The complex, frequently devastating, multi-organ pathophysiology of sickle cell disease has a single root cause: polymerization of deoxygenated sickle hemoglobin. A logical approach to disease modification is, therefore, to interdict this root cause. Ideally, such interdiction would utilize small molecules that are practical and accessible for worldwide application. Two types of such small molecule strategies are actively being evaluated in the clinic. The first strategy intends to shift red blood cell precursor hemoglobin manufacturing away from sickle hemoglobin and towards fetal hemoglobin, which inhibits sickle hemoglobin polymerization by a number of mechanisms. The second strategy intends to chemically modify sickle hemoglobin directly in order to inhibit its polymerization. Important lessons have been learnt from the pre-clinical and clinical evaluations to date. Open questions remain, but this review summarizes the valuable experience and knowledge already gained, which can guide ongoing and future efforts for molecular mechanism-based, practical and accessible disease modification of sickle cell disease.

Introduction

Sickle cell disease (SCD) demands practical, accessible oral therapies, since it is a problem of global scope. It afflicts millions of people worldwide, and has an especially high prevalence in pediatric populations in low-income, malaria-belt countries. Such therapies are technically plausible, since despite the complex and potentially devastating multi-organ pathophysiology of SCD, this condition has a single, well-characterized root cause: polymerization of deoxygenated sickle hemoglobin (HbS). The hemoglobin molecule is an assembly of two α-like protein subunits and two β-like protein subunits (α2β2), each with a heme moiety to transport an oxygen molecule. In SCD, the gene for the β sub-unit (HBB) of adult hemoglobin (HbA) contains an ‘A’ to ‘T’ mutation in the seventh codon. The β sub-units (βS) produced by this mutated gene substitute a hydrophilic glutamate with a hydrophobic valine, predisposing deoxygenated HbS (α2βS2) to polymerization and gelation in red blood cells (RBC). This affects RBC viability, rheology and adhesiveness, promoting hemolysis, endothelial damage, occlusion of small blood vessels, and thromboses of large vessels. The hemolytic anemia is frequently severe, and is only partially and non-sustainably compensated by >10-fold increases in erythropoiesis. The net consequence of this anemia and vaso-occlusion is decreased oxygen delivery and hypoxic injury to potentially all tissues of the body, manifest clinically as episodic pain, chronic pain, avascular necrosis of bones, infections, overt and silent strokes, renal/respiratory/cardiac/hepatic failure, and early death. In the USA >$1 billion in annual health care costs is attributed to SCD, and even so, the median life expectancy of affected individuals is shortened by two or more decades on average. Most children with SCD in low-income countries do not even survive to adulthood. By way of emphasis, all this morbidity and mortality begins with a single process, polymerization of deoxygenated HbS in RBC, and it is therefore logical to attempt to interdict this root cause. Two major small molecule drug approaches are in active clinical evaluation: (i) small molecules to shift the hemoglobin manufactured by RBC precursors from HbS to fetal hemoglobin (HbF), and (ii) small molecules to chemically modify HbS to impede its polymerization.
Interdicting HbS polymerization by pharmacological induction of HbF

At the fetal stage of life, RBC contain fetal hemoglobin (HbF), an assembly of two α-globin subunits and two γ-globin subunits (α2γ2), with the γ-globin subunits being encoded by duplicated γ-globin genes (HBG2 and HBG4). During human development, the switch from HbF to HbA production begins late in fetal gestation (~7 months), and the typical adult pattern of <1% HbF and >90% HbA in RBC is established by ~12 months post-conception. Several genetic polymorphisms or mutations in humans, some but not all identified, promote persistent, relatively high RBC HbF content beyond infancy. The phenotypes with particularly generous HbF levels (HbF >10%) are referred to as hereditary persistence of fetal hemoglobin (HPFH). SCD patients who co-inherit such genetic variants can, in the best cases, have asymptomatic, normal life-spans. Notably, HbF has benefits even at lower dynamic ranges than seen in HPFH: HbF levels correlate continuously with fewer vaso-occlusive pain crises, less renal damage, less pulmonary hypertension, fewer strokes and longer survival. In short, nature has demonstrated that HbF is a highly potent modulator of SCD.

