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Perspective

Treating Sickle Cell Disease by Targeting HbS Polymerization
(Running Title: Targeting HbS Polymerization)

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Although the root cause of sickle cell disease is the polymerization of HbS to form fibers that make red cells less flexible, most drugs currently in clinical trials are targeting the downstream sequelae of this primary event. Less attention has been devoted toward investigation of the multiple ways in which fiber formation can be inhibited. In this Perspective, we describe the molecular rationale for 5 distinct approaches to inhibit polymerization and also discuss progress with the few anti-polymerization drugs currently in clinical trials.

Introduction

There has been a recent explosion in the interest among investigators in both the hematology academic community and in the pharmaceutical industry to develop new treatments for sickle cell disease, as indicated by the large number of active clinical protocols¹⁻⁵(see clinicaltrials.gov) and the many talks and posters at the December 2016 ASH meeting in San Diego. The interest and progress in discovering new treatments for the first “molecular disease” ⁶,⁷ have been fueled by multiple factors. With an appropriate sibling match, the disease can be cured in both children and adults by stem cell transplantation.⁸,⁹ Moreover, sickle cell disease is a testing ground for the exciting emerging methods of gene therapy and gene editing.¹⁰⁻¹² While these are potential therapies for patients in the US, neither will be available for several decades for millions of patients in sub-Saharan Africa and elsewhere.¹³ What is needed, therefore, to treat the vast majority of patients is an affordable drug that can be taken orally. The development of hydroxyurea has been a major advance in the treatment of sickle cell disease, but is only partially effective in preventing vaso-occlusive crises¹⁴⁻¹⁶.

Most of the non-genetic approaches currently in clinical trials are aimed at using drugs to ameliorate the downstream sequelae of sickle hemoglobin (HbS) polymerization, such as adhesion of red cells to vascular endothelium, leukocytes and platelets, as well as inflammation, coagulation and nitric oxide scavenging.¹⁷⁻²¹ Indeed, there is strong rationale for thorough investigation of these phenomena, and therapeutic applications are beginning to bear fruit. For example, the administration of GMI-1070, a pan-selectin inhibitor, appears to shorten the duration of acute sickle pain crises accompanied by a marked reduction in opioid use.²² Moreover, a recent randomized double-blinded study found that monthly administration of a monoclonal antibody against P-selectin was effective in lowering the frequency of sickle pain crises.²³ Although these therapeutic interventions do not appear to be significantly superior to hydroxyurea, they could be effective in combination. Other attempts to target downstream sequelae have thus far proven to be even less effective.²⁴⁻³²
In this Perspective we argue that more bench research and clinical trials should be directed toward the polymerization process itself, the root cause of sickle cell pathology. The purpose of this article is to briefly describe the biochemical and biophysical basis of five distinct approaches to inhibit polymerization for treating sickle cell disease and to discuss their connection to the anti-polymerization drugs currently in clinical trials.

Before considering different ways of decreasing fiber formation, it is important to point out that therapeutic benefit does not require complete inhibition of HbS polymerization. The rates of oxygen binding and dissociation are so fast (milliseconds) that the fractional saturation of normal hemoglobin during the ~1 second duration of blood flow through the microcirculation is determined only by the oxygen pressure. Consequently, the equilibrium oxygen binding curves, which are measured on a minutes time scale, are relevant to the physiological situation in vivo. In contrast, kinetics plays a critical role in sickle cell disease because the system is very far from equilibrium. For most SS red cells, the rate of polymerization in vivo is much slower than the transit time through the microcirculation and therefore far less polymerization occurs in vivo than is observed at the same oxygen pressures at equilibrium in vitro. If polymerization were at equilibrium (i.e., instantaneous relative to the transit time), almost every cell in patients with homozygous SS disease would contain fibers in the tissues, while numerous studies show that only a fraction of red cells (often quite small) contain fibers in the veins (see 34 and references therein). Moreover, at equilibrium, sickle fibers would be present in the tissues in a large fraction of red cells of patients who are compound heterozygotes for HbS and hereditary persistence of fetal hemoglobin (HbS/HPFH). The red cells of these patients have a pancellular distribution of fetal hemoglobin and "have symptoms of neither sickle cell disease nor hemolytic anemia". What makes SS disease survivable and S/HPFH benign is the unusual kinetics of polymerization, with a marked delay period prior to the appearance of fibers that allows most cells to escape the small vessels of the tissues before fibers start to form (Fig. 1). Because the delay time is so sensitive to oxygen pressure and intracellular hemoglobin
Figure 1. Connection between kinetics and pathophysiology. (A) Schematic of kinetic progress curve for polymerization occurring on the seconds time scale measured by light scattering due to fiber formation (a.u. = arbitrary units). Prior to the appearance of fibers there is a delay (lag phase)38. The delay time is extraordinarily sensitive to HbS concentration, depending on the 30th power of the concentration40. Such a huge exponent means that a decrease of only 8% in the HbS concentration increases the delay time 10-fold. (B) Schematic of microcirculation - arteriole, capillary and venule. The vast majority of cells escape the microcirculation before fibers form and cause cellular distortion (“sickling”).34,41 (C) Schematic of vaso-obstruction. If the delay time is shorter than the transit time (or fibers have not completely dissolved upon oxygenation in the lungs42 and can grow without a delay34,43), fibers form within the small vessels and can cause vaso-obstruction. In this picture, factors that slow the transit of red cells through the microcirculation, such as increased adherence to the vascular endothelium by damaged red cells or increased leukocytes associated with infection will increase the probability of vaso-occlusion.

