1-1-2020

The Evolving Pharmacotherapeutic Landscape for the Treatment of Sickle Cell Disease.

Samir K Ballas

Mediterranean Journal of Hematology and Infectious Diseases

Review Article

The Evolving Pharmacotherapeutic Landscape for the Treatment of Sickle Cell Disease

Samir K. Ballas.

Cardeza Foundation for Hematologic Research, Department of Medicine, Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia, PA, USA.

Competing interests: The authors declare no conflict of Interest.

Abstract. Sickle cell disease (SCD) is an extremely heterogeneous disease that has been associated with global morbidity and early mortality. More effective and inexpensive therapies are needed. During the last five years, the landscape of the pharmacotherapy of SCD has changed dramatically. Currently, 54 drugs have been used or under consideration to use for the treatment of SCD. These fall into 3 categories: the first category includes the four drugs (Hydroxyurea, L-Glutamine, Crizanlizumab tmca and Voxelotor) that have been approved by the United States Food and Drug Administration (FDA) based on successful clinical trials. The second category includes 22 drugs that failed, discontinued or terminated for now and the third category includes 28 drugs that are actively being considered for the treatment of SCD. Crizanlizumab and Voxelotor are included in the first and third categories because they have been used in more than one trial. New therapies targeting multiple pathways in the complex pathophysiology of SCD have been achieved or are under continued investigation. The emerging trend seems to be the use of multimodal drugs (i.e. drugs that have different mechanisms of action) to treat SCD similar to the use of multiple chemotherapeutic agents to treat cancer.

Keywords: Sickle cell disease; Pharmacotherapeutic.

Citation: Ballas S.K. The evolving pharmacotherapeutic landscape for the treatment of Sickle Cell Disease. Mediterr J Hematol Infect Dis 2020, 12(1): e2020010, DOI: http://dx.doi.org/10.4084/MJHID.2020.010

Published: January 1, 2020 **Received: November 25, 2019** **Accepted: December 17, 2019**

Correspondence to: Samir K. Ballas MD FACP. Cardeza Foundation, Department of Medicine, Sidney Kimmel Medical College, Thomas Jefferson University, 1020 Locust Street, Philadelphia, PA 19107. Tel: 856 745 6380, Fax: 856 795 0809. E-mail: samir.ballas@jefferson.edu

Introduction. Sickle cell anemia (SCA) is among the most common inherited hemolytic anemias, and affects an estimated 100,000 persons in the US and probably millions worldwide.[1] The true global incidence of sickle cell disease (SCD) is unknown. The World Health Organization has estimated that each year 220,000 babies are born with SCD in Africa, and that SCD accounts for up to 16% of deaths of children aged < 5 years in some African countries.[2,3] The reported prevalence of the sickle cell trait in African Americans varies from 6.7 to 10.1% and in Africans the range is from 10 to 40% across equatorial Africa and decreases to between 1 and 2% on the North African coast and < 1% in South Africa.[4-6] The prevalence of the sickle cell trait varies widely worldwide and may be as high as 50% in certain regions.[6-8] The prevalence of SCA is ~ 1 in 600 newborn African American infants and 150,000 - 300,000 newborn Africans.[9-11]

Sickle cell anemia is a hereditary disorder of hemoglobin (Hb) where the sickle gene is inherited, homozygously, from both parents. The sickle mutation is the result of a single base change (GAG → GTG) in the sixth codon of exon 1 of the β-globin gene responsible for the synthesis of the β-globin polypeptide of the Hb molecule (α2β2). This change, in turn, results in replacement of a normal glutamic acid with valine at position 6 of the β-globin chain and the formation of sickle Hb. Sickle erythrocytes are rigid with decreased deformability and reduced life span resulting in hemolysis, vaso-occlusive disease, vasculopathy and subsequent inflammation and end organ damage.[12,13]

Clinical manifestations of SCD include pain syndromes, anemia and its sequelae, organ failure including infection/inflammation and comorbid conditions.[14] The painful acute vaso-occlusive crisis (VOC) is the hallmark of SCD and traditionally, has been thought to be to be due to sickle erythrocytes occluding the microvasculature, especially within bones, and causing tissue ischemia, injury, and pain. Recent studies, however, suggest that the mechanism is a more complex process that is multicellular, involving interactions with the vascular endothelium, as well as contributions from hemolysis, inflammation, and coagulation.[15] Despite having a common genetic basis and similar pathophysiology, individual patients with SCA have a highly variable clinical phenotype. The prevalence of these complications varies with age from infancy through adult life as shown in **Figure 1**. However, pain, infections and anemia requiring blood transfusion occur throughout the life span of affected patients.

Clinical care for affected individuals has been mostly palliative, including supportive, symptomatic, preventative and abortive approaches, as shown in **Table 1**.

Advances in the management of SCD beyond palliation include pharmacotherapy and curative cellular therapies. The latter include stem cell transplantation and gene therapy[15,16] and these will not be addressed in this review. In addition, some of the current approaches to the management of SCD could be pharmacologic or nonpharmacologic, especially when it comes to pain management. Examples of nonpharmacologic treatments include meditation, therapeutic massage, transcutaneous electrical nerve stimulation, heat and cold packs, distraction, relaxation, music, guided imagery, self-hypnosis, acupuncture and biofeedback.[13,17] Current examples of pharmacologic therapies include the use of non-steroidal anti-inflammatory drugs, opioids, adjuvants, steroids, and so on.[13] The aim of this study is to review the current status of pharmacotherapy for the treatment of SCD, Historically, pharmacotherapeutic drugs that have been tried to treat SCD fall into three groups. The first group includes the successful drugs approved by the FDA shown in **Table 2**. The second group includes the drugs that were tried but failed to show a beneficial effect shown in **Table 3**. The third group includes potential drugs that are being used in different phases of randomized clinical trials shown in **Table 4** and will be discussed below.

The Economic Burdens of SCD. Sickle cell disease is a global disease affecting millions of people worldwide and hundreds of thousands in the US. It affects not only those of African descent, but also persons of Middle Eastern, Indian, Latin American and Mediterranean descent. It has received very little attention and even less research funding. National Institute of Health (NIH) grants for sickle cell research were much less than that for less-common inherited diseases. In 1972, the National Sickle Cell Anemia Control Act was signed, which paved the way for more research funding

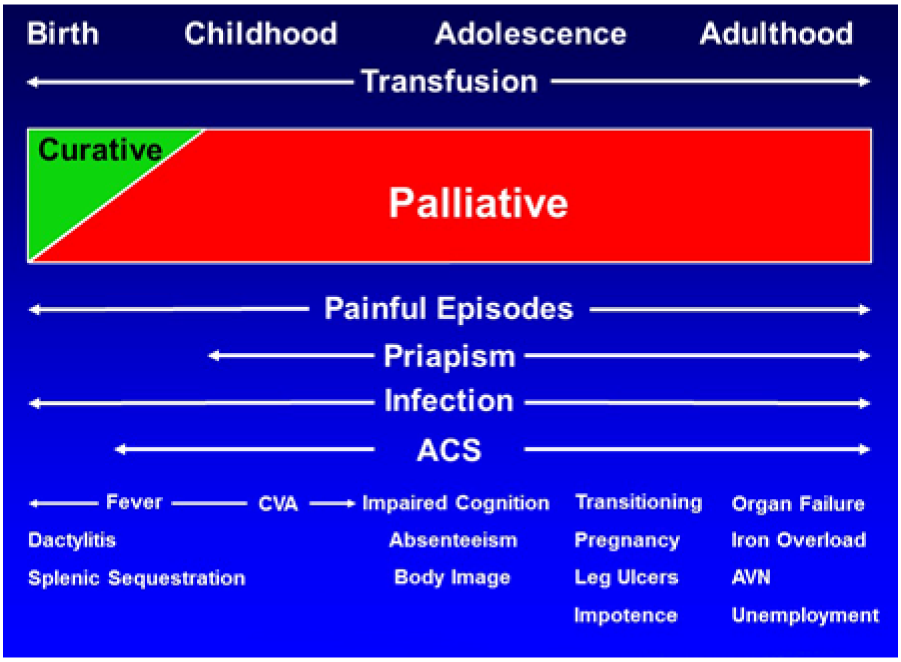


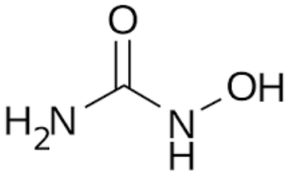
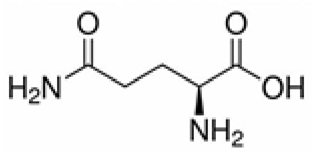
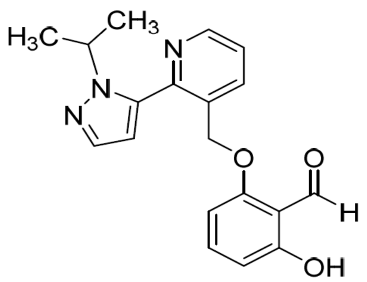
Figure 1. Sequence of complications of sickle cell anemia from birth through adult life. ACS = acute chest syndrome; AVN = Avascular necrosis; CVA = Cerebrovascular accident. From Hem Onc Clin North Am. 2005; 19:785-802. Used with permission.

Table 1. Palliative Management of Sickle Cell Disease and its Complications.

Management	Definition
1. Supportive	Management intended to maintain the essential requirements for good health such balanced diet, sleep, hydration, folic acid, etc.
2. Symptomatic	Management targeted to alleviate the symptoms of the disease as they occur. These include blood transfusion for symptomatic anemia, analgesics for pain, antibiotics for infections, etc.
3. Preventative	Approaches to prevent the occurrence of complications of the disease. These include things like vaccination, avoidance of stressful situations, transfusion to prevent the recurrence of stroke, etc.
4. Abortive	Major purpose of this approach is to abort painful crisis thus preventing them from getting worse or precipitating other complications. Nitric oxide aborted VOCs in the ED but not during hospitalization.

Adapted from Blood. 2012;120(18):3647-56. Used with permission.

Table 2. Approved Drugs.

Compound	Company	Structure	Mechanism of Action	Formulations	Indication
Hydroxyurea	Numerous	Hydroxycarbamide CH4N2O2 	An antineoplastic agent that inhibits DNA synthesis through the inhibition of ribonucleotide diphosphate reductase. Hb F Induction, reduces inflammation and hemolysis	Capsules: Hydrea and Droxia; 100, 200, 300, 400, 500 mg Tablets: Siklos;100 mg, 1000 mg Solution 100 mg/ml or higher as needed	Sickle cell anemia; Myeloproliferative disorders, certain cancers
L-Glutamine (Endari)	Emmaus Medical Inc.	2-Amino-4-carbamoylbutanoic acid 	Not well known. It may improve the NAD redox potential in sickle RBCs through increasing the availability of reduced glutathione.	Powder in packets containing 5g each	Reduction of the acute complications of sickle cell disease in adult and pediatric patients 5 years of age and older
Crizanlizumab-tmca	Novartis	Monoclonal antibody	Anti-P-selectin	Intravenous solution	Reduces the frequency of VOCs in adults and pediatric patients aged 16 years and older
Voxelotor (Oxbryta, GBT440)	Global Blood Therapeutics Inc	Benzaldehyde, 2-hydroxy-6-((2-(1-(1-methylethyl)-1H-pyrazol-5-yl)-3-pyridinyl)methoxy) 	Hb S Polymerization inhibitor	Tablets (500 mg) for oral use	Treatment of sickle cell disease in adults and pediatric patients 12 years of age and older

NAD = nicotinamide adenine dinucleotid.

and established screening and education programs. The NIH dedicated $10 million to be spent on SCD research at that time.[13] The economic burden to patients with SCD is significant.[18-22] Many patients are living in poverty with their illness due to chronic pain, and physical disability limiting their ability to work and contribute to society.[13] The economic burden on society was estimated at $1.1 billion in 2009.[18] This number is projected to increase as patients with SCD are living longer as we continue to improve supportive care. A solution to this problem is not simple, requiring multidisciplinary action with increased funding, legislation, research and supportive services. Simple therapy with hydroxyurea (HU) is still not available to

Table 3. Completed multicenter randomized double-blind placebo-controlled trials to prevent or treat sickle painful crises that failed, discontinued or terminated.

Compound	Company	Mechanism of Action	Indication	Stage of Development	Reference
Acetylsalicylic acid	Takeda	Benzoic acid, 2-(acetyloxy)-	General pain and thrombosis, SCD	Phase I and II study for SCD completed	[1]
AES-103	AesRx	Anti-sickling agent	Anemia; SCD	Phase I study for SCD completed. Phase II study for SCD terminated by the Sponsor due to unbinding between study drug and placebo groups at the subject, site and Sponsor levels	[2,3]
Dipyridamole	Boehringer Ingelheim Pharmaceuticals, Inc	RBC hydration	Thrombosis; SCD	Phase II study withdrawn	[4]
Eptifibatide	Millennium and Schering Plough	Antiplatelet agent Use as therapeutic agent for VOC	Acute myocardial infarction, unstable angina, abrupt closure following coronary angioplasty, stroke and other diseases associated with arterial thrombosis; treat VOC	Phase II for SCD terminated due to slow accrual and no cost extension not approved by NHLBI	[5]
HQK 1001	HemaQuest	γ globin gene promoter	SCA and β-Thalassemia	Phase II for SCD terminated	[6]
Inhaled Nitric Oxide (NO)	Ikaria	Vasodilator	Therapeutic for VOC	Phase III for SCD; Failure	[7]
L-citrulline	Asklepion	Vasodilator	Pediatric pulmonary hypertension, post-cardiopulmonary bypass surgery; SCD	Ceased; Phase I for SCD	[8]
Magnesium Sulfate ($MgSO_4$)	Numerous companies produce magnesium as magnesium oxide, magnesium citrate, magnesium sulfate, magnesium gluconate and magnesium pidolate	RBC hydration Therapeutic agent	Vitamin supplement; Treat VOC	Phase II and III for SCD; Failure	[9]
MP4CO	Sangart	Prevents microvascular stasis; Therapeutic agent	Anemia; Treat SCD	Discontinued; Phase I completed. Phase II withdrawn prior to enrollment for SCD	[10,11]
Nonionic polyoxyethylene-polyoxypropylene; Poloxamer 188 (Flocor)	CytRx	Oxirane, methyl-, polymer with oxirane, block, Therapeutic agent	Surfactant	Treat VOCs and ACS in SCD and acute myocardial infarction	[12-13]
Omega-3-acid ethyl esters	Glaxo Smith Kline	Anti-inflammatory agent	Improves several cardiovascular risk factors: lowers serum triglyceride concentration, lowers blood pressure, reduces resting heart rate, improves endothelial dysfunction; SCD	Phase II for SCD terminated due to manufacturing problem with study drug	[15,16]
Prasugrel (DOVE Trial)	Eli Lilly	Inhibition of platelet activation and aggregation	Prevention of VOC	Failure	[17]
Senicapoc	Pfizer	Gardos channel blocker, Preventive agent	Prevention of VOC	Phase II completed for SCD; Drug increased red cell survival and hematocrit and blood viscosity; Phase III trial failed	[18,19]
Sildenafil		Preventive agent	Prevention of VOC	Failure	[20, 21]
Sodium nitrite	Hope	Used to treat cyanide poisoning, Therapeutic agent for leg ulcers	Vasodilator; treat SCD leg ulcers	Phase I and II study for SCD terminated due to low enrollment	[22]

TRF-1101	TRF Pharma	Anti-sickling agent	SCD	Phase I study completed and successfully demonstrated improved microvascular blood flow in patients with SCD and revealed no drug-related side effects. Phase II study terminated due to perceived futility because the baseline pain score in first 40 patients was too low to be able demonstrate improvement.	[23]
Varespladib sodium	Shionogi	Inhibitor of secretory phospholipases A2 (sPLA2)	Therapy for acute chest syndrome in SCA	Discontinued; no current studies being conducted in relation to SCD	[24]
Vepoloxamer 18 (EPIC)	Mast Therapeutics	Similar to Poloxamer 188	Therapeutic for VOC	Failure	[25]
Vorintostat	Merck & Co.	Hb F induction	Cutaneous T-cell lymphoma; SCD	Phase II terminated due to slow accrual	[26]
Sevuparin	Modus therapeutics	Polysaccharide-based drug that is designed to retain the anti-adhesive properties of heparin. Therapeutic agent	Treatment of VOCs	Underwent Phase I and II Trials. Failed to Show Clinically Meaningful Improvements in Managing VOCs,	[27]
Rivipansel sodium; GMI-1070	GlycoMimetics	1,3,6-Naphthalenetrisulfonic acid, 8-[[13-[(1R,3R,4R,5S)-3-[[2-O-benzoyl-3-O-[(1S)-1-carboxy-2-cyclohexylethyl]-_-D-galactopyranosyl]oxy]-4-[(6-deoxya-L-galactopyranosyl)oxy]-5-[[(1,2,3,6-tetrahydro-2,6-dioxo-4 pyrimidinyl) carbonyl] amino] cyclohexyl]-	Inflammation and VOCs in SCD. Therapeutic agent	Phase III to treat VOC failed	[28,29]
Sanguinate	Prolong Pharmaceutical	Sanguinate is PEGylated Bovine Carboxyhemoglobin	Designed to prevent clumping of RBC and maintain blood flow. Therapeutic agent	Phase II trial to treat VOC failed	[30]

NHLBI: National Heart, Lung, and Blood Institute; RBC = Red blood cell; SCA = Sickle cell anemia; SCD = Sickle cell disease; VOC: Vaso-occlusive crisis.

the millions in Africa today. As we continue to push for new therapies for SCD, HU continues to have tremendous potential in the global marketplace.

