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Sickle Cell Disease: Advances in Treatment

Renée V. Gardner, MD

Department of Pediatrics, Louisiana State University Health Sciences Center, Children's Hospital of New Orleans, New Orleans, LA

Background: Sickle cell disease causes significant morbidity and mortality and affects the economic and healthcare status of many countries. Yet historically, the disease has not had commensurate outlays of funds that have been aimed at research and development of drugs and treatment procedures for other diseases.

Methods: This review examines several treatment modalities and new drugs developed since the late 1990s that have been used to improve outcomes for patients with sickle cell disease.

Results: Targeted therapies based upon the pathophysiologic mechanisms of sickle cell disease that result in organ dysfunction and painful episodes include hydroxyurea, L-glutamine, crizanlizumab, and other drugs that are currently on the market or are on the verge of becoming available. These agents have the potential to improve survival and quality of life for individuals with sickle cell disease. Also discussed is stem cell transplantation that, to date, is the only curative approach for this disease, as well as the current status of gene therapy.

Conclusion: These examples demonstrate how the current knowledge of sickle cell disease pathophysiology and treatment approaches intersect. Although interest in sickle cell research has blossomed, many more clinical trials need to be initiated and subjected to more strenuous examination and analysis than have been used in the past.

Keywords: *Anemia–sickle cell, genetic therapy, hydroxyurea, oxidative stress, poloxamer, stem cell transplantation*

Address correspondence to Renée V. Gardner, MD, Department of Pediatrics, Louisiana State University Health Sciences Center, Children's Hospital of New Orleans, 200 Henry Clay Ave., New Orleans, LA 70118. Tel: (504) 896-9740. Email: rgardn@lsuhsc.edu

INTRODUCTION

In 1910, sickle cell disease burst onto the Western medical scene as a "strange" or, as Herrick termed it, a "new, unknown disease."[1] Physicians were intrigued by the sickled appearance of the red cells in this disorder, and case reports and analytical papers detailing the clinical features of this disorder appeared to almost always involve people of color.[2-6] The disease then became known as a "black disease."[6-8] Not until 1949, however, was the molecular nature of sickle cell discovered.[9] In 1958, Ingram discovered the genetic basis of the disease and demonstrated that the disease originated from the substitution of a valine for glutamic acid at the sixth amino acid position of the hemoglobin beta chain.[10] This amino acid substitution, now known to be the result of a single point mutation of the hemoglobin gene, produces profound changes in the behavior and conformation of the hemoglobin molecule in individuals affected by the disease.[11]

In 1927, Hahn and Gillespie had reported on the mechanism of sickle formation, observing that the sickle hemoglobin in its deoxygenated state assumed the characteristic shape, the sickle, that gives the disorder its name.[12] Cells containing deoxygenated hemoglobin not only formed this rigid shape but also were dehydrated,[13] had abnormal cell surface and distinct migratory characteristics, were sticky and prone to adhesion, and had abnormal rheologic properties.[14,15] Clinically, not only did patients with sickle cell disease experience repeated painful episodes (crises), but because of recurrent episodes of vaso-occlusion, they ultimately suffered chronic organ damage. Physicians noted a paucity of individuals who survived into their adult years.[6]

Sickle cell disease, one of the most common inherited diseases worldwide, is now understood to be a disorder of global importance and economic as well as clinical significance. Those affected by the disease live in areas of sub-Saharan Africa, the Middle East, India, the Caribbean, South and Central America, some countries along the Mediterranean Sea, as well as in the United States and Europe.[16] The disease has, at times, through forced and unforced migration, been introduced to areas in which it was not endemic.[17] In the United States, 80,000-100,000 individuals are affected by the disorder; worldwide, more than 300,000 children are estimated to be born annually with sickle cell disease.[18-20] This number includes approximately 3,000 children born with the disease each year in the United States.[18]

