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NOVEL THERAPEUTIC APPROACHES IN THALASSEMIAS, SICKLE CELL DISEASE AND OTHER RED CELL DISORDERS

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Abstract:

In this last decade, a deeper understanding of the pathophysiology of hereditary red cell disorders and the development of novel classes of pharmacologic agents have provided novel therapeutic approaches to thalassemias, sickle cell disease (SCD) and other red cell disorders. Here, we analyze and discuss the novel therapeutic options according to their targets and taking into consideration the complex process of erythroid differentiation, maturation, and survival of erythrocytes in the peripheral circulation. We focus on active clinical exploratory and confirmatory trials on thalassemias, SCD and other red cell disorders. Beside α -thalassemia and SCD, we found that the development of new therapeutic strategies has allowed the design of clinic studies also for hereditary red cell disorders still lacking valuable therapeutic alternative such as β -thalassemias, congenital dyserythropoietic anemia or Blackfan Diamond anemia. In addition, reduction of heme synthesis, which can be achieved by the repurposed anti-psychotic drug Bitopertin, might affect not only hematological disorders but multiorgan diseases such as erythropoietic protoporphyria. Finally, our review highlights the current state of therapeutic scenarios, in which multiple indications targeting different red cell disorders are being considered for a single agent. This is welcome change which will hopefully expand therapeutic option for patients affected by thalassemias, SCD and other red cell disorders.

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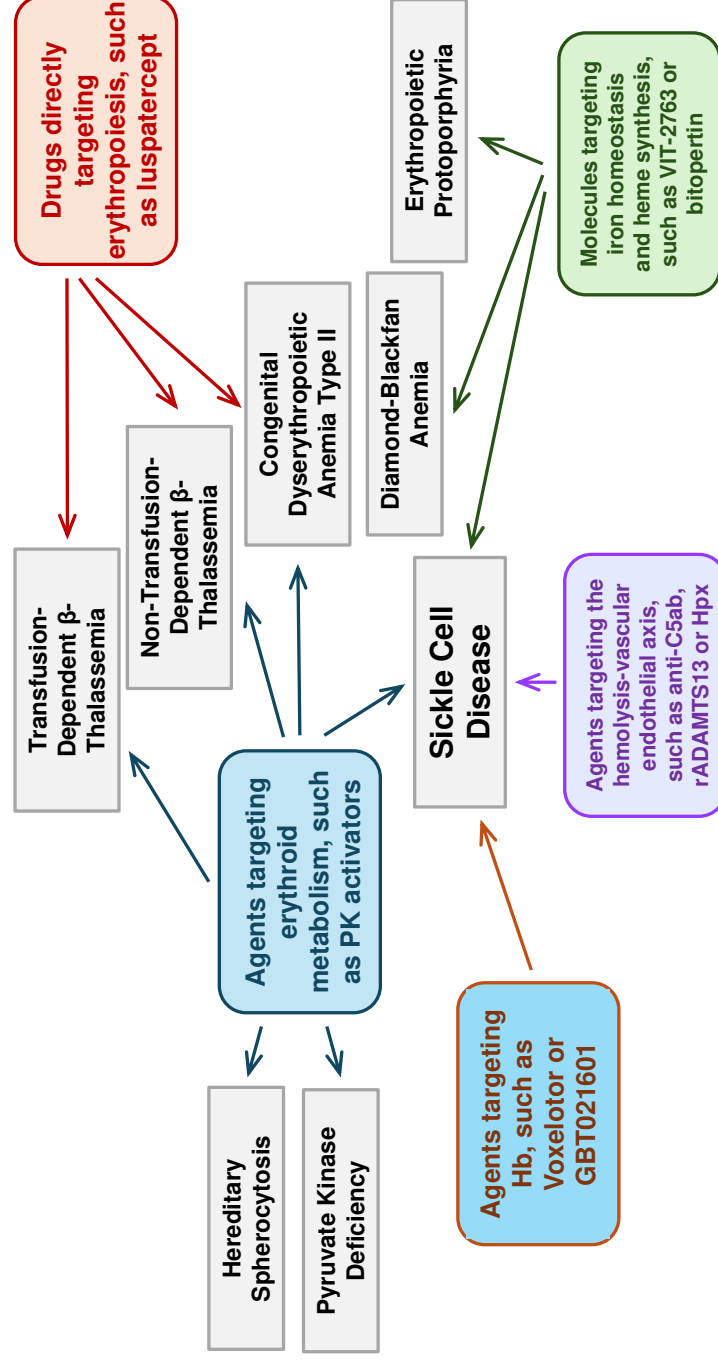
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Novel Therapeutic Approaches for Thalassemia, Sickle Cell Disease, and Other Inherited Red Cell Disorders



Conclusions: Identifying new druggable targets has enabled the development of novel pharmacologic agents for treating inherited red cell disorders. A few of these agents have already been approved for clinical use.

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NOVEL THERAPEUTIC APPROACHES IN THALASSEMIAS, SICKLE CELL DISEASE AND OTHER RED CELL DISORDERS

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ABSTRACT

In this last decade, a deeper understanding of the pathophysiology of hereditary red cell disorders and the development of novel classes of pharmacologic agents have provided novel therapeutic approaches to thalassemias, sickle cell disease (SCD) and other red cell disorders. Here, we analyze and discuss the novel therapeutic options according to their targets and taking into consideration the complex process of erythroid differentiation, maturation, and survival of erythrocytes in the peripheral circulation. We focus on active clinical exploratory and confirmatory trials on thalassemias, SCD and other red cell disorders. Beside β -thalassemia and SCD, we found that the development of new therapeutic strategies has allowed the design of clinic studies also for hereditary red cell disorders still lacking valuable therapeutic alternative such as α -thalassemias, congenital dyserythropoietic anemia or Blackfan Diamond anemia. In addition, reduction of heme synthesis, which can be achieved by the repurposed anti-psychotic drug Bitopertin, might affect not only hematological disorders but multiorgan diseases such as erythropoietic protoporphyria. Finally, our review highlights the current state of therapeutic scenarios, in which multiple indications targeting different red cell disorders are being considered for a single agent. This is welcome change which will hopefully expand therapeutic option for patients affected by thalassemias, SCD and other red cell disorders.

INTRODUCTION

The global burden of disease for thalassemias, sickle cell disease and red cell disorders has emerged as a significant public health problem due to the negative impact on quality of life and survival.¹⁻³ A better understanding of the pathophysiology of these disorders has allowed the identification of new druggable targets.⁴⁻⁶ In the present review, we analyzed active clinical exploratory and confirmatory trials on thalassemias, SCD and other red cell disorders. We exclude (i) paroxysmal nocturnal hemoglobinuria (PNH) and autoimmune hemolytic anemias (AIHAs);^{7,8} (ii) therapeutic strategies for ineffective erythropoiesis in low-grade myelodysplastic syndrome;⁹ and (iii) fetal hemoglobin inducers since they were recently reviewed in SCD,¹⁰ and a new review on reactivation of fetal hemoglobin (HbF) synthesis will be part of the present series of reviews. The novel therapeutic tools are discussed according to their targets and taking into consideration the complex process of erythroid differentiation, maturation, and survival of erythrocytes in the peripheral circulation.

Drug directly targeting erythropoiesis: luspatercept

Erythropoiesis is a dynamic and complex process going from committed erythroid progenitors throughout erythroblasts maturation to reticulocyte and mature red cells. After the approvals of multiple erythropoietic stimulating agents, Luspatercept, a ligand trap with high affinity for activin B, is the newest addition targeting both normal and pathologic erythropoiesis. The serendipitous observation of increased Hb in healthy women treated with Luspatercept for post-menopausal bone disease opened the way to its consideration for red cell disorders. Studies in mouse models for β -thalassemia have shown that luspatercept action goes behind activin B, possibly sequestering multiple activin-receptor ligands such as Growth Differentiation Factor 11 (GDF11) or Bone Morphogenetic Proteins (BMPs).^{4,11-13} Thus, Luspatercept has been proposed to indirectly affect (TGF- β)-Smads canonical pathways, possibly rebalancing the intracellular cell signaling and favoring erythroid maturation in late phase of erythropoiesis.^{4,14,15} Luspatercept was evaluated in transfusion dependent (TD) (BELIEVE, NCT02604433) and non-transfusion

dependent (NTD) (BEYOND, NCT03342404) β -thalassemic patients. Luspatercept significantly improved transfusion burden and anemia in TDT and NTDT, respectively with good safety and tolerability profile (BELIEVE and BEYOND studies respectively, Table 1S).¹⁵⁻¹⁹ Although the BEYOND in NTDT study did not achieve the secondary endpoint of patient reported outcomes, the improvement of symptomatic anemia and the lack of valuable therapeutic alternative supported the approval of luspatercept for treatment of NTDT patients by European Medicines Agency (EMA). This is different from Food and Drug Administration (FDA) position, which highlights the absence of agreement on benefit and risk of treatment of this heterogeneous patient population.^{4,16}

More recently, Wobus et al. reported modulation of mesenchymal cell function, suggesting a possible action of luspatercept also in the early stage of erythropoiesis.²⁰ These data have allowed the design of clinical studies to evaluate the effect of luspatercept in the following patients population: (i) pediatric β -thal/HbE (NCT04143724); (ii) α -thalassemia HbH (NTDT e TDT, NCT05664737); and (iii) CD41 (EudraCT Number: 2020-005736-30) (Table 1, Table 2S).

Molecules targeting iron-homeostasis or heme synthesis

In erythropoiesis, iron is crucial for hemoglobinization, but cytotoxic as free iron. In red cell disorders such as thalassemias or SCD, free iron negatively impact erythropoiesis, red cell features and survival in the peripheral circulation (Figure 1, 2).²¹⁻²⁴ Thus, modulation of iron homeostasis or heme biosynthesis might represent an interesting approach to improve anemia in hereditary red cell disorders.