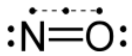
Evolution of the Approaches to Treat SCD. Since sickled cells were first described in 1910 and the mutation causing abnormal Hb S was identified in 1949, the complex mechanism underlying its pathophysiology continues to evolve.[23] A cascade of events driven by endothelial damage and inflammation leads to vasculopathy. The inciting event is injury to the red blood cell (RBC) membrane. Hemoglobin S polymerization impairs deformability of the RBC and causes oxidative injury and destruction of the RBC. RBC injury exposes phosphatidyl serine and releases Hb and other intracellular contents. This in turn depletes NO, increases endothelial adherence, releases proinflammatory cytokines and activates the coagulation cascade causing ischemia, reperfusion injury and vascular damage.[12,17,23]

Damaged sickle cells are prone to adhere to the endothelium by adhesion molecules. The RBC membrane receptors VLA-4/a4b1 bind to endothelial receptors directly to vascular cell adhesion molecule 1 (VCAM-1) and interacts with subendothelial matrix proteins (BCAM/LU, a4b1 with the laminin and von Willebrand factor).[24,25] Red blood cell interactions with the vascular endothelium also lead to the production of oxygen radicals by activating transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB). NF-kB upregulates the production of endothelial adhesion molecules such as E-selectin, VCAM-1 and intracellular adhesion molecule-1 (ICAM-1). P-selectin and E-selectin on endothelial cells have been suggested to participate in.[26,27]

In preclinical studies an anti-P-selectin molecule

Table 4. Potential drug therapies for the management of SCD.

Compound	Company	Structure	Indication	Stage of Development	Mechanism of Action
Decitabine	Astex Pharmaceuticals	5-aza-2'-deoxycytidine	Myelodysplastic syndrome, SCD	Phase II study completed for SCD	DNA methylase inhibitor; Hb F induction
Sodium butyrate	Sigma Aldrich	$C_4H_7NaO_2$	Inhibit tumor cell growth, SCD	No current studies being conducted in relation to SCD	Hb F induction
Pomalidomide	Celgene Corporation	$C_{13}H_{11}N_3O_4$	Graft versus host disease, myelofibrosis, scleroderma and idiopathic pulmonary Fibrosis, SCD	Phase I study completed for SCD	Hb F induction
Panobinostat	Novartis	(2E)-N-Hydroxy-3-[4-[[[2-(2-methyl-1H-indol-3-yl) ethyl]amino]methyl]phenyl]-2-propenamide	Treatment of multiple myeloma and other cancers, SCD	Phase I for SCD	Hb F induction
Intravenous Ig	GRIFOLS BIOLOGICALS, Inc.	$C_{6332}H_{9826}N_{1692}O_{1980}S_{42}$	Plasma protein replacement Therapy, SCD	Recruiting for Phase I/II for SCD	Inhibits cellular adhesion
Low-molecular weight heparin	Sanofi	$(C_{26}H_{40}N_2O_{36}S_5)n$	Deep vein thrombosis, Myocardial infarction, unstable angina, SCD	Phase II for SCD	Inhibits cellular adhesion
Dalteparin	Pfizer	2-O-sulpho-α-L-idopyranosuronic acid structure at the nonreducing end and a 6-O-sulpho-2,5-anhydro-D-mannitol structure at the reducing end of their chain	Acute venous thromboembolism, SCD	Phase II completed for SCD	Inhibits cellular adhesion
SelG1 (Crizanlizumab)	Selexys Pharmaceuticals; Now Novartis	Humanized P-selectin antibody	VOCs in children and adults with SCD	Sustain Trial Phase II completed for VOC; Four other trials are ongoing	Inhibits cellular adhesion
Propranolol	Forest Laboratories	2-Propanol, 1-[(1-methylethyl) amino]-3-(1-naphthalenyloxy)	Hypertension, SCD	Phase II completed for SCD	Inhibits cellular adhesion
Regadenoson: Adenosine 2A receptor antagonist	Gilead Sciences	1-(6-Amino-9-β-D-ribofuranosyl-9H-purin-2-yl)-N-methyl-1H-pyrazole-4-carboxamide	Vasodilator	Phase II completed for SCD	Anti-inflammatory Agent
NKTT120	NKT Therapeutics	Humanized antibody to iNKT	Rapid and sustained iNKT cell depletion in adults with SCD	Phase I study for SCD completed	Reduce chronic inflammation associated with SCD
Atorvastatin Statins	FA Davis	(C33H34FN2O5)2Ca. 3H2O	Improves endothelial dysfunction in SCD with nephropathy	Phase II trial has just been completed	3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor
Zileutin	Abbott Laboratories and now Cornerstone Therapeutics Inc.	C11H12N2O2S	Approved for the prophylaxis and treatment of chronic asthma for patients who are age 12 and older. Beneficial in SCD animal model	Phase I trial completed	Inhibits 5- lipoxygenase, a potent inflammatory leukotriene
N-acetyl cysteine	Galleon Pharmaceuticals	L-Cysteine, N-acetyl C5H9NO3S	Rad/chemotherapy-induced mucositis; Radio/chemotherapy induced injury, bone marrow, thrombocytopenia; apnea, SCD	Phase III for SCD completed	Anti-inflammatory Agent, reduces oxidative stress in sickle cell patients
α-Lipoic acid	Meda Biotech Inc.	1,2-Dithiolane-3-pentanoic acid C8H14O2S2	Diabetic neuropathy, SCD	In an open randomized trial, the ALA dose used was not effective to prevent oxidative damage in patients with SCD	Anti-inflammatory and anti-oxidant agent

Canakinumab	Novartis	Canakinumab is a recombinant, human anti-human-IL-1β monoclonal antibody that belongs to the IgG1/κ isotype subclass	For Familial Cold Autoinflammatory Syndrome (FCAS), Muckle-Wells Syndrome (MWS) and SCD	Phase II ongoing	Anti-inflammatory
Ambrisentan	Augusta University with Gilead Sciences and NHLBI as collaborators	Endothelin Receptor antagonist (ERA)	Determination of its safety and tolerability in treating SCD	Phase I	Anti-inflammatory, analgesic and improves pulmonary blood low
Rivaroxaban	Bayer	5-Chloro-N-({(5S)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl}methyl) thiophene-2-carboxamide	For treatment of thrombosis including stroke, prevent VOC	Phase I for SCD completed	Direct oral anti-coagulant
Arginine	Numerous companies produce since this is a vitamin supplement	2-Amino-5 guanidinopentanoic acid	Chest pain, high blood pressure and peripheral arterial disease, SCD	Phase III study for SCD completed in Brazil. Phase II study for SCD completed in US. Another Phase II study for SCD is recruiting in the US.	Vasodilatation
Inhaled Nitrix Oxide (NO) in the ED	INO Therapeutics		Therapeutic for VOC in the ED	double-blind, randomized, placebo controlled clinical trial	Vasodilatation
PF 04447943	Pfizer	6-[(3S,4S)-4-methyl-1-(pyrimidin-2-ylmethyl)pyrrolidin-3-yl]-1-(tetrahydro-2H-pyran-4-yl)-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one	Evaluate change from baseline in potential SCD-related biomarkers	Phase 1b for patients with SCD completed	Vasodilatations; Phosphodiesterase 9A inhibitor
IMR-687	Imara Inc	6-[(3S,4S)-4-Methyl-1-(2-pyrimidinylmethyl)-3-pyrrolidinyl]-3-(tetrahydro-2H-pyran-4-yl)imidazo[1,5-a]pyrazin-8(7H)-one Molecular formula C21 H26 N6 O2	SCA (Homozygous HbSS or Sickle-β0 Thalassemia)	Phase 1a completed. Phase 2a is recruiting with an open extension study	Phosphodiesterase inhibitor with multimodal mechanism of action: Vasodilatation Inhibition of white blood cell adhesion Increase Hb F level
Riociguat	Bayer	Methyl 4,6-diamino-2-[1-(2-fluorobenzyl)-1H-pyrazolo [3,4-b]pyridin-3-yl]-5-pyrimidinyl(methyl) carbamate	Multicenter study in patients with SCD	Phase 2 multi-center, randomized, double-blind, placebo-controlled, parallel groups study. Recruiting	Riociguat is a stimulator of soluble guanylate cyclase (sGC), an enzyme in the cardiopulmonary system and the receptor for NO resulting in vasodilatation
Oinciguat (IW-1701)	Ironwood, Cyclerion Therapeutics	OlinciguatUNII-PD5F4ZXD2 $C_{21}H_{16}F_5N_7O_3$	Patients with SCD	STRONG SCD to evaluate the safety and tolerability of different dose levels in SCD patients. Recruiting	Stimulates guanylate cyclase (sGC), known to play a key role in the production of nitric oxide. Vasodilatation
Voxelotor (GBT-440)	Global Blood Therapeutics Inc	Benzaldehyde, 2-hydroxy-6-((2-(1-(1-methylethyl)-1H-pyrazol-5-yl)-3-pyridinyl)methoxy)	Prevention of VOCs and treatment of other complications	After successful phase I and II trials, Phase 3 HOPE trial Voxelotor significantly increased Hb levels compared to placebo and reduced markers of hemolysis. Exploratory post-hoc trial showed that Voxelotor resolved or improved leg ulcers in some patients.	Inhibition of Hb S polymerization. Increases the affinity of Hb S to oxygen

FT-4202 Pyruvate Kinase Activator (PKR)	Forma Therapeutics, Inc.; Medpace, Inc.	An oral small-molecule agonist of pyruvate kinase red blood cell isozyme (PKR)	Treatment of hemolytic anemias	Phase I	Agonist of pyruvate kinase enzyme
Niacin (Vitamin B3)	AbbVie Ltd	$C_6NH_5O_2$	Reduces risk of heart disease, improves blood flow in people with SCD	Phase II study completed for SCD	Increases levels of HDL and improves blood flow
Cholecalciferol (Vitamin D3)	Numerous companies	25-Hydroxyvitamin D_3	Vitamin supplement, SCD	Phase study with adult patients with SCD completed. Phase I and II completed with pediatric patients with SCD. Phase III for pediatric patients with SCD not yet recruiting	Supplementary vitamin

ADP: Adenosine diphosphate; ED = Emergency department; Hb: Hemoglobin; HDL = High-density lipoproteins; kDa: Kilodalton; NO: Nitric oxide; RBC: Red blood cell; SCA: Sickle cell anemia; SCD: Sickle cell disease; VOCs: Vaso-occlusive crises.

showed increased microvascular flow and reduced adhesion of leukocytes to the endothelium.[26] ICAM-4, another RBC membrane protein, which participates in adhesion, can be activated by epinephrine to adhere to endothelial membrane and exacerbate vaso-occlusive disease and also increased leukocyte adhesion to endothelium.[27] When treated with propranolol (a b-adrenergic receptor antagonist) VOCs were diminished.[28,29]

In addition to adherence to endothelial cells, RBCs in SCA also adhered strongly to leukocytes in VOCs via interactions with P-selectin and E selectin. This interaction is propagated by TNF-a. Selectins function in adhesion to the vessel wall by recruiting rolling particles and cells and also contribute to cell activation. Patients with SCD have chronic elevation of proinflammatory cytokines at baseline, including C-reactive protein, TNF, IL-1 and IL-8. Damaged RBCs, activated endothelial cells, leukocytes and platelets (PLTs) contribute to a proinflammatory environment. Sickled RBCs stimulate endothelial cells to release TNF-α and IL-1β. There is increased production of placental growth factor, which activates monocytes to release reactive oxygen species (ROS), which enhances inflammation.

Additionally, invariant natural killer T (iNKT) cells are activated in patients with SCD, suggesting that iNKT cells may play a critical role in mediating inflammation. Intravascular hemolysis results in release of cell-free Hb in plasma, and hemin release that contribute to the inflammation.[25,30] Nitric oxide (NO) is produced by the endothelium from arginine and causes vasodilation by binding to endothelin-1, a vasoconstrictor. Intravascular hemolysis releases Hb, which scavenges NO in the plasma and subendothelial spaces.

Depletion of NO leads to vasoconstriction and formation of ROS. Nitric oxide also downregulates adhesion molecules, VCAM-1, ICAM-1 and E-selectin. Erythrocyte arginase released during hemolysis decreases arginine levels and decreases NO production. The byproducts of these reactions, urea, proline, polyamines and free radicals, cause vascular remodeling and vasculopathy. Patients with SCD have elevated asymmetric dimethylarginine, which inhibits arginine transport and promotes endothelial dysfunction.[17,31,32]

These inflammatory processes activate the coagulation cascade. Phosphatidylserine expression on RBC surface and microparticles activates tissue factor and, in turn, the extrinsic coagulation cascade. Tissue factor also promotes inflammation and endothelial damage. In preclinical studies in transgenic sickle mice, lowering tissue factor levels resulted in lower plasma levels of IL-6 and soluble VCAM-1.[33] Sickle cell disease is a chronic inflammatory state and ROS are increased at baseline compared with normal controls. Hemolysis releases Hb, and iron products, which increase ROS that generate superoxide (O2-) and peroxynitrate (ONOO-), which promotes an inflammatory response and causes cell death. Patients with SCD have impaired buffer system with decreased glutathione, and other antioxidants.[34-36]

Approved Pharmacotherapeutic Drugs. The ideal drug for SCD would have analgesic properties, be able to prevent VOCs or abort them with a rapid onset of action, would decrease the severity and frequency of VOCs, have limited hazardous side-effect profile and be effective in all patients, and available globally. Currently HU, L-glutamine, Crizanlizumab tmca and Voxelotor shown in **Table 2**, are the only agents that fit some of these criteria and are approved by the FDA.

Hydroxyurea. Hydroxyurea has many qualities of the ideal drug for SCD. It was first synthesized in 1869 and used in myeloproliferative disorders. Chemically it is a synthetic urea analog; also referred to as hydroxycarbamide (HC) that functions as an antineoplastic agent. In this review HU and HC are used synonymously. There is seemingly a tendency to use the HU acronym in the US and HC acronym in the UK. Hydroxyurea was identified as a potent Hb F inducer and was subsequently found to be both a feasible and effective treatment option for SCA.[13] It decreases the frequency of VOCs, acute chest syndrome (ACS), and the frequency of blood transfusion. In addition, HU improves the quality of life and decreases mortality in patients with SCA.[37] However, HU is not effective in about 25% of those with SCA, an acronym that also includes sickle-β^0-thalassemia (S-β^0-T).[38] Currently, it was found to be teratogenic and possibly carcinogenic in animal studies[39] but not in humans so far. It was the first pharmacotherapeutic drug to be approved by the FDA and by the European Medicines Agency (EMA) for the treatment of SCA.

Hydroxyurea is cell cycle specific for the S phase and inhibits DNA synthesis as a ribonucleotide reductase inhibitor. It induces the production of Hb F in the majority of patients with SCA who are compliant with therapy and thus prevents the formation of Hb S polymers.

The molecular mechanisms by which HU induces Hb F production are not fully clear. Proposed mechanisms include selectively killing cells in the bone marrow, and increasing the number of early erythroid progenitors such as fetal erythroblasts that lead to production of Hb F. It also reduces the number of adhesive reticulocytes[40] and circulating inflammatory cells such as monocytes and neutrophils. It alters circulating monocyte subsets and dampens the inflammatory potential of SCD.[41,42] It also improves RBC deformability.[43] More recently, HU was reported to have antioxidant activity.[44] It appears that patients whose high neutrophil and reticulocyte counts decrease significantly after HU therapy have a higher increase in Hb F levels.[3,21,45] In addition, HC affects the plasma proteome of children with SCA resulting in reduced inflammation and decreased activation of the coagulation factors.[46] The increased Hb F induced by HU decreases the biomarkers of oxidative stress and the scavenging of NO in both sickle cell mice and in patients with SCD.[44,47,48]

More complex effects of HU involve the production of NO, guanylyl cyclase and cGMP dependent protein kinase pathway important in inducing expression of the γ-globin gene. Additionally, HU improves erythrocyte deformability, lowering of circulating leukocytes and reticulocytes, and reduces hemolysis.[3,49,50] Since its first clinical application reported in 1984 by Platt et al., many trials were performed.[51] The Multicenter Study of HU in SCA, a placebo-controlled randomized Phase III trial of 299 adults with severe SCA, terminated early due to significant reductions in frequency of VOC, ACS, need for blood transfusion and delayed onset of first VOC.[52,53] This study led to the FDA approval of HU for therapy on February 25, 1998 for moderately or severely affected adults with SCA. The Pediatric Hydroxyurea Phase III Clinical Trial (BABY HUG), involving infants with SCA randomized either to HU (fixed dose 20 mg/kg/day) or placebo. This trial showed that HU did not clearly prevent organ damage, the primary endpoint of the 2-year treatment period, but significantly decreased the secondary endpoints: pain, ACS, hospitalizations, and transfusions in children.[54-59]

Formulations of HU are shown in **Table 2**. It is available as capsules or tablets. Solutions of 100 mg/ml or higher can be prepared by pharmacist as needed.[60] The usual staring dose is 15 mg/k/day. This may be increased gradually every month as needed to achieve the maximum tolerable dose. Some providers maintain a dose that increases Hb F to a desirable level before achieving the maximum tolerable dose.