Since the 1980s, novel approaches for the treatment of sickle cell disease have included the introduction of penicillin prophylaxis for children with sickle cell,[21] the institution of newborn screening programs,[22] and the use of transcranial Doppler screening for detection of cerebral vasculopathy and stroke prevention.[23] Hematologists have long recognized the need for better treatments of sickle cell. Optimally, a treatment approach was needed that did not just address

pain or treat and prevent sequelae of the disease (eg, susceptibility to infection from asplenia). What was needed instead was a treatment approach that worked by undercutting the pathophysiology of the disease. Research efforts previously concentrated on understanding the pathogenesis of the disease rather than on providing greater relief for the patients having the disorder. Progress in arriving at satisfactory treatment of individuals with sickle cell has often seemed to be a slow, halting process. Also, funding for research of sickle cell disease lagged behind that of other genetic diseases, fueling a suspicion that racial bias prevented significant outlays of moneys for study of the disorder.[24-27] The innovations enumerated above did result in stepwise improvements in survival, so the median life expectancy for those with homozygous disease is now into the fourth and fifth decades.[28]

Beyond hydroxyurea, which was introduced into clinical practice in the 1980s for adults,[29,30] few new drugs have been investigated or placed on the market for the treatment of the disorder until recently. This review investigates areas of potential intervention and promise that have evolved since the late 1990s.

Notably, 2017-2018 have been heralded as the most productive years, yielding novel initiatives aimed at this disease. In 2017, the American Society of Hematology (ASH) introduced its Advocacy Efforts Related to Sickle Cell Disease and Sickle Cell Trait.[31] In February 2018, United States Senators Tim Scott and Cory Booker advanced the Sickle Cell Disease Surveillance, Prevention, and Treatment Act of 2018.[32] ASH's efforts signaled a commitment to ensuring that individuals with sickle cell disease have access to care, as well as a concerted effort to train and educate physicians about the disease. ASH would also work with federal agencies such as the National Institutes of Health to expand, assess, and prioritize research of the disorder. The legislation introduced by the senators aims to "improve understanding of health care utilization by individuals with sickle cell disease and to establish cost-effective practices to improve and extend the lives of patients."[32] The legislation, if passed, would award grants to enable a better understanding of the prevalence and distribution of sickle cell disease. The bill is still being considered by the Senate. Those who work in the field of sickle cell disease viewed these two initiatives as an indication of interest in the disorder by the general community and a promise of much-needed funding for the study of a hitherto neglected disease. Because many practitioners, patients, and their families have long felt that the lack of funding or interest in sickle cell disease was an indication of neglect from the general medical community, these initiatives were heartening.[25,33-36]

PATHOPHYSIOLOGY OF SICKLE CELL DISEASE

The Figure depicts some of the pathophysiologic components of the disorder in simplified form. New insights into the pathophysiology of the disease are summarized in several comprehensive reviews.[37-41] No longer valid is the simplistic explanation of sickle cells being solely responsible for causing vascular blockage or vaso-occlusion once red cells assume the pathognomonic sickle cell shape following exposure of the cell to deoxygenation. While vaso-occlusion is central to the understanding of the disease and can cause local hypoxemia with ensuing direct tissue injury and inflammation, the single gene mutation seen in sickle cell disease leads to complex physiologic changes. These changes result in the protean clinical manifestations of the disease. We now recognize sickle cell disease as a condition not only characterized by vaso-occlusion, anemia, and hemolysis but also one with heightened inflammation, hypercoagulability, increased oxidative stress, and defective arginine metabolism. Sickle cell disease is a vasculopathy and also features the presence of multiple nutritional and micronutrient deficiencies that adversely affect the patient.[42]

Upon deoxygenation, the sickle hemoglobin is insoluble and undergoes polymerization and aggregation of the polymers into tubulin fibers that then produce sickling.[43,44] Because of their rigid shape, the cells are prone to being trapped in the microcirculation, while tissues downstream of this blockage are deprived of blood flow and oxygen and suffer ischemic damage or death. This blood flow deprivation in turn leads to tissue necrosis or reperfusion injury.

