



Review Article

The Evolving Pharmacotherapeutic Landscape for the Treatment of Sickle Cell Disease

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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.

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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 ($\alpha\beta_2$). 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 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

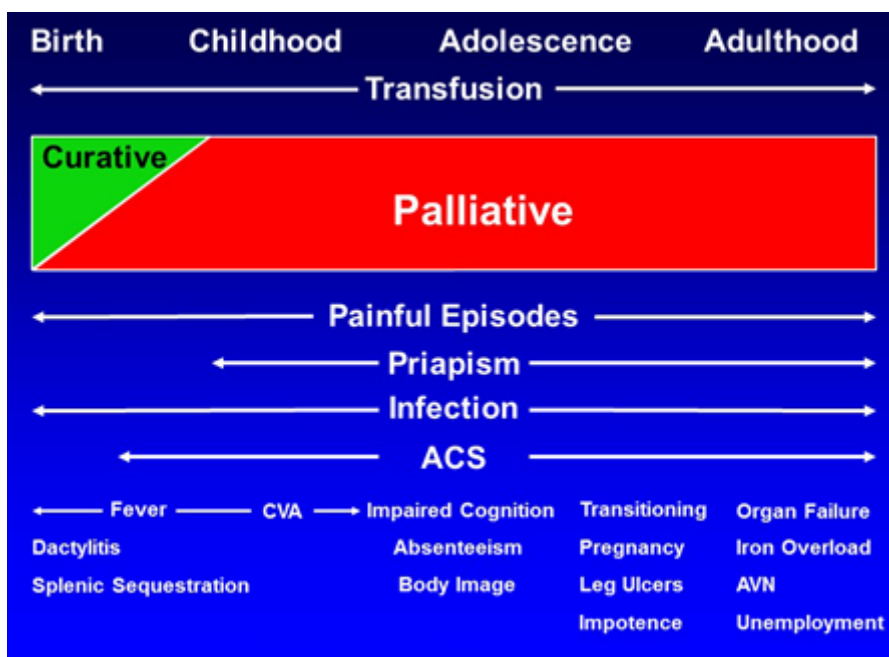


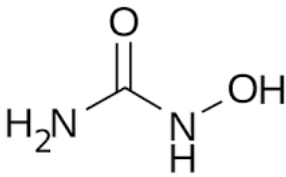
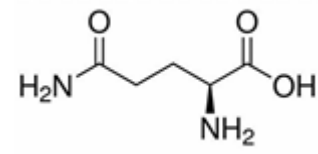
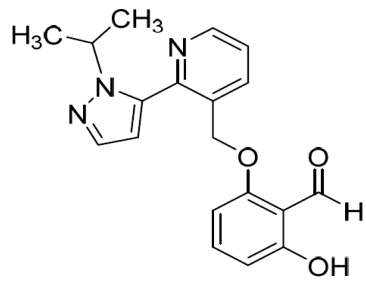
Figure 1. Sequence of complications of sickle cell anemia from birth through adult life. ACS = acute chest syndrome; AVN = Avascular necrosis; CVA = Cerebrovascular accident. From Hem Onc Clin North Am. 2005; 19:785-802. Used with permission.

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 $\text{CH}_4\text{N}_2\text{O}_2$  | 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-deoxy-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 ₄ H ₇ NaO 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 ₃ O 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 ₂ O 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 | (C ₃₃ H ₃₄ FN ₂ O ₅) ₂ Ca. 3H ₂ O | 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. | C ₁₁ H ₁₂ N ₂ O ₂ S | 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 C ₅ H ₉ N ₃ O ₃ S | 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 C ₈ H ₁₄ O ₂ S ₂ | 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-1 β 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 flow |
| 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 Nitric Oxide (NO) in the ED | INO Therapeutics | $\text{:}\ddot{\text{N}}=\ddot{\text{O}}\text{:}$ | 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 C ₂₁ H ₂₆ N ₆ O ₂ | SCA (Homozygous HbSS or Sickle- β 0 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 | Olinciguat UNII-PD5F4ZXD2 C ₂₁ H ₁₆ F ₅ N ₇ O 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 ₆ NH ₅ O 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: Sick cell anemia; SCD: Sick 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 β -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- α . 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-1 β . 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 heme 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 Sick 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 tmc 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 studies³⁹ 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 reticulocytes⁴⁰ 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 starting dose is 15 mg/kg/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 |

