <https://doi.org/10.1164/ajrccm.156.1.9611085> PMID:9230761
 92. Koehl B, Nivoit P, El Nemer W, et al. The endothelin B receptor plays a crucial role in the adhesion of neutrophils to the endothelium in sickle cell disease. *Haematologica* 2017;102:1161-1172. <https://doi.org/10.3324/haematol.2016.156869> PMID:28385784 PMCID:PMC5566019
 93. Taylor C, Kasztan M, Tao B, et al. Combined hydroxyurea and ETA receptor blockade reduces renal injury in the humanized sickle cell mouse. *Acta Physiol (Oxf)* 2018:e13178. <https://doi.org/10.1111/apha.13178> PMID:30144292
 94. Smith TP, Haymond T, Smith SN, et al. Evidence for the endothelin system as an emerging therapeutic target for the treatment of chronic pain. *Journal of Pain Research* 2014;7:531-545. <https://doi.org/10.2147/JPR.S65923> PMID:25210474 PMCID:PMC4155994
 95. Lutz BM, Wu S, Gu X, et al. Endothelin type A receptors mediate pain in a mouse model of sickle cell disease. *Haematologica* 2018;103:1124-1135. <https://doi.org/10.3324/haematol.2017.187013> PMID:29545351 PMCID:PMC6029538
 96. 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;90:2041-2046. PMID:9292541
 97. 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;286:2099-2106. <https://doi.org/10.1001/jama.286.17.2099> PMID:11694150
 98. Koo S, Yang Y, Neu B. Poloxamer 188 reduces normal and phosphatidylserine-exposing erythrocyte adhesion to endothelial cells in dextran solutions. *Colloids and surfaces B, Biointerfaces* 2013;112:446-451. <https://doi.org/10.1016/j.colsurfb.2013.07.035> PMID:24055859
 99. Archer N, Galacteros F, Brugnara C. 2015 Clinical trials update in sickle cell anemia. *Am J Hematol* 2015;90:934-950. <https://doi.org/10.1002/ajh.24116> PMID:26178236 PMCID:PMC5752136
 100. Kaul DK, Liu XD, Zhang X, et al. Peptides based on alphaV-binding domains of erythrocyte ICAM-4 inhibit sickle red cell-endothelial interactions and vaso-occlusion in the microcirculation. *Am J Physiol Cell Physiol* 2006;291:C922-930. <https://doi.org/10.1152/ajpcell.00639.2005> PMID:16738001
 101. Kaul DK, Liu XD, Zhang X, et al. Inhibition of sickle red cell adhesion and vasoocclusion in the microcirculation by antioxidants. *Am J Physiol Heart Circ Physiol* 2006;291:H167-175. <https://doi.org/10.1152/ajpheart.01096.2005> PMID:16443674
 102. Pan J, Xia L, McEver RP. Comparison of promoters for the murine and human P-selectin genes suggests species-specific and conserved mechanisms for transcriptional regulation in endothelial cells. *J Biol Chem* 1998;273:10058-10067. <https://doi.org/10.1074/jbc.273.16.10058> PMID:9545353
 103. Matsui NM, Borsig L, Rosen SD, et al. P-selectin mediates the adhesion of sickle erythrocytes to the endothelium. *Blood* 2001;98:1955-1962. <https://doi.org/10.1182/blood.V98.6.1955> PMID:11535535
 104. Kutlar A, Ataga KI, McMahon L, et al. A potent oral P-selectin blocking agent improves microcirculatory blood flow and a marker of endothelial cell injury in patients with sickle cell disease. *Am J Hematol* 2012;87:536-539. <https://doi.org/10.1002/ajh.23147> PMID:22488107
 105. Turhan A, Weiss LA, Mohandas N, et al. Primary role for adherent leukocytes in sickle cell vascular occlusion: a new paradigm. *Proc Natl Acad Sci U S A* 2002;99:3047-3051. <https://doi.org/10.1073/pnas.052522799> PMID:11880644 PMCID:PMC122470