These sickle cells are also prone to dehydration because of abnormalities in the Gardos channel.[13,45,46] These cells are characterized by abnormal activation of intracellular signaling pathways and have less nitric oxide[47] and adenosine triphosphate content.[48] These cells also have less antioxidant capacity.[49,50] As a result, many of the cellular components may have oxidative damage.[51] Oxidative damage to the cellular membrane proteins and aggregation of proteins along the inner surface of plasma membranes can lead to intracellular abnormalities at the red cell surface; such changes lead ultimately to increased phosphatidylserine exposure and the formation of microparticles that allow procoagulant activity by the red cell itself.[52]

With hemolysis, free hemoglobin is released into the plasma, acting as a scavenger of nitric oxide.[53,54] Because arginase-1 activity, necessary for production of nitric oxide, is lower in the sickle cell than in the normal red cell, nitric oxide cannot readily be made de novo, especially in individuals who tend to hemolyze at high rates. Another result of hemolysis is the formation of reactive oxygen species by reactions involving free hemoglobin.[55]

In addition, dysregulation of microRNA occurs in the sickle cell, small noncoding RNA molecules function to silence RNA, and posttranscriptional regulation of gene expression occurs.[56] Hence, gene expression is abnormal during erythropoiesis.

The abnormal adhesive properties of the sickle erythrocyte can lead to activation of adhesion receptors, such as those of the intercellular adhesion molecule-4.[57] Similarly, the glycoprotein basal cell adhesion molecule (Lutheran blood group), a transmembrane adhesion molecule found in the vascular endothelium, interacts with the unique integrin alpha 4 beta 1 expressed on sickle cells, mediating their adhesion to the endothelium.[58,59] The result is abnormal interactions between red cells, leukocytes, platelets, endothelium, and extracellular matrix proteins. Such abnormal cell-cell interactions lead to a steady process of adherent interactions, driving endothelial cell expression of procoagulant proteins. The mitogen-activated protein kinase ERK 1/2 and the upstream kinase responsible for its activation, MEK 1/2, are constitutively activated in sickle red cells, leading to increased adhesion.[60-62] The selectins E-selectin and P-selectin are upregulated in sickle cell disease and also mediate adhesion, with the degree of red cell adhesion correlating with greater severity of disease.[63]