The common side effects of HU are listed in **Table 5**. Toxic effects are dose and time dependent and can be prevented by careful monitoring and surveillance. Side effects are generally reversible with cessation or decrease of the drug dose. Hydroxyurea is myelosuppressive and leukopenia is the most common manifestation followed by thrombocytopenia and anemia. Macrocytosis is common and may mask folic acid deficiency, so folic acid supplementation is recommended during treatment with HU. Idiosyncratic side effects are rare, reversible and more common in generic formulations.[61] **Figure 2** shows an example of HU-induced melanonychia.

Phase IV of the HU study which refers to its use in the general population post-approval by the FDA, showed a plethora of publications globally addressing various aspects of its pros and cons. Most important among these are as described below.

a. Adherence to HU Therapy. The BABY HUG trial, which demonstrated safety and efficacy of

Table 5. Side Effects of Hydroxyurea.

Myelosuppression
Leukopenia/Neutropenia Thrombocytopenia Anemia
Megaloblastic Erythropoiesis
Idiosyncratic Nausea, Vomiting Stomatitis, Anorexia, Diarrhea Constipation Skin rash Erythema, Pruritus Hair Loss Hyperpigmentation, horizontal & Longitudinal **Melanonychia** Decreased Libido Partial complex seizure
Long Term Effects Unknown

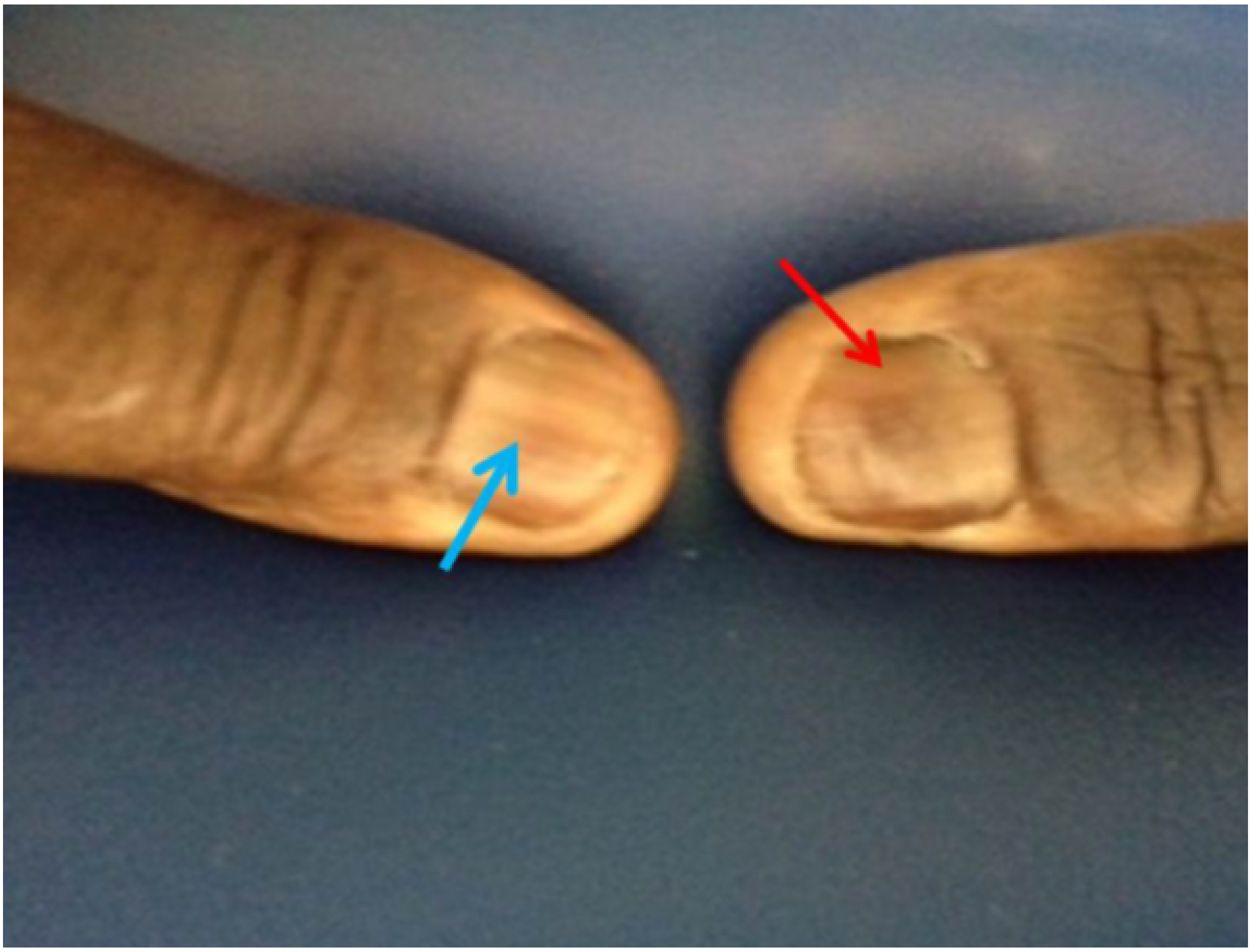


Figure 2. Fingernails of a 38-year-old man with sickle cell anemia and hydroxyurea-induced melanonychia characterized by longitudinal (blue arrow) and diffuse (red arrow) bands. From J Blood Disorders Transf. 2013;4:5. Used with permission.

starting HU in infancy contributed to a robust increase in HU prescribing for children with SCD.[62] Hydroxyurea use in infants 5-12 months old resulted in a better response compared with use in older patients.[63] Moreover**,** prospective longitudinal follow-up of children with SCD treated with HU since infancy was highly effective in preventing complications of SCD.[64] Pediatric hematologists strongly recommend the use of HU in children with SCD early and frequently.[65]

Unfortunately, access to specialist care for adolescents and adults with SCD is limited and associated with many barriers. Most important among these include appointment non-adherence.[66] Factors that seem to influence these barriers may be provider- or patient-related. Thus, patients who felt their providers were not listening to their concerns tended to be non-adherent to HU therapy.[67]

Similarly, at the global level the use of HU for the treatment of patients with SCD varied considerably. The universal administration of HU to children with SCD was successful in Malawi[68] but not in Nigeria[69] where concerns about its long-term safety and toxicity limited its prescription by physicians and acceptability by patients. The major barriers to the use of HU in the treatment of SCD in Nigeria included lack of national guidelines for the use of HU, concerns for infertility and safety profile of HU in pregnancy and lactation.[69]

b. Hydroxyurea and Stroke. According to the Cooperative Study of SCD (CSSCD), stroke occurred in 11% of children with SCA younger than 20 years of age and 24% of adults by the age 45.[70] However, the use of transcranial Doppler (TCD) in the Stroke Prevention in SCA (STOP 1) trial to identify persons at higher risk for ischemic stroke, along with the prophylactic management of those patients with chronic transfusion (simple or RBC exchange), has dramatically reduced the incidence of childhood primary stroke to 2% to 3%.[71,72] The STOP 2 trial determined that regular transfusion for primary stroke prevention could not be halted safely, even in patients with a normal magnetic resonance angiogram whose TCD results have normalized.[72,73]

Discontinuation of transfusions after 30 months resulted in a high rate of reversion to abnormal TCD velocity and stroke.[72,73] A number of studies indicate that transfusion to prevent the recurrence of strokes should be performed indefinitely, even after transition to adult programs.[74-76] The advent of HU raised the possibility if it could replace or decrease the need for transfusion to prevent the recurrence of stroke. However, the Stroke with transfusions changing to HU

(SWITCH) trial and the Transcranial doppler with transfusions changing to HU (TWITCH) trial were not successful [77,78] and blood transfusion and iron chelation therapy remain the better choice for the prevention of primary and secondary stroke in patients with SCA. Nevertheless, HU treatment of children with SCA is associated with more intact brain white matter integrity by using quantitative MRI[79] and prevents the conversion to abnormal transcranial doppler in SCA.[80] The NIH guidelines for the management of SCD indicated that if it is not possible to implement a transfusion program in children and adults who have had a stroke, then HU therapy is recommended.[38]

c. *Hydroxyurea and Leg ulcers.* The effect of HU on leg ulcers in patients with SCD is controversial, though it has been reported to cause leg ulcers in patients with myeloproliferative syndromes.[81] Data on leg ulcers from the Cooperative Study of Sickle Cell Disease (CSSCD) identified five risk factors associated with leg ulcers in patients with SCD.[82] Leg ulcers were more common in males and older patients and less common in patients with α-gene deletion, high total Hb level and high levels of Hb F. Since HU is known to increase total Hb level and Hb F, one would expect that HU would be protective against the development of leg ulcers. Nevertheless, there are anecdotes of leg ulcers occurring after therapy with HU and of healed old ulcers reactivated after HU therapy.[83] de Montalembert et al followed a cohort of 101 children with SCD treated with HU for a median of 22 months; among these only one 18 year-old patient had leg ulcers 23 months after treatment.[84]

d. *Hydroxyurea: pregnancy and lactation.* The FDA developed a system to rate medications and drugs based on potential benefits and risks to the fetus. Drugs are classified into pregnancy categories A, B, C, D, and X where A is safe and X contraindicated. Hydroxyurea is classified as a category D drug; these drugs have positive evidence human fetal risk but use may be justified in some circumstances. Because HU, an S-phase antineoplastic drug, is known to be carcinogenic, mutagenic, and teratogenic in animals, a major inclusion criterion in the Multicenter Study of HU in SCA (MSH) was the use of contraceptives both by females and males, to avoid fetal exposure to HU. Despite this precautionary measure, some women have become pregnant while they or their male partners were taking HU. Surviving patients enrolled in the original MSH trial for up to 17 years post randomization were followed.[37] The findings suggested that exposure of the fetus to HU did not cause teratogenic changes in those pregnancies that terminated in live birth, whether full term or premature.[39] This appears to be true whether the parent taking HU was the mother or the father. Safety of HU during pregnancy and SCD was also reported in 3 other patients.[85,86] Safety of HU during pregnancy was also reported in other hematologic disorders.[86]

The NHLBI evidence-based SCD guidelines identified the safety of HU during gestation and subsequent lactation as an important knowledge gap that requires further investigation. A clinical trial for that purpose is underway.[87]

Similarly, breastfeeding is usually contraindicated during maternal therapy with antineoplastic drugs, but the evidence of this recommendation for HU is very weak.[38,88] Current recommendations state that breastfeeding should be avoided for at least 3 hours after the mother takes HU.[89] Currently, clinical trial [NCT02990598]: Hydroxyurea Exposure in Lactation A Pharmacokinetics Study (HELPS) (HELPS) is underway to examine the pharmacokinetics and distribution of oral HU when administered as a single dose to lactating women.[90]

L-Glutamine (Endari). L-glutamine is an amino acid used in the synthesis of protein. It is the most abundant amino acid in human blood.[91] The body can usually synthesize sufficient amounts of L-glutamine, but in some instances of stress, the body's demand for glutamine increases, and glutamine must be obtained from the diet. Accordingly, it is a non-essential and conditionally essential amino acid in humans. Reduced glutathione is the primary buffer for reactive oxygen species (ROS).

L-glutamine is metabolized to glutamate, the glutathione precursor, and preserves intracellular nicotinamide adenine dinucleotide (NAD), which is necessary for glutathione recycling. Oral supplementation of glutamine in SCD increases the NAD redox potential and may reduce sickle erythrocyte adhesiveness.[32,33] Decreased NAD redox potential due to low level of L-glutamine was a major mechanism for the presence sickle RBCs under oxidant stress conditions.[92] Oral glutamine was developed by Emmaus Medical for the treatment of short bowel syndrome and in SCA and β thalassemia. It decreases the resting energy expenditure in children with SCD. A multicenter Phase III trial of L-glutamine supplementation in 230 children to prevent VOC is completed; results wed that the median number of pain crises over 48 weeks was lower among those who received oral therapy with L-glutamine, administered alone or with HU, than among those who received placebo, with or without HU.[92-95] Two Phase II trials are also completed.[96,97]

Endari was approved by the FDA on July 7, 2017 to reduce the acute complications of SCD in adult and pediatric patients 5 years of age and older.[98] It is available as an oral powder: 5 grams of L–glutamine as a white crystalline powder in paper-foil-plastic laminate packets. It should be administered orally, twice per day at the dose based on body weight as follows: 5 g twice daily for patients weighing < 30 Kg, 10g twice daily for patients weighing 30-65 Kg and 15 g twice daily for patients weighing > 65 kg. Side effects of Endari included low-grade nausea, noncardiac chest pain, fatigue, and musculoskeletal pain occurred more frequently in the l-glutamine group than in the placebo group. There are no available data on Endari use during pregnancy and lactation.

The efficacy of L-Glutamine in the management of SCD awaits the data generated in phase IV post approval in the general population of patients with SCD.

Crizanlizumab tmca (ADAKVEO). The efficacy of SelG1 (Crizanlizumab), a humanized anti-P-selectin monoclonal antibody, in preventing VOCs was evaluated in Phase II SUSTAIN trial in combination with or without HU.[99] Crizanlizumab intravenous therapy resulted in a significantly lower rate of sickle cell-related VOCs than placebo and was associated with a low incidence of adverse events.[99] The FDA approved crizanlizumab-tmca (ADAKVEO, Novartis) on November 15, 2019 to reduce the frequency of VOCs in adults and pediatric patients aged 16 years and older with SCD.[100] The recommended dose is 5 mg/kg intravenously over a period of 30 minutes on week 0, 2, and every 4 weeks thereafter. The most common side effects (>10%) were nausea, arthralgia, back pain, and pyrexia.

Voxelotor (Oxbryta, GBT440). Voxelotor is an inhibitor of Hb S polymerization indicated for the treatment of SCD in adults and children 12 years of age and older. It exerts its action by biding to the amino acid terminal of both α chains of Hb. The efficacy and safety of Voxelotor (OXBRYTA) in SCD was evaluated in a Phase III randomized, double-blind, placebo-controlled multicenter trial in combination with and without HU (HOPE Trial).[101,102] It was approved by the US FDA on November 19, 2019.[103] The approval was accelerated based on increase in Hb. Continued approval for this indication may be contingent upon verification and description of clinical benefit in confirmatory trial(s).

Efficacy was based on Hb response rate defined as a Hb increase of >1 g/dL from baseline to Week 24 in patients treated with OXBRYTA 1,500 mg versus placebo. The response rate for OXBRYTA 1,500 mg was 51.1% (46/90) compared to 6.5% (6/92) in the placebo group ($p < 0.001$).

Recommended dosage of OXBRYTA is 1,500 mg orally once daily with or without food. Recommended dosage for severe hepatic impairment is 1,000 mg orally once daily with or without food. The daily dose of OXBRYTA has to be adjusted in the presence of concomitant medications. Thus, in the presence of strong CYP3A4 inhibitors or fluconazole, the dose should be decreased to 1000 mg once daily. On the other hand, in the presence of strong or moderate CVP3A4 inducers the recommended dose should be increased to 2,500 mg once daily.[103]

Pending Pharmacotherapeutic Drugs for the Treatment of SCD. Currently, there are at least 50 unapproved pharmacotherapeutic drugs that were or are being used or tried to treat SCD during the last two decades. Most of these were multicenter randomized double-blind placebo-controlled trials to prevent or treat sickle painful VOCs. Preventive pharmacotherapy includes drugs that are taken routinely as outpatients with the hope that may decrease the frequency of VOCs that require treatment in the emergency department or hospital. Therapeutic pharmacotherapy includes drugs that are administered after admission to the hospital with the hope that they may abort the VOC and decrease the length of hospital stay and the amount of analgesics used. Twenty-two of these drugs, shown in **Table 3**, failed, discontinued or terminated.

Among the 22 drugs listed in **Table 3**, Rivipansel sodium (GMI-1070), has an interesting history that demonstrates the steps a drug has to go through in order to achieve approval. It is a small-molecule pan-selectin inhibitor that binds to E, P and L selectin that was developed by Glycomimetic to target inflammation in sickle VOCs. It improves blood flow by inhibiting E-selectin and neutrophil activation. A randomized, double-blind, placebo-controlled Phase II trial in 76 subjects hospitalized for sickle cell VOC assessing GMI-1070 is complete. Data showed that the patients treated with rivipansel sodium experienced reduction in duration of VOC, length of hospital stay and reduction in the use of opioids for pain relief. Both adult and pediatric patients demonstrated improvement and adverse event rates were comparable between rivipansel sodium and placebo.[104,105] However, Phase III of the study failed.

Failure of the 22 drugs listed in **Table 3** teaches us at least two important lessons. First, most of the drugs that went through phase III trials failed to treat or abort VOCs or ACS. The approved drugs prevented or decreased the frequency of VOCs. The second lesson is that hydration of sickle RBC does not seem to be an adequate approach in the management of SCD. In the last 2-3 decades hydration of sickle RBC was one of the major approaches to treat SCD. The phase III Senicapoc trial showed that hydration of sickle erythrocytes is counterproductive. This study concluded that hydration of sickle RBC improves their survival which, in turn, increases the blood hematocrit. Consequently, higher hematocrit is associated with increased blood viscosity that promotes vaso-occlusion and the precipitation of a new VOC.