Detailed biochemical studies have demonstrated how: the intracellular concentration of HbS is a major determinant of polymerization kinetics, and HbF substitution for HbS decreases this concentration. Moreover, HbF does not polymerize with deoxygenated HbS for reasons of molecular structure (the sophisticated biophysics underlying this have recently been reviewed in detail). By contrast, HbA can polymerize with deoxygenated HbS. In short, HbF interdicts the root-cause pathophysiology of SCD. It is logical therefore to attempt to use pharmacology to recapitulate such naturally demonstrated, powerful disease modulation.

The earliest efforts at HbF induction

The earliest efforts built on the observation that HbF is enriched in RBC produced during the recovery phase of
bone marrow from severe insults or stress. 

One way of creating such stress is to administer cytotoxic (cell killing) drugs, leading to clinical evaluation in SCD of the oral ribonucleotide reductase inhibitor hydroxyurea. In the pivotal trial, hydroxyurea (15-35 mg/kg) increased HbF for 2 years in ~50% of the adult SCD patients treated. As predicted, HbF increases with hydroxyurea correlated strongly with longer RBC half-life, fewer pain crises, and better quality of life (the benefits of hydroxyurea therapy in sickle cell mice also depended on HbF induction). Trial patients with HbF levels >0.5 g/dL also survived longer although a caveat to these analyses was that it was not known whether the higher HbF levels were intrinsic to the patients or a result of the hydroxyurea therapy.

There were, however, noteworthy limitations to the induction of HbF by hydroxyurea: (i) average HbF increases at 2 years were modest (3.6%); (ii) HbF increases were particularly unlikely in patients with the lowest baseline HbF levels and thus at highest risk of morbidity and mortality, and (iii) HbF increases diminished over time, even in the ~50% of patients with excellent initial HbF induction.

A shared basis for these several limitations was suggested by the correlation between lower HbF increases and fewer reticulocytes (<300,000 x 10^9/L) and neutrophils (<7.5 x 10^9/L) at baseline: this correlation underscored that HbF induction by cytotoxicity requires sufficient reserves of hematopoietic precursors to mount repeated recoveries from the stress that destroys their counterparts. Such reserves are circumscribed, subject to attrition via vaso-occlusion in the marrow and kidneys, and decline with aging. A declining capacity to compensate for hemolytic anemia is a problem even separate from considerations of sustainable HbF induction via cytotoxicity: SCD patients require erythropoiesis at >10-fold the normal rate simply to sustain hemoglobin levels compatible with life, and dwindling compensatory reticulocytosis is a major cause of early death. Therefore, alternative, non-cytotoxic, durable, and more potent methods of inducing HbF are needed.

**Directly targeting the enzymes that silence the γ-globin gene**

DNA in nuclei is packaged together with RNA and structural proteins – histones - to form chromatin. Chromatin regulates gene transcription by determining accessibility of genes to the massive machinery (~150 proteins) that transcribes genes. Reorganization (‘remodeling’) of chromatin, to facilitate or hinder this machinery, is signaled via post-translational modifications to histones - methylation and acetylation of lysine residues, phosphorylation of threonines and serines – and by modifications to DNA, mainly, methylation of cytosines that precede guanines (CpG). These signals determine whether ATP-dependent chromatin remodelers shift histones towards or away from gene transcription start sites, repositioning these physical barriers to either welcome or obstruct the gene transcribing basal transcription factor machinery.

Thus, induction of HbF, even when it is indirectly via bone marrow stress, implies remodeling γ-globin and β-globin gene loci, to activate one and not the other. Specifically, persistent HbF expression requires: (i) decreased operation at HBG2/HBG1 of epigenetic enzymes that create ‘off’ marks and that reposition histones to obstruct transcription start sites, and (ii) increased function of the epigenetic enzymes that create epigenetic ‘on’ marks and that reposition histones away from transcription start sites, with *vice versa* at HBB. Cytotoxic methods of inducing HbF achieve such chromatin remodeling crudely and indirectly, via bone marrow stress. 