 106. Blann AD, Mohan JS, Barefoot D, et al. Soluble P-selectin and vascular endothelial growth factor in steady state sickle cell disease: relationship to genotype. *J Thromb Thrombolysis* 2008;25:185-189. <https://doi.org/10.1007/s11239-007-0177-7> PMID:18080800
 107. Wun T, Styles L, DeCastro L, et al. Phase I study of the E-selectin inhibitor GMI 1070 in patients with sickle cell anemia. *PLoS One* 2014;9:e101301. <https://doi.org/10.1371/journal.pone.0101301> PMID:24988449 PMCID:PMC4079300
 108. Chang J, Patton JT, Sarkar A, et al. GMI-1070, a novel pan-selectin antagonist, reverses acute vascular occlusions in sickle cell mice. *Blood* 2010;116:1779-1786. <https://doi.org/10.1182/blood-2009-12-260513> PMID:20508165 PMCID:PMC2947397
 109. Telen MJ, Batchvarova M, Shan S, et al. Sevuparin binds to multiple adhesive ligands and reduces sickle red blood cell-induced vaso-occlusion. *Br J Haematol* 2016;175:935-948. <https://doi.org/10.1111/bjh.14303> PMID:27549988
 110. Gutsaeva DR, Parkerson JB, Yeri genahally SD, et al. Inhibition of cell adhesion by anti-P-selectin aptamer: a new potential therapeutic agent for sickle cell disease. *Blood* 2011;117:727-735. <https://doi.org/10.1182/blood-2010-05-285718> PMID:20926770 PMCID:PMC3031491
 111. Ataga KI, Kutlar A, Kanter J. Crizanlizumab in Sickle Cell Disease. *N Engl J Med* 2017;376:1796. <https://doi.org/10.1056/NEJMoa1611770> PMCID:PMC5481200
 112. Slomski A. Crizanlizumab Prevents Sickle Cell Pain Crises. *JAMA* 2017;317:798. <https://doi.org/10.1001/jama.2017.0355>
 113. 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;125:2656-2664. <https://doi.org/10.1182/blood-2014-06-583351> PMID:25733584 PMCID:PMC4408290
 114. White J, Lindgren M, Liu K, et al. Sevuparin blocks sickle blood cell adhesion and sickle-leucocyte rolling on immobilized L-selectin in a dose dependent manner. *Br J Haematol* 2018.
 115. Field JJ, Nathan DG. Advances in sickle cell therapies in the hydroxyurea era. *Mol Med* 2014;20 Suppl 1:S37-42.
 116. Field JJ, Ataga KI, Majerus E, Eaton CA, Mashal R, Nathan DG. A phase I single ascending dose study of NKT120 in stable adult sickle cell patients. In: *Blood*, editor. American Society of Hematology; 2014. p a977.
 117. Field JJ, Nathan DG, Linden J. The role of adenosine signaling in sickle cell therapeutics. *Hematology/Oncology Clinics of North America* 2014;28:287-299. <https://doi.org/10.1016/j.hoc.2013.11.003> PMID:24589267 PMCID:PMC3997263
 118. Field JJ, Majerus E, Gordeuk VR, et al. Randomized phase 2 trial of regadenoson for treatment of acute vaso-occlusive crises in sickle cell disease. *Blood Adv* 2017;1:1645-1649.

- <https://doi.org/10.1182/bloodadvances.2017009613>
PMid:29296811 PMCID:PMC5728341
119. Massaro M, Scoditti E, Carluccio MA, et al. Basic mechanisms behind the effects of n-3 fatty acids on cardiovascular disease. *Prostaglandins Leukot Essent Fatty Acids* 2008;79:109-115. <https://doi.org/10.1016/j.plefa.2008.09.009> PMid:18951002
 120. Calder PC. Omega-3 fatty acids and inflammatory processes. *Nutrients* 2010;2:355-374. <https://doi.org/10.3390/nu2030355> PMid:22254027 PMCID:PMC3257651
 121. Rangel-Huerta OD, Aguilera CM, Mesa MD, et al. Omega-3 long-chain polyunsaturated fatty acids supplementation on inflammatory biomarkers: a systematic review of randomised clinical trials. *Br J Nutr* 2012;107 Suppl 2:S159-170.