In the last two decades, a much-expanded knowledge of iron homeostasis has resulted in the identifications of multiple functional targets.²⁵⁻²⁷ Heparin (Hamp) and ferroportin (FPN) play an essential role in regulating iron absorption and iron organ accumulation. In the presence of ineffective erythropoiesis, erythroblasts release excessive amounts of erythroferrone (Erfe), which acts as Hamp suppressor. Reduced Hamp affects the duodenal FPN externalization, establishing an iron over-absorption vicious cycle that sustains organ iron accumulation (Figure 2).²⁸ Studies in animal models of β -thalassemia show amelioration of anemia and ineffective erythropoiesis as well as improvement of organ iron overload when treated with (i) Hamp-mimetic; (ii) Hamp-agonist such as Vamifeport (VIT-2793), an oral FPN

inhibitor, or the IONIX-TMPRSS6 ligand conjugated antisense agent; and (iii) apotransferrin (Figure 2).^{29-31 32} However, clinical studies on iron restriction in TDT or NTDT subjects were either unsuccessful or terminated early (Table 3S).^{33,34} Bitopertin, a glycine transport inhibitor that reduces heme synthesis (Figure 2) had a similar fate. Bitopertin was expected to re-balance heme and globin synthesis and to reduce erythroid oxidation.^{35,36} Although Bitopertin improved ineffective erythropoiesis and red cell survival in β -thalassemic mice, the clinical study in patients with NTDT (NCT03271541) was discontinued due to the lack of efficacy as determined by *an interim* analysis (Table 3S).³⁴ Diamond- Blackfan anemia (DBA) and erythropoietic protoporphyria (EPP) seem to offer new possibilities for bitopertin. In the first case, the rationale is represented by accumulation of free heme that overcomes the expression of cyto-protective systems in cell models and *ex vivo* in CD34⁺ derived cells from patients with DBA.³⁷ A phase 1-2 intra-patient dose-escalation study of bitopertin for steroid-refractory DBA (NCT 05828108) has been recently activated (Table 1, Table 2S). In EPP, *in vitro* evidence of improvement of EPP cell metabolism and reduced protoporphyrin IX (PPIX) synthesis in presence of bitopertin.³⁸ This generated the rationale to design the open-label clinical trial BEACON (ACTRN12622000799752) and the phase-2 AURORA double blind placebo controlled trial (NCT05308472) in adult subjects with EPP and X-linked porphyria (Table 1, Table 2S). Encouraging *ad interim* data comes from both studies, showing dose-dependent, and sustained reductions of plasma PPIX levels, amelioration of sunlight tolerance, stable Hb and grade 1-2 adverse events (e.g. limited dizziness, lightheadedness, headache, and nausea). In AURORA study, a significant improvement in the patient global impression of change (PGIC) was also reported in EPP patient treated with bitopertin 60 mg once daily compared to placebo group. Considering the heterogeneous group of porphyrias, givosiran, a small interfering (siRNA) reducing the activity of liver ALA-synthase-1 (ALAS-1) has been recently approved by FDA and EMA for the treatment of acute hepatic porphyrias (AHP).^{39,40}

Altogether, the failures of molecules targeting iron homeostasis or heme synthesis in β -thalassemia when translated from pre-clinical to clinical studies can be explained by functional differences in iron homeostasis between human and murine β -thalassemic erythropoiesis. Multiple factors in addition to free iron determine the

severity of oxidation in β -thalassemic cells, which ultimately overwhelms adaptive mechanisms such as autophagy and drives β -thalassemic cells towards energy depletion and apoptosis.^{36,41,42} These agents could be successful in disorders of erythropoiesis with preserved maturation profile such as polycythemia vera (PV).⁴³⁻⁴⁶

Finally, the modulation of iron homeostasis as therapeutic option has been also explored in patients with SCD.⁴⁷⁻⁵¹ In SCD, iron restriction decreases HbS synthesis, resulting in reduction of the cellular HbS concentration, which in turn modulates the kinetic of HbS polymerization with a secondary beneficial impact on red cell oxidative stress (Figure 1).⁴⁷⁻⁵⁰ In observational studies in patients with SCD, iron-restriction obtained by either phlebotomy or erythropheresis resulted in a marked reduction of pain crisis rate and an improvement of patient's quality of life.⁴⁷⁻⁵⁰ Recently, humanized mouse model for SCD exposed to iron restriction by either diet or Vamifeport (VIT-2793, oral FPN inhibitor) showed an improvement of sickle red cell features, a reduction of sickling, with a beneficial effect on inflammatory vasculopathy.^{52,53} These data further support the rationale for ViSionSerenity (NCT04817670), a phase 2a, double-blind, randomized, placebo-controlled study to evaluate efficacy and safety of multiple doses of Vamifeport in subjects with SCD (Table 1, Table 2S).

Agents targeting erythroid metabolism

The long lifespan of erythrocytes into the peripheral circulation requires efficient metabolic and antioxidant machineries. In red cells, the metabolic keystone is adenosine triphosphate (ATP), which is produced by the glycolytic degradative process, involving pyruvate kinase (PK) in the final step. 2-3 diphosphoglycerate (2,3-DPG) is an intermediate metabolite of the glycolytic pathway (Figure 3). The 2,3-DPG /ATP ratio is critical to ensure normal functioning and survival of red cells into the peripheral circulation.^{6,54} In addition, the existing cross-connection between glycolysis and the pentose phosphate shunt crucially contributes the production of nicotinamide adenine dinucleotide phosphate (NADPH) and nicotinamide adenine dinucleotide (NADH) (Figure 2, 3). Of note a substantial portion of key anti-oxidant systems such as glutathione (GSH), are NADH or NADPH dependent.^{6,55-57} In SCD or β -thalassemia, despite enhanced glycolysis in response to increased NADH demand, anti-oxidant systems may not be able to cope with severe and persistent

oxidative stress.^{6,55-57} This might also worsen the red cell membrane mechanical instability, contributing to the decrease lifespan of pathologic erythrocytes. The situation becomes more complex in hereditary spherocytosis (HS), which is characterized by reduced red cell deformability due to defects in membrane protein(s) and a relative PK deficiency.⁵⁸⁻⁶¹

The development of PK activators has changed the scenario of the treatment of patients with PK deficiency, but also of other red cell disorders characterized by either severe oxidation as in thalassemias and SCD (Figure 1) or relative PK deficiency as in HS.^{54,62,63} Three oral drugs targeting PK function have been recently evaluated (i) mitapivat and AG-946, which bind the PK enzyme pocket at the dimer-dimer interface, resulting in PK activation of both PK-R and PK-M2; and (ii) etavopivat, a selective oral PK-R activator.⁵⁴ Mitapivat has been approved by FDA and the EMA for treatment of hemolytic anemia in adult patients with PK deficiency (Table 4S).^{64,65}

Pre-clinical studies in a mouse model for β -thalassemia have shown the beneficial effects of mitapivat on both ineffective erythropoiesis and chronic hemolytic anemia with an accompanying improvement of iron homeostasis.^{66,67} In a phase-2 proof of concept study (NCT03692052) the effects of mitapivat were evaluated in a cohort of patients with non-transfusion dependent thalassemias (NTDT), including both α - and β -thalassemic genotypes (Table 1S).^{68,69} Mitapivat improved anemia in 80% of NTDT patients within the first 6 weeks of treatment (sustained increase of Hb >1.0 g/dL) and reduced markers of ineffective erythropoiesis and hemolysis.⁶⁹ Of note, all α -thal patients responded to mitapivat treatment. The adverse events were grade 1-2 (e.g. insomnia, dizziness, or headache) similar to those reported in patients with PK deficiency treated with mitapivat.⁶⁹ Two phase-3 multicenter, randomized, double-blind, placebo-controlled trials to assess the efficacy and safety of mitapivat in adults with α - or β -NTDT (ENERGIZE, NCT04770753) or transfusion dependent thalassemias (TDT) (ENERGIZE-T, NCT04770779) are ongoing and have recently completed patient enrollment (Table 1, Table 2S).

The beneficial effects of mitapivat on β -thal ineffective erythropoiesis has led to the consideration of mitapivat in other hereditary red cell disorders associated with perturbation of erythropoiesis such as the congenital dyserythropoietic anemia type-

II (CDA-II).⁷⁰⁻⁷² An European/Canadian prospective, multicenter, single-arm phase 2 trial (SATISFY, NCT05935202) on patients with CDAII is expected to start enrollment in 2024 (Table 1, Table 2S). Of note, SATISFY also involves membranopathies such as HS, based on (i) abnormalities in metabolome of HS red cell;⁵⁸⁻⁶¹ and (ii) the improvement of anemia in a mouse model for mild HS.⁷³

In SCD, the abnormal the increased 2,3-DPG and the reduced ATP are powerful determinant of Hb S deoxygenation/sickling, with a negative impact also on responsiveness to oxidant damage related to reduced energy availability.^{74,75} Both mitapivat and etavopivat have then been tested in mouse models for SCD, displaying an improvement of 2,3-DPG/ATP ratio, increased Hb oxygen affinity and reduced sickling. This was associated with decreased markers of chronic hemolysis and amelioration of some red cell features (e.g.: markers of red cell oxidation, mitochondria red cell retention, extramedullary erythropoiesis).^{76,77}

In a proof-of-concept study (NCT04000165, Table 1S), mitapivat improved anemia in patients with SCD, reducing markers of hemolysis, increasing ATP/2,3-DPG ratio and the activation of the Lands cycle involved in membrane lipid remodeling.^{78,79} A phase-2 open-label study with mitapivat in patients with SCD (ESTIMATE study; www.trial-register.nl; NL8517, Table 1S) showed a significant increased Hb (>1 g/dl in 75% of SCD patients) and a decrease of markers of hemolysis associated with the encouraging observation of reduced annualized VOCs compared to patients' historical baseline. Mitapivat was well tolerated, adverse events were mainly grade 1-2 (e.g. headache, increase AST, ALT, dyspepsia).⁸⁰ As shown in Table 1, there are two ongoing clinical studies with mitapivat in patients with SCD. The first is the extension study (NCT04610866) of a previous phase 1 study (NCT04000165) (Table 1S).⁷⁸ Data for up to 2 years show that mitapivat is safe and well tolerated in patients with SCD with evidence of sustained long-term improvements in Hb, hemolytic and sickling kinetics.⁸¹ The second is a 2/3 phase double-blind, randomized, placebo controlled trial (RISE UP, NCT05031780) evaluating the efficacy and safety of mitapivat in patients with SCD. Results from the phase 2 double-blind period show that treatment with mitapivat produced statistically significant and clinically meaningful improvement in Hb response at both dose levels (50 mg BID and 100 mg BID) compared to placebo, with a safety profile consistent with previous studies (Table 1, Table 2S).⁸² Indeed, the primary endpoint was achieved in 46.2% of patients at 50 mg BID and in 50% of patients at 100 mg BID.⁸²

Of note a reduction in the annualized rate of sickle cell pain was observed in SCD patients from both mitapivat treated groups compared to placebo (0.83 and 0.51 respectively in mitapivat 50 BID and 100 BID groups vs 1.71 in placebo group). Although the study was not powered for this endpoint, these results are extremely promising for clinical management of SCD patients and needs to be confirmed in the phase-3 study.

A next generation PK activator with improved metabolic profile (AG-946) compared to mitapivat has been developed. A phase 1 clinical trial with AG-946 in healthy volunteers and in patients with SCD independently from the genotype has been recently completed (NCT04536792) (Table 1).⁸³⁻⁸⁵

Etavopivat has been first tested in a phase 1 clinical study (NCT03815695, Table 1S) in healthy individuals and volunteers with SCD. Increased ATP and decreased 2-3 DPG contents were observed in red cells from healthy controls.⁸⁶ In SCD patients, this was associated with a sustained increase in Hb in 73% of SCD subjects treated with etavopivat (400 mg QD) and a trend towards reduction of VOCs requiring hospitalization.⁸⁷ Two clinical studies on etavopivat in SCD patients (GLADIOLUS, NCT04987489, and HIBISCUS, NCT04624659, phase 2 and 2/3 respectively) are open to enrollment (Table 1, Table 2S). Two phase 2 pediatric studies (NCT05953584 and NCT05725902, Table 1, Table 2S) will evaluate respectively the activity of etavopivat on transcranial doppler velocities in children with SCD, who are at increased risk for primary stroke and the effect of etavopivat on cerebral hemodynamic response in children with SCD.