So why not identify repressing epigenetic enzymes and inhibit them directly (Figure 2)? Cells contain dozens of epigenetic enzymes mediating gene activation and repression, and not all repressing epigenetic enzymes (corepressors).
isor protein complexes) are logical molecular targets for therapy. Sequence-specific DNA-binding factors are particular in their epigenetic co-regulator usage, e.g., even distinguishing between closely similar BAF and PBAF coacti-

Table 1. Scientifically validated molecular targets for HbF induction and candidate drugs

| Target          | Recruited by BCL11A | Drugs                                                                 | Stage                                                                 |
|-----------------|----------------------|----------------------------------------------------------------------|----------------------------------------------------------------------|
| HDAC*           | Yes                  | - Depsipeptide (HDAC1,2,4,6)                                         | - Marketed for peripheral T-cell lymphomas                           |
|                 |                      | - Belinostat (broad HDAC inhibitor)                                   | - Phase I in SCD (panobinostat)                                      |
|                 |                      | - Panobinostat (broad)                                                | (ClinicalTrials.gov identifier: NCT01245179)                         |
|                 |                      | - Vorinostat (broad)                                                  | - Phase II in SCD and β-thalassemia                                  |
|                 |                      |                                                                      | (HQK-1001) (ClinicalTrials.gov identifiers: NCT01642758, NCT01601340) |
| DNMT1           | Yes                  | - Decitabine                                                          | - Marketed for myelodysplastic syndromes                             |
|                 |                      | - 5-azacytidine (decitabine pro-drug)                                 | - Oral forms, including in combination with inhibitors of degradation, are in phase I/II for liquid/solid malignancies, and SCD (ClinicalTrials.gov identifier: NCT01665315) |
| KDM1A           | Yes                  | - ORY-1001 (related to RN-1)                                          | - Phase I/II in liquid/solid malignancies                            |
|                 |                      | - GSK2379552                                                         | - Phase I in SCD (INCB059872)                                        |
|                 |                      | - 4SC-202                                                            | (ClinicalTrials.gov identifier: NCT0313324) (terminated, results not publicly available) |
|                 |                      | - INCB05872                                                          |                                                                      |
| PRMT5           | Not reported         | - GSK3326595                                                         | - Phase I in liquid/solid malignancies                               |
| EHMT2           | Not reported         | - UNC0638                                                            | - Pre-clinical in vitro                                              |
| ISWI (CHD4, SMARCA5) | Yes                | - not officially designated, patent issued                          | - Pre-clinical in vitro                                              |

*Only histone deacetylase (HDAC) inhibitors approved in the USA are listed, several other HDAC inhibitors are in clinical trials. **Only KDM1A inhibitors registered in clinical trials in the USA are listed, several other compounds are in development. SCD: sickle cell disease.
ring covalently binds to DNMT1 and causes its degradation. By depleting DNMT1 protein, decitabine disrupts its scaffolding functions for other epigenetic enzymes such as KDM1A. That is, decitabine does not just inhibit the enzyme function of DNMT1 but produces a broad corepressor disrupting effect. Because the deoxyribose moiety of decitabine is unmodified, it can incorporate into the elongating DNA strand during the S-phase without terminating chain extension or causing cytotoxicity, contrasting with most nucleoside analogs used in the clinic to treat cancer. High concentrations of decitabine do, however, produce off-target anti-metabolite effects and cytotoxicity, in significant part via its uridine moiety degradation products that can misincorporate into DNA or inhibit thymidylate synthase. We designed decitabine dose, schedule and route-of-administration regimens to produce non-cytotoxic depletion of DNMT1 in vivo. These regimens increased HbF by >10% in SCD patients who had no HbF response (~0.3%) to hydroxyurea in the pivotal clinical trial. That is, very small, non-cytotoxic doses of ~0.2 mg/kg twice weekly were sufficient to produce large increases in HbF and total hemoglobin, even in patients in whom hydroxyurea ~20 mg/kg/day, >1000-fold the molar amount of decitabine, did not induce HbF (Figure 2).