 122. Russo C, Olivieri O, Girelli D, et al. Omega-3 polyunsaturated fatty acid supplements and ambulatory blood pressure monitoring parameters in patients with mild essential hypertension. *J Hypertens* 1995;13:1823-1826. <https://doi.org/10.1097/00004872-199512010-00059> PMid:8903660
 123. Ren H, Obike I, Okpala I, et al. Steady-state haemoglobin level in sickle cell anaemia increases with an increase in erythrocyte membrane n-3 fatty acids. *Prostaglandins Leukot Essent Fatty Acids* 2005;72:415-421. <https://doi.org/10.1016/j.plefa.2005.03.005> PMid:15876528
 124. Ren H, Ghebremeskel K, Okpala I, et al. Abnormality of erythrocyte membrane n-3 long chain polyunsaturated fatty acids in sickle cell haemoglobin C (HbSC) disease is not as remarkable as in sickle cell anaemia (HbSS). *Prostaglandins Leukot Essent Fatty Acids* 2006;74:1-6. <https://doi.org/10.1016/j.plefa.2005.10.002> PMid:16314081
 125. Daak AA, Ghebremeskel K, Hassan Z, et al. Effect of omega-3 (n-3) fatty acid supplementation in patients with sickle cell anemia: randomized, double-blind, placebo-controlled trial. *Am J Clin Nutr* 2013;97:37-44. <https://doi.org/10.3945/ajcn.112.036319> PMid:23193009
 126. Daak AA, Dampier CD, Fuh B, et al. Double-blind, randomized, multicenter phase 2 study of SC411 in children with sickle cell disease (SCOT trial). *Blood Adv* 2018;2:1969-1979. <https://doi.org/10.1182/bloodadvances.2018021444> PMid:30097463 PMCID:PMC6093734
 127. Daak A, Rabinowicz A, Ghebremeskel K. Omega-3 fatty acids are a potential therapy for patients with sickle cell disease. *Nat Rev Dis Primers* 2018;4:15. <https://doi.org/10.1038/s41572-018-0012-9> PMid:30093627
 128. Tomer A, Kasey S, Connor WE, et al. Reduction of pain episodes and prothrombotic activity in sickle cell disease by dietary n-3 fatty acids. *Thromb Haemost* 2001;85:966-974. <https://doi.org/10.1055/s-0037-1615948> PMid:11434703
 129. Cabannes R, Lonsdorfer J, Castaigne JP, et al. Clinical and biological double-blind-study of ticlopidine in preventive treatment of sickle-cell disease crises. *Agents and actions Supplements* 1984;15:199-212. PMid:6385647
 130. Heeney MM, Hoppe CC, Abboud MR, et al. A Multinational Trial of Prasugrel for Sickle Cell Vaso-Occlusive Events. *N Engl J Med* 2016;374:625-635. <https://doi.org/10.1056/NEJMoa1512021> PMid:26644172
 131. Hsu LL, Sarnaik S, Williams S, et al. A dose-ranging study of ticagrelor in children aged 3-17 years with sickle cell disease: a two-part phase 2 study. *Am J Hematol* 2018. <https://doi.org/10.1002/ajh.25273>
 132. Reid M, Badaloo A, Forrester T, et al. In vivo rates of erythrocyte glutathione synthesis in adults with sickle cell disease. *American journal of physiology Endocrinology and metabolism* 2006;291:E73-79. <https://doi.org/10.1152/ajpendo.00287.2005> PMid:16434557
 133. Silva DG, Belini Junior E, de Almeida EA, et al. Oxidative stress in sickle cell disease: an overview of erythrocyte redox metabolism and current antioxidant therapeutic strategies. *Free Radic Biol Med* 2013;65:1101-1109. <https://doi.org/10.1016/j.freeradbiomed.2013.08.181> PMid:24002011
 134. Joep W.R. Sins XF, Karin Fijnvandraat, Melissa Dominguez, A. W. Rijnveld, Jean-Louis Kerkhoffs, A van Meurs, M. R. De Groot, H Heijboer, Erfan Nur, Brenda M Luken, Sacha S Zeerleder, Marie-Françoise Dresse, Phu-Quoc Le, Philippe Hermans, Anna Vanderfaeillie, Eric Van Den Neste, Fleur Samantha Benghiat, Rachel Kesse-Adu, Andre Delannoy, Andre Efira, Marie-Agnes Azerad, C A de Borgie, Junmei Chen, Jose A. Lopez and Bart J. Biemond. Effects of Oral N -Acetylcysteine on Oxidative Stress in Patients with Sickle Cell Disease. In: *Blood*, editor. ASH. Atlanta; 2017.
 135. Sins JWR, Fijnvandraat K, Rijnveld AW, et al. Effect of N-acetylcysteine on pain in daily life in patients with sickle cell disease: a randomised clinical trial. *Br J Haematol* 2017. PMid:28643376
 136. Joep W.R. Sins KF, Anita W. Rijnveld, Martine B. Boom, Jean-Louis Kerkhoffs, Alfred H. van Meurs, Marco R De Groot, Harriet Heijboer, Marie-Françoise Dresse, Alina Ferster, Philippe Hermans, Anna Vanderfaeillie, Eric W Van Den Neste, Fleur Samantha Benghiat, Jo Howard, Rachel Kesse-Adu, Andre Delannoy, Andre Efira, Marie-Agnes Azerad, Corianne A.J.M. de Borgie and Bart J. Biemond. N-Acetylcysteine in Patients with Sickle Cell Disease: A Randomized Controlled Trial. In: *Blood*, editor. ASH: Blood; 2016.