The remaining 28 drugs that are not approved by the

FDA so far but are being used in different stages of clinical trials to prevent or treat VOCs are listed in **Table 4** and discussed below. The mechanism of action of these drugs includes Hb F induction, inhibition of cellular adhesion, anti-inflammatories, surfactants, anti-platelets, vasodilators, anti-adhesives, inhibition of Hb S polymerization, etc. It is rather unfortunate that the majority of these drugs as well as HU were developed for indications other than SCD. This is unlike other rare diseases such as hemophilia and cystic fibrosis for which a few, if any, repurposed drugs are used. The reasons for this disparity are not known. The complex pathophysiology of SCD, its protean clinical manifestations and the suboptimal interest from funders and scientists may be some of the reasons.

Potential Pharmacotherapeutic Drugs for the Treatment of SCDl.

a. Targeting Hb F production: Decitabine is an intravenous cytosine analog 5-aza-2'-deoxycytidine, which hypomethylates DNA by inhibiting DNA methyltransferase. It is approved for treatment of myelodysplastic syndrome. It increases fetal Hb by reactivating the silenced γ-globin through hypomethylation at its promoter site. In a small study of eight patients refractory or intolerant to HU, it increased Hb F and Hb levels when administered subcutaneously.[106] Ongoing trials will further clarify its efficacy and tolerability. A Phase II study with planned enrollment of 40 patients with high-risk SCD is recruiting.[107] A Phase I combination study of oral decitabine with tetrahydrouridine,[108] a competitive inhibitor of cytidine deaminase, is also recruiting and its aim is to evaluate oral bioavailability of decitabine in combination therapy.[109,110]

Pomalidomide is an orally active thalidomide analog developed by Celgene for the treatment of graft versus host disease, SCA, myelofibrosis, scleroderma and idiopathic pulmonary fibrosis. Preclinical studies showed that it induced Hb F production in an SCD model with similar efficacy as HU. Surprisingly, pomalidomide improved erythropoiesis in comparison to myelosuppression seen with HU. However, when given in combination with HU, this effect was lost and fetal Hb levels were suppressed.[111] A Phase I study of pomalidomide in SCD was completed. Twelve patients enrolled and data have not been published.[112]

Panobinostat is a recently approved histone deacetylase (HDAC) inhibitor.[113] A study of panobinostat in patients with SCD is active but not recruiting yet.[114] L-arginine, a substrate for NO, was evaluated in combination with HU in a small randomized trial of 21 adult patients with SCD. There was a greater response in fetal Hb levels and reticulocyte count in the group receiving combination therapy versus HU alone. This study suggests that fetal Hb synthesis depends on NO effect on erythroid progenitors.[115]

b. Targeting adhesion: Intravenous Ig (IVIg) also inhibits leukocyte adhesion and activation by binding to FcγRIII expressed on neutrophils.[116] A Phase I/II trial is currently recruiting to evaluate Gamunex (Intravenous gamma globulin) versus normal saline in sickle cell acute pain.[117]

Low-molecular weight heparins (LMWH). In a randomized clinical trial of 253 patients, *Tinzaparin*, an LMWH, showed reduced duration of VOC and no severe bleeding complications.[118] These results need to be validated in a multicenter study. A recent Phase II trial of an oral P-selectin inhibitor (pentosan polysulfate sodium) similar to heparin but with greater P-selectin blocking ability than heparin showed improved microvascular flow in SCD patients in a Phase I study.[119] Another LMWH, *Dalteparin*, was used in a completed phase II trial.[120]

Crizanlizumab. The efficacy of SelG1 (Crizanlizumab), a humanized anti-P-selectin monoclonal antibody, in preventing VOC was evaluated in five different trials. The first was the successful SUSTAIN trial that was approved by the FDA on November 15, 2019 as described above. The remaining four trials are as follows:

- The STAND trial whose purpose is to compare the efficacy and safety of 2 doses of crizanlizumab (5.0 mg/kg and 7.5 mg/kg) versus placebo in adolescent and adult SCD patients with a history of VOCs leading to healthcare visit.[121]
- The SPARTAN trial to evaluate the safety and efficacy of crizanlizumab in SCD related priapism.[122]
- Phase II CSEG101B2201 study is to confirm and to establish the appropriate dosing and to evaluate the safety in pediatric patients ages 6 months to <18 years with a history of VOC with or without HU, receiving ranibizumab for 2 years. The approach is to extrapolate from the PK/pharmacodynamics already established in the adult population. The study is designed as a Phase II, multicenter, open-label study.[123]
- Phase II multicenter open label study to determine the pharmacokinectics and pharmacodynamics study of SEG101 (criznalizumab) in SCD patients with VOCs.[124]

Propranolol significantly reduced RBC adhesion in a dose-dependent manner. Adverse events were not severe, did not vary with the dose administered and no elevation in heart rate was noted. These results imply that β-blockers have a potential role in inhibiting RBC adhesion.[125] A Phase II study of propranolol in SCD has been completed and no data have been reported at the time that this manuscript was written.[126]

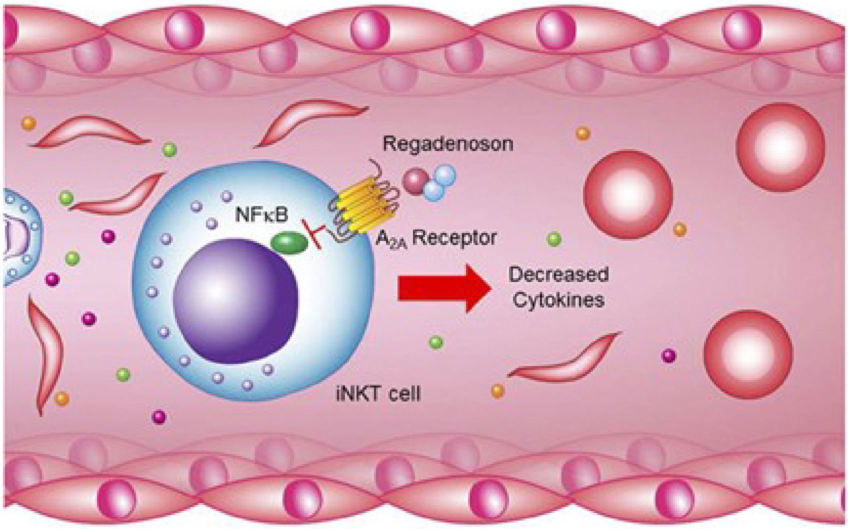


Figure 3: Randomized phase 2 trial of Regadenoson for treatment of acute vaso-occlusive crises in sickle cell disease. From Blood Adv. 2017;1(20):1645-9. Used with permission.

c. *Targeting inflammation*

*Regadenoson***.** In SCA patients there is increase in the number of activated Invariant Natural Killer T (iNKT) cells. Regadenoson is an A2A receptor agonist that reduces the iNKT cells activation and thus decreases inflammation (**Figure 3**). It was developed by CV Therapeutics, now Gilead Sciences, as an adjunct in cardiac perfusion imaging. A Phase I study in 27 adults with SCD showed a 48% decrease in activation of iNKT cells compared to baseline after Regadenoson was administered with no toxicities identified.[127] Randomized phase 2 trial of Regadenoson for treatment of acute VOCs in SCD did not reduce iNKT cell activation to a prespecified level when administered to patients with SCD. Since iNKT cell activation was not reduced, the benefit of iNKT cell-based therapies in SCD cannot be determined.[128] Further studies may be needed.

NKTT-120 is an investigational drug developed by NKT Therapeutics to treat the symptoms of SCA. It is a humanized monoclonal antibody designed to target iNKT cells. Preclinical studies showed rapid and sustained iNKT cell depletion in adults with SCD after the administration of NKTT-120. Depletion of iNKT cells had no effect on other natural killer cells. The T-cell antibody response was not impaired in response to a Keyhole Limped Hemocyanin (KLH) challenge.[129] An open-label, multi-center, single-ascending-dose study of NKTT120 to determine its pharmacokinetics, pharmacodynamics and safety in patients with SCA in the steady state showed rapid, specific and sustained iNKT cell depletion without any toxicity or attributed serious adverse events.[130]

Statins. The vascular injury seen in SCD has been described to share similarities with that of atherosclerosis. Statins decrease inflammation and improve endothelial function in cardiovascular disease and are under study in SCD. They slow the production of cholesterol in the body that may build up on the walls of the arteries and block blood flow to the heart, brain, and other parts of the body. A pilot study of 26 patients treated with atorvastatin showed a dose-related decrease in inflammatory biomarkers (C-reactive protein and IL-6 levels) and increased NO metabolite levels.[131] A Phase II trial of atorvastatin to determine its effect on blood vessels in patients with SCD was first posted in November 2012. The primary hypothesis is that endothelial dysfunction is an important contributor to the pathophysiology of albuminuria in SCD. The investigators propose that atorvastatin will improve endothelial dysfunction, decrease levels of soluble fms-like tyrosine kinase-1 (sFLT-1), and decrease albuminuria in patients with SCD. The study was completed on November 14, 2019. Results not available yet.[132]

Zileuton. Sickle cell disease patients have elevated levels of 5-lipoxygenase, a potent inflammatory leukotriene. Zileuton, a specific inhibitor of 5-lipoxygenase, is FDA approved for asthma. Beneficial effects in the SCD animal model have led to a completed Phase I trial in SCD. It showed that higher dose of Zileuton was safely tolerated by SCD patients with good compliance.[133]

N-acetylcysteine. N-acetylcysteine (NAC) is an inexpensive amino acid derivative that replenishes intracellular levels of the glutathione and it is the rate-limiting substrate for glutathione generation, an important antioxidant with pleiotropic effects on inflammation.[134] NAC inhibits dense cell formation and restores glutathione levels toward normal, which enables the cell to fight damage from ROS. It was used 30 years ago as a mucolytic agent in cystic fibrosis and asthma. In the oral and parenteral routes, it treats acetaminophen toxicity. In pilot studies, the administration of NAC resulted in a reduction of oxidative stress. A Phase II, double-blind, randomized clinical trial was completed to determine the efficacy of NAC in decreasing dense cell and irreversible sickle cell formation and VOC episodes in SCD. NAC inhibited dense cell formation, restored glutathione levels toward normal and decreased VOC episodes.[135] A Phase III trial is underway.[136]

*Canakinumab***.** Canakinumab has already been approved by the FDA in 2009 as ILARIS, an interleukin-1β blocker indicated for the treatment of Cryopyrin-Associated Periodic Syndromes (CAPS), in adults and children 4 years of age and older including: Familial Cold Autoinflammatory Syndrome (FCAS) and Muckle-Wells Syndrome (MWS).[137] Because of its anti-inflammatory potential it is being considered in a study to determine its efficacy, safety and tolerability in pediatric and young adult patients with SCA.[138]

A recent presentation at the 2019 American Society of Hematology annual meeting described a multi-center, randomized, parallel group, double-blind, placebo-controlled trial that recruited patients with SCA (HbSS or HbS/$ß^0$thalassemia) with history of ≥2 major pain episodes/year, screening baseline detectable pain (using pain e-diaries) and serum high sensitivity CRP level ≥1.0 mg/L. Patients were randomized with 1:1 ratio to receive six monthly subcutaneous injections of either canakinumab 300 mg (4 mg/kg for patients ≤40 kg) or placebo. The concurrent use of hydroxyurea was a stratification factor at randomization. Outcomes were measured at baseline and at weeks 4, 8, 12, 16, 20, 24, after which all patients moved to open label canakinumab treatment for additional 6 months.

Interim analysis for futility and safety was performed on the first 30 enrolled patients (canakinumab, n=16; placebo, n=14), of whom 26 patients completed the Week 12 assessments (canakinumab, n=14; placebo, n=12), and 13 patients completed the Week 24 assessments. Enrolled patients (median age 17 years, range 12-20; 19 males, 11 females) were evenly distributed in the arms of the study. Results showed that Futility criteria were not met and no canakinumab-associated safety issues were identified in this first interim analysis. A second interim analysis is pending.[139]

*Ambrisentan***.** Ambriseentan (Letairis) is an endothelin receptor antagonist which has already been approved by the FDA in 2007 for the treatment of pulmonary arterial hypertension (PAH) (WHO Group 1): To improve exercise ability and delay clinical worsening. In combination with tadalafil to reduce the risks of disease progression and hospitalization for worsening PAH, and to improve exercise ability. Preliminary data about its potential role in SCD suggest that These data suggest that endothelin receptor blockade is safe, well tolerated and has the potential to impact various aspects of disease pathophysiology in SCD.[140-142]

d. Targeting oxidative Injury

α-Lipoic acid. Alpha-lipoic acid (ALA) is a potent antioxidant that is employed in the treatment of several diseases. It augments cellular stress response by increasing the transcription of antioxidant genes, decreasing NF-kB, and increasing glutathione synthesis. Acetyl-l-carnitine is an essential nutrient that facilitates the entry of long-chain fatty acids into the mitochondria and decreases lipid peroxidation in tissue. α-Lipoic acid and acetyl-L-carnitine have a synergistic antioxidant effect.[143] A recent Phase II trial combining antioxidants enrolled 42 patients to determine whether α-lipoic acid and acetyl-L-carnitine will lower systemic inflammation in patients with SCD. This study is complete; however, data is not available for review.[144] In an open randomized trial ALA treatment protected normal individuals from oxidative damage to lipids and proteins. In SCD patients, the dose applied were not effective to prevent the oxidative damage.[145] Further trials are not planned at the present.

e. Targeting anti-coagulation

*Rivaroxaban***.** The direct oral anticoagulants (DOACs) include Rivaroxaban. Investigational therapies targeting multiple pathways are being studied for the treatment of SCD. Rivaroxaban, an orally active direct Factor-Xa inhibitor and serine protease inhibitor, was FDA approved in the US as an anticoagulant for prophylaxis and treatment in acute coronary syndromes, cerebral ischemia, pulmonary embolism and venous thrombosis. It is currently being evaluated in a Phase II clinical trial in SCD to reduce inflammation, coagulation and endothelial cell activation, and improve microvascular blood flow in patients during the non-VOC steady state.[146]

f. Targeting vasodilatation.

*Arginine***.** Arginine is depleted in hemolysis due to the release of arginase and leads to decreased NO formation. In SCD patients with pulmonary hypertension, arginine supplementation increases plasma NO and rapidly decreases pulmonary artery pressure by 15%.[147] A recent randomized, double-blind, placebo-controlled study of high-dose arginine supplementation in hospitalized SCD patients with VOC was completed and found a > 56% reduction in opioid use in patients receiving arginine compared with controls.[148] A Phase II, randomized trial in 38 children showed a significant reduction in opioid use and lower pain scores at discharge in those treated with arginine in comparison to the placebo arm. There was no significant difference in hospital length of stay and no toxicity was noted.[149] A study was completed in children with SCD to evaluate the effectiveness of arginine at increasing NO levels, improving RBC function and reducing hospitalizations and pain medication use. This was done by measuring gardos channel activity, mean corpuscular Hb concentration (MCHC) and NO levels. There was only statistically significant difference in low-dose arginine with decreased MCHC versus placebo. Data is available but has not been published.[150] Other studies have been completed and awaiting analysis and two are currently recruiting.[151-154]

Inhaled NO. As mentioned before NO failed as a therapeutic agent for hospitalized patients with SCD and VOC.[155] Interestingly, the use of inhaled NO in the emergency department significantly reduced pain scores compared with placebo ($P < 0.02$) at the end of NO inhalation although both groups had similar baseline pain scores.[156,157] Moreover, NO has been reported to reduces sickle Hb polymerization.[158]

PF 04447943 (Phosphodiesterase 9A Inhibitor). A randomized, double-blinded, Phase 1b trial [159] at 18 centers in the U.S. and Europe evaluated the safety and tolerability of PF-04447943 over 29 days in people with stable SCD. Multiple doses of PF-04447943, with or without HU, administered to patients with SCD were generally well tolerated and showed pharmacodynamics parameters suggestive of a protective effect against vaso-occlusion. In addition, possible biomarkers to measure efficacy for use in future SCD studies were noted.[160] Inhibition of PDE9A is required to treat diseases that lower the level of cGMP which, in turn, regulates signal transduction[161] and mediates vasodilatation.

IMR-687 is a highly selective, potent inhibitor of phosphodiesterase 9. It has a multimodal mechanism of action that acts primarily on RBC and has the potential to act on white blood cells, adhesion mediators and other cell types that are implicated in SCD. Currently, it is an open-label extension study in adult patients with SCA who were previously participants in the Phase 2a study titled "A Phase 2a, Randomized, Double-Blind, Placebo-Controlled Study of IMR-687 in Adult Patients with SCA".[162] This open-label extension study will evaluate the long-term safety and tolerability of IMR-687 in adult SCA patients. Exploratory long-term parameters will also be examined.

Riociguat is used in a Phase 2 multi-center, randomized, double-blind, placebo-controlled, parallel groups study aimed to evaluate its safety, tolerability and efficacy compared with placebo in patients with SCD.[163]

Olinciguat is use in the STRONG SCD in patients with SCD. The primary aim of the study is to evaluate the safety and tolerability of different dose levels of Olinciguat compared with placebo when administered daily for approximately 12 weeks to patients with stable SCD. Exploratory objectives include evaluation of pharmacokinetic as well as evaluation of its effect on symptoms of SCD, health-related quality of life, and biomarkers of pharmacodynamic activity.[164]

g. Targeting Polymerization

Voxelotor (OXBRYTA), previously known as GBT440, has the potential to selectively bind to Hb, and increase its affinity for oxygen. It also inhibits Hb polymerization and prevents RBCS from becoming deformed. This should restore normal RBC function and oxygen delivery. It should also help reduce the risk of VOCs caused by sickle cells blocking blood vessels.