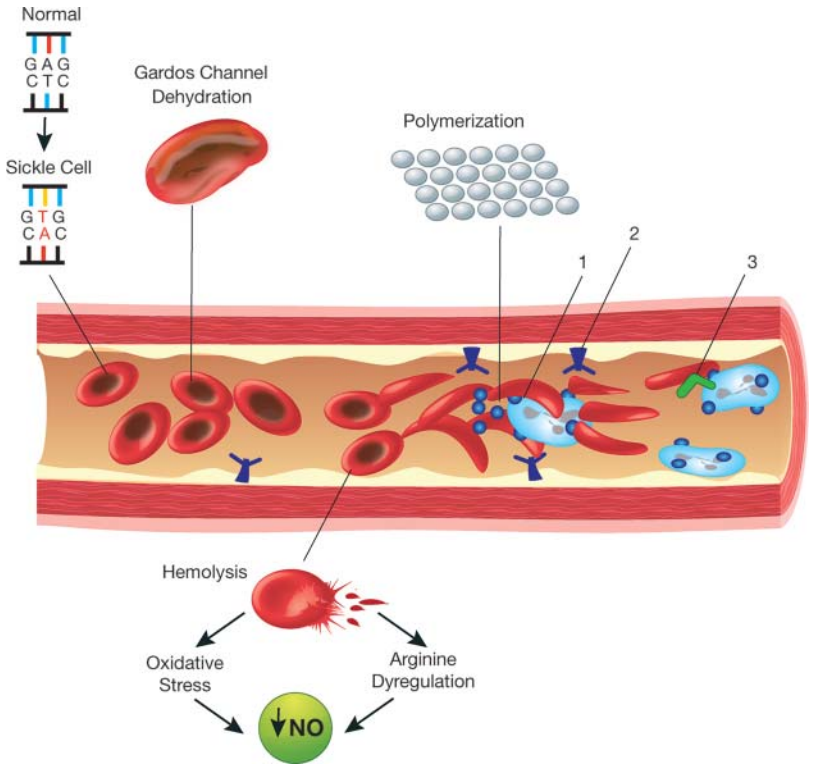

Figure. Schematic representation of the pathophysiology (in part) of sickle cell anemia. A single gene mutation (GAG→GTG and CTC→CAC) results in a defective hemoglobin that when exposed to deoxygenation (depicted in the right half of the diagram) polymerizes (upper right of the diagram), resulting in the formation of sickle cells. Vaso-occlusion can then occur. The disorder is also characterized by abnormal adhesive properties of sickle cells; peripheral blood mononuclear cells (depicted in light blue; shown as the large cells under the sickle cells) and platelets (depicted in dark blue; shown as the dark circular shapes on the mononuclear cells) adhere to the sickled erythrocytes. This aggregate is labeled 1. The mononuclear cells have receptors (eg, CD44 [labeled 3 and depicted in dark green on the cell surface]) that bind to ligands, such as P-selectin (labeled 2 and shown on the endothelial surface), that are upregulated. The sickle erythrocytes can also adhere directly to the endothelium. Abnormal movement or rolling and slowing of cells in the blood also can occur. These changes result in endothelial damage. The sickled red cells also become dehydrated as a result of abnormalities in the Gardos channel. Hemolysis contributes to oxidative stress and dysregulation of arginine metabolism, both of which lead to a decrease in nitric oxide (NO) that, in turn, contributes to the vasculopathy that characterizes sickle cell disease.

In addition to these changes, the cell containing sickle hemoglobin is stiffer than a normal red cell would be in circulation.[15,64-66] Such abnormal deformability persists even when the cell has assumed an apparently normal ovoid shape. Morphologically normal sickle hemoglobin-containing erythrocytes are just as adherent-prone as irreversibly sickled cells.

Inflammation is also key to the initiation of vaso-occlusion.[67,68] Even in steady state, leukocytes and platelets are activated, and markers of inflammation are elevated. Multiple inflammatory cytokines, such as interleukin (IL)-10, IL-4, macrophage-inflammatory protein (MIP-1α), and tumor necrosis factor alpha (TNF-α), are elevated even at baseline.[69,70] The leukotriene synthetic enzyme 5-lipoxygenase activates both monocytic and endothelial cells, leading to production of leukotrienes that are increased in steady state to the extent that elevated levels correlate with a higher painful event rate.[70]

Invariant natural killer T-cells are also activated and present in increased numbers.[71] As an example of their importance, they may play a role in the pathogenesis of ischemia/reperfusion injury in sickle cell disease.

All these changes show how the disorder is a complicated patchwork of contributory pathologies that are fascinating but make it difficult to create an all-encompassing therapeutic strategy.

Table. Treatments Targeting Specific Pathogenetic Mechanisms of Sickle Cell Disease

Pathogenetic Mechanism	Counteragent
P-selectin inhibition	Crizanlizumab
Polymerization	Voxelotor
Upregulation of fetal hemoglobin production	Hydroxyurea Butyrate 5-Azacytidine, Decitabine
Oxidative stress	L-glutamine
Genetic mutation	CRISPR/Cas 9 technology and transplantation
Abnormal rheology	Poloxamer 188

CRISPR/Cas 9, clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9.

TREATMENT OF SICKLE CELL DISEASE

Hemoglobin F Production

Hydroxyurea. Patients of Arab-Indian haplotype generally manifest high hemoglobin F levels (approximately 20%) and have a mild clinical phenotype of sickle cell disease.[72,73] Likewise, patients who are compound heterozygotes for hereditary persistence of fetal hemoglobin (up to 30% hemoglobin F) have few if any manifestations of the disorder.[74] Therefore, the assumption seemed reasonable that inducing hemoglobin F in individuals in which it had been turned off might accrue considerable benefit to patients with sickle cell disease.

Hydroxyurea induces the production of hemoglobin F. Hemoglobin F in turn reduces hemoglobin S polymerization and subsequent sickling. For this reason, hydroxyurea has been the standard of care for patients with sickle cell disease since the late 1980s.[28,29,75-78] Until 2008-2013, no other drugs carried such promise or were on the horizon. While the efficacy of hydroxyurea is principally attributable to its ability to turn on production of hemoglobin F, other salutary effects include its reduction of the expression of adhesion molecules on red blood cells and the decrease in neutrophil, monocyte, platelet, and reticulocyte numbers that may translate into decreased blood viscosity, fewer deleterious cell-cell interactions, and a reduction in hemolysis.[76-78] The drug has been quite effective in bringing about a reduction in the number of vaso-occlusive pain or acute chest syndrome episodes, the number of hospitalizations, and the number of transfusions required by patients.[79,80] Most important, the demonstration of a definite survival advantage for those taking the drug would seem to be a persuasive finding for healthcare providers and patients and an inducement to take it.[80] However, as Brandow and Panepinto noted in a discussion of hydroxyurea use in sickle cell disease, "true effectiveness [of any drug] is dependent upon utilization in real clinical practice."[81] In one study they reviewed, only 75% of providers used hydroxyurea in their patients who had 3 or more painful episodes per year, and only 30% of individuals who might be eligible for the drug were taking it.[81] Not all barriers to the use of hydroxyurea are known, but some that have been identified include fear of side effects including teratogenesis, effects on fertility, and the possibility of increased risk of malignancy.[81,82]