In SCD, another therapeutic approach to sustain the antioxidant machinery is represented by oral supplementation with L-glutamine. In red cells, the L-glutamine conversion into glutamate supports the GSH antioxidant system and the production of NAD⁺ by NAD synthase (Figure 3). L-Glutamine supplementation has been shown to beneficially impact sickle cell related vaso-occlusive crisis (VOC) pain rate, resulting in FDA approval for patients with SCD.⁸⁸ However, the lack of pharmacodynamic/kinetic data and the uncertainties about mechanisms of action require additional studies to better understand the indications for L-glutamine in SCD.^{89,90} EMA did not share the positive view of FDA and rejected the application for L-Glutamine in SCD, expressing concerns on the methodology used in the clinical study

Molecules targeting hemoglobin are still on track in SCD

Voxelotor is the only oral approved anti-sickling therapeutic molecule that covalently binds to HbS, weakening HbS fiber contacts. In the HOPE study, Voxelotor ameliorated chronic hemolysis in the absence of a significant impact on the rate of VOCs in patients with SCD. FDA approved Voxelotor for adults and children ≥ 4 years of age with SCD, while EMA approved it for SCD patients ≥ 12 years of age.⁹¹ The open label extension of the HOPE study (HOPE-OLE) and the PROSPECT registry will help to determine whether Voxelotor might prevent sickle cell related organ complications.^{92,93} A phase 2-3 study on second generation Voxelotor-like anti-sickling agent GBT021601 in patients with SCD is now enrolling (Table 5S). The *ad interim* data support the tolerability and the safety of GBT021601 in patients with SCD. This was associated with an improvement of anemia (2.7 g/dL Hb increase in 100 mg/day group and 3.17 g/dL in the 150 mg/day group), a reduction in adherent cells in flow adhesion assay in presence of VCAM-1, without major change in the pain rate.⁹⁴

Agents targeting the hemolysis-vascular endothelial axis

Chronic hemolysis is a distinguishing feature of thalassemias, SCD and hereditary red cells disorders such as HS or PK deficiency. Although erythrophagocytosis plays a key role in early removal of pathologic red cells, a smaller component of hemolysis takes place intra-vascularly. This might be further increased by splenectomy.⁹⁵⁻⁹⁸ Consumption of hemopexin (Hpx), the physiologic buffer of free heme, increases plasma free heme. In SCD, the detrimental effect of free heme results in (i) local NO deficiency and plasma pro-oxidant environment; (ii) activation of complement system; (iii) inhibition of ADAMST13 activity, with relative ADAMST13 deficiency and accumulation of large multimers of von Willebrand factor (vWF) (Figure 4). All together these changes contribute to chronic inflammatory vasculopathy, resulting in up-regulation of pro-inflammatory cytokines and markers of vascular endothelial activation such as vascular cell adhesion molecule-1 (VCAM-1) or Selectin (Figure 4).⁹⁹⁻¹⁰²

Hpx has been explored as candidate therapeutic option for hemolytic anemias in pre-clinical studies in mouse models for SCD.¹⁰³⁻¹⁰⁶ A phase-1 clinical study (NCT04285827) has been designed to evaluate the safety and tolerability of single dose of recombinant Hpx (CSL889) in patients with SCD without or with active

VOCs. *Ad interim* results indicate no serious adverse events (AE) related to CSL889 (Table 1, Table 2S).¹⁰⁷

An attempt to target the free heme related reduced NO bioavailability and to improve the “arginine deficiency syndrome” during acute events in SCD, is represented by the acute administration of L-Arginine, a precursors of NO synthesis.^{108,109} Despite many published studies, there is still uncertainty on the role of arginine therapy in SCD. Two new ongoing studies on L-Arginine supplementation may provide some needed clarity (Table 1, Table 2S). STArT (NCT04839354) is a double-blind, placebo-controlled, randomized, phase 3, multicenter trial on the efficacy of intravenous (IV) L-arginine administration during VOCs in children, adolescents, and young adults with SCD. R34 pK/PD (NCT02447874), a phase 1/2 study, evaluates pharmacokinetics of L-arginine infusion during acute VOCs in children, adolescents, and young adults with SCD as well as change in NO metabolites.

Growing evidence indicates that overactivation of complement plays a role in sickle cell related acute and chronic clinical manifestations. Indeed, free heme, dense red cells, erythroid microparticles, and heme induced inhibitory effect on Factor I (FI), a complement regulatory protein,¹¹⁰⁻¹¹³ synergize towards complement activation in patients with SCD.^{110,111,114} In pre-clinical studies, anti-C5-antibody or a 14E1-mouse specific properdin inhibitor were shown to protect against acute sickle cell related clinical manifestations, generating the rationale for designing clinical trials with complement inhibitors in patients with SCD.¹¹¹ Up to now, three clinical studies on the effects of complement inhibitors have been recruiting (Table 1, Table 2S). Crosswalk-a (NCT04912869, phase 1) and Crosswalk-c (NCT05075824, phase 2) trials evaluate the effects of Crovalimab, an anti C5-antibody, respectively on safety and efficacy vs placebo of intravenous administration in adult patients with SCD (Table 1). Another strategy to control complement overactivation in SCD is based on the block of properdin, a positive regulator of the complement system. The clinical trial Phoenix (NCT05565092) evaluates the safety of ALXN1820, a humanized bispecific variable heavy domain of heavy chain (VHH) antibody that simultaneously binds albumin and properdin in patients with SCD. Although the therapeutic strategy seemed promising, this latter clinical trial has been recently suspended due to change in company development strategies.

Considering the intense crosstalk between free heme and vascular endothelial compartment, evidence in cell- and animal-based studies supports the protective effects of recombinant ADAMST13 (rADAMST13) against acute sickle cell related hemolysis and organ damage.^{115,116} A phase 1 randomized double-blind placebo controlled multicenter ascending single dose study with rADAMST13 in patients with SCD has been designed (NCT03997760) (Table 1, Table 2S). No rADAMST13-related serious treatment-emergent adverse events (TEAEs) were recorded, supporting the design of future studies on patients with SCD in acute setting.¹¹⁷

An holistic approach to treat inflammatory vasculopathy and unresolved inflammation has been proposed in diseases other than SCD such as in cardiovascular disease or atherosclerosis by administering ω -3 fatty acids (ω -3 PUFAs).¹¹⁸⁻¹²⁰ Previous studies in both mouse models and patients with SCD have shown beneficial effects of dietary supplementation with ω -3 PUFAs preparations.¹²¹⁻¹²⁴ As shown in Table 1, two clinical studies with omega(ω)-3 fatty acid supplementation are actively recruiting SCD patients. The first one (NCT05758766) is an interventional randomized crossover trial, which studies the impact of ω 3 fatty acid supplementation derived from plant (Flaxseed) on both acute and chronic pain rate and on patients' quality of life. The second has been designed as phase 2 open-label study (NCT05861453), which evaluates Epeleuton, a second-generation synthetic ω 3 fatty acid on adult patients with SCD (Table 1, Table 2S).¹²⁵ Epeleuton shows an advantageous functional profile compared to other formulations of ω -3 fatty acids tested in SCD.¹²¹⁻¹²⁴ A phase 2, randomized, double-blind, placebo-controlled, parallel-group, dose-finding study showed that the a DHA ethyl ester SC411, increased Hb, improved markers of inflammatory vasculopathy and reduced use of analgesic at home and the number of days of school absence related to sickle cell pain.¹²⁴ There is an open-label extension of SC411 in children with SCD (NCT02973360), (Table 6S).

Other therapeutic strategies to control or limit sickle cell related inflammatory vasculopathy are represented by the P-selectin blockers, crizanlizumab or inclacumab.⁶² Crizanlizumab, successfully reduced VOCs in patients with SCD in the SUSTAIN study, with rates of treatment-emergent adverse events similar between treatment arms across all subgroups.⁶² However it was not superior to placebo in

modifying the pain crisis rate in adult patients with SCD in the STAND phase 3 study (NCT03814746).¹²⁶ This induced EMA to recommend the suspension of marketing authorization for Crizanlizumab as treatment of patients with SCD (<https://www.ema.europa.eu/en/medicines/human/referrals/adakveo>). As shown in Table 5S, additional clinical studies on Crizanlizumab in special SCD setting are still ongoing. Among them, primary analyses from the SPARTAN trial (NCT03938454) on SCD-related priapism show that patients treated with crizanlizumab over 26 weeks exhibited approximately half as many priapic events compared with baseline with safety profile similar to that reported in registration trials (Table 5S).¹²⁷ Promising *in vitro*, *ex-vivo* data on Inclacumab, an anti-P selectin IgG4 antibody, support the ongoing phase-3 clinical trial in adult patients with SCD (THRIVE-131, NCT04935879;-132, NCT04927247; -133OLE, NCT05348915) (Table 1, table 2S).^{128,129}

The attempt to interfere with inflammasome has been explored in a phase 2 study with canakinumab, an antibody against IL1, in children-young adult patients with SCD (Table 1S). Canakinumab improved markers of chronic inflammation and patients' fatigue, but failed to reduce VOC rate.¹³⁰ Up to now, tocilizumab, an anti-IL6 antibody, is under evaluation in a phase 2 study (NCT05640271) in adult patients with SCD admitted to the emergency department for acute chest syndrome (ACS). Tocilizumab is expected to beneficial impact both inflammatory response and pain during ACS (Table 1, Table 2S).

CONCLUSIONS

The therapeutic portfolio for thalassemias, SCD and other red cell disorders is constantly developing, considering a new scenario where single agents target common physiologies in different red cell disorders. This is extremely interesting in the context of rare diseases, since it increases the size of the population using the same compounds and makes these orphan disorders more attractive for pharma investments.¹³¹

The increase life expectancy of patients with hereditary red cell disorders might prompt to consider therapeutic strategies targeting inflammatory vasculopathy also in disorders other than SCD such as NTDT or HS in elderly patients, based on the synergy between hemolysis-related inflammatory vasculopathy⁹⁶ and aging. Finally, we are seeing some light at the end of the tunnel when we consider

luspatercept and mitapivat in clinical management of two orphan conditions such as α -thalassemia and CDA-II, whose treatment is still based on blood transfusion and iron chelation therapy. Collectively these new therapeutic approaches should be considered not only as single agent but possibly in combination between them or with already standard of care treatments such as hydroxycarbamide in SCD.