concentration, even small degrees of polymerization inhibition can produce large increases in the delay time, allowing many more cells to escape the tissues without sickling.44 Moreover, transient changes in the delay time probably contributes importantly to the episodic nature and unpredictability of sickle cell crises.45 Thus, the probability of sickling in the microcirculation is decreased if either the delay time is increased43,44 or the transit time through the microcirculation is decreased, which is the therapeutic rationale for reducing adhesion of blood cells to the vascular endothelium.17-19,21

Five approaches to inhibiting HbS polymerization

I. Block intermolecular contacts in the sickle fiber.

One of the important early milestones in sickle cell research was the construction of a detailed molecular model of the fiber structure (Fig. 2). The structure is based on image reconstruction of transmission electron micrographs (Fig. 2A),46 the X-ray structure of deoxy HbS47 (Fig. 2B) and the determination of residues that participate in an intermolecular contact from polymerization studies on mixtures of HbS with other naturally occurring hemoglobins containing mutated residues on the molecular surface48,49. The 21 nm diameter fiber is constructed of 14 strands, consisting of 7 helically twisted strand pairs found in the X-ray structure of deoxyHbS (Fig. 2C). The polymerization studies of mixtures positioned the X-ray determined double strands in the fiber to define the inter-double strand contacts.
A common approach to drug development is to identify a protein target and screen compounds that bind tightly to a specific site on the protein, such as the active or, more recently, allosteric site of an enzyme. The analogous approach for treating sickle cell disease would be to find a small molecule that has a high affinity for a site on the surface of the HbS molecule that is involved in an intermolecular contact in the fiber (see ref. 44 for a complete list of contact sites). The problem in using this approach for sickle cell disease is three-fold. First, the drug must have a high degree of specificity for binding to hemoglobin. Secondly, there is almost one pound of hemoglobin in the average patient with homozygous sickle cell disease, so unless the binding is extremely strong, as in a covalent bond, a very large amount of drug would be required. The third concern is that the surface of the hemoglobin molecule is smooth, with no residues involved in an intermolecular contact available for stereospecific covalent attachment of an inhibitor and no apparent deep clefts or crevices that would be required for tight, non-covalent binding (Fig. 2D). However, transient openings in the structure for drug targeting might be discovered using all-atom molecular dynamics simulations, a method currently being used to find binding sites for
drug targeting that are not obvious from static X-ray structures.\textsuperscript{50} Consequently, although much more challenging than the more common empirical drug targeting, this approach should not be discounted.