Voxelotor is oral, once-daily drug that binds to the α-chain of HbS, stabilizing the molecule in the R-state conformation, which is known to interrupt HbS polymerization.[101,165,166] The target for HbS modification with voxelotor is 20%-30%. In phase 1/2 trials, Voxelotor inhibited HbS polymerization, RBC sickling, and hemolysis, with a consequent increase in Hb concentration, while also demonstrating an acceptable safety profile and was well tolerated.[167] Phases 1/2 completed and Phase 3 randomized, placebo-controlled HOPE trial involving patients with SCD, Voxelotor (1500 mg and 900 mg) significantly increased and sustained Hb levels compared to placebo and reduced markers of hemolysis. These findings are consistent with inhibition of HbS polymerization and indicate a disease-modifying potential. The secondary endpoints pertaining to frequency of VOC, hospitalization stay, etc. we're not significantly different from placebo. Moreover, exploratory post-hoc trial showed that Voxelotor resolved or improved leg ulcers in some patients. The new drug application (NDA) for Voxelotor is currently under priority review by the FDA which provides for a six-month review, and has been assigned a Prescription Drug User Fee Act (PDUFA) target action date of February 26, 2020.

Besides the HOPE trial, Voxelotor is being considered for other future trials. These include the following:

- Hemoglobin oxygen affinity modulation to inhibit Hb S polymerization (HOPE-KIDS 2, GBT 440-032) trial. The objective of this trial is to investigate the effect of Voxelotor on Transcranial Doppler (TCD) flow velocity in pediatric patients with SCD with conditional TCD.
- Actigraphy improvement with Voxelotor (Active) trial. The objective of this trial is to assess the impact of Voxelotor on physical activity, sleep quality, and overall patient wellbeing in individuals with SCD. Part 1 of this trial will be a phase 4 open-label, single-arm, within-subject comparison followed by Part 2 trial which is a randomized withdrawal placebo-controlled trial.

FT-4202 (PKR Activator). FT-4202 is a selective RBC pyruvate kinase-R activator (PKR) to be used as a modifying therapy for the treatment of SCD. Its mechanism of action includes activating the RBC's natural PKR activity to decrease 2,3-DPG levels which results in shifting the oxygen dissociation curve to the left causing Hb to hold on to oxygen molecules longer to decrease RBC sickling. In addition, the downstream action of FT-4202 increases ATP levels that provide energy to RBCs health and survival. These effects would increase Hb levels and possibly decrease the frequency of VOCs.[168,169]

h. Targeting Supplements

Niacin (Vitamin B3). Niacin is a drug that has been used to increase high density cholesterol (HDL), the "good cholesterol". It improves the blood flow in people with SCD.[170]

Niacin, a drug that has been used to increase HDL (good cholesterol) levels, improves blood flow in people without SCD. This study will see if it can do the same in people with the disease.

Cholecalciferol (Vitamin D3). About 98% of patients with SCD have vitamin D deficiency, defined as a 25-hydroxyvitamin D level (25(OH)D) less than or equal to 20 ng/mL. As a result of low bone density, patients may develop osteonecrosis, chronic inflammation and related pain.[171] Since vitamin D regulates calcium levels and supports bone health, its deficiency may worsen musculoskeletal health problems already present in people with SCD. However, a Cochrane review study showed that the evidence for vitamin D3 supplementation in patients with SCD is not of sufficient quality to guide clinical practice. Evidence of vitamin D supplementation in sickle cell disease from high quality studies is needed.[172]

Conclusions. There has been tremendous advance in our knowledge of the pathophysiology of sickle cell vascular injury over the past decade resulting in new therapeutic targets. The field is witnessing promising translational studies hoping to replace or use with HU as the primary pharmacologic therapy for patients with SCD. This review includes therapies targeting increases in fetal Hb and the complex pathways in adhesion, inflammation, oxidative damage and polymerization.

Hydroxyurea is an oral agent that has decreased morbidity and mortality in adults and children with SCA. It decreases recurrent VOCs, ACS and blood transfusion requirements, and improves quality of life mainly through increasing fetal Hb production. It is inexpensive and potentially available worldwide. It is cytotoxic, which may cause myelosuppression and its carcinogenic effects are unknown and long-term studies have failed to document this. Traditionally, it has been. contraindicated in pregnancy and during lactation due to potential teratogenicity. Recent anecdotes and case reports indicated its safety during pregnancy and lactation. Its role in pregnancy and lactation is currently the subject of clinical trials. It seems it should not be taken during the first two trimesters of pregnancy.

L-glutamine is metabolized to glutamate, the glutathione precursor, and preserves intracellular NAD, which is necessary for glutathione recycling. Oral supplementation of glutamine in SCD increases the NAD redox potential and may improve sickle erythrocyte adhesiveness. Oral glutamine was developed by Emmaus Medical for the treatment of short bowel syndrome and in SCA and β thalassemia. It decreases the resting energy expenditure in children with SCD. A multicenter Phase III trial of L- glutamine supplementation in 230 children to prevent VOC is completed Results showed that the median number of pain crises over 48 weeks was lower among those who received oral therapy with L-glutamine, administered alone or with HU, than among those who received placebo, with or without HU.

Decitabine is an attractive agent as it induced fetal Hb with similar disadvantageous risk profile like HU with potential myelosuppression, teratogenicity and carcinogenicity. It is an already approved therapy for myelodysplastic syndrome and acute myeloid leukemia, conditions more prevalent in the elderly. It is being evaluated in oral form and in combination therapy currently and further testing is warranted in the pediatric population. Unlike HU, its effect to increase Hb F level occurs much sooner than that for HU. N-acetylcysteine has reached Phase III trials. It targets inflammation. A combination with a fetal Hb-inducing agent such as HU is a potential strategy to combat SCD. Studies involving NO so far have been disappointing in the sickle cell population. It is surprising that arginine therapy. was more promising than NO since its role is to increase NO. Nevertheless, this natural amino acid is an ideal agent for a combination regimen.

In the sickle cell population, there are challenges with clinical trial enrollment since it is a relatively rare and clinically heterogeneous disease. A paradigm shift in clinical trial design would improve outcome. Due to the complex pathophysiology of the disease, clinical trials targeting a multi-agent approach may be more successful as in oncology where combination chemotherapy regimens have been more efficacious. Trial design in SCD over the past three decades has historically incorporated all patients with SCA. Recently, this

approach is being modified to reassess endpoints to determine benefits in targeted phenotypes, including quality-of-life measures and incorporating biomarkers in patient selection.

In summary, our greater understanding of the pathophysiology of SCD has led to many new targets for drug therapy, and with a paradigm shift in clinical trial design. We are in an exciting position to improve care for the millions who suffer from SCD. It is very probable that in the near future we may witness new trials to treat SCD that contain two or more drugs that have different mechanism of action. My prediction is that such trials may have acronyms such as FOC, FOV, FOCV, etc. trials where F refers to a drug that increases Hb F, O refers to an antioxidant drug, C refers to anti-adhesion drug and V to anti-polymerization drug or other possible combinations.

References:

1. Piel FB, Hay SI, Gupta S, Weatherall DJ, Williams TN. Global burden of sickle cell anaemia in children under five, 2010-2050: modelling based on demographics, excess mortality, and interventions. PLoS Med. 2013;10(7):e1001484. https://doi.org/10.1371/journal.pmed.1001484 PMid:23874164 PMCid:PMC3712914
2. Odame I. Developing a global agenda for sickle cell disease: report of an international symposium and workshop in Cotonou, Republic of Benin. Am J Prev Med. 2010;38(4 Suppl):S571-5. https://doi.org/10.1016/j.amepre.2009.12.021 PMid:20331960
3. McGann PT, Ware RE. Hydroxyurea for sickle cell anemia: what have we learned and what questions still remain? Curr Opin Hematol. 2011;18(3):158-65. https://doi.org/10.1097/MOH.0b013e32834521dd PMid:21372708 PMCid:PMC3181131
4. Castro O, Rana SR, Bang KM, Scott RB. Age and prevalence of sickle-cell trait in a large ambulatory population. Genet Epidemiol. 1987;4(4):307-11. https://doi.org/10.1002/gepi.1370040409 PMid:3666437
5. Steinberg M, H., Forget BG, Higgs D, R., Weatherall DJ. Disorders of hemoglobin: Genetics, Pathophysiology, and Clinical Management, Second Edition. 2nd ed. Cambridge: Cambridge University Press; 2009. 826 p. https://doi.org/10.1017/CBO9780511596582
6. Goldsmith JC, Bonham VL, Joiner CH, Kato GJ, Noonan AS, Steinberg MH. Framing the research agenda for sickle cell trait: building on the current understanding of clinical events and their potential implications. Am J Hematol. 2012;87(3):340-6. https://doi.org/10.1002/ajh.22271 PMid:22307997 PMCid:PMC3513289
7. Serjeant GR, Serjeant BE. Sickle cell disease, 3rd edition. Oxford: Oxfird University Press; 2001. 772 p. https://doi.org/10.1046/j.1365-2141.2001.02557.x PMid:11167776
8. Bunn HF, Forget BG. Hemoglobin: Molecular, Genetic and Clinical Aspects. Philadelphia: WB Saunders; 1986.
9. Ballas SK, Park D, Wapner RJ. Neonatal screening for sickle cell disease in a metropolitan university hospital: efficacy and problems. J Med Screen. 1994;1(4):229-32. https://doi.org/10.1177/096914139400100409 PMid:8790526
10. Shafer FE, Lorey F, Cunningham GC, Klumpp C, Vichinsky E, Lubin B. Newborn screening for sickle cell disease: 4 years of experience from California's newborn screening program. J Pediatr Hematol Oncol. 1996;18(1):36-41. https://doi.org/10.1097/00043426-199602000-00007 PMid:8556368
11. Diallo DA. Sickle cell disease in Africa: current situation and strategies for improving the quality and duration of survival. Bull Acad Natl Med. 2008;192(7):1361-72; discussion 72-3.
12. Vichinsky E. Emerging 'A' therapies in hemoglobinopathies: agonists, antagonists, antioxidants, and arginine. Hematology Am Soc Hematol Educ Program. 2012;2012:271-5. https://doi.org/10.1182/asheducation.V2012.1.271.3798318 PMid:23233591
13. Ballas SK. Sickle Cell Pain, 2nd Edition. Washington DC: International Association for the Study of Pain; 2014.
14. Zhang D, Xu C, Manwani D, Frenette PS. Neutrophils, platelets, and inflammatory pathways at the nexus of sickle cell disease pathophysiology. Blood. 2016;127(7):801-9. https://doi.org/10.1182/blood-2015-09-618538 PMid:26758915 PMCid:PMC4760086
15. Motta I, Ghiaccio V, Cosentino A, Breda L. Curing Hemoglobinopathies: Challenges and Advances of Conventional and New Gene Therapy Approaches. Mediterr J Hematol Infect Dis. 2019 Nov 1;11(1):e2019067. doi: 10.4084/MJHID.2019.067. eCollection 2019. Review. PubMed PMID: 31700592; PubMed Central PMCID:PMC6827604.
16. Ballas SK. Sickle cell anaemia: progress in pathogenesis and treatment. Drugs. 2002;62(8):1143-72. https://doi.org/10.2165/00003495-200262080-00003 PMid:12010077
17. Kotiah SD, Ballas SK. Investigational drugs in sickle cell anemia. Expert Opin Investig Drugs. 2009;18(12):1817-28. https://doi.org/10.1517/13543780903247463 PMid:19780709
18. Kauf TL, Coates TD, Huazhi L, Mody-Patel N, Hartzema AG. The cost of health care for children and adults with sickle cell disease. Am J Hematol. 2009;84(6):323-7. https://doi.org/10.1002/ajh.21408 PMid:19358302
19. Lanzkron S, Haywood C, Segal JB, Dover GJ. Hospitalization rates and costs of care of patients with sickle-cell anemia in the state of Maryland in the era of hydroxyurea. Am J Hematol. 2006;81(12):927-32. https://doi.org/10.1002/ajh.20703 PMid:16924648
20. Moore RD, Charache S, Terrin ML, Barton FB, Ballas SK. Cost-effectiveness of hydroxyurea in sickle cell anemia. Investigators of the Multicenter Study of Hydroxyurea in Sickle Cell Anemia. Am J Hematol. 2000;64(1):26-31. https://doi.org/10.1002/(SICI)1096-8652(200005)64:1<26::AID-AJH5>3.0.CO;2-F
21. Benjamin LJ, Swinson GI, Nagel RL. Sickle cell anemia day hospital: an approach for the management of uncomplicated painful crises. Blood. 2000;95(4):1130-6. https://doi.org/10.1182/blood.V95.4.1130.003k03a_1130_1136 PMid:10666181
22. Ballas SK. The cost of health care for patients with sickle cell disease. Am J Hematol. 2009;84(6):320-2. https://doi.org/10.1002/ajh.21443 PMid:19415728
23. Manwani D, Frenette PS. Vaso-occlusion in sickle cell disease: pathophysiology and novel targeted therapies. Hematology Am Soc Hematol Educ Program. 2013;2013:362-9. https://doi.org/10.1182/asheducation-2013.1.362 PMid:24319205
24. Kaul DK, Finnegan E, Barabino GA. Sickle red cell-endothelium interactions. Microcirculation. 2009;16(1):97-111. https://doi.org/10.1080/10739680802279394 PMid:18720225 PMCid:PMC3059190
25. Madigan C, Malik P. Pathophysiology and therapy for haemoglobinopathies. Part I: sickle cell disease. Expert Rev Mol Med. 2006;8(9):1-23. https://doi.org/10.1017/S1462399406010659
26. Gutsaeva DR, Parkerson JB, Yerigenahally SD, Kurz JC, Schaub RG, Ikuta T, et al. Inhibition of cell adhesion by anti-P-selectin aptamer: a new potential therapeutic agent for sickle cell disease. Blood. 2011;117(2):727-35. https://doi.org/10.1182/blood-2010-05-285718 PMid:20926770 PMCid:PMC3031491
27. Turhan A, Weiss LA, Mohandas N, Coller BS, Frenette PS. Primary role for adherent leukocytes in sickle cell vascular occlusion: a new paradigm. Proc Natl Acad Sci U S A. 2002;99(5):3047-51. https://doi.org/10.1073/pnas.052522799 PMid:11880644 PMCid:PMC122470
28. Zennadi R, Moeller BJ, Whalen EJ, Batchvarova M, Xu K, Shan S, et al. Epinephrine-induced activation of LW-mediated sickle cell adhesion and vaso-occlusion in vivo. Blood. 2007;110(7):2708-17. https://doi.org/10.1182/blood-2006-11-056101 PMid:17609430 PMCid:PMC1988948
29. Hines PC, Zen Q, Burney SN, Shea DA, Ataga KI, Orringer EP, et al. Novel epinephrine and cyclic AMP-mediated activation of BCAM/Lu-dependent sickle (SS) RBC adhesion. Blood. 2003;101(8):3281-7. https://doi.org/10.1182/blood-2001-12-0289 PMid:12506027