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AUTHORS CONTRIBUTION

VMP, LDF analyzed the studies and wrote the paper. FM contributed to the literature analysis.

AUTHORS DISCLOSURES

VMP: consultant for Bluebird Bio, advisory board Bluebird Bio, BMS and Vertex; speakers bureau for Novartis. LDF: research grants: Agios, Bristol, Advisory Board: Roche.

REFERENCES

1. Kassebaum NJ, Jasrasaria R, Naghavi M, et al. A systematic analysis of global anemia burden from 1990 to 2010. *Blood*. 2014;123(5):615-624.
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(9859):2197-2223.
3. Piel FB, Rees DC, DeBaun MR, et al. Defining global strategies to improve outcomes in sickle cell disease: a Lancet Haematology Commission. *Lancet Haematol*. 2023;10(8):e633-e686.
4. Musallam KM, Taher AT, Kattamis A, Kuo KHM, Sheth S, Cappellini MD. Profile of Luspatercept in the Treatment of Anemia in Adults with Non-Transfusion-Dependent beta-Thalassemia (NTDT): Design, Development and Potential Place in Therapy. *Drug Des Devel Ther*. 2023;17:1583-1591.
5. Roessler HI, Knoers N, van Haelst MM, van Haften G. Drug Repurposing for Rare Diseases. *Trends Pharmacol Sci*. 2021;42(4):255-267.
6. Fattizzo B, Cavallaro F, Marcello A, Vercellati C, Barcellini W. Pyruvate Kinase Deficiency: Current Challenges and Future Prospects. *J Blood Med*. 2022;13:461-471.

7. Risitano AM, Peffault de Latour R. How we('ll) treat paroxysmal nocturnal haemoglobinuria: diving into the future. *Br J Haematol*. 2022;196(2):288-303.
8. Berentsen S, Barcellini W. Autoimmune Hemolytic Anemias. *N Engl J Med*. 2021;385(15):1407-1419.
9. Cazzola M. Ineffective erythropoiesis and its treatment. *Blood*. 2022;139(16):2460-2470.
10. Steinberg MH. Fetal hemoglobin in sickle cell anemia. *Blood*. 2020;136(21):2392-2400.
11. Guerra A, Oikonomidou PR, Sinha S, et al. Lack of Gdf11 does not improve anemia or prevent the activity of RAP-536 in a mouse model of beta-thalassemia. *Blood*. 2019;134(6):568-572.
12. Jann J, Gascon S, Roux S, Fauchoux N. Influence of the TGF-beta Superfamily on Osteoclasts/Osteoblasts Balance in Physiological and Pathological Bone Conditions. *Int J Mol Sci*. 2020;21(20).
13. Dussiot M, Maciel TT, Fricot A, et al. An activin receptor IIA ligand trap corrects ineffective erythropoiesis in beta-thalassemia. *Nat Med*. 2014;20(4):398-407.
14. Sanchez-Duffhues G, Hiepen C, Knaus P, Ten Dijke P. Bone morphogenetic protein signaling in bone homeostasis. *Bone*. 2015;80:43-59.
15. Cappellini MD, Viprakasit V, Taher AT, et al. A Phase 3 Trial of Luspatercept in Patients with Transfusion-Dependent beta-Thalassemia. *N Engl J Med*. 2020;382(13):1219-1231.
16. Taher AT, Cappellini MD, Kattamis A, et al. Luspatercept for the treatment of anaemia in non-transfusion-dependent beta-thalassaemia (BEYOND): a phase 2, randomised, double-blind, multicentre, placebo-controlled trial. *Lancet Haematol*. 2022;9(10):e733-e744.
17. Piga A, Longo F, Gamberini MR, et al. Long-term safety and erythroid response with luspatercept treatment in patients with beta-thalassemia. *Ther Adv Hematol*. 2022;13:20406207221134404.
18. Hermine O CM, Taher AT, Coates TD, Viprakasit V, Kattamis A, Shetty JK, Weisskopf MB, Holot N, Vodala S, Kuo WL, Porter JB. . Effect of Luspatercept on Red Blood Cell (RBC) Transfusion Burden, Iron Chelation Therapy (ICT), and Iron Overload in Adults with Transfusion-Dependent β -Thalassemia (TDT) from the BELIEVE Trial: A Long-Term Analysis. In: *Blood* ed. ASH. New Orleans: Blood; 2022:8215-8217.
19. Piga A, Perrotta S, Gamberini MR, et al. Luspatercept improves hemoglobin levels and blood transfusion requirements in a study of patients with beta-thalassemia. *Blood*. 2019;133(12):1279-1289.
20. Wobus M, Mies A, Asokan N, et al. Luspatercept restores SDF-1-mediated hematopoietic support by MDS-derived mesenchymal stromal cells. *Leukemia*. 2021;35(10):2936-2947.
21. Shalev O, Repka T, Goldfarb A, et al. Deferiprone (L1) chelates pathologic iron deposits from membranes of intact thalassemic and sickle red blood cells both in vitro and in vivo. *Blood*. 1995;86(5):2008-2013.
22. Shalev O, Hebbel RP. Catalysis of soluble hemoglobin oxidation by free iron on sickle red cell membranes. *Blood*. 1996;87(9):3948-3952.
23. Browne P, Shalev O, Hebbel RP. The molecular pathobiology of cell membrane iron: the sickle red cell as a model. *Free Radic Biol Med*. 1998;24(6):1040-1048.

24. de Franceschi L, Shalev O, Piga A, et al. Deferiprone therapy in homozygous human beta-thalassemia removes erythrocyte membrane free iron and reduces KCl cotransport activity. *J Lab Clin Med.* 1999;133(1):64-69.
25. Rivella S. Iron metabolism under conditions of ineffective erythropoiesis in beta-thalassemia. *Blood.* 2019;133(1):51-58.
26. Kautz L, Jung G, Valore EV, Rivella S, Nemeth E, Ganz T. Identification of erythroferrone as an erythroid regulator of iron metabolism. *Nat Genet.* 2014;46(7):678-684.
27. Muckenthaler MU, Rivella S, Hentze MW, Galy B. A Red Carpet for Iron Metabolism. *Cell.* 2017;168(3):344-361.
28. Kattamis A, Papassotiriou I, Palaiologou D, et al. The effects of erythropoietic activity and iron burden on hepcidin expression in patients with thalassemia major. *Haematologica.* 2006;91(6):809-812.
29. Casu C, Aghajan M, Oikonomidou PR, Guo S, Monia BP, Rivella S. Combination of Tmprss6- ASO and the iron chelator deferiprone improves erythropoiesis and reduces iron overload in a mouse model of beta-thalassemia intermedia. *Haematologica.* 2016;101(1):e8-e11.
30. Preza GC, Ruchala P, Pinon R, et al. Minihepcidins are rationally designed small peptides that mimic hepcidin activity in mice and may be useful for the treatment of iron overload. *J Clin Invest.* 2011;121(12):4880-4888.
31. Manolova V, Nyffenegger N, Flace A, et al. Oral ferroportin inhibitor ameliorates ineffective erythropoiesis in a model of beta-thalassemia. *J Clin Invest.* 2019;130(1):491-506.
32. Li H, Rybicki AC, Suzuka SM, et al. Transferrin therapy ameliorates disease in beta-thalassemic mice. *Nat Med.* 2010;16(2):177-182.
33. Taher A. K-SA, Tantiworawit A., Wong P., Szecsody P. Safety and preliminary pharmacodynamic effects of the ferroportin inhibitor vamifeport (VIT-2763) in patients with non-transfusion-dependent beta thalassemia (NTDT): results from phase 2A study. In: Hemasphere ed. EHA. Wein: Hemasphere; 2022:173-174.
34. Taher AT, Viprakasit V, Cappellini MD, et al. Haematological effects of oral administration of bitopertin, a glycine transport inhibitor, in patients with non-transfusion-dependent beta-thalassaemia. *Br J Haematol.* 2021;194(2):474-477.
35. Garcia-Santos D, Hamdi A, Saxova Z, et al. Inhibition of heme oxygenase ameliorates anemia and reduces iron overload in a beta-thalassemia mouse model. *Blood.* 2018;131(2):236-246.
36. Matte A, Federti E, Winter M, et al. Bitopertin, a selective oral GLYT1 inhibitor, improves anemia in a mouse model of beta-thalassemia. *JCI Insight.* 2019;4(22).
37. Rio S, Gastou M, Karboul N, et al. Regulation of globin-heme balance in Diamond-Blackfan anemia by HSP70/GATA1. *Blood.* 2019;133(12):1358-1370.
38. Halloy F, Iyer PS, Ghidini A, et al. Repurposing of glycine transport inhibitors for the treatment of erythropoietic protoporphyria. *Cell Chem Biol.* 2021;28(8):1221-1234 e1226.
39. Kuter DJ, Bonkovsky HL, Monroy S, et al. Efficacy and safety of givosiran for acute hepatic porphyria: Final results of the randomized phase III ENVISION trial. *J Hepatol.* 2023;79(5):1150-1158.
40. Balwani M, Sardh E, Ventura P, et al. Phase 3 Trial of RNAi Therapeutic Givosiran for Acute Intermittent Porphyria. *N Engl J Med.* 2020;382(24):2289-2301.
41. Knight ZA, Schmidt SF, Birsoy K, Tan K, Friedman JM. A critical role for mTORC1 in erythropoiesis and anemia. *Elife.* 2014;3:e01913.