II. **Induce fetal hemoglobin synthesis.**

The symptoms of sickle cell disease do not appear until several months after birth when most of the fetal hemoglobin (HbF) is replaced by HbS. Moreover, as mentioned previously, the compound heterozygous condition of sickle cell disease with pancellular persistence of fetal hemoglobin (S/HPFH) is a relatively benign condition. Importantly, in S/HPFH, the HbF is homogeneously distributed. These "F cells" all contain about 30\% HbF and 70\% HbS.\textsuperscript{37} In contrast, in SS disease HbF is heterogeneously distributed with about 35\% F cells and no detectable HbF in the remaining 65\%.\textsuperscript{51} Following hydroxyurea therapy at maximum tolerated doses, the fraction of F cells rises to nearly 50\% while the fraction of HbF increases even more. Thus hydroxyurea therapy results in an increase in HbF per F cell. The drug would be more effective if the increase in HbF were distributed among a greater fraction of red cells.\textsuperscript{52} The focus of current research in this area, therefore, is to induce HbF synthesis in as many red cells as possible.\textsuperscript{53} One promising approach is the selective inhibition of the transcription factor BC11A which dramatically increases the expression of $\gamma$ globin.\textsuperscript{54,55}

Because of the enormous sensitivity of the kinetics of polymerization to $\alpha_2\beta^S_2$ concentration, the beneficial effect of HbF results primarily from the decrease in the intracellular concentration of the HbS homotetramer, $\alpha_2\beta^S_2$.\textsuperscript{56} This inhibitory effect, however, is quite complex\textsuperscript{35} (Fig. 3). Dissociation of tetramers into $\alpha\beta$ dimers and random
Figure 3. Mechanism of inhibition of polymerization by fetal hemoglobin. (A) Dissociation of tetramers into dimers and reassociation in mixtures of HbS and HbF results in 3 tetramers in a binomial distribution, thereby further lowering the fraction of the HbS homotetramer (α_{2}β_{S}^{2}).57 (B) Cartoon of polymerization equilibrium in HbS/HbF mixture. As in a crystallization reaction, hemoglobin tetramers are present in two phases - the solution phase (left) or the fiber phase (right). The fibers that form in these mixtures are primarily composed of the HbS homotetramers, but there is also some copolymerization of hybrid tetramers (α_{2}β_{S}^{5}γ) (half-green, half-empty circles).35,58 (C) Cartoon of F cell with 30% HbF and 70% HbS.
The excluded volume effect of the non-copolymerizing $\alpha_2\gamma_2$ tetramer (full green-filled circles) and partially copolymerizing tetramer $\alpha_2\beta^S\gamma$ (half-green, half-empty circles) increases the activity of the polymerizing $\alpha_2\beta_2^S$ homotetramer (empty circles) (D) Activity coefficient ($\gamma$) as a function of total Hb concentration. The unitless activity coefficient is the factor that multiplies the measured concentration (i.e. moles per liter or grams per deciliter) to obtain the “activity”, which is the thermodynamically-effective concentration.\(^5^9\) reassociation results in a binomial distribution of tetramers, reducing the concentration of the $\alpha_2\beta_2^S$ homotetramer further.\(^5^7\) (Fig. 3A). A mixture of 70% HbS and 30% HbF, for example, contains 3 tetramers with a binomial distribution, 49% $\alpha_2\beta_2^S$, 9% $\alpha_2\gamma_2$, and 42% $\alpha_2\gamma\beta^S$.

Two additional effects must be considered in addition to tetramer-dimer dissociation and reassociation. One is copolymerization of the $\alpha_2\gamma\beta^S$ hybrid tetramer (Fig.3B),\(^5^8,6^0\) which can enter the fiber, but to a much lesser extent than the $\alpha_2\beta_2^S$ homotetramer or even the $\alpha_2\beta_2^S\beta^A$ hybrid tetramer because in the $\gamma$ subunit threonine at position 87 is replaced by a glutamine, which forms a much less favorable critically-important intermolecular lateral contact with valine 6 (Fig. 2B). The second effect on the $\alpha_2\beta_2^S$ concentration decrease is the large non-ideality in the concentrated HbS solution within the red cell. The thermodynamically effective concentration of $\alpha_2\beta_2^S$, called the activity, is not the measured concentration in moles or grams per unit volume, but rather the concentration multiplied by a correction factor, the activity coefficient ($\gamma$) (Fig. 3D). The activity coefficient accounts for the fact that the non-copolymerizing tetramers take up space in the solution and decrease the volume accessible to the polymerizing tetramers. This so-called “excluded volume” effect is extremely large and must be considered in all thermodynamic and kinetic descriptions of polymerization.\(^5^9,6^1-6^4\) The activity at the concentration of 35 g/dl, a typical MCHC, for example, is almost 100-times greater than the measured concentration. The net result of this effect is that the activity of $\alpha_2\beta_2^S$ in the solution phase at equilibrium is increased, making the decrease in the concentration of the $\alpha_2\beta_2^S$ tetramer less effective in increasing the delay time than by simply increasing red cell volume by means of osmotic or ionophoric dilution discussed below.