 137. Niihara Y, Miller ST, Kanter J, et al. A Phase 3 Trial of L-Glutamine in Sickle Cell Disease. *N Engl J Med* 2018;379:226-235. <https://doi.org/10.1056/NEJMoa1715971> PMid:30021096
 138. Niihara Y KH, Tran L, Razon R, Macan H, Stark C, Wun T, Adams-Graves P. A phase 3 study of L-Glutamine Therapy for sickle cell anemia and sickle b0-thalassemia. In: *Blood*, editor. American Society of Hematology: Blood; 2014. p a86.
 139. Niihara Y, Zerez CR, Akiyama DS, et al. Oral L-glutamine therapy for sickle cell anemia: I. Subjective clinical improvement and favorable change in red cell NAD redox potential. *Am J Hematol* 1998;58:117-121. [https://doi.org/10.1002/\(SICI\)1096-8652\(199806\)58:2<117::AID-AJH5>3.0.CO;2-V](https://doi.org/10.1002/(SICI)1096-8652(199806)58:2<117::AID-AJH5>3.0.CO;2-V)
 140. Quinn CT. L-Glutamine for sickle cell anemia: more questions than answers. *Blood* 2018;132:689-693. <https://doi.org/10.1182/blood-2018-03-834440>
 141. Lagresle-Peyrou C, Lefrere F, Magrin E, et al. Plerixafor enables safe, rapid, efficient mobilization of hematopoietic stem cells in sickle cell disease patients after exchange transfusion. *Haematologica* 2018;103:778-786. <https://doi.org/10.3324/haematol.2017.184788> PMid:29472357 PMCID:PMC5927997
 142. Esrick EB, Bauer DE. Genetic therapies for sickle cell disease. *Semin Hematol* 2018;55:76-86. <https://doi.org/10.1053/j.seminhematol.2018.04.014> PMid:29958563
 143. Leonard A, Tisdale JF. Stem cell transplantation in sickle cell disease: therapeutic potential and challenges faced. *Expert Rev Hematol* 2018;11:547-565. <https://doi.org/10.1080/17474086.2018.1486703> PMid:29883237
 144. Angelucci E, Matthes-Martin S, Baronciani D, et al. Hematopoietic stem cell transplantation in thalassemia major and sickle cell disease: indications and management recommendations from an international expert panel. *Haematologica* 2014;99:811-820. <https://doi.org/10.3324/haematol.2013.099747> PMid:24790059 PMCID:PMC4008115
 145. Saraf SL, Oh AL, Patel PR, et al. Haploidentical Peripheral Blood Stem Cell Transplantation Demonstrates Stable Engraftment in Adults with Sickle Cell Disease. *Biol Blood Marrow Transplant* 2018;24:1759-1765. <https://doi.org/10.1016/j.bbmt.2018.03.031> PMid:29656137
 146. Ribeil JA, Hacein-Bey-Abina S, Payen E, et al. Gene Therapy in a Patient with Sickle Cell Disease. *N Engl J Med* 2017;376:848-855. <https://doi.org/10.1056/NEJMoa1609677> PMid:28249145
 147. Antoniani C, Meneghini V, Lattanzi A, et al. Induction of fetal hemoglobin synthesis by CRISPR/Cas9-mediated editing of the human beta-globin locus. *Blood* 2018;131:1960-1973. <https://doi.org/10.1182/blood-2017-10-811505> PMid:29519807
 148. Sato M, Saitoh I, Inada E. Efficient CRISPR/Cas9-based gene correction in induced pluripotent stem cells established from fibroblasts of patients with sickle cell disease. *Stem Cell Investig* 2016;3:78. <https://doi.org/10.21037/sci.2016.11.05> PMid:28066780 PMCID:PMC5182212
 149. Ye L, Wang J, Tan Y, et al. Genome editing using CRISPR-Cas9 to create the HPFH genotype in HSPCs: An approach for treating sickle cell disease and beta-thalassemia. *Proc Natl Acad Sci U S A* 2016;113:10661-10665. <https://doi.org/10.1073/pnas.1612075113> PMid:27601644 PMCID:PMC5035856