Market decitabine, however, is a parenteral drug with trivial oral bioavailability, undermining potential for worldwide application. We have therefore combined oral decitabine with tetrahydroydridine to inhibit the enzyme that limits its oral bioavailability, cytidine deaminase. This combination was well-tolerated and safe in a phase 1 study in patients with severe SCD. The target decitabine dose of 0.16 mg/kg produced a wide decitabine concentration-time profile (low Cmax, long T1/2) ideal for non-cytotoxic DNMT1 depletion and decreased DNMT1 protein in peripheral blood mononuclear cells by >75% and repetitive element Cpg methylation by ~10%. This increased HbF by 4-9%, doubling HbF-enriched RBC (F-cells) up to ~80% of total RBC. Total hemoglobin increased by 1.2-1.9 g/dL (~0.2%).

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patients with myeloid malignancies. A corollary of the above is that although inhibiting silencing epigenetic enzymes can produce cell fate or function shifts, these relate to what the cells were to begin with and are not drastic. This is of course critical clinically, since a candidate epigenetic therapeutic for SCD will be distributed systemically.

What then about the cellular/transcription factor context of erythropoiesis enables inhibition of DNMT1 etc., to activate \( HBG2/\)HBB? Several groups have found that the developmental switch from \( HBG2/\)HBB to HBB activation is recapitulated, albeit very rapidly, during erythroid lineage maturation (a ‘maturational switch’ during routine erythropoiesis). The maturational switch entails removal of activating and acquisition of repressive epigenetic marks at \( HBG2/\)HBB.\(^{106,110}\) with physical migration of the shared enhancer, the locus control region, from the \( HBG2/\)HBB to the HBB locus.\(^{111}\) These dynamics at \( HBG2/\)HBB and HBB during erythropoiesis creates an opportunity for pharmacological/biochemical intervention to prevent enhancer migration, to stall the massive gene activating machinery at \( HBG2/\)HBB. That is, HBF induction by inhibiting epigenetic ‘off’ enzymes such as DNMT1 is not predicated on returning the enhancer from HBB back to \( HBG2/\)HBB (turning a gene that is ‘off’ to ‘on’), but on preventing a switch from \( HBG2/\)HBB to HBB in the first place (preventing a gene that is ‘on’ from being turned ‘off’). Accordingly, HBF induction by an inhibitor of the silencing epigenetic enzyme EHMT2 (UNC0638) depended on the timing of its addition to cultures of synchronously maturing erythroid progenitors,\(^{112}\) with similar observations in our hands with DNMT1-depleting drugs (personal communication).

**Why are these drugs being evaluated for, or used, to treat cancer?**

Some of the most recurrently mutated, deleted or amplified genes in cancers encode for chromatin remodelers. Thus, another concern with epigenetic targeting is whether it might mimic some of these genetic changes and favor activation of oncogenic programs. It is reassuring to some extent, however, that the epigenetic targets and drugs discussed above have been or are being developed to treat and/or prevent cancer. We recently reviewed the biological rationale for this, and it is briefly summarized here: cancer cells, including self-replicating cancer cells (cancer or leukemia ‘stem’ cells), contain high amounts of the lineage master transcription factors that normally activate terminal lineage-fates, and depend on specific corepressors (‘addictions’) in order to avoid these terminal fates.\(^{111}\) The pathway of action is activation of the terminal lineage-fates intended by cancer cell lineage master transcription factor content. The same chromatin-‘relaxing’ treatments that trigger terminal lineage-fates of cancer/leukemia stem cells preserve self-renewal of uncommitted tissue stem cells, since these cells express stem cell master transcription factors, not high levels of lineage-specifying transcription factors.\(^{110,111}\) This therapeutic index explains why non-cytotoxic doses and schedules of decitabine can suppress malignant clones and simultaneously improve functional blood counts even in elderly patients with myeloid malignancies.\(^{111,112}\) Stated simply, several corepressor components (repressing epigenetic enzymes), e.g., DNMT1, HDAC, KDM1A, have been biologically validated as molecular targets for the treatment and prevention of cancer.\(^{111}\)

**Teratogenic risks**

Another concern is the potential for teratogenicity: this should be assumed for individual agents, unless shown otherwise by formal toxicological studies.