Hydroxyurea is recommended for patients with sickle cell disease who meet the following criteria:[83]

- Patients who have ≥3 moderate to severe pain episodes in a 12-month period
- Patients who have a history of stroke and a contraindication to chronic transfusions (as an alternative to receiving no transfusion)
- Children who have a history of acute chest syndrome or symptomatic anemia
- Infants and children 9 months of age or older who are asymptomatic or have infrequent pain episodes

Interestingly, these recommendations were made for pediatric usage even though no large, randomized trials have been conducted with children. Current usage is based on efficacy studies performed in children that include a randomized, placebo-controlled crossover trial with a small number of children and open-label single-arm studies.[84-86] Because the hydroxyurea arm showed a significant decline in pain crises, the use of hydroxyurea in children appeared to be validated and children could then be treated with this drug, despite its not having US Food and Drug Administration (FDA) approval for this patient population. Hydroxyurea has been safe with minimal side effects and has resulted in a significant decrease in mortality in both adults and children.

The primary reason for ineffectiveness with this drug seems to be noncompliance, but some individuals genuinely are nonresponders. Patient response is also variable. Reasons for the lack of consistency and for the lack of response are not known. Vascular and other changes associated with the disorder that might presage major sickle-related complications may still occur despite the use of hydroxyurea and despite any apparent beneficial effects of the drug.[87] Further, the drug can, over time, have a diminished ability to induce hemoglobin F, perhaps because of marrow exhaustion.[88] Data from 2007 suggest that polymorphisms in genes that regulate hemoglobin F expression, metabolism of the drug, and erythroid progenitor proliferation (individuals having higher degrees of reticulocytosis seem to respond better to hydroxyurea) may also be factors determining the responsiveness of an individual to hydroxyurea.[89]

The fact that not everyone will be a candidate for or respond to hydroxyurea increases the exigency to explore other approaches to the treatment of sickle cell, including preventive measures. Efforts have been underway for years to take advantage of the new understanding of the pathophysiology of the disease. Therapeutic candidates (Table) have included drugs that are aimed at (1) finding alternative pathways for turning on hemoglobin F production; (2) preventing cellular adhesion and aggregation; (3) altering blood flow dynamics in the vasculature; (4) preventing hemoglobin S polymerization; (5) enhancing the hemoglobin's oxygen affinity; (6) decreasing inflammation; and (7) targeting directly the genetic mutation of the sickle cell gene. As noted, complete elimination of the mutant gene is not required for clinical improvement to be seen because diminution of hemoglobin S gene expression to 50% has been demonstrated to be sufficient to allow a phenotype similar to sickle trait.[90]

Butyric Acid and Butyrate (HQK-1001). Other drugs besides hydroxyurea have been proposed that lead to an

increase in hemoglobin F. One such drug is butyric acid, a short chain fatty acid.[91-93] Its mechanism of action is not known. Although butyric acid showed early promise, formidable drawbacks to its use included the large amounts required for effect and the necessity for the drug to be given intravenously and in large volume for 4 days every 4 weeks. The inconvenience and the necessity for utilization of a central venous catheter were impediments to its use in patients.