30. Schaer DJ, Buehler PW, Alayash AI, Belcher JD, Vercellotti GM. Hemolysis and free hemoglobin revisited: exploring hemoglobin and hemin scavengers as a novel class of therapeutic proteins. Blood. 2013;121(8):1276-84.
https://doi.org/10.1182/blood-2012-11-451229
PMid:23264591 PMCid:PMC3578950
31. Morris CR, Kato GJ, Poljakovic M, Wang X, Blackwelder WC, Sachdev V, et al. Dysregulated arginine metabolism, hemolysis-associated pulmonary hypertension, and mortality in sickle cell disease. JAMA. 2005;294(1):81-90.
https://doi.org/10.1001/jama.294.1.81
PMid:15998894 PMCid:PMC2065861
32. Kato GJ, Wang Z, Machado RF, Blackwelder WC, Taylor JGt, Hazen SL. Endogenous nitric oxide synthase inhibitors in sickle cell disease: abnormal levels and correlations with pulmonary hypertension, desaturation, haemolysis, organ dysfunction and death. Br J Haematol. 2009;145(4):506-13.
https://doi.org/10.1111/j.1365-2141.2009.07658.x
PMid:19344390 PMCid:PMC2935697
33. Chantrathammachart P, Pawlinski R. Tissue factor and thrombin in sickle cell anemia. Thromb Res. 2012;129 Suppl 2:S70-2.
https://doi.org/10.1016/j.thromres.2012.02.038
PMid:22398014 PMCid:PMC3335974
34. Chirico EN, Pialoux V. Role of oxidative stress in the pathogenesis of sickle cell disease. IUBMB Life. 2012;64(1):72-80.
https://doi.org/10.1002/iub.584
PMid:22131167
35. Gizi A, Papassotiriou I, Apostolakou F, Lazaropoulou C, Papastamataki M, Kanavaki I, et al. Assessment of oxidative stress in patients with sickle cell disease: The glutathione system and the oxidant-antioxidant status. Blood Cells Mol Dis. 2011;46(3):220-5.
https://doi.org/10.1016/j.bcmd.2011.01.002
PMid:21334230
36. Nur E, Biemond BJ, Otten HM, Brandjes DP, Schnog JJ. Oxidative stress in sickle cell disease; pathophysiology and potential implications for disease management. Am J Hematol. 2011;86(6):484-9.
https://doi.org/10.1002/ajh.22012
PMid:21544855
37. Steinberg MH, McCarthy WF, Castro O, Ballas SK, Armstrong FD, Smith W, et al. The risks and benefits of long-term use of hydroxyurea in sickle cell anemia: A 17.5 year follow-up. Am J Hematol. 2010;85(6):403-8.
https://doi.org/10.1002/ajh.21699
PMid:20513116 PMCid:PMC2879711
38. Expert Panel Report. Evidence-Based Management of Sickle Cell Disease Bethesda MD: National Heart, Lung, and Blood Institute; 2014 [Available from: http://www.nhlbi.nih.gov/health-pro/guidelines/sickle-cell-disease-guidelines/].
39. Ballas SK, McCarthy WF, Guo N, DeCastro L, Bellevue R, Barton BA, et al. Exposure to hydroxyurea and pregnancy outcomes in patients with sickle cell anemia. J Natl Med Assoc. 2009;101(10):1046-51.
https://doi.org/10.1016/S0027-9684(15)31072-5
40. Borba R, Lima CS, Grotto HZ. Reticulocyte parameters and hemoglobin F production in sickle cell disease patients undergoing hydroxyurea therapy. J Clin Lab Anal. 2003;17(2):66-72.
https://doi.org/10.1002/jcla.10070
PMid:12640630 PMCid:PMC6807693
41. Guarda CC, Silveira-Mattos PSM, Yahouedehou S, Santiago RP, Aleluia MM, Figueiredo CVB, et al. Hydroxyurea alters circulating monocyte subsets and dampens its inflammatory potential in sickle cell anemia patients. Sci Rep. 2019;9(1):14829.
https://doi.org/10.1038/s41598-019-51339-x
PMid:31616024 PMCid:PMC6794261
42. Penkert RR, Hurwitz JL, Thomas P, Rosch J, Dowdy J, Sun Y, et al. Inflammatory molecule reduction with hydroxyurea therapy in children with sickle cell anemia. Haematologica. 2018;103(2):e50-e4.
https://doi.org/10.3324/haematol.2017.177360
PMid:29146708 PMCid:PMC5792285
43. Ballas SK, Connes P. Rheological properties of sickle erythrocytes in patients with sickle-cell anemia: The effect of hydroxyurea, fetal hemoglobin, and alpha-thalassemia. Eur J Haematol. 2018;101(6):798-803.
https://doi.org/10.1111/ejh.13173
PMid:30204261 PMCid:PMC6224298
44. Torres Lde S, da Silva DG, Belini Junior E, de Almeida EA, Lobo CL, Cancado RD, et al. The influence of hydroxyurea on oxidative stress in sickle cell anemia. Rev Bras Hematol Hemoter. 2012;34(6):421-5.
https://doi.org/10.5581/1516-8484.20120106
PMid:23323065 PMCid:PMC3545428
45. Gardner K, Bell C, Bartram JL, Allman M, Awogbade M, Rees DC, et al. Outcome of adults with sickle cell disease admitted to critical care - experience of a single institution in the UK. Br J Haematol. 2010;150(5):610-3.
https://doi.org/10.1111/j.1365-2141.2010.08271.x
PMid:20560967
46. Brewin J, Tewari S, Menzel S, Kirkham F, Inusa B, Renney G, et al. The effects of hydroxycarbamide on the plasma proteome of children with sickle cell anaemia. Br J Haematol. 2019;186(6):879-86.
https://doi.org/10.1111/bjh.15996
PMid:31140594
47. Dasgupta T, Fabry ME, Kaul DK. Antisickling property of fetal hemoglobin enhances nitric oxide bioavailability and ameliorates organ oxidative stress in transgenic-knockout sickle mice. Am J Physiol Regul Integr Comp Physiol. 2010;298(2):R394-402.
https://doi.org/10.1152/ajpregu.00611.2009
PMid:20007516 PMCid:PMC2828175
48. Kaul DK, Liu XD, Chang HY, Nagel RL, Fabry ME. Effect of fetal hemoglobin on microvascular regulation in sickle transgenic-knockout mice. J Clin Invest. 2004;114(8):1136-45.
https://doi.org/10.1172/JCI200421633
PMid:15489961 PMCid:PMC522244
49. Rees DC. The rationale for using hydroxycarbamide in the treatment of sickle cell disease. Haematologica. 2011;96(4):488-91.
https://doi.org/10.3324/haematol.2011.041988
PMid:21454878 PMCid:PMC3069221
50. Davies S, Olujohungbe A. Hydroxyurea for sickle cell disease. Cochrane Database Syst Rev. 2001(2):CD002202.
51. Platt OS. Hydroxyurea for the treatment of sickle cell anemia. N Engl J Med. 2008;358(13):1362-9.
https://doi.org/10.1056/NEJMct0708272
PMid:18367739
52. Ballas SK, Marcolina MJ, Dover GJ, Barton FB. Erythropoietic activity in patients with sickle cell anaemia before and after treatment with hydroxyurea. Br J Haematol. 1999;105(2):491-6.
https://doi.org/10.1111/j.1365-2141.1999.01339.x
PMid:10233426
53. Charache S, Terrin ML, Moore RD, Dover GJ, Barton FB, Eckert SV, et al. Effect of hydroxyurea on the frequency of painful crises in sickle cell anemia. Investigators of the Multicenter Study of Hydroxyurea in Sickle Cell Anemia. N Engl J Med. 1995;332(20):1317-22.
https://doi.org/10.1056/NEJM199505183322001
PMid:7715639
54. Charache S, Barton FB, Moore RD, Terrin ML, Steinberg MH, Dover GJ, et al. Hydroxyurea and sickle cell anemia. Clinical utility of a myelosuppressive "switching" agent. The Multicenter Study of Hydroxyurea in Sickle Cell Anemia. Medicine. 1996;75(6):300-26.
https://doi.org/10.1097/00005792-199611000-00002
PMid:8982148
55. Alvarez O, Miller ST, Wang WC, Luo Z, McCarville MB, Schwartz GJ, et al. Effect of hydroxyurea treatment on renal function parameters: results from the multi-center placebo-controlled BABY HUG clinical trial for infants with sickle cell anemia. Pediatr Blood Cancer. 2012;59(4):668-74.
https://doi.org/10.1002/pbc.24100
PMid:22294512 PMCid:PMC3396762
56. Armstrong FD, Elkin TD, Brown RC, Glass P, Rana S, Casella JF, et al. Developmental function in toddlers with sickle cell anemia. Pediatrics. 2013;131(2):e406-14.
https://doi.org/10.1542/peds.2012-0283
PMid:23296434 PMCid:PMC3557401
57. Lebensburger JD, Miller ST, Howard TH, Casella JF, Brown RC, Lu M, et al. Influence of severity of anemia on clinical findings in infants with sickle cell anemia: analyses from the BABY HUG study. Pediatr Blood Cancer. 2012;59(4):675-8.
https://doi.org/10.1002/pbc.24037
PMid:22190441 PMCid:PMC3337342
58. McGann PT, Flanagan JM, Howard TA, Dertinger SD, He J, Kulharya AS, et al. Genotoxicity associated with hydroxyurea exposure in infants with sickle cell anemia: results from the BABY-HUG phase III clinical trial. Pediatr Blood Cancer. 2012;59(2):254-7.
https://doi.org/10.1002/pbc.23365
PMid:22012708 PMCid:PMC3277805
59. Wang WC, Ware RE, Miller ST, Iyer RV, Casella JF, Minniti CP, et al. Hydroxycarbamide in very young children with sickle-cell anaemia: a multicentre, randomised, controlled trial (BABY HUG). Lancet. 2011;377(9778):1663-72.

https://doi.org/10.1016/S0140-6736(11)60355-3
60. Lam MS. Extemporaneous compounding of oral liquid dosage formulations and alternative drug delivery methods for anticancer drugs. Pharmacotherapy. 2011;31(2):164-92. https://doi.org/10.1592/phco.31.2.164 PMid:21275495
61. Ballas SK, Singh P, Adams-Graves P, Wordell CJ. Idiosyncratic Side Effects of Hydroxyurea in Patients with Sickle Cell Anemia. J Blood Disorders Transf. 2013;4:5.
62. Su ZT, Segal JB, Lanzkron S, Ogunsile FJ. National trends in hydroxyurea and opioid prescribing for sickle cell disease by office-based physicians in the United States, 1997-2017. Pharmacoepidemiol Drug Saf. 2019;28(9):1246-50. https://doi.org/10.1002/pds.4860 PMid:31328369
63. Schuchard SB, Lissick JR, Nickel A, Watson D, Moquist KL, Blaylark RM, et al. Hydroxyurea use in young infants with sickle cell disease. Pediatr Blood Cancer. 2019;66(7):e27650. https://doi.org/10.1002/pbc.27650 PMid:30729675
64. Thomas R, Dulman R, Lewis A, Notarangelo B, Yang E. Prospective longitudinal follow-up of children with sickle cell disease treated with hydroxyurea since infancy. Pediatr Blood Cancer. 2019;66(9):e27816. https://doi.org/10.1002/pbc.27816 PMid:31157521
65. Ware RE, McGann PT, Quinn CT. Hydroxyurea for children with sickle cell anemia: Prescribe it early and often. Pediatr Blood Cancer. 2019;66(8):e27778. https://doi.org/10.1002/pbc.27778 PMid:31038282
66. Creary SE, Modi AC, Stanek JR, Chisolm DJ, O'Brien SH, Nwankwo C, et al. Allocation of Treatment Responsibility and Adherence to Hydroxyurea Among Adolescents With Sickle Cell Disease. J Pediatr Psychol. 2019. https://doi.org/10.1093/jpepsy/jsz061 PMid:31403687
67. Jabour SM, Beachy S, Coburn S, Lanzkron S, Eakin MN. The Role of Patient-Physician Communication on the Use of Hydroxyurea in Adult Patients with Sickle Cell Disease. J Racial Ethn Health Disparities. 2019;6(6):1233-43. https://doi.org/10.1007/s40615-019-00625-5 PMid:31410784
68. Mvalo T, Topazian HM, Kamthunzi P, Chen JS, Kambalame I, Mafunga P, et al. Real-world experience using hydroxyurea in children with sickle cell disease in Lilongwe, Malawi. Pediatr Blood Cancer. 2019;66(11):e27954. https://doi.org/10.1002/pbc.27954 PMid:31397075
69. Adeyemo TA, Diaku-Akinwunmi IN, Ojewunmi OO, Bolarinwa AB, Adekile AD. Barriers to the use of hydroxyurea in the management of sickle cell disease in Nigeria. Hemoglobin. 2019;43(3):188-92. https://doi.org/10.1080/03630269.2019.1649278 PMid:31462098
70. Ohene-Frempong K, Weiner SJ, Sleeper LA, Miller ST, Embury S, Moohr JW, et al. Cerebrovascular accidents in sickle cell disease: rates and risk factors. Blood. 1998;91(1):288-94.
71. Hogan AM, Vargha-Khadem F, Saunders DE, Kirkham FJ, Baldeweg T. Impact of frontal white matter lesions on performance monitoring: ERP evidence for cortical disconnection. Brain. 2006;129(Pt 8):2177-88. https://doi.org/10.1093/brain/awl160 PMid:16815874
72. Pegelow CH, Macklin EA, Moser FG, Wang WC, Bello JA, Miller ST, et al. Longitudinal changes in brain magnetic resonance imaging findings in children with sickle cell disease. Blood. 2002;99(8):3014-8. https://doi.org/10.1182/blood.V99.8.3014 PMid:11929794
73. el Gammal T, Adams RJ, Nichols FT, McKie V, Milner P, McKie K, et al. MR and CT investigation of cerebrovascular disease in sickle cell patients. AJNR Am J Neuroradiol. 1986;7(6):1043-9.
74. Abboud MR, Yim E, Musallam KM, Adams RJ. Discontinuing prophylactic transfusions increases the risk of silent brain infarction in children with sickle cell disease: data from STOP II. Blood. 2011;118(4):894-8. https://doi.org/10.1182/blood-2010-12-326298 PMid:21633086 PMCid:PMC3148169
75. Scantlebury N, Mabbott D, Janzen L, Rockel C, Widjaja E, Jones G, et al. White matter integrity and core cognitive function in children diagnosed with sickle cell disease. J Pediatr Hematol Oncol. 2011;33(3):163-71. https://doi.org/10.1097/MPH.0b013e3182036f33 PMid:21325970
76. Wang WC, Pavlakis SG, Helton KJ, McKinstry RC, Casella JF, Adams RJ, et al. MRI abnormalities of the brain in one-year-old children with sickle cell anemia. Pediatr Blood Cancer. 2008;51(5):643-6. https://doi.org/10.1002/pbc.21612 PMid:18478575
77. Ware RE, Helms RW. Stroke With Transfusions Changing to Hydroxyurea (SWiTCH). Blood. 2012;119(17):3925-32. https://doi.org/10.1182/blood-2011-11-392340 PMid:22318199 PMCid:PMC3350359
78. Ware RE, Davis BR, Schultz WH, Brown RC, Aygun B, Sarnaik S, et al. Hydroxycarbamide versus chronic transfusion for maintenance of transcranial doppler flow velocities in children with sickle cell anaemia-TCD With Transfusions Changing to Hydroxyurea (TWiTCH): a multicentre, open-label, phase 3, non-inferiority trial. Lancet. 2016;387(10019):661-70. https://doi.org/10.1016/S0140-6736(15)01041-7
79. Kapustin D, Leung J, Odame I, Williams S, Shroff M, Kassner A. Hydroxycarbamide treatment in children with Sickle Cell Anaemia is associated with more intact white matter integrity: a quantitative MRI study. Br J Haematol. 2019;187(2):238-45. https://doi.org/10.1111/bjh.16063 PMid:31215028
80. Hankins JS, McCarville MB, Rankine-Mullings A, Reid ME, Lobo CL, Moura PG, et al. Prevention of conversion to abnormal transcranial Doppler with hydroxyurea in sickle cell anemia: A Phase III international randomized clinical trial. Am J Hematol. 2015;90(12):1099-105. https://doi.org/10.1002/ajh.24198 PMid:26414435 PMCid:PMC4715740
81. Sirieix ME, Debure C, Baudot N, Dubertret L, Roux ME, Morel P, et al. Leg ulcers and hydroxyurea: forty-one cases. Arch Dermatol. 1999;135(7):818-20. https://doi.org/10.1001/archderm.135.7.818 PMid:10411157
82. Koshy M, Enstuah R, Koranda A. Leg ulcers in patients in sickle cell disease. Blood. 1989;74:1403-8. https://doi.org/10.1182/blood.V74.4.1403.1403 PMid:2475188
83. Soya E, Makowski C, Blaise S. Leg ulcer induced by hydroxycarbamide in sickle cell disease: What is the therapeutic impact? Int Wound J. 2019;16(4):897-902. https://doi.org/10.1111/iwj.13115 PMid:30916480
84. de Montalembert M, Begue P, Bernaudin F, Thuret I, Bachir D, Micheau M. Preliminary report of a toxicity study of hydroxyurea in sickle cell disease. French Study Group on Sickle Cell Disease. Arch Dis Child. 1999;81(5):437-9. https://doi.org/10.1136/adc.81.5.437 PMid:10519721 PMCid:PMC1718114
85. Byrd DC, Pitts SR, Alexander CK. Hydroxyurea in two pregnant women with sickle cell anemia. Pharmacotherapy. 1999;19(12):1459-62. https://doi.org/10.1592/phco.19.18.1459.30901 PMid:10600098
86. Diav-Citrin O, Hunnisett L, Sher GD, Koren G. Hydroxyurea use during pregnancy: a case report in sickle cell disease and review of the literature. Am J Hematol. 1999;60(2):148-50. https://doi.org/10.1002/(SICI)1096-8652(199902)60:2<148::AID-AJH12>3.0.CO;2-I
87. Children's Hospital Medical Center Cincinnati. Hydroxyurea Exposure Limiting Pregnancy and Follow-Up Lactation (HELPFUL) (NCT04093986): ClinicalTrials.gov; [Available from: https://clinicaltrials.gov/ct2/show/NCT04093986 (Accessed on November 18, 2019)].
88. Pistilli B, Bellettini G, Giovannetti E, Codacci-Pisanelli G, Azim HA, Jr., Benedetti G, et al. Chemotherapy, targeted agents, antiemetics and growth-factors in human milk: how should we counsel cancer patients about breastfeeding? Cancer Treat Rev. 2013;39(3):207-11. https://doi.org/10.1016/j.ctrv.2012.10.002 PMid:23199900
89. Ware RE, Marahatta A, Ware JL, al. e. Hydroxyurea exposure in lactation-a pharmacokinetics study (HELPS). Blood. 2018;132(Suppl 1):3677. https://doi.org/10.1182/blood-2018-99-114142