**Drug metabolism is central to the clinical profile of activity**

Drugs, being biologically active, are metabolized, and this too can contribute substantially to their in vivo profile of activity. For example, DNMT1-depleting decitabine is a pyrimidine nucleoside analog pro-drug that depends absolutely for its activity on the pyrimidine metabolism enzyme deoxycytidine kinase: Deoxycytidine kinase executes the initial phosphorylation of decitabine in cells, which rate-limits its conversion into the nucleotide form that actually depletes DNMT1. Serendipitously, deoxycytidine kinase is most highly expressed in the nucleotide form that actually depletes DNMT1. Serendipitously, deoxycytidine kinase is most highly expressed in the nucleotide form that actually depletes DNMT1. Thus, another concern with epigenetic targeting is whether it might mimic some of these genetic changes and favor activation of oncogenic programs. It is reassuring to some extent, however, that the epigenetic targets and drugs discussed above have been or are being developed to treat and/or prevent cancer. We recently reviewed the biological rationale for this, and it is briefly summarized here: cancer cells, including self-replicating cancer cells (cancer or leukemia ‘stem’ cells), contain high amounts of the lineage master transcription factors that normally activate terminal lineage-fates, and depend on specific corepressors (‘addictions’) in order to avoid these terminal fates. The pathway of action is activation of the terminal lineage-fates intended by cancer cell lineage master transcription factor content. The same chromatin-‘relaxing’ treatments that trigger terminal lineage-fates of cancer/leukemia stem cells preserve self-renewal of uncommitted tissue stem cells, since these cells express stem cell master transcription factors, not high levels of lineage-specifying transcription factors. This therapeutic index explains why non-cytotoxic doses and schedules of decitabine can suppress malignant clones and simultaneously improve functional blood counts even in elderly patients with myeloid malignancies. Stated simply, several corepressor components (repressing epigenetic enzymes), e.g., DNMT1, HDAC, KDM1A, have been biologically validated as molecular targets for the treatment and prevention of cancer. Another concern is the potential for teratogenicity: this should be assumed for individual agents, unless shown otherwise by formal toxicological studies.

**Baseline HBF levels dictate final HBF levels**

There is a wide variation in baseline HBF levels in patients with SCD and even in the general population, reflecting the influence of various genetic polymorphisms on the regulation of this locus. Even if a molecular targeted therapy produces similar rates of increase in HBF% (the percentage of total hemoglobin that is HBF) in all patients, the final HBF% will be dictated by the level at which HBF% began. Moreover, in clinical trials we have conducted with DNMT1-depleting decitabine, we have noticed a slightly lower slope to the rate of increase in HBF% in SCD patients with lower HBF% at baseline. Fortunately and importantly, however, HBF induced by this epigenetic strategy was well-distributed among RBC, and the rates of increase of HBF-enriched RBC (F-cells) was actually higher in patients with low F-cells at baseline.

At some time-point after starting therapy, F-cells entering the circulation are matched by a similar number of F-cells leaving the circulation, producing plateaus in HBF% and F-cell%.

**Small molecules to chemically modify HbS to impede polymerization**

The scientific foundation for efforts to chemically modify HbS is the two-state allosteric Monod-Wyman-Changeux structural model which characterizes the rapidly reversible equilibrium between the quaternary structure of hemoglobin with low oxygen affinity (fully deoxygenated hemoglobin, “T” quaternary structure) and the hemoglobin quaternary structure with high affinity for oxygen (deoxygenated hemoglobin, ‘R’ quaternary structure). The Monod-Wyman-Changeux model demonstrated incompatibility of the R conformation with polymerization, creating a foundation to propose molecules to favor the high oxygen affinity R conformation, as a method to delay HbS polymerization. The basic concern with such an approach is that SCD is a disease of decreased oxygen delivery to tissues and...
thus, if a chemical modification produces a high oxygen affinity hemoglobin molecule, there is a necessary play off between decreased oxygen supply from increased oxygen affinity of hemoglobin versus increased oxygen supply from less HbS polymerization/higher total hemoglobin. This balancing act is discussed in more detail in the section ‘Lessons learned so far’ below. Ultimately, however, rigorous clinical evaluation is key,5,118 and clinical evaluation has started or is underway for a number of candidate drugs that exploit these principles:

