

**Review Article****New Therapeutic Options for the Treatment of Sickle Cell Disease**Alessandro Matte¹, Francesco Zorzi¹, Filippo Mazzi¹, Enrica Federti¹, Oliviero Olivieri¹ and Lucia De Franceschi¹.¹ Department of Medicine, University of Verona and AOUI Verona, Verona. Italy**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.

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Correspondence to: Lucia De Franceschi. Department of Medicine, University of Verona and AOUI Verona, Verona; Italy. Fax: +39 045 8027473; phone: +39045 8124401. E-mail: lucia.defranceschi@univr.it

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

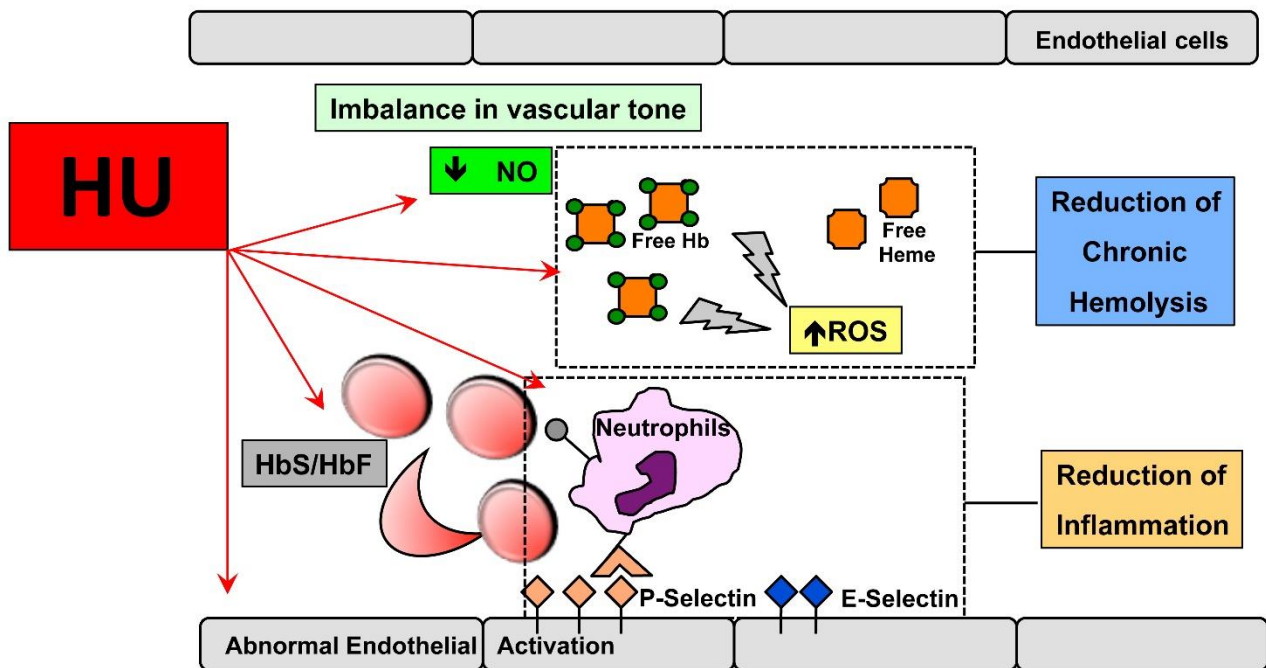


Figure 2. Schematic diagram of multimodal therapeutic action of hydroxyurea (HU) in sickle cell disease. ROS: reactive oxygen species; Hb: hemoglobin; NO: nitric oxide; HbS: sickle hemoglobin; HbF: fetal hemoglobin.

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.</i> <i>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

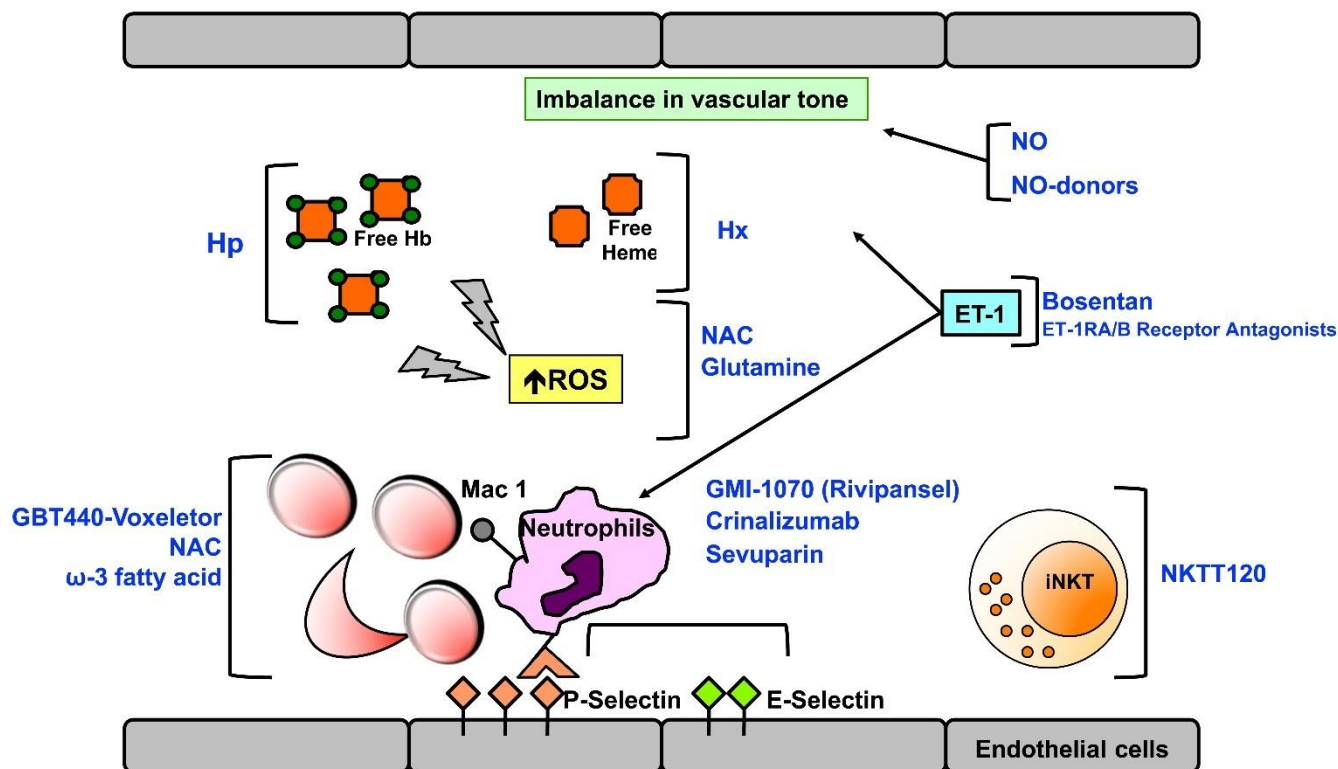


Figure 3. Schematic diagram of the mechanisms of action of pathophysiology based new therapeutic options for treatment of sickle cell disease and sickle cell vasculopathy. Hp: haptoglobin; Hx: hemopexin; NAC: N-Acetyl-cysteine; Ab: antibody; ROS: reactive oxygen species; iNKT: invariant natural killer 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.

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