90. Children's Hospital Medical Center Cincinnati. Hydoxyurea Exposure in Lactation A Pharmacokinetics Study (HELPS) (HELPS) (NCT02990598): ClinicalTrials.gov; [Available from: https://clinicaltrials.gov/ct2/show/NCT02990598 (Accessed on November 18, 2019)].
91. Brosnan JT. Interorgan amino acid transport and its regulation. J Nutr. 2003;133(6 Suppl 1):2068s-72s. https://doi.org/10.1093/jn/133.6.2068S PMid:12771367
92. Niihara Y, Miller ST, Kanter J, Lanzkron S, Smith WR, Hsu LL, et al. A Phase 3 Trial of l-Glutamine in Sickle Cell Disease. N Engl J Med. 2018;379(3):226-35. https://doi.org/10.1056/NEJMoa1715971 PMid:30021096
93. Niihara Y, Smith WR, Stark CW. A Phase 3 Trial of l-Glutamine in Sickle Cell Disease. N Engl J Med. 2018;379(19):1880. https://doi.org/10.1056/NEJMoa1715971
94. Minniti CP. l-Glutamine and the Dawn of Combination Therapy for Sickle Cell Disease. N Engl J Med. 2018;379(3):292-4. https://doi.org/10.1056/NEJMe1800976 PMid:30021091
95. Emmaus Medical Inc. A Phase III Safety and Efficacy Study of L-Glutamine to Treat Sickle Cell Disease or Sickle βo-thalassemia (NCT01179217): ClinicalTrials.gov; [Available from: https://clinicaltrials.gov/ct2/show/NCT01179217 (Accessed on November 18, 2019)].
96. Emmaus Medical Inc. L-Glutamine Therapy for Sickle Cell Anemia and Sickle ß0 Thalassemia (NCT00125788) ClinicalTrials.gov [Available from: https://clinicaltrials.gov/ct2/show/NCT00125788 (Accessed on November 18, 2019)].
97. St. Jude Children's Research Hospital. Trial of Oral Glutamine in Patients with Sickle Cell Anemia (NCT00131508): ClinicalTrials.gov; [Available from: https://clinicaltrials.gov/ct2/show/NCT00131508 (Accessed on November 18, 2019)].
98. Emmaus Medical Inc. ENDARI (L-glutamine oral powder) [Package insert]. Torrance, CA.2017.
99. Ataga KI, Kutlar A, Kanter J, Liles D, Cancado R, Friedrisch J, et al. Crizanlizumab for the Prevention of Pain Crises in Sickle Cell Disease. N Engl J Med. 2017;376(5):429-39. https://doi.org/10.1056/NEJMoa1611770 PMid:27959701 PMCid:PMC5481200
100. Novartis Pharmaceuticals. ADAKVEO (crizanlizumab-tmca) injection [Package insert]. East Hanover, NJ.2019.
101. Vichinsky E, Hoppe CC, Ataga KI, Ware RE, Nduba V, El-Beshlawy A, et al. A Phase 3 Randomized Trial of Voxelotor in Sickle Cell Disease. N Engl J Med. 2019;381(6):509-19. https://doi.org/10.1056/NEJMoa1903212 PMid:31199090
102. Global Blood Therapeutics. Study to Evaluate the Effect of Voxelotor Administered Orally to Patients With Sickle Cell Disease (GBT_HOPE) (GBT_HOPE) [NCT03036813] ClinicalTrials.gov [Available from: https://clinicaltrials.gov/ct2/show/NCT03036813 (Accessed on December 16, 2019)].
103. Global Blood Therapeutics. OXBRYTA (voxelotor) tablets [Package insert]. San Francisco, CA.2019.
104. GlycoMimetics, editor GlycoMimetics Announces Presentation of Rivipansel Data in Pediatric Patients at American Society of Pediatric Hematology Oncology 27th Annual Meeting2014 May 15; Palmer House Hilton Hotel, Chicago.
105. Telen MJ, Wun T, McCavit TL, De Castro LM, Krishnamurti L, Lanzkron S, et al. Randomized phase 2 study of GMI-1070 in SCD: reduction in time to resolution of vaso-occlusive events and decreased opioid use. Blood. 2015;125(17):2656-64. https://doi.org/10.1182/blood-2014-06-583351 PMid:25733584 PMCid:PMC4408290
106. Saunthararajah Y, Hillery CA, Lavelle D, Molokie R, Dorn L, Bressler L, et al. Effects of 5-aza-2'-deoxycytidine on fetal hemoglobin levels, red cell adhesion, and hematopoietic differentiation in patients with sickle cell disease. Blood. 2003;102(12):3865-70. https://doi.org/10.1182/blood-2003-05-1738 PMid:12907443
107. National Heart Lung and Blood Institute (NHLBI). Decitabine for High-Risk Sickle Cell Disease (NCT01375608) ClinicalTrials.gov [Available from: https://clinicaltrials.gov/ct2/show/NCT01375608 (Accessed on November 18, 2019)].
108. Molokie R, Lavelle D, Gowhari M, Pacini M, Krauz L, Hassan J, et al. Oral tetrahydrouridine and decitabine for non-cytotoxic epigenetic gene regulation in sickle cell disease: A randomized phase 1 study. PLoS Med. 2017;14(9):e1002382. https://doi.org/10.1371/journal.pmed.1002382 PMid:28880867 PMCid:PMC5589090
109. Lavelle D, Vaitkus K, Ling Y, Ruiz MA, Mahfouz R, Ng KP, et al. Effects of tetrahydrouridine on pharmacokinetics and pharmacodynamics of oral decitabine. Blood. 2012;119(5):1240-7. https://doi.org/10.1182/blood-2011-08-371690 PMid:22160381 PMCid:PMC3277356
110. Yogen Saunthararajah. Study of Decitabine and Tetrahydrouridine (THU) in Patients With Sickle Cell Disease (NCT01685515) ClinicalTrials.gov [Available from: https://clinicaltrials.gov/ct2/show/NCT01685515 (Accessed on November 18, 2019)].
111. Meiler SE, Wade M, Kutlar F, Yerigenahally SD, Xue Y, Moutouh-de Parseval LA, et al. Pomalidomide augments fetal hemoglobin production without the myelosuppressive effects of hydroxyurea in transgenic sickle cell mice. Blood. 2011;118(4):1109-12. https://doi.org/10.1182/blood-2010-11-319137 PMid:21536862 PMCid:PMC3148160
112. Celgene. Study to Determine the Maximum Tolerated Dose, Safety and Effectiveness of Pomalidomide for Patients With Sickle Cell Disease (SCD-001) [NCT01522547] ClinicalTrials.gov [Available from: https://clinicaltrials.gov/ct2/show/NCT01522547 (Accessed on November 18, 2019)].
113. Srinivas NR. Clinical pharmacokinetics of panobinostat, a novel histone deacetylase (HDAC) inhibitor: review and perspectives. Xenobiotica; the fate of foreign compounds in biological systems. 2017;47(4):354-68.
114. Kutlar A. Study of Panobinostat (LBH589) in Patients With Sickle Cell Disease (LBH589) [NCT01245179] Clinical Trials.gov [Available from: https://clinicaltrials.gov/ct2/show/NCT01245179 (Accessed on November 18, 2019)].
115. Elias DB, Barbosa MC, Rocha LB, Dutra LL, Silva HF, Martins AM, et al. L-arginine as an adjuvant drug in the treatment of sickle cell anaemia. Br J Haematol. 2013;160(3):410-2. https://doi.org/10.1111/bjh.12114 PMid:23157285
116. Chang J, Shi PA, Chiang EY, Frenette PS. Intravenous immunoglobulins reverse acute vaso-occlusive crises in sickle cell mice through rapid inhibition of neutrophil adhesion. Blood. 2008;111(2):915-23. https://doi.org/10.1182/blood-2007-04-084061 PMid:17932253 PMCid:PMC2200843
117. Albert Einstein College of Medicine. Intravenous Gammaglobulin for Sickle Cell Pain Crises (NCT01757418) ClinicalTrials.gov [Available from: https://clinicaltrials.gov/ct2/show/NCT01757418 (Accessed on November 18, 2019)].
118. Qari MH, Aljaouni SK, Alardawi MS, Fatani H, Alsayes FM, Zografos P, et al. Reduction of painful vaso-occlusive crisis of sickle cell anaemia by tinzaparin in a double-blind randomized trial. Thromb Haemost. 2007;98(2):392-6. https://doi.org/10.1160/Th06-12-0718 PMid:17721622
119. Kutlar A, Ataga KI, McMahon L, Howard J, Galacteros F, Hagar W, et al. A potent oral P-selectin blocking agent improves microcirculatory blood flow and a marker of endothelial cell injury in patients with sickle cell disease. Am J Hematol. 2012;87(5):536-9. https://doi.org/10.1002/ajh.23147 PMid:22488107
120. Duke University. Treatment of Sickle Cell Patients Hospitalized in Pain Crisis With Prophylactic Dose Low-molecular-weight Heparin (LMWH) Versus Placebo (NCT01419977) ClinicalTrials.gov [Available from: https://clinicaltrials.gov/ct2/show/NCT01419977 (Accessed on November 18, 2019)].
121. Novartis Pharmaceuticals. Study of Two Doses of Crizanlizumab Versus Placebo in Adolescent and Adult Sickle Cell Disease Patients (STAND) [NCT03814746] ClinicalTrials.gov [Available from: https://clinicaltrials.gov/ct2/show/NCT03814746 (Accessed on November 21, 2019)].
122. Novartis Pharmaceuticals. A Study to Evaluate the Safety and Efficacy of Crizanlizumab in Sickle Cell Disease Related Priapism (SPARTAN) [NCT03938454] ClinicalTrials.gov [Available from: https://clinicaltrials.gov/ct2/show/NCT03938454 (Accessed on November 21, 2019)].
123. Novartis Pharmaceuticals. Study of Dose Confirmation and Safety of Crizanlizumab in Pediatric Sickle Cell Disease Patients (NCT03474965) ClinicalTrials.gov [Available from:

https://clinicaltrials.gov/ct2/show/NCT03474965 (Accessed on November 21, 2019)].
124.Novartis Pharmaceuticals. Pharmacokinetics and Pharmacodynamics Study of SEG101 (Crizanlizumab) in Sickle Cell Disease (SCD) Patients With Vaso- Occlusive Crisis (VOC) [NCT03264989] ClinicalTrials.gov [Available from: https://clinicaltrials.gov/ct2/show/NCT03264989 (Accessed on November 21, 2019)].
125.De Castro LM, Zennadi R, Jonassaint JC, Batchvarova M, Telen MJ. Effect of propranolol as antiadhesive therapy in sickle cell disease. Clin Transl Sci. 2012;5(6):437-44. https://doi.org/10.1111/cts.12005 PMid:23253664 PMCid:PMC3762678
126.DeCastro LM. Study of Propranolol as Anti-Adhesive Therapy in Sickle Cell Disease (SCD) [NCT01077921] ClinicalTrials.gov [Available from: https://clinicaltrials.gov/ct2/show/NCT01077921 (Accessed on November 18, 2019)].
127.Field JJ, Lin G, Okam MM, Majerus E, Keefer J, Onyekwere O, et al. Sickle cell vaso-occlusion causes activation of iNKT cells that is decreased by the adenosine A2A receptor agonist regadenoson. Blood. 2013;121(17):3329-34. https://doi.org/10.1182/blood-2012-11-465963 PMid:23377438 PMCid:PMC3637009
128.Field JJ, Majerus E, Gordeuk VR, Gowhari M, Hoppe C, Heeney MM, et al. Randomized phase 2 trial of regadenoson for treatment of acute vaso-occlusive crises in sickle cell disease. Blood Adv. 2017;1(20):1645-9. https://doi.org/10.1182/bloodadvances.2017009613 PMid:29296811 PMCid:PMC5728341
129.Scheuplein F, Thariath A, Macdonald S, Truneh A, Mashal R, Schaub R. A humanized monoclonal antibody specific for invariant Natural Killer T (iNKT) cells for in vivo depletion. PLoS One. 2013;8(9):e76692. https://doi.org/10.1371/journal.pone.0076692 PMid:24086759 PMCid:PMC3785425
130.Field JJ, Majerus E, Ataga KI, Vichinsky EP, Schaub R, Mashal R, et al. NNKTT120, an anti-iNKT cell monoclonal antibody, produces rapid and sustained iNKT cell depletion in adults with sickle cell disease. PLoS One. 2017;12(2):e0171067. https://doi.org/10.1371/journal.pone.0171067 PMid:28152086 PMCid:PMC5289534
131.Hoppe C, Kuypers F, Larkin S, Hagar W, Vichinsky E, Styles L. A pilot study of the short-term use of simvastatin in sickle cell disease: effects on markers of vascular dysfunction. Br J Haematol. 2011;153(5):655-63. https://doi.org/10.1111/j.1365-2141.2010.08480.x PMid:21477202 PMCid:PMC3601917
132.University of North Carolina Chapel Hill. Effect of Atorvastatin on Endothelial Dysfunction and Albuminuria in Sickle Cell Disease (ENDO) [NCT01732718] ClinicalTrials.gov [Available from: https://clinicaltrials.gov/ct2/show/NCT01732718 (Accessed on November 18, 2019)].
133.Children's Hospital Medical Center Cincinnati. Trial of Zileuton CR in Children and Adults with Sickle Cell Disease ClinicalTrials.gov [Available from: http://clinicaltrials.gov/ct2/show/NCT01136941?term=nct01136941&rank=1 (Accessed on November 18, 2019)].
134.Zafarullah M, Li WQ, Sylvester J, Ahmad M. Molecular mechanisms of N-acetylcysteine actions. Cell Mol Life Sci. 2003;60(1):6-20. https://doi.org/10.1007/s000180300001 PMid:12613655
135.Pace BS, Shartava A, Pack-Mabien A, Mulekar M, Ardia A, Goodman SR. Effects of N-acetylcysteine on dense cell formation in sickle cell disease. Am J Hematol. 2003;73(1):26-32. https://doi.org/10.1002/ajh.10321 PMid:12701116
136.Academisch Medisch Centrum - Universiteit van Amsterdam (AMC-UvA). N-Acetylcysteine in Patients With Sickle Cell Disease (NAC) [NCT01849016] ClinicalTrials.gov [Available from: https://clinicaltrials.gov/ct2/show/NCT01849016 (Accessed on November 18, 2019)].
137.Novartis Pharmaceuticals. ILARIS (canakinumab) [Package insert]. East Hanover, NJ.2012.
138.Novartis Pharmaceuticals. Study of Efficacy, Safety and Tolerability of ACZ885 (Canakinumab) in Pediatric and Young Adult Patients With Sickle Cell Anemia (NCT02961218) ClinicalTrials.gov [Available from: https://clinicaltrials.gov/ct2/show/NCT02961218 (Accessed on December 16, 2019)].
139.Rees DC, Kilinc Y, Unal S, Dampier C, Pace BS, Kaya B, et al. Double-Blind, Randomized Study of Canakinumab Treatment in Pediatric and Young Adult Patients with Sickle Cell Anemia. Blood. 2019;134 (Suppl_1):615.
140.Gilead Sciences Inc. Letairis (ambrisentan) tablets [Package insert]. Foster City, CA.2015.
141.Augusta University. The Role of Endothelin-1 in Sickle Cell Disease (NCT02712346) ClinicalTrials.gov [Available from: https://clinicaltrials.gov/ct2/show/NCT02712346 (Accessed on December 16, 2019)].
142.Kutlar A, Pollock J, Meiler SE, Harris R, Hongyan X, Wells L, et al. Phase-I Study of ETA Receptor Antagonist Ambrisentan in Sickle Cell Disease. Blood. 2019;134 (Suppl_1):617.
143.Lal A, Atamna W, Killilea DW, Suh JH, Ames BN. Lipoic acid and acetyl-carnitine reverse iron-induced oxidative stress in human fibroblasts. Redox Rep. 2008;13(1):2-10. https://doi.org/10.1179/135100008X259150 PMid:18284845
144.UCSF Benioff Children's Hospital Oakland. Antioxidant Therapy to Reduce Inflammation in Sickle Cell Disease (NCT01054768) ClinicalTrials.gov [Available from: https://clinicaltrials.gov/ct2/show/NCT01054768 (Accessed on November 18, 2019)].
145.Martins VD, Manfredini V, Peralba MC, Benfato MS. Alpha-lipoic acid modifies oxidative stress parameters in sickle cell trait subjects and sickle cell patients. Clin Nutr. 2009;28(2):192-7. https://doi.org/10.1016/j.clnu.2009.01.017 PMid:19231043
146.Christen JR, Bertolino J, Jean E, Camoin L, Ebbo M, Harle JR, et al. Use of Direct Oral Anticoagulants in Patients with Sickle Cell Disease and Venous Thromboembolism: A Prospective Cohort Study of 12 Patients. Hemoglobin. 2019:1-4. https://doi.org/10.1080/03630269.2019.1689997 PMid:31724442
147.Morris CR, Morris SM, Jr., Hagar W, Van Warmerdam J, Claster S, Kepka-Lenhart D, et al. Arginine therapy: a new treatment for pulmonary hypertension in sickle cell disease? Am J Respir Crit Care Med. 2003;168(1):63-9. https://doi.org/10.1164/rccm.200208-967OC PMid:12626350
148.Morris CR, Ansari M, Lavrisha L, et al. Arginine therapy for vaso-occlusive pain episodes in sickle cell disease. Blood (ASH Annual Meeting Abstracts). 2009;114:573. https://doi.org/10.1182/blood.V114.22.573.573
149.Morris CR, Kuypers FA, Lavrisha L, Ansari M, Sweeters N, Stewart M, et al. A randomized, placebo-controlled trial of arginine therapy for the treatment of children with sickle cell disease hospitalized with vaso-occlusive pain episodes. Haematologica. 2013;98(9):1375-82. https://doi.org/10.3324/haematol.2013.086637 PMid:23645695 PMCid:PMC3762093
150.UCSF Benioff Children's Hospital Oakland. Effectiveness of Arginine as a Treatment for Sickle Cell Anemia (Arginine) [NCT00513617] ClinicalTrials.gov [Available from: https://clinicaltrials.gov/ct2/show/NCT00513617 (Accessed on November 18, 2019)].
151.Hospital de Clinicas de Porto Alegre. L-Arginine and Sickle Cell Disease (NCT01142219) ClinicalTrials.gov [Available from: https://clinicaltrials.gov/ct2/show/NCT01142219 (Accessed on November 18, 2019)].
152.National Institutes of Health Clinical Center. Evaluation of Hydroxyurea Plus L-arginine or Sildenafil to Treat Sickle Cell Anemia (NCT00056433) ClinicalTrials.gov [Available from: https://clinicaltrials.gov/ct2/show/NCT00056433 (Accessed on November 18, 2019)].
153.UCSF Benioff Children's Hospital Oakland. Arginine Treatment of Acute Chest Syndrome (Pneumonia) in Sickle Cell Disease Patients (NCT00029731) ClinicalTrials.gov [Available from: https://clinicaltrials.gov/ct2/show/NCT00029731 (Accessed on November 18, 2019)].
154.Perrine SP. Phase II Randomized Trial:Arginine Butyrate Plus Standard Local Therapy in Patients With Refractory Sickle Cell Ulcers (NCT00004412) ClinicalTrials.gov [Available from: https://clinicaltrials.gov/ct2/show/NCT00004412 (Accessed on November 18, 2019)].
155.Gladwin MT, Kato GJ, Weiner D, Onyekwere OC, Dampier C, Hsu L, et al. Nitric oxide for inhalation in the acute treatment of sickle cell pain crisis: a randomized controlled trial. JAMA. 2011;305(9):893-902. https://doi.org/10.1001/jama.2011.235 PMid:21364138 PMCid:PMC3403835