42. Lupo F, Tibaldi E, Matte A, et al. A new molecular link between defective autophagy and erythroid abnormalities in chorea-acanthocytosis. *Blood*. 2016;128(25):2976-2987.
43. Bruchova H, Yoon D, Agarwal AM, Swierczek S, Prchal JT. Erythropoiesis in polycythemia vera is hyper-proliferative and has accelerated maturation. *Blood Cells Mol Dis*. 2009;43(1):81-87.
44. Nemeth E, Ganz T. Hepcidin and Iron in Health and Disease. *Annu Rev Med*. 2023;74:261-277.
45. Aschemeyer S, Qiao B, Stefanova D, et al. Structure-function analysis of ferroportin defines the binding site and an alternative mechanism of action of hepcidin. *Blood*. 2018;131(8):899-910.
46. Bennett C, Jackson VE, Pettikiriarachchi A, et al. Iron homeostasis governs erythroid phenotype in polycythemia vera. *Blood*. 2023;141(26):3199-3214.
47. Haddy TB, Castro O. Overt iron deficiency in sickle cell disease. *Arch Intern Med*. 1982;142(9):1621-1624.
48. Castro O, Poillon WN, Finke H, Massac E. Improvement of sickle cell anemia by iron-limited erythropoiesis. *Am J Hematol*. 1994;47(2):74-81.
49. Bouchair N, Manigne P, Kanfer A, et al. [Prevention of sickle cell crises with multiple phlebotomies]. *Arch Pediatr*. 2000;7(3):249-255.
50. Rombos Y, Tzanetea R, Kalotychou V, et al. Amelioration of painful crises in sickle cell disease by venesections. *Blood Cells Mol Dis*. 2002;28(2):283-287.
51. Castro OL, De Franceschi L, Ganz T, et al. Iron restriction in sickle cell disease: When less is more. *Am J Hematol*. 2024.
52. Parrow NL, Violet PC, George NA, et al. Dietary iron restriction improves markers of disease severity in murine sickle cell anemia. *Blood*. 2021;137(11):1553-1555.
53. Nyffenegger N, Zennadi R, Kalleda N, et al. The oral ferroportin inhibitor vamifeport improves hemodynamics in a mouse model of sickle cell disease. *Blood*. 2022;140(7):769-781.
54. Matte A, Federti E, De Franceschi L. Erythrocyte pyruvate kinase activation in red cell disorders. *Curr Opin Hematol*. 2023;30(3):93-98.
55. Ogasawara Y, Funakoshi M, Ishii K. Glucose metabolism is accelerated by exposure to t-butylhydroperoxide during NADH consumption in human erythrocytes. *Blood Cells Mol Dis*. 2008;41(3):237-243.
56. De Franceschi L, Bertoldi M, Matte A, et al. Oxidative stress and beta-thalassemic erythroid cells behind the molecular defect. *Oxid Med Cell Longev*. 2013;2013:985210.
57. Zanella A, Fermo E, Bianchi P, Valentini G. Red cell pyruvate kinase deficiency: molecular and clinical aspects. *Br J Haematol*. 2005;130(1):11-25.
58. Andres O, Loewecke F, Morbach H, et al. Hereditary spherocytosis is associated with decreased pyruvate kinase activity due to impaired structural integrity of the red blood cell membrane. *Br J Haematol*. 2019;187(3):386-395.
59. Al-Samkari H, van Beers EJ. Mitapivat, a novel pyruvate kinase activator, for the treatment of hereditary hemolytic anemias. *Ther Adv Hematol*. 2021;12:20406207211066070.
60. Cappellini MD, Marcon A, Fattizzo B, Motta I. Innovative Treatments for Rare Anemias. *Hemasphere*. 2021;5(6):e576.
61. De Franceschi L, Olivieri O, Miraglia del Giudice E, et al. Membrane cation and anion transport activities in erythrocytes of hereditary spherocytosis: effects of different membrane protein defects. *Am J Hematol*. 1997;55(3):121-128.

62. Matte A, Cappellini MD, Iolascon A, Enrica F, De Franceschi L. Emerging drugs in randomized controlled trials for sickle cell disease: are we on the brink of a new era in research and treatment? *Expert Opin Investig Drugs*. 2020;29(1):23-31.
63. D'Alessandro A, Anastasiadi AT, Tzounakas VL, et al. Red Blood Cell Metabolism In Vivo and In Vitro. *Metabolites*. 2023;13(7).
64. Al-Samkari H, Galacteros F, Glenthøj A, et al. Mitapivat versus Placebo for Pyruvate Kinase Deficiency. *N Engl J Med*. 2022;386(15):1432-1442.
65. Glenthøj A, van Beers EJ, Al-Samkari H, et al. Mitapivat in adult patients with pyruvate kinase deficiency receiving regular transfusions (ACTIVATE-T): a multicentre, open-label, single-arm, phase 3 trial. *Lancet Haematol*. 2022;9(10):e724-e732.
66. Matte A, Federti E, Kung C, et al. The pyruvate kinase activator mitapivat reduces hemolysis and improves anemia in a beta-thalassemia mouse model. *J Clin Invest*. 2021;131(10).
67. Matte A, Kosinski PA, Federti E, et al. Mitapivat, a pyruvate kinase activator, improves transfusion burden and reduces iron overload in beta-thalassemic mice. *Haematologica*. 2023;108(9):2535-2541.
68. Musallam KM, Taher AT, Cappellini MD. Right in time: Mitapivat for the treatment of anemia in alpha- and beta-thalassemia. *Cell Rep Med*. 2022;3(10):100790.
69. Kuo KHM, Layton DM, Lal A, et al. Safety and efficacy of mitapivat, an oral pyruvate kinase activator, in adults with non-transfusion dependent alpha-thalassaemia or beta-thalassaemia: an open-label, multicentre, phase 2 study. *Lancet*. 2022;400(10351):493-501.
70. Iolascon A, Andolfo I, Russo R. Congenital dyserythropoietic anemias. *Blood*. 2020;136(11):1274-1283.
71. Aizawa S, Harada T, Kanbe E, et al. Ineffective erythropoiesis in mutant mice with deficient pyruvate kinase activity. *Exp Hematol*. 2005;33(11):1292-1298.
72. Aizawa S, Kohdera U, Hiramoto M, et al. Ineffective erythropoiesis in the spleen of a patient with pyruvate kinase deficiency. *Am J Hematol*. 2003;74(1):68-72.
73. Matte A, Wilson AB, Gevi F, et al. Mitapivat reprograms the RBC metabolome and improves anemia in a mouse model of hereditary spherocytosis. *JCI Insight*. 2023;8(20).
74. Jensen M, Shohet SB, Nathan DG. The role of red cell energy metabolism in the generation of irreversibly sickled cells in vitro. *Blood*. 1973;42(6):835-842.
75. Poillon WN, Robinson MD, Kim BC. Deoxygenated sickle hemoglobin. Modulation of its solubility by 2,3-diphosphoglycerate and other allosteric polyanions. *J Biol Chem*. 1985;260(26):13897-13900.
76. Rab MAE, Bos J, van Oirschot BA, et al. Decreased activity and stability of pyruvate kinase in sickle cell disease: a novel target for mitapivat therapy. *Blood*. 2021;137(21):2997-3001.
77. Quezado ZMN, Kamimura S, Smith M, et al. Mitapivat increases ATP and decreases oxidative stress and erythrocyte mitochondria retention in a SCD mouse model. *Blood Cells Mol Dis*. 2022;95:102660.
78. Xu JZ, Conrey A, Frey I, et al. A phase 1 dose escalation study of the pyruvate kinase activator mitapivat (AG-348) in sickle cell disease. *Blood*. 2022;140(19):2053-2062.
79. D'Alessandro A, Le K, Lundt M, et al. Functional and multi-omics signatures of mitapivat efficacy upon activation of pyruvate kinase in red blood cells from patients with sickle cell disease. *Haematologica*. 2024.

80. van Dijk MJ, Rab MAE, van Oirschot BA, et al. Safety and efficacy of mitapivat, an oral pyruvate kinase activator, in sickle cell disease: A phase 2, open-label study. *Am J Hematol*. 2022;97(7):E226-E229.
81. Conrey A FI, Asomaning N, Charles RP, Parekh D, Xu J, Le K, Kruah B, Li Q, Dunkelberger E, Cellmer T, Yates AM, Wind-Rotolo M, Jeffries N, Eaton WA, Thein SL. Long-Term Safety and Efficacy of Mitapivat, an Oral Pyruvate Kinase Activator, in Adults with Sickle Cell Disease: Extension of a Phase 1 Dose Escalation Study. In: Blood ed. ASH. San Diego: Blood; 2023:273.
82. Idowu M OL, Dumitriu B, Lobo C, Thein SL, Andemariam B, Nnodu OE, Inati A, Glaros AK, Saad STO, Bartolucci P, Colombatti C, Taher AH, Abboud MR, Oluyadi A, Iyer V, Yin O, Morris S, Yates AM, Shao H, Patil S, Urbstonaitis R, Zaidi AU, Smith WR. A Phase 2/3, Double-Blind, Randomized, Placebo-Controlled, Multicenter Study of Mitapivat in Patients with Sickle Cell Disease: RISE UP Phase 2 Results. In: Blood ed. ASH. San Diego Blood; 2023:271.
83. Rab MAE, Van Oirschot BA, Kosinski PA, et al. AG-348 (Mitapivat), an allosteric activator of red blood cell pyruvate kinase, increases enzymatic activity, protein stability, and ATP levels over a broad range of PKLR genotypes. *Haematologica*. 2021;106(1):238-249.
84. Xu JZ, Vercellotti GM. Pyruvate kinase activators: targeting red cell metabolism in sickle cell disease. *Hematology Am Soc Hematol Educ Program*. 2023;2023(1):107-113.
85. Gurov X.D. ME, Iyer V., Claeys A., Patil S., Urbstonaitis U., Xiao J., Callaghan M.U. Results from the single and multiple ascending dose study to assess the safety, tolerability, pharmacokinetics and pharmacodynamics of AG-946 in healthy volunteers. In: ASH ed. ASH. New Orleans: ASH; 2022:5426-5427.
86. Forsyth S, Schroeder P, Geib J, et al. Safety, Pharmacokinetics, and Pharmacodynamics of Etavopivat (FT-4202), an Allosteric Activator of Pyruvate Kinase-R, in Healthy Adults: A Randomized, Placebo-Controlled, Double-Blind, First-in-Human Phase 1 Trial. *Clin Pharmacol Drug Dev*. 2022;11(5):654-665.
87. Telen M. BR, Hagar R., Idowu M., Osunkwo I., Kalfa T., Kuypers F., Geib J., Schroeder P., Wu E., Kelly P., Saraf S. Etavopivat treatment for up to 12 weeks in patients with sickle cell disease is well tolerated and improves red blood cell health. In: HemaSphere ed. EHA. Wein; 2022:02-03.
88. 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(3):226-235.
89. Sadaf A, Quinn CT. L-glutamine for sickle cell disease: Knight or pawn? *Exp Biol Med (Maywood)*. 2020;245(2):146-154.
90. Quinn CT. L-Glutamine for sickle cell anemia: more questions than answers. *Blood*. 2018;132(7):689-693.
91. Vichinsky E, Hoppe CC, Ataga KI, et al. A Phase 3 Randomized Trial of Voxelotor in Sickle Cell Disease. *N Engl J Med*. 2019;381(6):509-519.
92. Achebe M.O. HHMA, Al-Kindi S., Brown c., Tlfer P., Biemond B.J., Lipato t., Davis M., Gray S., Gordeuk V.R. Over 4 years of safety and efficacy with Voxelotor treatment for patients with sickle cell disease: update results from an open-label extension of the phase 3 HOPE trial. In: ASH ed. ASH San Diego: ASH; 2023:2527.
93. Andemariam B. BHH, Ershler W.B., Bergmann S., Sakhalkar V.S., Chang A., Desai P., Shah N., Pennington S., Miler R.E., Decastro L.M., Xu M., Hayward B., Yu C., Liles D.k. Real-world experience of individuals with sickle cell disease treated with voxelotor: initial report from the multicenter, prospective prospect study. In: ASH ed. ASH. San Diego: ASH; 2023:2499.