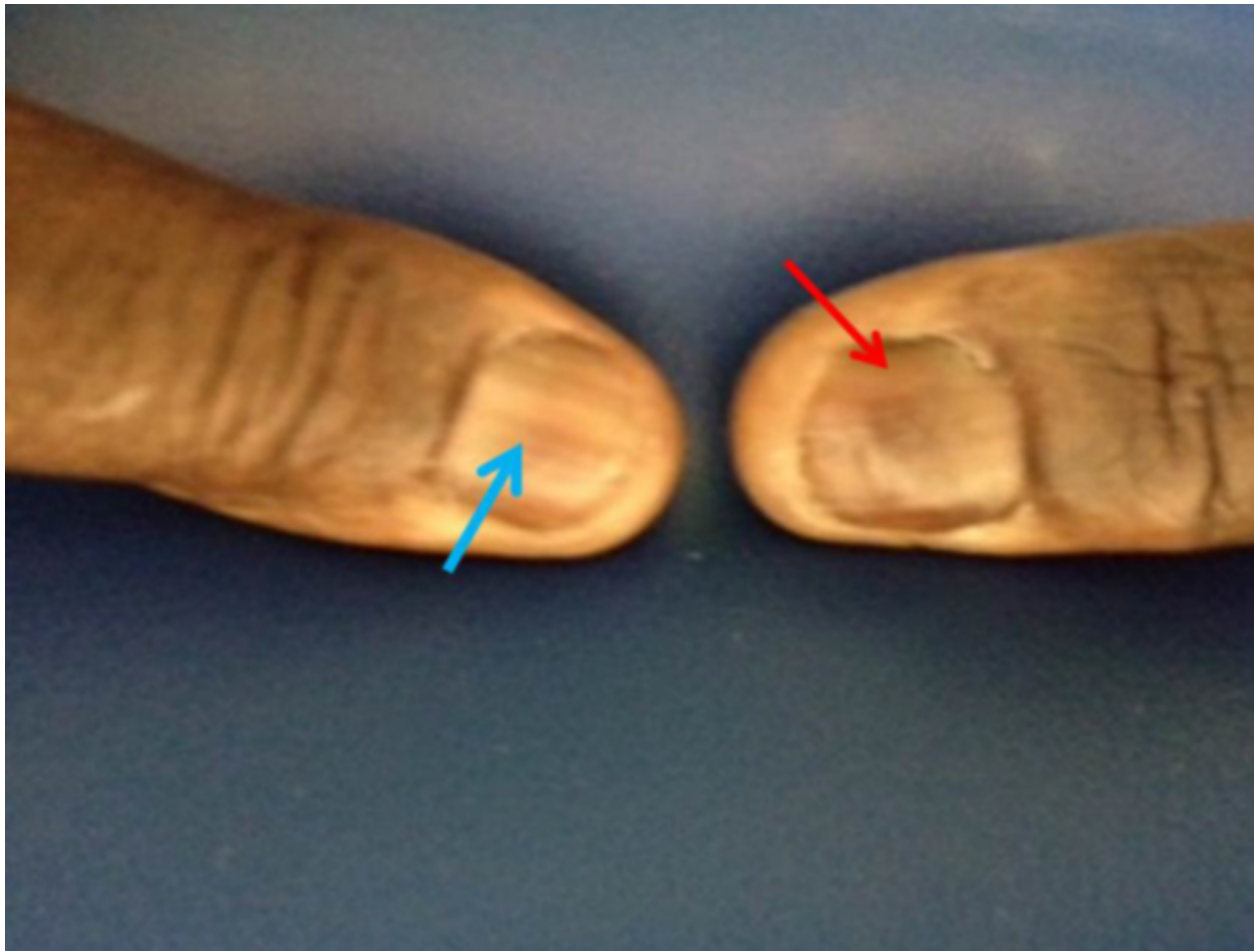


Figure 2. Fingernails of a 38-year-old man with sickle cell anemia and hydroxyurea-induced melanonychia characterized by longitudinal (blue arrow) and diffuse (red arrow) bands. From *J Blood Disorders Transf.* 2013;4:5. Used with permission.

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 Malawi⁶⁸ but not in Nigeria⁶⁹ 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 MRI⁷⁹ 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 SelGI (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 binding 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 CYP3A4 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 SCDI.

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 Fc γ RIII 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 pharmacokinetics and pharmacodynamics study of SEG101 (crizanlizumab) 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

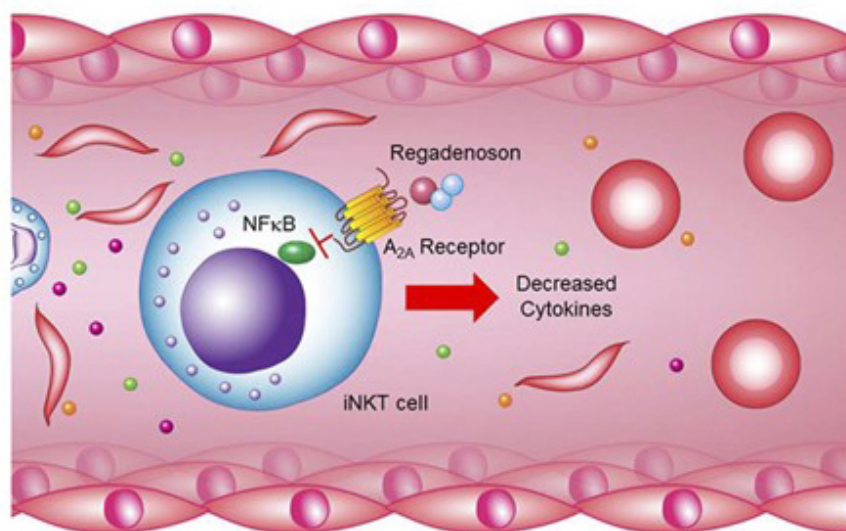


Figure 3: Randomized phase 2 trial of Regadenoson for treatment of acute vaso-occlusive crises in sickle cell disease. From Blood Adv. 2017;1(20):1645-9. Used with permission.

c. Targeting inflammation

Regadenoson. In SCA patients there is increase in the number of activated Invariant Natural Killer T (iNKT) cells. Regadenoson is an A_{2A} 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-1 β 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 multicenter, randomized, parallel group, double-blind, placebo-controlled trial that recruited patients with SCA (HbSS or HbS/ β^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. Ambrisentan (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 reduce 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 transduction¹⁶¹ 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

Oliniguat is used 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 Oliniguat 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. were 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.

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