### III. Increase oxygen affinity.

The results of studies on the control polymerization by oxygen can be explained by applying the famous two-state allosteric model of Monod Wyman and Changeux (MWC) (Fig. 4).\(^6^5,6^6\) According to the model of MWC, there is an equilibrium between a low oxygen affinity arrangement of the 4 subunits of fully deoxyhemoglobin, called the T quaternary structure, and a high affinity arrangement of fully oxygenated Hb, called the R quaternary structure (Fig. 4A). Solubility measurements of concentrated solutions of HbS as a function of the saturation of hemoglobin with oxygen in the solution phase can be almost quantitatively explained by not allowing the R quaternary structure to enter the fiber. (The solubility is the concentration of Hb in the solution phase and corresponds to the measured concentration of Hb in the supernatant after sedimenting the fibers in an ultracentrifuge. The solubility is an accurate measure of the stability of the fiber - the lower the solubility, the more stable is the fiber\(^6^7,6^8\). Moreover, the sickle fiber binds oxygen with an affinity that is very similar to the T quaternary structure in the crystal.\(^5^9,7^0\) These results are consistent with a structural analysis, which shows that the arrangement of the subunits in the R structure produces many steric clashes\(^7^1\) that preclude incorporation into the T-containing fiber.
Figure 4. Mechanism of polymerization inhibition by increasing oxygen affinity. (A) Hemoglobin exists in a rapidly reversible equilibrium between low and high affinity quaternary conformations, called T and R, respectively. They differ primarily by a ~15° relative rotation of αβ dimers. Location of beta 6 valine is shown as a yellow dot on the surface of the molecule. Preferential binding of a small molecule such as a drug (red circle) to R shifts the quaternary equilibrium toward R. (B) Cartoon of polymerization equilibrium. Only the T quaternary structure (empty circles) enters the fiber. R quaternary conformations (filled circles) are completely excluded. (C) Oxygen binding curves. Preferential binding of a drug to the R quaternary structure causes a left shift (increased oxygen affinity).
The MWC analysis of the control of polymerization by oxygen strongly suggests that increasing oxygen affinity by shifting the T-R equilibrium toward R, could be a sound way to inhibit HbS polymerization in vivo and therefore be an effective treatment strategy. Indeed, years ago, this rationale prompted Ernest Beutler to increase the fraction of R hemoglobin in a small number of sickle cell patients by induction of either methemoglobin or carboxyhemoglobin. He found that either intervention resulted in a significant prolongation of red cell life span. However neither could be adapted for practical and safe long-term therapy. Current advances in drug discovery and high throughput screening offer hope of developing small molecules that bind to R preferentially and with high specificity and would therefore be a compelling therapeutic approach. The potential downside of altering the T-R equilibrium is that the resulting “left shift” in the oxygen binding curve (Fig. 4C) could potentially decrease oxygen delivery in a disease where the patients already suffer from impaired blood flow and oxygen transport. The question, however, is what is the net effect – reduced oxygen delivery from the left shift or improved oxygen delivery from decreased sickling and therefore decreased vaso-occlusive events. The physiology is too complicated to make any predictions based on biophysical studies, but as discussed below, the results of clinical studies currently underway, should provide the answer to this question.

IV. Reduce concentration of 2,3-diphosphoglycerate

2,3-diphosphoglycerate (DPG) is the major allosteric effector for hemoglobin and has three effects on HbS polymerization. It binds in the cleft between the β subunits (Fig. 5A,B) to stabilize the deoxy (T) quaternary structure and thereby decreases oxygen affinity by shifting the T-R quaternary equilibrium towards T (Fig. 5C). Thus, lowering DPG concentration would increase the fraction of HbS in the non-polymerizing R quaternary structure. A second effect of DPG is its stabilization of the fiber, as shown by a decrease in the HbS solubility. Although the decrease in solubility is only ~ 10 %, the presence of DPG will have a dramatic effect on the kinetics of polymerization, i.e. the delay time, which depends on about the 30th power of the solubility. Finally, lowering DPG concentration results in a third, albeit smaller, but therapeutically significant increase in solubility because of an accompanying rise in the intracellular pH via the Gibbs-Donnan equilibrium. Over the physiologic intracellular pH range 7.2-7.3 the solubility of deoxyHbS increases significantly with rising pH. Thus, reduction of red cell DPG increases both the solubility and delay times by three independent mechanisms.