Small molecules to convert hemoglobin to methemoglobin
The earliest clinical effort in this field evaluated the conversion of hemoglobin to methemoglobin following the administration of sodium nitrite or para-amino-propiophenone (PAPP) to five patients.119 Both agents were able to increase methemoglobin. Methemoglobin levels of >20% (but not less) produced by sodium nitrite extended RBC survival as measured by chromium-labeling. The methemoglobinemia itself was apparently well-tolerated, but there was no evidence of any clinical benefit. Instead, there were significant side-effects from the administered drugs.119

Interestingly, higher methemoglobin levels produced by PAPP did not extend RBC survival, possibly because PAPP was directly hemolytic.

Small molecules to convert hemoglobin to carboxyhemoglobin
Carbon monoxide can be used to convert hemoglobin to carboxyhemoglobin. Infusion of free pegylated carboxyhemoglobin (MP4CO), as a hemoglobin-based carbon monoxide carrier, was evaluated in a phase I study.120 In an abstract description of results in 18 patients, the maximum increase in carboxyhemoglobin was to 2%, which returned to pre-dosing levels within 8 h of completion of the MP4CO infusion. There was no significant increase in total hemoglobin. No further studies have been reported.

Small molecules that delay HbS polymerization by unclear mechanisms
Niprisan (Nix-0699) and related small molecules (SCD-101) are plant-derived molecules that have been found to delay polymerization of deoxygenated HbS, but by unclear mechanisms.121 SCD-101 has been evaluated in a phase IB clinical trial in 26 SCD patients. There were no major adverse events attributed to the drug taken for 28 days, and it appeared to decrease chronic pain and fatigue at higher doses. However, there were no laboratory data providing evidence of decreased hemolysis or increased total hemoglobin, although analysis of peripheral smears suggested improvements in RBC shape.122

Small molecules to increase hemoglobin oxygen affinity
Specific small molecule aldehydes have been found to form reversible Schiff base linkages with the N-terminal amino group of hemoglobin α chains to lock in the high oxygen affinity R conformation, and the polyaromatic aldehyde GBT440 (voxelotor) has been developed through to phase III clinical trial evaluation. In phase I/II randomized, double-blind, placebo-controlled evaluation in SCD patients, some of whom were receiving concurrent therapy with hydroxyurea, there were increases in total hemoglobin of ≥1 g/dL in six of 12 patients who received the drug for 90 days or more.123 There were concurrent decreases in markers of hemolysis (lactate dehydrogenase, total bilirubin). There were no significant adverse events attributed to study drug. Oxygen delivery was evaluated by measurement of oxygen consumption during cardiopulmonary exercise testing, erythropoietin levels, resting heart rate and heart rate during peak exercise, and these parameters did not suggest decreased oxygen delivery to tissues.125 A subsequent double-blind, randomized, placebo-controlled phase III clinical trial evaluated two different doses of the study drug (900 and 1500 mg per day) in 274 SCD patients, two-thirds of whom remained on stable doses of hydroxyurea initiated well before study enrollment.124 A hemoglobin response, defined as an increase from baseline of >1 g/dL at week 24, occurred in 51% of the patients on the 1500 mg dose, 35% on the 900 mg dose, and 7% on placebo, in intention-to-treat analyses. There were also improvements in biomarkers of hemolysis. The frequency of vaso-occlusive crises did not differ between the treatment arms. Breakdown of vaso-occlusive crisis frequency according to whether or not the patients were taking hydroxyurea was not reported. Erythropoietin levels (as a surrogate for oxygen delivery) as well as grade 3 and serious adverse events were similar between the treatment arms.124