An orally bioavailable form of butyrate, 2,2-dimethylbutyrate (HQK-1001), has been studied. In a phase 1/2 trial of HQK-1001, 21 patients having sickle cell disease completed the study.[92] Increases in hemoglobin F >1.1% above the baseline percentage were observed in 50% of subjects receiving the higher escalating doses of 20 and 30 mg/kg/day. The study period was relatively brief, so the effect on erythropoiesis was not analyzed. Also, as a phase 1/2 study, the study's purpose was not to assess effectiveness in ameliorating sickle cell disease symptomatology. In a phase 2 trial conducted by Reid and others, the drug was given in a dose of 15 mg/kg twice daily.[93] The mean absolute increase in hemoglobin F was 0.9% with no significant difference in mean changes of hemoglobin. Of note, the mean annualized rate of pain crises for those receiving HQK-1001 was 3.5, whereas the rate for those receiving placebo was 1.7. Adverse effects included gastritis (the dose-limiting side effect), nausea, headache, and fatigue. The study terminated after a planned interim analysis, and the authors concluded that "additional studies of HQK-1001 at this dose and schedule are not recommended in [sickle cell disease]."[93]

Decitabine and 5-Azacytidine. The human gamma globin gene is silenced in most individuals during early childhood and through adulthood through epigenetic gene regulation, signifying that modification of gene expression rather than alteration of the genetic code is responsible for controlling or suppressing gene expression levels. DNA methylation, carried out by the enzyme DNA methyltransferase 1 (DNMT1), then enables the developmental switch from the production of the gamma globin to the beta globin chain.[94]

Researchers have proposed that interference with or depletion of DNMT1 might prevent the switchoff of hemoglobin F production.[95-100] The drug decitabine and its prodrug 5-azacytidine have been found to deplete DNMT1 levels.[101] In animals, 5-azacytidine produced increases in hemoglobin F levels up to 20 times those produced by hydroxyurea, even in animals that derived minimal benefit from hydroxyurea by being poor responders.[101] In this study with the primary endpoint of hemoglobin F production, hemoglobin F production increased in a dose-dependent fashion with the use of decitabine, and the rate of pain crisis was lower in almost all groups studied.[101] However, the drug has several shortcomings, including poor bioavailability, negligible solid tissue distribution, a very brief half-life, and formation of uridine degradation products that could potentially cause DNA damage and cytotoxicity. Teratogenesis and carcinogenesis are real concerns.

Prevention of Oxidative Stress

L-Glutamine. In 2017, considerable excitement was generated by the announcement of the commercial availability of L-glutamine (Endari), touted as the first new drug approved by the FDA for treatment of sickle cell disease in 30 years.[102,103] This agent's use is based upon the fact that the sickle red cell, because of decreased redox potential, is more susceptible to oxidant stress or damage than a normal red cell. Sickle red cells absorb and utilize L-glutamine to a far greater extent than normal red cells, having rates of L-glutamine utilization that exceed de novo synthesis. Supplementation with L-glutamine therefore leads to improved transport and utilization of glutamine in the sickled erythrocyte and to a subsequent rise in the levels of the naturally occurring redox agents nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide hydrogenase. The increase in redox agents in turn improves the cellular defenses against oxidative stress.

In an open-label pilot clinical trial of the drug, all patients achieved normalization of nicotinamide adenine dinucleotide redox potential and a decrease in permanently sickled cells in peripheral blood.[104] Only 7 adult patients participated in the trial, and no clinical benefit was seen. However, Niihara and associates demonstrated in subsequent clinical trials that all patients experienced normalization of their nicotinamide adenine dinucleotide redox potential and had a decrease in clinical symptoms when given L-glutamine.[104,105] In a phase 3 study published in 2014 that included both adults and children, the median cumulative hospital days were lowered by 41%.[105] The frequency of vaso-occlusive episodes was decreased by 25%, and the incidence of acute chest syndrome decreased by more than 50%.[105] Side effects were negligible. However, the rate of withdrawal from the study was unexpectedly high. Patients receiving the drug were given an oral dose of 0.3 g/kg twice daily for 48 weeks, and 62% withdrew in the placebo group vs 49% in the medication arm. Such a large withdrawal of subjects affected the power of analysis for the study and possibly was responsible for the failure of the drug effect to remain statistically significant for the duration of the study. For these reasons, the data have been critically appraised by others as lacking in quality.[106] Another criticism is that the administration of the drug is "onerous"; therefore, long-term compliance might be difficult to maintain since the patient would have "to take a lot of it and mix it up and drink it down two to three times a day."[106] Another concern is whether insurance will cover the cost of the drug because Medicaid and other insurance providers have been reluctant to cover supplements of any type.[106,107] The drug is expensive. A 5 g packet of L-glutamine costs $580-$620 and must be given two or three times daily.[107] While provisions are being made for patient assistance and third-party coverage, the cost is considerable. Last, efficacy is only seen after weeks or months.