The importance of DPG in the pathophysiology of sickle cell disease is underscored by two recent and highly relevant case reports. Individuals with sickle trait (AS), which is normally a totally benign condition, have a clinical phenotype almost as severe as SS disease when they also inherit a deficiency in red cell pyruvate kinase that causes a marked elevation of red cell DPG. Because DPG plays such a critical role in potentiating HbS polymerization, there is compelling rationale for the development of drugs that target the enzymatic pathway responsible for its remarkably high (5 mM) concentration in red cells. A number of anions stimulate the “phosphatase” activity of DPG synthase, thereby lowering DPG levels. In particular, in the presence of 0.02 mM phosphoglycolate, the dephosphorylation of DPG is activated more than 1000-fold. Accordingly, the addition of 30–40 mM glycolate to a suspension of normal human red cells results in a rapid decrease in DPG without any impact on ATP levels.
Figure 5. Binding of 2,3-diphosphoglycerate (DPG) to hemoglobin. (A and B) DPG binds in the cleft between the beta (yellow) subunits of the T quaternary structure. (C) Reduction of DPG concentration shifts the quaternary equilibrium toward R to produce a left-shift in the binding curve and increases the solubility (the concentration of Hb in the solution phase (Figs. 3B and 4B, left)). Both factors decrease sickling.
V. Reduce intracellular hemoglobin concentration.

The discovery of the enormous sensitivity of the kinetics of fiber formation to HbS concentration\textsuperscript{38,44} led several investigators to exploit this finding in both physiological and clinical studies. The red cell behaves like a micro-osmometer with a volume that depends on the osmolality of the plasma.\textsuperscript{88} Consequently, sensitivity of the delay time to concentration suggested that therapeutic benefit could result from an increase in red cell volume sufficient to reduce the intracellular Hb concentration by as little as 10\%.\textsuperscript{89} An early unblinded, limited clinical study did in fact report that lowering of plasma osmolarity via induction of hyponatremia reduced the frequency of pain crises.\textsuperscript{90} Although maintenance of sustained low sodium diet is impractical, an important result of this study was that red cells could be swollen without serious side effects that might result from osmotic effects on other cells.\textsuperscript{90}

Reduction in intracellular hemoglobin concentration is commonly encountered in patients who develop iron deficiency. In mouse models of sickle cell disease, induction of iron deficiency by suppression of intestinal expression of HIF-2\textsubscript{α} resulted in increased hemoglobin levels accompanied by reduction in MCHC and hemolytic rate.\textsuperscript{91} In SS patients who have sustained blood loss either by hemorrhage or by iatrogenic phlebotomy sufficient to result in iron deficiency, the decrease in MCHC was accompanied by increased hemoglobin and less hemolysis.\textsuperscript{92}

When red cells sickle, damage to the membrane results in calcium influx which triggers enhanced potassium efflux via the so-called Gardos channel. As a result, the red cell hemoglobin concentration increases along with a marked increase in the probability of further sickling. Therefore, inhibition of this channel is a plausible therapeutic approach. Treatment with Senicapoc (ICA-17043), a highly potent Gardos inhibitor, resulted in a decrease in red cell density and MCHC, increased hemoglobin levels and reduced hemolysis in both a sickle mouse model\textsuperscript{93} and in sickle cell patients.\textsuperscript{94} However, a Phase 3 clinical study was terminated early because administration of this drug appeared to cause a slight increase in the rate of sickle pain crises.\textsuperscript{1} Although the rationale for Gardos channel inhibitors is sound, their efficacy depends on damage to the red cell membrane caused by cycles of cell sickling and unsickling from HbS polymerization and depolymerization.