156. Lopez BL, Davis-Moon L, Ballas SK, Ma XL. Sequential nitric oxide measurements during the emergency department treatment of acute vasoocclusive sickle cell crisis. Am J Hematol. 2000;64(1):15-9. https://doi.org/10.1002/(SICI)1096-8652(200005)64:1<15::AID-AJH3>3.0.CO;2-P
157. Head CA, Swerdlow P, McDade WA, Joshi RM, Ikuta T, Cooper ML, et al. Beneficial effects of nitric oxide breathing in adult patients with sickle cell crisis. Am J Hematol. 2010;85(10):800-2. https://doi.org/10.1002/ajh.21832 PMid:20799359
158. Ikuta T, Thatte HS, Tang JX, Mukerji I, Knee K, Bridges KR, et al. Nitric oxide reduces sickle hemoglobin polymerization: potential role of nitric oxide-induced charge alteration in depolymerization. Arch Biochem Biophys. 2011;510(1):53-61. https://doi.org/10.1016/j.abb.2011.03.013 PMid:21457702 PMCid:PMC3889650
159. Pfizer. Safety, Tolerability, Pharmacokinetics, And Pharmacodynamics Study Of PF-04447943, Co-Administered With And Without Hydroxyurea, In Subjects With Stable Sickle Cell Disease (NCT02114203) ClinicalTrials.gov [Available from: https://clinicaltrials.gov/ct2/show/NCT02114203 (Accessed on November 18, 2019)].
160. Charnigo RJ, Beidler D, Rybin D, Pittman DD, Tan B, Howard J, et al. PF-04447943, a Phosphodiesterase 9A Inhibitor, in Stable Sickle Cell Disease Patients: A Phase Ib Randomized, Placebo-Controlled Study. Clin Transl Sci. 2019;12(2):180-8. https://doi.org/10.1111/cts.12604 PMid:30597771 PMCid:PMC6440678
161. Singh N, Patra S. Phosphodiesterase 9: insights from protein structure and role in therapeutics. Life Sci. 2014;106(1-2):1-11. https://doi.org/10.1016/j.lfs.2014.04.007 PMid:24746902
162. Imara Inc. An Extension Study of IMR-687 in Adult Patients With Sickle Cell Anemia (NCT04053803) ClinicalTrials.gov [Available from: https://clinicaltrials.gov/ct2/show/NCT04053803 (Accessed on November 22, 2019)].
163. Kato GJ. A Multi-Center Study of Riociguat in Patients With Sickle Cell Diseases (NCT02633397) ClinicalTrials.gov [Available from: https://clinicaltrials.gov/ct2/show/NCT02633397 (Accessed on November 22, 2019)].
164. Cyclerion Therpeutics. A Study of the Effect of IW-1701 (Olinciguat), a Stimulator of Soluble Guanylate Cyclase (sGC), on Patients With Sickle Cell Disease (SCD) (STRONG SCD) [NCT03285178] ClinicalTrials.gov [Available from: https://clinicaltrials.gov/ct2/show/NCT03285178 (Accessed on November 22, 2019)].
165. Eaton WA, Bunn HF. Treating sickle cell disease by targeting HbS polymerization. Blood. 2017;129(20):2719-26. https://doi.org/10.1182/blood-2017-02-765891 PMid:28385699 PMCid:PMC5437829
166. Geng X, Dufu K, Hutchaleelaha A, Xu Q, Li Z, Li CM, et al. Increased hemoglobin-oxygen affinity ameliorates bleomycin-induced hypoxemia and pulmonary fibrosis. Physiological reports. 2016;4(17). https://doi.org/10.14814/phy2.12965 PMid:27624688 PMCid:PMC5027366
167. Howard J, Hemmaway CJ, Telfer P, Layton DM, Porter J, Awogbade M, et al. A phase 1/2 ascending dose study and open-label extension study of voxelotor in patients with sickle cell disease. Blood. 2019;133(17):1865-75. https://doi.org/10.1182/blood-2018-08-868893 PMid:30655275 PMCid:PMC6484388
168. Forma Therapeutics Inc. A SAD/MAD to Assess the Safety, Pharmacokinetics and Pharmacodynamics of FT-4202 in Healthy Volunteers and Sickle Cell Disease Patients (NCT03815695) ClinicalTrials.gov [Available from: https://clinicaltrials.gov/ct2/show/NCT03815695 (Accessed on December 16, 2019)].
169. Kalfa TA, Kuypers FA, Telen MJ, Malik P, Konstantinidis DG, Estepp JH, et al. Phase 1 Single (SAD) and Multiple Ascending Dose (MAD) Studies of the Safety, Tolerability, Pharmacokinetics (PK) and Pharmacodynamics (PD) of FT-4202, an Allosteric Activator of Pyruvate Kinase-R, in Healthy and Sickle Cell Disease Subjects. Blood. 2019;134 (Suppl_1):616.
170. National Heart Lung and Blood Institute (NHLBI). Niacin to Improve Blood Flow in People With Sickle Cell Disease (NCT00508989) ClinicalTrials.gov [Available from: https://clinicaltrials.gov/ct2/show/NCT00508989 (Accessed on November 18, 2019)].
171. Icahn School of Medicine at Mount Sinai. Vitamin D3 in Patients With Sickle Cell Disease (NCT03012555) ClinicalTrials.gov [Available from: https://clinicaltrials.gov/ct2/show/NCT03012555 (Accessed on November 18, 2019)].
172. Soe HH, Abas AB, Than NN, Ni H, Singh J, Said AR, et al. Vitamin D supplementation for sickle cell disease. Cochrane Database Syst Rev. 2017;1:Cd010858. https://doi.org/10.1002/14651858.CD010858.pub2 PMCid:PMC6464759

Table References:

1. Greenberg J, Ohene-Frempong K, Halus J, Way C, Schwartz E. Trial of low doses of aspirin as prophylaxis in sickle cell disease. J Pediatr. 1983;102(5):781-4. https://doi.org/10.1016/S0022-3476(83)80258-3
2. Baxalta now part of Shire. A Single Dose Study of the Safety, Blood Levels and Biological Effects of Aes-103 Compared to Placebo in Subjects With Stable Sickle Cell Disease (NCT01597401) ClinicalTrials.gov [Available from: https://clinicaltrials.gov/ct2/show/NCT01597401 (Accessed on November 18, 2019).
3. Xu GG, Pagare PP, Ghatge MS, Safo RP, Gazi A, Chen Q, et al. Design, Synthesis, and Biological Evaluation of Ester and Ether Derivatives of Antisickling Agent 5-HMF for the Treatment of Sickle Cell Disease. Mol Pharm. 2017;14(10):3499-511. https://doi.org/10.1021/acs.molpharmaceut.7b00553 PMid:28858508 PMCid:PMC5871537
4. Joiner CH, Jiang M, Claussen WJ, Roszell NJ, Yasin Z, Franco RS. Dipyridamole inhibits sickling-induced cation fluxes in sickle red blood cells. Blood. 2001;97(12):3976-83. https://doi.org/10.1182/blood.V97.12.3976 PMid:11389043
5. Desai PC, Brittain JE, Jones SK, McDonald A, Wilson DR, Dominik R, et al. A pilot study of eptifibatide for treatment of acute pain episodes in sickle cell disease. Thromb Res. 2013;132(3):341-5. https://doi.org/10.1016/j.thromres.2013.08.002 PMid:23973010 PMCid:PMC3791139
6. HemaQuest Pharmaceuticals Inc. A Study of HQK-1001 in Patients With Sickle Cell Disease (NCT01322269) ClinicalTrials.gov [Available from: https://clinicaltrials.gov/ct2/show/NCT01322269 (Accessed on November 18, 2019).
7. Gladwin MT, Kato GJ, Weiner D, Onyekwere OC, Dampier C, Hsu L, et al. Nitric oxide for inhalation in the acute treatment of sickle cell pain crisis: a randomized controlled trial. JAMA. 2011;305(9):893-902. https://doi.org/10.1001/jama.2011.235 PMid:21364138 PMCid:PMC3403835
8. Waugh WH, Daeschner CW, 3rd, Files BA, McConnell ME, Strandjord SE. Oral citrulline as arginine precursor may be beneficial in sickle cell disease: early phase two results. J Natl Med Assoc. 2001;93(10):363-71.
9. Brousseau DC, Scott JP, Badaki-Makun O, Darbari DS, Chumpitazi CE, Airewele GE, et al. A multicenter randomized controlled trial of intravenous magnesium for sickle cell pain crisis in children. Blood. 2015;126(14):1651-7. https://doi.org/10.1182/blood-2015-05-647107 PMid:26232172 PMCid:PMC4591790
10. Belcher JD, Young M, Chen C, Nguyen J, Burhop K, Tran P, et al. MP4CO, a pegylated hemoglobin saturated with carbon monoxide, is a modulator of HO-1, inflammation, and vaso-occlusion in transgenic sickle mice. Blood. 2013;122(15):2757-64. https://doi.org/10.1182/blood-2013-02-486282 PMid:23908468 PMCid:PMC4067504
11. Sangart. Phase 2 Study of MP4CO to Treat Vaso-occlusive Sickle Crisis (NCT01925001) ClinicalTrials.gov [Available from: https://clinicaltrials.gov/ct2/show/NCT01925001 (Accessed on November 18, 2019).
12. Orringer EP, Casella JF, Ataga KI, Koshy M, Adams-Graves P, Luchtman-Jones L, et al. Purified poloxamer 188 for treatment of acute vaso-occlusive crisis of sickle cell disease: A randomized controlled trial. JAMA. 2001;286(17):2099-106.

https://doi.org/10.1001/jama.286.17.2099
PMid:11694150
13. Ballas SK, Files B, Luchtman-Jones L, Benjamin L, Swerdlow P, Hilliard L, et al. Safety of purified poloxamer 188 in sickle cell disease: phase I study of a non-ionic surfactant in the management of acute chest syndrome. Hemoglobin. 2004;28(2):85-102.
https://doi.org/10.1081/HEM-120035919
PMid:15182051
14. Mast Therapeutics Inc. Phase III Randomized Study of Poloxamer 188 for Vaso-Occlusive Crisis of Sickle Cell Disease (NCT00004408) ClinicalTrials.gov
[Available from: https://clinicaltrials.gov/ct2/show/NCT00004408
(Accessed on November 18, 2019).
15. Daak AA, Ghebremeskel K, Hassan Z, Attallah B, Azan HH, Elbashir MI, et al. Effect of omega-3 (n-3) fatty acid supplementation in patients with sickle cell anemia: randomized, double-blind, placebo-controlled trial. Am J Clin Nutr. 2013;97(1):37-44.
https://doi.org/10.3945/ajcn.112.036319
PMid:23193009
16. Miller RE. Omega-3 Fatty Acids in Sickle Cell Disease (NCT02947100) ClinicalTrials.gov
[Available from: https://clinicaltrials.gov/ct2/show/NCT02947100
(Accessed on November 18, 2019).
17. Heeney MM, Hoppe CC, Abboud MR, Inusa B, Kanter J, Ogutu B, et al. A Multinational Trial of Prasugrel for Sickle Cell Vaso-Occlusive Events. N Engl J Med. 2016;374(7):625-35.
https://doi.org/10.1056/NEJMoa1512021
PMid:26644172
18. Ataga KI, Reid M, Ballas SK, Yasin Z, Bigelow C, James LS, et al. Improvements in haemolysis and indicators of erythrocyte survival do not correlate with acute vaso-occlusive crises in patients with sickle cell disease: a phase III randomized, placebo-controlled, double-blind study of the Gardos channel blocker senicapoc (ICA-17043). Br J Haematol. 2011;153(1):92-104.
https://doi.org/10.1111/j.1365-2141.2010.08520.x
PMid:21323872
19. Icagen. A Study Evaluating the Long-Term Safety of ICA-17043 in Sickle Cell Disease Patients With or Without Hydroxyurea Therapy (NCT00294541) ClinicalTrials.gov
[Available from: https://clinicaltrials.gov/ct2/show/NCT00294541
(Accessed on November 18, 2019).
20. Machado RF, Martyr S, Kato GJ, Barst RJ, Anthi A, Robinson MR, et al. Sildenafil therapy in patients with sickle cell disease and pulmonary hypertension. Br J Haematol. 2005;130(3):445-53.
https://doi.org/10.1111/j.1365-2141.2005.05625.x
PMid:16042696 PMCid:PMC2063570
21. Machado RF, Barst RJ, Yovetich NA, Hassell KL, Kato GJ, Gordeuk VR, et al. Hospitalization for pain in patients with sickle cell disease treated with sildenafil for elevated TRV and low exercise capacity. Blood. 2011;118(4):855-64.
https://doi.org/10.1182/blood-2010-09-306167
PMid:21527519 PMCid:PMC3148167
22. Montefiore Medical Center. Topical Sodium Nitrite in Sickle Cell Disease and Leg Ulcers (NCT02863068) ClinicalTrials.gov [Available from: https://clinicaltrials.gov/ct2/show/NCT02863068 (Accessed on November 18, 2019).
23. TRF Pharma Inc. TRF-1101 Assessment in Sickle Cell Disease (NCT00773890) ClinicalTrials.gov
[Available from: https://clinicaltrials.gov/ct2/show/NCT00773890
(Accessed on November 18, 2019).
24. Anthera Pharmaceuticals. A Study of Varespladib Infusion in Subjects With Sickle Cell Disease. (IMPACTS-2) [NCT01522196] ClinicalTrials.gov
[Available from: https://clinicaltrials.gov/ct2/show/NCT01522196
(Accessed on November 18, 2019).
25. Mast Therapeutics Inc. Evaluation of Purified Poloxamer 188 in Vaso-Occlusive Crisis of Sickle Cell Disease (EPIC) [NCT01737814] ClinicalTrials.gov [Available from:
https://clinicaltrials.gov/ct2/show/NCT01737814?term=purified+poloxamer+188&rank=1 (Accessed on November 18, 2019).
26. Dana-Faber Cancer Institute. Efficacy of Vorinostat to Induce Fetal Hemoglobin in Sickle Cell Disease (NCT01000155) ClinicalTrials.gov
[Available from: https://clinicaltrials.gov/ct2/show/NCT0100015 5
(Accessed on November 18, 2019).
27. Modus Therapeutics AB. Sevuparin Infusion for the Management of Acute VOC in Subjects With SCD (NCT02515838) ClinicalTrials.gov
[Available from:
https://clinicaltrials.gov/ct2/show/record/NCT02515838
(Accessed on November 18, 2019).
28. Kutlar A, Embury SH. Cellular adhesion and the endothelium: P-selectin. Hematol Oncol Clin North Am. 2014;28(2):323-39.
https://doi.org/10.1016/j.hoc.2013.11.007
PMid:24589269
29. Pfizer. Efficacy and Safety of Rivipansel (GMI-1070) in the Treatment of Vaso-Occlusive Crisis in Hospitalized Subjects With Sickle Cell Disease (NCT02187003) ClinicalTrials.gov
[Available from: https://clinicaltrials.gov/ct2/show/NCT02187003
(Accessed on November 18, 2019).
30. Prolong Pharmaceuticals. Study of SANGUINATE™ In the Treatment of Sickle Cell Disease Patients With Vaso-Occlusive Crisis (NCT02411708) ClinicalTrials.gov
[Available from: https://clinicaltrials.gov/ct2/show/NCT02411708
(Accessed on November 18, 2019).