94. Saraf S.L. ASU, Akinsete A.M., Fasola F. A., Idowu M., Pennington S., Ershler W.B., Stagg M.P., Chin W., Zimmerman E., Adenola A., Pochron M.P., Lisbon E.A. Preliminary results from a multicenter phase 2/3 study of next-generation HbS polymerization inhibitor GBT021601 for the treatment of patients with sickle cell disease. In: *Blood* ed. ASH. San Diego: Blood; 2023:274.
95. Muller-Eberhard U, Javid J, Liem HH, Hanstein A, Hanna M. Plasma concentrations of hemopexin, haptoglobin and heme in patients with various hemolytic diseases. *Blood*. 1968;32(5):811-815.
96. Vinchi F, Sparla R, Passos ST, et al. Vasculo-toxic and pro-inflammatory action of unbound haemoglobin, haem and iron in transfusion-dependent patients with haemolytic anaemias. *Br J Haematol*. 2021;193(3):637-658.
97. Cancado RD. Pyruvate kinase deficiency: novel mutations and a better understanding of the genotype-to-phenotype correlation in Brazilian patients. *Rev Bras Hematol Hemoter*. 2018;40(1):1-2.
98. Kittivorapart J, Crew VK, Wilson MC, Heesom KJ, Siritanaratkul N, Toye AM. Quantitative proteomics of plasma vesicles identify novel biomarkers for hemoglobin E/beta-thalassemic patients. *Blood Adv*. 2018;2(2):95-104.
99. Schaer DJ, Buehler PW, Alayash AI, Belcher JD, Vercellotti GM. Hemolysis and free hemoglobin revisited: exploring hemoglobin and heme scavengers as a novel class of therapeutic proteins. *Blood*. 2013;121(8):1276-1284.
100. Gbotosho OT, Kapetanaki MG, Kato GJ. The Worst Things in Life are Free: The Role of Free Heme in Sickle Cell Disease. *Front Immunol*. 2020;11:561917.
101. Scully M, Knobl P, Kentouche K, et al. Recombinant ADAMTS-13: first-in-human pharmacokinetics and safety in congenital thrombotic thrombocytopenic purpura. *Blood*. 2017;130(19):2055-2063.
102. Schnog JJ, Kremer Hovinga JA, Krieg S, et al. ADAMTS13 activity in sickle cell disease. *Am J Hematol*. 2006;81(7):492-498.
103. Gentinetta T, Belcher JD, Brugger-Verdon V, et al. Plasma-Derived Hemopexin as a Candidate Therapeutic Agent for Acute Vaso-Occlusion in Sickle Cell Disease: Preclinical Evidence. *J Clin Med*. 2022;11(3).
104. 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(3):377-390.
105. Vinchi F, Costa da Silva M, Ingoglia G, et al. Hemopexin therapy reverts heme-induced proinflammatory phenotypic switching of macrophages in a mouse model of sickle cell disease. *Blood*. 2016;127(4):473-486.
106. 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(12):1317-1329.
107. Biemond BJS, B.J.; Wilson, F.; Jochems, J.; Lindqvist, L.M.; Jung, K.; Gentinetta, T.; Costin, S.; Kato, G.J.; Eleftheriou, P.; Fok, H.; Wahab, E.; Leung, P.M.; Sharif, J.; Boucher, A.; Fitzgerald, R.; Keese-Adu, R.; Azbell, R.; Liles, D.; Bergmann, S.; Lanzkron, S.; Gordeuk, V. A phase 1 study of csl888 (hemopexin) in adult patients with sickle cell disease. . In: *Hemasphere* ed. EHA. Frankfurt: Hemasphere; 2023:15.
108. Morris CR, Brown LAS, Reynolds M, et al. Impact of arginine therapy on mitochondrial function in children with sickle cell disease during vaso-occlusive pain. *Blood*. 2020;136(12):1402-1406.

109. Morris CR, Hamilton-Reeves J, Martindale RG, Sarav M, Ochoa Gautier JB. Acquired Amino Acid Deficiencies: A Focus on Arginine and Glutamine. *Nutr Clin Pract*. 2017;32(1_suppl):30S-47S.
110. Merle NS, Grunenwald A, Rajaratnam H, et al. Intravascular hemolysis activates complement via cell-free heme and heme-loaded microvesicles. *JCI Insight*. 2018;3(12).
111. Vercellotti GM, Dalmasso AP, Schaid TR, Jr., et al. Critical role of C5a in sickle cell disease. *Am J Hematol*. 2019;94(3):327-337.
112. Ivy ZK, Belcher JD, Khasabova IA, et al. Cold exposure induces vaso-occlusion and pain in sickle mice that depend on complement activation. *Blood*. 2023;142(22):1918-1927.
113. Lombardi E, Matte A, Risitano AM, et al. Factor H interferes with the adhesion of sickle red cells to vascular endothelium: a novel disease-modulating molecule. *Haematologica*. 2019;104(5):919-928.
114. Roumenina LT, Chadebecq P, Bodivit G, et al. Complement activation in sickle cell disease: Dependence on cell density, hemolysis and modulation by hydroxyurea therapy. *Am J Hematol*. 2020;95(5):456-464.
115. Rossato P, Glantschnig H, Canneva F, et al. Treatment with recombinant ADAMTS13, alleviates hypoxia/reoxygenation-induced pathologies in a mouse model of human sickle cell disease. *J Thromb Haemost*. 2023;21(2):269-275.
116. Rossato P, Federti E, Matte A, et al. Evidence of protective effects of recombinant ADAMTS13 in a humanized model of sickle cell disease. *Haematologica*. 2022;107(11):2650-2660.
117. Kanter J PP, Desai P, Ataga KI, Crary SE, Lanzkron S, Field J, Chung YC, Wang LT, Mellgård B, Nguyen VAV, Gordeuk VR. Safety and Pharmacokinetics of Recombinant ADAMTS13 in Patients with Sickle Cell Disease: A Phase 1 Randomized, Double-Blind, Placebo-Controlled Study. In: *Blood* ed. ASH. San Diego: Blood; 2023:149.
118. Sherratt SCR, Libby P, Bhatt DL, Mason RP. A biological rationale for the disparate effects of omega-3 fatty acids on cardiovascular disease outcomes. *Prostaglandins Leukot Essent Fatty Acids*. 2022;182:102450.
119. Kotlyarov S, Kotlyarova A. Molecular Pharmacology of Inflammation Resolution in Atherosclerosis. *Int J Mol Sci*. 2022;23(9).
120. Matte A, Recchiuti A, Federti E, et al. Resolution of sickle cell disease-associated inflammation and tissue damage with 17R-resolvin D1. *Blood*. 2019;133(3):252-265.
121. 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(7):870-880.
122. 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(1):15.
123. Wu CYC, Lopez-Toledano MA, Daak AA, et al. SC411 treatment can enhance survival in a mouse model of sickle cell disease. *Prostaglandins Leukot Essent Fatty Acids*. 2020;158:102110.
124. 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(15):1969-1979.
125. Climax J, Newsome PN, Hamza M, et al. Effects of Epeleuton, a Novel Synthetic Second-Generation n-3 Fatty Acid, on Non-Alcoholic Fatty Liver Disease, Triglycerides, Glycemic Control, and Cardiometabolic and Inflammatory Markers. *J Am Heart Assoc*. 2020;9(16):e016334.

126. Abboud MR CR, De Montalembert M, Smith WR, Rimawi H, Voskaridou E, Guvenc B, Keefe D, Grosch K, Nassin ML, Watson J, Yssel J, Reshetnyak E, Dei Adomakoh Y. Efficacy, Safety, and Biomarker Analysis of 5 Mg and 7.5 Mg Doses of Crizanlizumab in Patients with Sickle Cell Disease: Primary Analyses from the Phase III STAND Study. . In: *Blood* ed. ASH. San Diego: Blood; 2023:272.
127. Idowu M DM, Burnett A, Darbari DS, Adam S, Anderson AR, Kanter J, Billett HH, Liles DK, Nickel RS, Decastro LM, Andemariam B, Blyden GT, Heeney MM, Ramadas P, McLemore ML, Kolekar Y, Laine D, Paulose J, Sarkar R, Vignogna MB, El Rassi F. . Primary Analysis of Spartan: A Phase 2 Trial to Assess the Efficacy and Safety of Crizanlizumab in Patients with Sickle Cell Disease Related Priapism. In: *Blood* ed. ASH. San Diego: Blood; 2023:146.
128. Andemariam B. IA, Colombatti R., Minniti C., Brown C., Hottman M., Gray S., Hoppe c., Davis M., Yue P. Trials in progress: the THRIVE studies evaluating the efficacy, safety, and long-term treatment with inclacumab, a P-Selecting inhibitor, in patients with sickle cell disease. In: *Hemasphere* ed. EHA. Frankfurt: Hemasphere; 2023:23.
129. Federici C. SM, Kovshovik P., Opheim K., Comba G., Sertel S., Braxton S.J., Bode A., Zak J., Geng X., Gurkan U.a. Inclacumab reduce preexisting red blood cell adhesion to activated endothelial cells: in vitro assessment of the microfluidic platform endothelium on chip. In: *Blood* ed. ASH. San Diego: ASH publication; 2023:5267.
130. Rees DC, Kilinc Y, Unal S, et al. A randomized, placebo-controlled, double-blind trial of canakinumab in children and young adults with sickle cell anemia. *Blood*. 2022;139(17):2642-2652.
131. Pierzynowska K, Kaminska T, Wegrzyn G. One drug to treat many diseases: unlocking the economic trap of rare diseases. *Metab Brain Dis*. 2020;35(8):1237-1240.