Another approach to decreasing intracellular HbS concentration, proposed many years ago, is to use ionophores.\textsuperscript{95} These agents transport extracellular sodium ions into the red cell, accompanied by water influx to maintain osmotic equilibrium and, in doing so, swell the red cell. A new and sensitive sickling time assay has shown that potentially therapeutic levels of inhibition can be achieved at sub-nanomolar concentrations of ionophores, making increase in red cell volume a viable approach to therapy.\textsuperscript{96}

\textbf{Figure 6.} Schematic of normal and swollen red cell. A small increase in red volume to decrease the intracellular HbS concentration dramatically increases the delay time of sickling because of its enormous dependence on the HbS concentration. Even a 10\% increase in cell volume is predicted to have a therapeutic effect.\textsuperscript{99,96}
Anti-polymerization drugs currently in clinical trials

The website www.clinicaltrials.gov lists an impressive number of recent and on-going investigations into various aspects of sickle cell disease. In a review published last year Telen covered a wide range of treatments that focus on downstream sequelae of sickle vaso-occlusion, including 5 agents that target adhesion, 7 that target inflammation, 5 involving anticoagulants, and 6 anti-platelet agents. In contrast, Telen’s review covers fewer drugs that directly target HbS polymerization. These include 4 that induce HbF expression. However none of them currently appear to offer convincing advantages over hydroxyurea.

Among the drugs listed as an anti-sickling agent is SCD-101, a plant extract similar to Niprisan (Nix-0699). Niprisan is an ethanol-water extract from seeds, stems, fruit and leaves of four different plants, has been used among diverse folk groups in Nigeria, and reported to be effective in lowering the frequency of pain crises in sickle cell patients. Niprisan inhibits in vitro sickling and increases both the solubility and delay time of solutions of deoxyhemoglobin S, albeit in a highly non-physiological buffer. Moreover, the extract prolonged the survival of transgenic sickle mice following acute hypoxic challenge. A recent Phase 1B dose escalation study of 26 SS and S/β thalassemia patients found that SCD-101 was well tolerated and, at higher doses, appeared to relieve chronic pain and fatigue but had no impact on hemoglobin levels or hemolysis.

Telen’s review lists only one anti-sickling agent undergoing clinical trials that is known to directly modify hemoglobin structure: 5 hydroxymethylfurfural (Aes-103). This agent is an aldehyde that forms a reversible Schiff base linkage primarily with the N-terminal amino of α-globin resulting in a dose-dependent increase in oxygen affinity. A significant concern with aldehyde drugs is their potential to covalently modify other cellular and plasma proteins. More recently a polyaromatic aldehyde, GBT440, has been developed that also binds via a Schiff base to α-globin N-terminus with enhancement of oxygen affinity similar to Aes-103, but with higher specificity and at much lower concentrations. At doses that achieve an optimal increase in oxygen affinity, the partition between levels of GBT440 in the red cell and plasma is an impressive 70:1 ratio. In initial clinical trials in normal volunteers and in sickle cell patients, once a day oral administration of GBT440 is well tolerated with no significant adverse effects. Patients with SS disease have a dose-dependent increase in hemoglobin levels within two weeks of initiating therapy, accompanied by a decrease in reticulocyte count, serum non-conjugated bilirubin and fraction of irreversibly sickled cells in peripheral blood films. Thus, GBT440 results in a significant prolongation of red cell life span in sickle cell patients. The crucial question, of course, is the impact of this drug on the incidence and severity of pain crises and on vaso-occlusive organ damage. The efficacy and safety of the drug is now being investigated in a double blind multi-center Phase 3 study of approximately 300 patients. The progress thus far with this drug offers a strong impetus to discover and evaluate other drugs that directly target HbS polymerization.

Conclusion

Because there are so many ways to inhibit HbS polymerization, there is cause for optimism. Of course once a polymerization inhibitor is discovered, many hurdles must be overcome, including issues of toxicity, bioavailability, pharmacokinetics, etc., before it can become an FDA-approved drug. It would therefore be prudent to first investigate the anti-polymerization effect of the numerous molecules, in addition to FDA-approved drugs, for which toxicity information
exists. Major therapeutic effects could result by administering a combination of drugs, which, by acting on different molecular targets, would be non-competitive. For therapeutic strategies other than the promotion of HbF synthesis, drug discovery could be accelerated by carrying out screens with intact red cells, such as the recently reported laser-photolysis method for measuring sickling times of sickle trait cells. A method that is less technically demanding is currently under development at NIH.

**Authorship:**

Contribution: W.A.E. and H.F.B. contributed equally to writing the manuscript.

Disclosure: H.F. Bunn is a consultant for Global Blood Therapeutics Inc, the producer of GBT440.

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