Chemical modification of HbS – lessons learned so far and open questions

Balancing acts
The clinical trial results with GBT440 thus illustrate that chemical modification of hemoglobin to increase its oxygen affinity (promote the hemoglobin R conformation) can indeed significantly decrease hemolysis and significantly increase total hemoglobin. The hope and goal is that higher hemoglobin increases oxygen supply by amounts that exceed any decrease in oxygen supply from the higher oxygen affinity of the modified hemoglobin molecule,5,118 as per the equation:

\[
\text{Oxygen Supply} = \text{Blood Flow (mL blood/100 g tissue/min)} \times \text{Arterial Oxygen Saturation (％)} \times \text{Total Hemoglobin (g/dL)}.125
\]

Thus, increasing total hemoglobin increases oxygen supply, but chemical modification of some of these hemoglobin molecules to increase oxygen affinity decreases effective arterial oxygen saturation and oxygen supply. Some tissues, e.g., the brain, have limited capacity to increase the ‘blood flow’ component in the equation, and hence, are particularly dependent on the ‘arterial oxygen saturation’ x ‘total hemoglobin’ components, as extensively modeled recently.118,125 Under scoring this point, most silent cerebral infarctions in SCD children have been found to be caused by disruption to oxygen supply that is not caused by large vessel vasculopathy, implying anemia and/or blood oxygen saturation are critical drivers of this hypoxic damage.136-138

Even the ‘blood flow’ component of the equation is a balancing act in SCD patients: whole blood viscosity is a key determinant of blood flow; less HbS polymerization, by increasing (improving) RBC deformability, can decrease whole blood viscosity and thus increase blood flow. On the other hand, higher total hemoglobin/hematocrit can increase blood viscosity which can decrease blood flow, even with hematocrits in an anemia range, because of the contribution of baseline low RBC deformability of SCD to viscosity. This blood flow calculus needs
to be considered with small molecules aiming to chemically modify HbS, and with small molecules aiming to substitute HbS with HbF.

Ultimately, the risk/benefit calculus for any therapeutic approach requires careful clinical trial determination.

**Combinatorial approaches**

In oncology, combinations of drugs are almost mandatory, because the target cell population is evolving, and will select to evade the effects of drugs. Although target cells in SCD are not evolving, other biological realities compel consideration of combination therapies. One reality is that most SCD patients will already have tissue/organ damage that can undermine the potential benefits of novel small molecule therapeutics. For example, diminished bone marrow reserve from vaso-occlusive damage and/or replication-mediated exhaustion, which decreases compensatory reticulocytosis, and which contributes to early death. If high doses of compounds that can produce compensatory reticulocytosis (nicotinamide, glutamine, etc.) could limit the scope of potential benefit that can be produced by HbF inducers or HbS modifiers. Another biological reality, but potentially positive, is demonstrated by the approval by the FDA of Gardos channel blocker, senicapoc (ICA-17043), in patients taking GBT440 1500 mg alone versus ~55% of patients taking GBT440 1500 mg + hydroxyurea in the phase III trial, but whether vaso-occlusive crisis frequency and other adverse events varied between these two groups was not described. The efficacy calculus and hope is that increases in oxygen delivery from better RBC deformability and higher total hemoglobin will exceed decreases in oxygen delivery caused by greater blood viscosity and chemical modification of HbS.

**Conclusions**

Clinical proof-of-principle that substantial total hemoglobin increases can be produced by non-cytotoxic inhibition of specific epigenetic enzymes, to shift RBC precursor hemoglobin manufacturing from HbS to HbF, and by chemical modification of hemoglobin to promote the high oxygen affinity ‘R’ quaternary structure of the hemoglobin molecule, has already been generated in SCD patients. Clinical evaluation to determine the long-term safety, the impact on symptoms and multi-organ pathophysiology, and the durability of any benefits, is ongoing. There is hope that one or more of the small molecules being evaluated will pass rigorous scrutiny and culminate in practical, accessible, cost-effective, safe and potent disease-modifying therapy for SCD patients worldwide.

**Funding source:** National Heart, Lung and Blood Institute UO1 HL117658.

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