Metabolic targets	Mitapivat (AG-348)	<p>ENERGIZE (NCT04770753) Population: β-thal with or without α-globin gene mutations, HbE/β-thal, or α-thal/HbH disease Age: \geq 18 yrs. Enrolled N=194 pts</p>	<p>Phase 3 Efficacy and safety of mitapivat in non-transfusion-dependent alpha- or beta-thalassemia (α- or β-NTDT) pts</p>	Active, not recruiting
		<p>ENERGIZE-T (NCT04770779) Population: β-thal with or without α-globin gene mutations, HbE/β-thal, or α-thal/HbH disease Age: \geq 18 yrs. Enrolled N=258 pts</p>	<p>Phase 3 Efficacy and safety of mitapivat in transfusion-dependent Thalassemias (α- or β-TDT) pts</p>	Active, not recruiting
		<p>SATISFY (NCT05935202) Population: RBC membranopathy or CDAll Age: \geq 18 yrs. Enrollment (Estimated) N=25 pts</p>	<p>Phase 2 Safety and efficacy of Mitapivat in adult patients with membranopathies</p>	Not yet recruiting
		<p>NCT04610866 Population: HbSS Age: 18 to 70 yrs. Enrolled N=15 pts</p>	<p>Phase 1/2 Extension of a Phase 1 Pilot Study of Mitapivat Safety, tolerability, pharmacokinetics, and pharmacodynamics of long-term Mitapivat dosing in subjects with stable SCD</p>	Active, not recruiting
		<p>RISE-UP (NCT05031780) Population: HbSS, HbSC, HbS/βtal⁰, HbS/βtal⁺, or other SCD variants Age: \geq 16 yrs. Enrolled N=277 pts</p>	<p>Phase 2/3 Double-blind, randomized, placebo-controlled, multicenter study to evaluate the efficacy and safety of mitapivat in subjects with SCD</p>	Active, not recruiting
AG-946	<p>NCT04536792 Population: SCD Age: 18 to 55 yrs. Enrollment (Estimated) N=118 (Actual) N=122</p>	<p>Phase 1 A Phase 1 study for safety, tolerability, pharmacokinetics, and pharmacodynamics of AG-946 in healthy volunteers and in subjects with SCD</p>	Completed	
	<p>HIBISCUS (NCT04624659) Population: SCD</p>	<p>Phase 2/3 An adaptive, randomized, placebo-controlled,</p>	Recruiting	

	<p>Etavopivat (FT-4202)</p>	<p>Age: 12 to 65 yrs Enrollment (Estimated) N=344 pts</p> <p>GLADIOLUS (NCT04987489) Population: SCD; β-thal, HbE/ β-thal or HbH (α-thal), or other thal variant Age: 12 to 65 yrs. Enrollment (Estimated) N=60 pts</p> <p>NCT05953584 Population: HbSS, HbSβ^0 thal Age: 12 to 16 yrs. Enrollment (Estimated) N=46 pts</p> <p>NCT05725902 Population: HbSS or HbS/β^0 thal Age: 12 to 21 yrs. Enrollment (Estimated) N=12 pts</p>	<p>double-blind, multi-center study of oral etavopivat, a pyruvate kinase activator in patients with SCD</p> <p>Phase 2 Open-label study to evaluate safety and clinical activity of etavopivat in patients with thalassemia or SCD</p> <p>Phase 2 Open-label study to evaluate the activity of etavopivat on transcranial doppler velocities in pediatric patients with SCD who are at increased risk for primary stroke</p> <p>Phase 2 Effect of etavopivat on cerebral hemodynamic response in children with SCD</p>	<p>Recruiting</p> <p>Recruiting</p> <p>Not yet recruiting</p>
<p>Targeting iron homeostasis or heme synthesis</p>	<p>FPN blocker</p> <p>Bitopertin</p>	<p>ViSionSerenity (NCT04817670) Population: HbSS or HbS/β^0 thal Age: 18 to 60 yrs. Enrollment (Estimated) N=24 pts</p> <p>NCT05828108 Population: Steroid-Refractory DBA Age: 18 to 100 yrs. Enrollment (Estimated) N=30 pts</p> <p>BEACON (ACTRN12622000799752) Population: Erythropoietic protoporphyria and X-linked protoporphyria Age: \geq 18 yrs. Enrollment (Estimated) N = 22 pts</p>	<p>Phase 2 Double-blind, randomized, placebo-controlled, efficacy, and safety study of multiple doses of VIT-2763 in subjects with SCD</p> <p>Phase 1/2 Intra-patient dose-escalation study of the selective glyt1 inhibitor, bitopertin for steroid-refractory Diamond-Blackfan Anemia.</p> <p>Phase 2 Randomized, open label study of bitopertin to evaluate the safety, tolerability, efficacy, and Protoporphyrin IX (PPIX) concentration in participants with erythropoietic protoporphyria (EPP) and X-linked protoporphyria (XLP)</p>	<p>Recruiting</p> <p>Recruiting</p> <p>Recruiting</p>

		AURORA (NCT05308472) Population: Erythropoietic protoporphyria Age: ≥ 18 yrs. Enrollment N = 75 pts	Phase 2 Randomized, double-blind, placebo-controlled study of bitopertin to evaluate the safety, tolerability, efficacy, and Protoporphyrin IX (PPIX) concentrations in participants with erythropoietic protoporphyria (EPP)	Active, not recruiting
Targeting hemolysis and the vascular	Hpx (CSL889)	NCT04285827 Population: SCD Age: 18 to 60 yrs. Enrolled N=28 pts	Phase 1 A 2-part, Phase 1, multi-center, single-dose, open label, study to evaluate the safety, tolerability, and pharmacokinetics of csl889 in adult patients with SCD	Completed
	L-Arginine	STArT (NCT04839354) Population: SCD (any genotype) Age: 3 to 21 yrs. Enrollment (Estimated) N=360 pts	Phase 3 SCD Treatment with Arginine Therapy (STArT) Trial	Recruiting
		R34 pK/PD (NCT02447874) Population: HbSS, HbSβ ⁰ thal, Age: 7 to 21 yrs. Enrollment (Estimated) N=21 pts	Phase 1/2 Arginine therapy for the treatment of VOCs in children with severe SCD	Recruiting
	Complement (AP)	CROSSWALK-a (NCT04912869) Population: HbSS or HbSβ ⁰ thal Age: 12 to 55 yrs. Enrollment (Estimated) N=30 pts	Phase 1 A phase IB randomized, placebo-controlled study evaluating the safety, pharmacokinetics,-dynamics, and efficacy of Crovalimab for the management of acute uncomplicated VOCs in patients with SCD	Recruiting
Crovalimab	CROSSWALK-c (NCT05075824) Population: HbSS or HbSβ ⁰ thal Age:12 to 55 yrs. Enrollment (Estimated) N=90 pts	Phase 2 A randomized double-blind phase 2a study evaluating the efficacy, safety, pharmacokinetics, and pharmacodynamics of Crovalimab as adjunct treatment in prevention of VOCs in SCD	Recruiting	

endothelial axis	r-ADAMST13 (SHP665)	RAISE (NCT03997760) Population: HbSS or HbSβ ⁰ thal Age: 18 to 65 yrs. Enrolled N= 9 pts	Phase 1 A Study of SHP655 (rADAMTS13) in SCD	Completed
	ω-3 fatty acid	NCT05758766 Population: HbSS or HbSβ ⁰ thal at steady state Age: 5 to 18 yrs. Enrollment (Estimated) N=30 pts	Interventional, not applicable Study on use of plat extracts of ω -3 fatty acids to improve outcomes in individuals with SCD	Recruiting
	Epeleuton (DS102)	NCT05861453 Population: HbSS or HbSβ ⁰ thal Age: ≥ 18 yrs. Enrollment (Estimated) N=30 pts	Phase 2 Pharmacokinetics, pharmacodynamics, and safety of epeleuton in patients with SCD	Recruiting
	Inclacumab	THRIVE-131 (NCT04935879) Population: HbSS, HbSC, HbS/beta ⁰ thal, HbS/ beta ⁺ thal Age: ≥ 12 yrs. Enrollment N=232	Phase 3 A randomized, double-blind, placebo-controlled, multicenter study to assess the safety and efficacy of Inclacumab in participants with SCD experiencing VOCs	Active, not recruiting
		THRIVE-132 (NCT04927247) Population: SCD (any genotype) Age: ≥ 12 yrs. Enrollment N=72	Phase 3 A randomized, double-blind, placebo-controlled, multicenter study of a single dose of Inclacumab to reduce re-admission in participants with SCD and recurrent VOCs.	Completed
		THRIVE-133 OLE (NCT05348915) Population: SCD Age: ≥ 12 yrs. Enrollment (Estimated) N=520	Phase 3 An open-label extension study to evaluate the long-term safety of Inclacumab administered to participants with SCD, who have participated in an Inclacumab clinical trial	Recruiting
	Tocilizumab	NCT05640271 Population: HbSS, HbSC, HbS/beta ⁰ thal, HbS/ beta ⁺ thal Age: ≥ 18 yrs. Enrollment (Estimated) N=200	Phase 2 Low-Dose tocilizumab for acute chest syndrome in SCD pts.	Recruiting

*Studies Outcomes and dosages are reported in Supplementary Table 2S.

NCT: National Clinical Trial; thal: thalassemia; HbE: hemoglobin E; HbH: hemoglobin H; Hb: Hemoglobin; N: number; pts: patients; NTD: non-transfusion dependent; CDAlI: congenital dyserythropoietic anemia type II; SCD: sickle cell disease; RBC: red blood cells; DBA Diamond-Blackfan Anemia; VOC: vaso-occlusive crisis; TD: transfusion dependent, pts: patients, yrs: years.

FIGURE LEGENDS

Figure 1. Effects of free iron on red cells from patients with either β -thalassemia or sickle cell disease (SCD). In pathologic red cells free iron sustains chronic oxidation with generation of reactive oxygen species (ROS) throughout the Fenton reaction. This requires an efficient antioxidant machinery with the metabolic support of ATP (see also Figure 3). The chronic and severe red cell membrane damage is further amplified respectively by membrane association of free alpha chains in β -thalassemic erythrocytes and cyclic polymerization/depolymerization events in sickle red cells. In both disorders, red cell membrane oxidation results in (i) increased membrane mechanical instability favoring to abnormally clusterization of oxidized band 3 (B3); (ii) exposition of phosphatidyl serine (PS); and (iii) generation of erythroid microparticles (E-MP), carrying also PS. The cumulative effects of oxidation are the premature red cell aging with accelerated removal by erythrophagocytosis mediated by both PS exposure and naturally occurring anti-band 3 antibody (N-Ab). In addition, in sickle erythrocytes, membrane damage is associated with increase permeability to Ca^{2+} with the activation of the Gardos channel (KCNN4) coupled with the oxidation induced activation of the K-Cl (KCC) cotransport. This ends in sickle red cell dehydration, relative increase in HbS concentration with a negative impact on HbS polymerization kinetic. Of note, in SCD a smaller component of hemolysis takes place intra-vascularly with saturation of physiologic binding proteins (e.g. hemopexin) allowing the presence of free heme and hemoglobin in the peripheral circulation.

Figure 2. Therapeutic strategies targeting iron homeostasis or heme synthesis. Hepcidin (Hamp) agonist(s) block Tmprss6 function (SLN124, Sapablursen), resulting in Smad phosphorylation and nuclear translocation. This ends in up-regulation of Hamp expression. Hamp mimetics (LJPC-401, PTG300) increase Hamp level. Hamp inhibits the iron export activity of ferroportin in enterocytes and macrophages. Ferroportin blocker(s) (VIT-2763), mimic Hamp function. Apotransferrin binds circulating free iron (Fe^{2+}). Collectively, these strategies

targeting iron homeostasis have been proposed to reduce free iron to re-balance iron/heme synthesis and globin synthesis in β -thalassemia, which is characterized by ineffective erythropoiesis. Bitopertin as Glyt1 inhibitor blocks the import of glycine, which is the first step in the metabolic cascade of heme biosynthesis. Bitopertin treatment has been shown to reduce protoporphyrin-IX erythroblasts content. Hamp: hepcidin, Tmprss6: transmembrane protease serine 6; HJV: hemojuvelin; BMP6: bone morphogenic protein 6; BMPR: bone morphogenic protein-receptor; Smad: suppressor of mothers against decapentaplegic.