Prevention of Adhesion

Crizanlizumab. As noted previously, adhesion of platelets to red cells, monocytes, and neutrophils is an integral component of the pathogenesis of sickle cell disease.[108,109] The degree of red cell adhesion correlates with the severity of disease. Selectins, especially P-selectin which is upregulated in sickle cell disease, are responsible for initiation of the static adhesion of the sickle red cells to the vessel surface and the ensuing vascular obstruction that is seen in crisis or inflammation.[110]

For this reason, effort has been devoted to the development of methods to block P-selectin activity. Crizanlizumab, a humanized monoclonal antibody, is one such agent. It

blocks cell-cell adhesion by targeting P-selectin.[111] In a double-blind, randomized phase 2 trial, 198 patients were given either high- or low-dose crizanlizumab or placebo.[111] The annual median crisis rate decreased 45.3% in patients who received the high dose of the drug and decreased 32.6% in patients who received the low dose. Eighteen percent of the patients enrolled in the study experienced no crises at all during the treatment phase. The drug was administered by intravenous infusion and was shown to have a relatively long 30-day half-life. However, the fact that the drug is administered by intravenous infusion could possibly prove to be a drawback to its use. Also, the study's impact and importance were diminished somewhat by not having included children.

Improvement in Flow Dynamics

Poloxamer 188. Although most of the agents that have been discussed to this point involve a preventive approach to sickle cell disease, poloxamer 188, a nonionic block copolymer surfactant, has been shown to improve microvascular blood flow in sickle cell disease by decreasing blood viscosity.[112-115] How it does so is not well understood. However, poloxamer 188 seems to block aggregating interactions of cells to cells and cells to protein in the blood. In a randomized, double-blind, placebo-controlled trial examining the effects of poloxamer 188 on patients in active crisis, the duration of crisis was decreased in those taking the drug, with 52% reporting crisis resolution vs 37% of those on placebo.[112] Renal dysfunction, however, was reported in early trials of poloxamer 188 for treatment of patients with myocardial infarction.[113,114] In an early phase 2 study using poloxamer 188 in sickle cell disease, one patient (of 28) receiving the drug developed renal dysfunction (defined by a rise in serum creatinine), but he had preexistent mild renal impairment.[115] Renal involvement was subsequently presumed to be the result of the presence of low molecular weight substances in the early and less homogeneous formulations of the drug. After purification of poloxamer 188, far fewer cases of renal toxicity were reported.[116,117] Orringer and his coinvestigators also showed that the safety profile was acceptable.[112]

Prevention of Polymerization

Voxelotor (GBT-440). Most studies focused on preventing polymerization of the sickle erythrocyte have involved the use of drugs that could turn back the hands of the clock and switch on the production of hemoglobin F. However, a novel drug that inhibits polymerization through a groundbreaking technique has generated considerable excitement among hematologists. Voxelotor (GBT-440) is a small molecule that in binding to hemoglobin S increases the oxygen affinity of the hemoglobin S molecule.[118,119] Voxelotor thereby inhibits polymerization of the molecule and can prevent damage to the red cell. Clinical trials involving this drug have been quite promising. In a 90-day trial, a marked decrease in hemolysis from baseline to day 90 was observed, along with a sustained decline in the number of irreversibly sickled cells, a median decrease of about 70%.[120] Preliminary results of a phase 2a clinical trial showed that teenagers given the drug at a dose of 900 mg daily experienced improvement in the disease, while 55% had improvement of hematologic parameters such as hemoglobin and reticulocyte count.[121] In a single-center experience published in 2017, 7 patients who would not have met the strict inclusion criteria established by Global Blood Therapeutics for the company's phase 3 trial were given the drug for periods up to 15 months.[122] One patient was noncompliant, but all patients taking the drug as instructed had increases in hemoglobin. Hospital admissions for vaso-occlusive crisis declined by 60%, chronic pain described as "background pain" decreased, all patients reported reduced fatigue, and those who required transfusion saw a decrease in transfusions by approximately 50%. For patients who required chronic supplemental oxygen, oxygen saturation increased to the extent that they were able to stop the oxygen. Of course, the experience of 7 people is not conclusive evidence of a drug's efficacy, and a larger study is needed to prove whether the drug has true effectiveness. Such a phase 3 study is underway, and results are being accrued in an international, multicenter trial (NCT03036813).[123] The FDA has designated the drug as a "breakthrough therapy."[124]