Figure 3. Pathways targeted by pyruvate kinase(s) activators and L-Glutamine

in red cells. The glycolytic pathway generates ATP and interfaces the pentose phosphate shunt, which is the main source of NADPH. This is required by antioxidant systems and the Rapoport-Luebering shunt that generates 2,3-DPG. Pyruvate kinase (PK) is the last enzyme in the glycolytic pathway. In SCD, the intense and sustain oxidation results in consumption of NADH and glutathione, favoring hemolysis. L-Glutamine as glutamate might support NADH and GSH systems, reducing red cell oxidation.

2,3-DPG: 2,3-Diphosphoglycerate; ATP: adenosine triphosphate; ADP: adenosine diphosphate; NADP: nicotinamide adenine dinucleotide phosphate, NAD: nicotinamide adenine dinucleotide; GSH; glutathione-SH.

Figure 4. Agents targeting the hemolysis-vascular endothelial axis

In sickle cell disease (SCD), one-third of the chronic hemolysis happens to be intravascular. This is also associated with the release of erythroid microparticles, which also contains heme. Thus, the consumption of physiologic binding proteins respectively haptoglobin for hemoglobin and hemopexin (Hpx) for heme, results increased level of free hemoglobin and free heme into the peripheral circulation, promoting a plasmatic pro-oxidant environment. This contributes to chronic, unresolved inflammation characterizing SCD resulting in up-regulation of pro-inflammatory cytokines (IL-1 β , IL-6, TNF- α) and makers of vascular endothelial activation such as vascular cell adhesion molecule-1 (VCAM-1) or Selectin To counteract the detriment effects of free heme and to limit inflammatory vasculopathy, the following novel therapeutic strategies are under evaluation in

clinical trials in patients with SCD (see Table 1): (i) recombinant ADASMT13 (r-ADAMST13) for the relative ADAMST13 functional deficiency; (ii) crovalimab as anti-C5 inhibitor to block the overactivation of the complement alternative pathway; (iii) L-Arginine to support nitric oxide (NO) synthesis (iv) anti-P- selectin antibodies to prevent cell-cell adhesion between red cells and neutrophils to vascular endothelial cells; (v) blockers of the pro-inflammatory cytokines (anti- IL1b, IL-6 or TNF-a antibodies); and (vi) multi-target approach by ω -3 fatty acid supplementation.

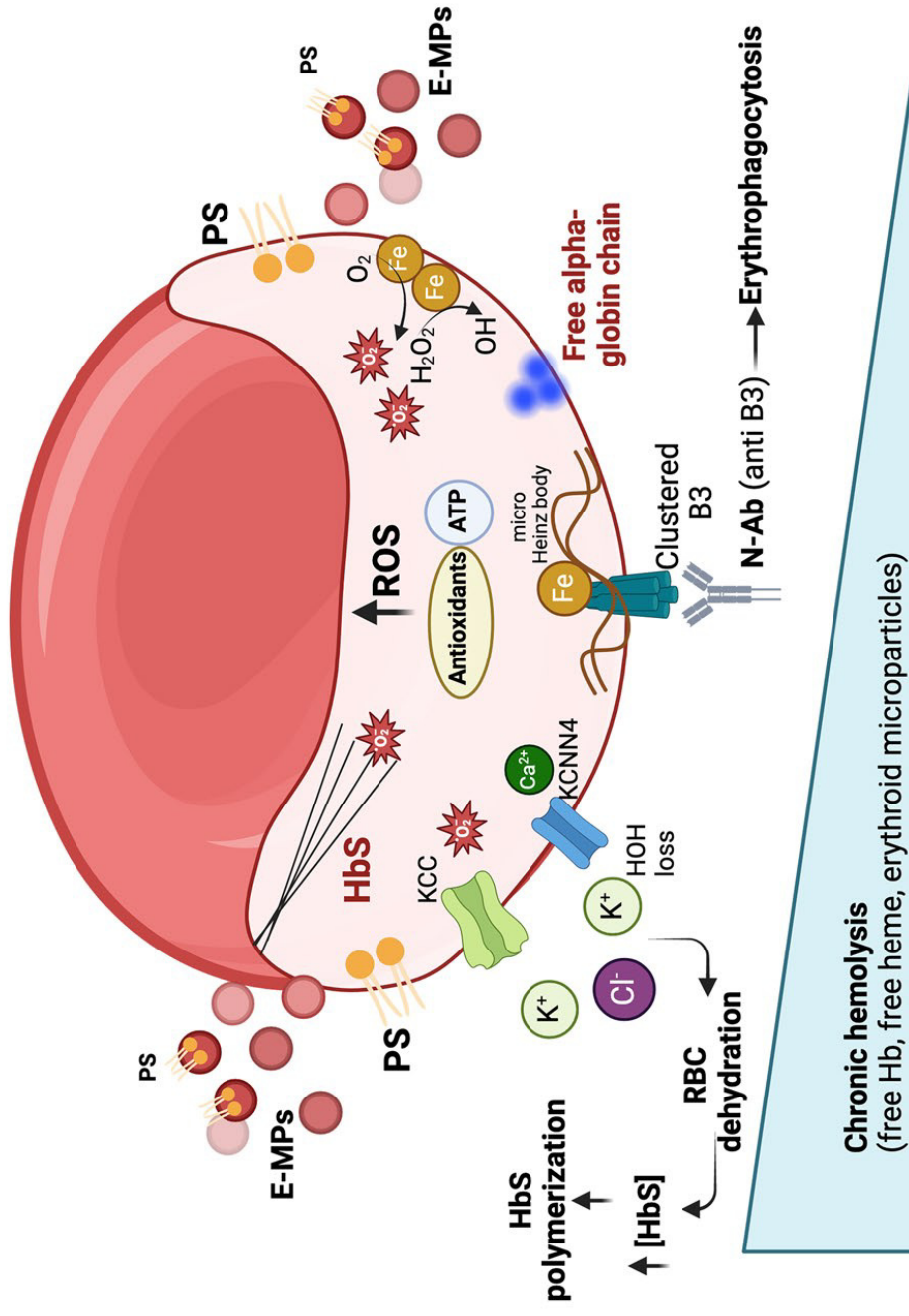


Figure 2

Figure 2

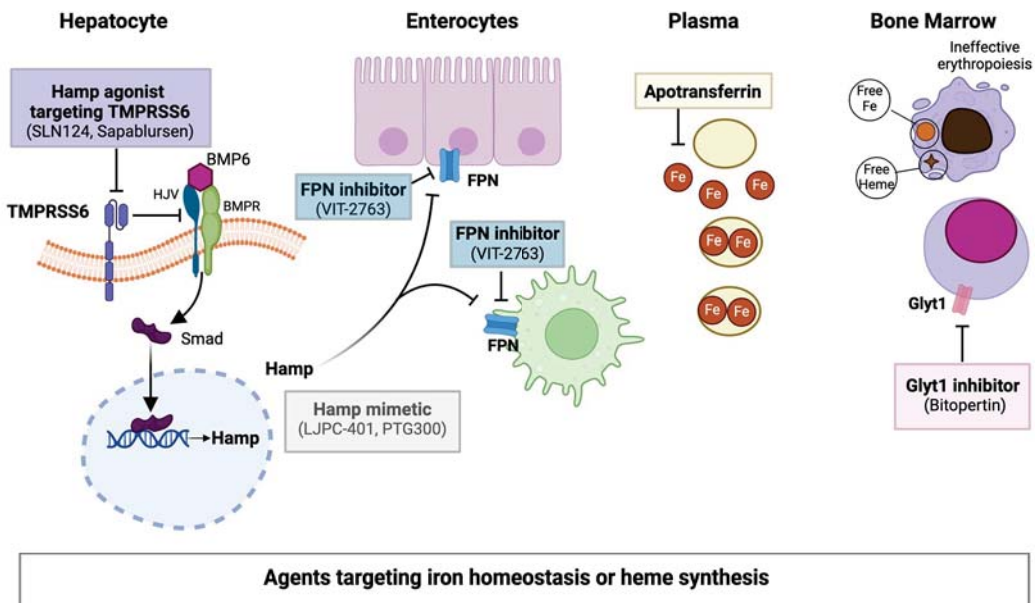


Figure 3

Figure 3

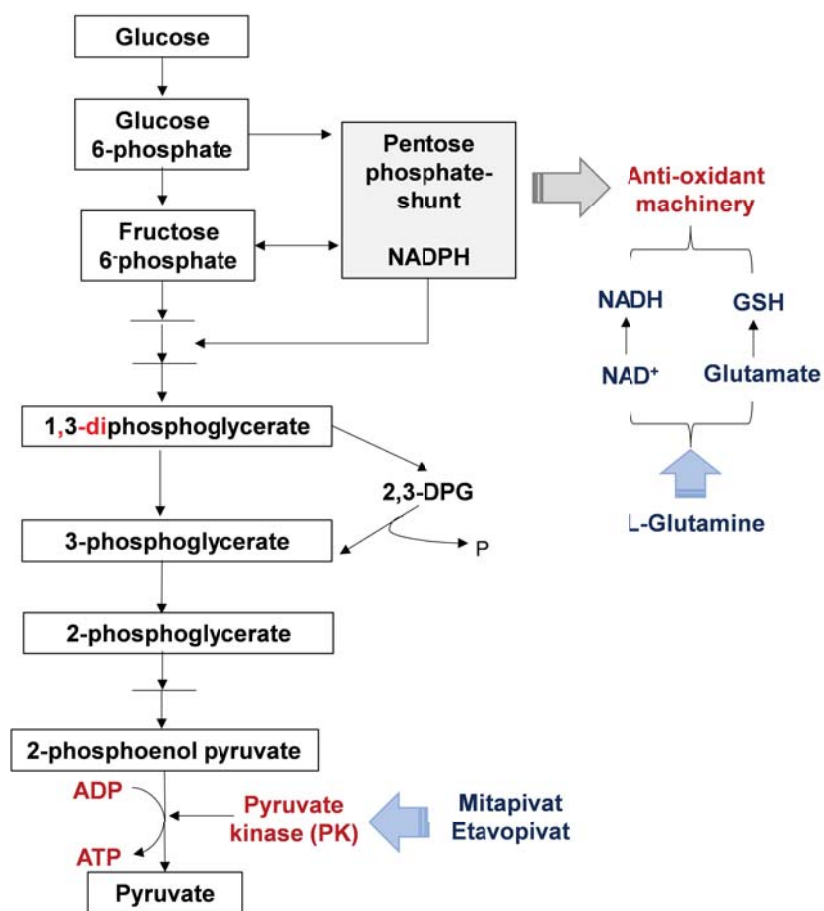


Figure 4

Figure 4