CURES FOR SICKLE CELL DISEASE

Stem Cell Transplantation

The only cure available to patients with sickle cell disease is stem cell transplantation. However, the selection of patients who should benefit from this treatment modality is controversial. Transplant has been performed, for the most part, in patients who have suffered a stroke, have had multiple episodes of acute chest syndrome, or have had recurrent vaso-occlusive crises (≥3 episodes requiring hospitalization per year), ie, patients considered to have the worst disease severity.[125] Controversies have arisen not only about whom to transplant but also about the optimal age to transplant, source of donor cells, and type of conditioning regimen.[126-130] Most stem cell transplants thus far have relied upon myeloablative conditioning regimens and have been bone marrow–derived with human leukocyte antigen (HLA)-matched sibling donors as the source of stem cells.[131,132] But the probability of an individual having a matched sibling donor is only 16%-20% among minorities if an 8 of 8 allele match is sought.[126,133] The effort to expand the availability of transplant for most patients with sickle cell disease has led to consideration of alternative donor sources, such as cord blood, matched unrelated, and haploidentical cells.

Gluckman et al conducted a survey of 1,000 recipients of HLA-identical sibling transplants from European, American, and non-European centers.[134] Sixty percent of patients underwent myeloablative conditioning, and the unadjusted overall survival rate after 5 years and event-free survival rate were 92.9% and 91.4%, respectively.[134] Transplant led to stabilization of organ function, gradually ameliorated complications of sickle cell disease such as cardiovascular and pulmonary dysfunction, and reduced the occurrence of vaso-occlusive episodes. In another series, results from HLA-identical sibling transplants after myeloablative conditioning with antithymocyte globulin were reported.[135] The event-free survival rate for sibling transplants after myeloablative regimens was approximately 95% in this series.[135] While myeloablative conditioning has remained the standard of care for hematopoietic stem cell transplantation, it has been associated with toxicities that have included veno-occlusive disease of the liver and neurotoxicities such as seizures, stroke, and brain hemorrhage.[133] Late effects of

transplant such as growth failure, hypogonadism, sterility, and secondary malignancies have also been reported.[128-133] The median age for transplantation has been 9-10 years; individuals who are older have not fared as well, with a lower probability of survival in general and of graft-vs-host-disease (GVHD)–free survival in particular.[130]

Attempts at decreasing the toxicities associated with transplantation have resulted in the use of less-rigorous conditioning regimens (reduced-intensity conditioning regimens). For these transplants, the goal became producing a state of mixed chimerism in which recipient marrow is incompletely replaced by donor cells, producing in some instances a trait-like phenotype. These regimens have been better tolerated, especially in patients with preexisting comorbidities, and have resulted in an 86%-90% disease-free survival rate.[133,136-138] Indications of what the lower limit of red cell donor chimerism is to allow improvement of disease manifestations have varied. In 2017, Fitzhugh and colleagues published a paper in which they stated that chimerism of 20% is necessary to abrogate the sickle phenotype.[139] However, the earlier experience of Walters and coauthors cited results in which one individual with as few as 11% donor cells expressed a hemoglobin S level of 7% and ceased to have a transfusion requirement; he also did not have symptoms consistent with sickle cell disease any longer.[140] One significant problem associated with reduced-intensity transplantation remains the higher likelihood of loss of donor cells or engraftment failure.

The search for alternative sources of stem cells has also led to the use of unrelated donors. Unrelated donor marrow transplants have had less success, with 1- and 2-year event-free survival rates of 76% and 69%, respectively, and overall survival of 86% and 79%, respectively.[140] The rate of GVHD was relatively high (62%), and more GVHD-related deaths occurred than would be ordinarily seen with related donors.[140]

Unrelated cord blood has also been proposed as a source of donor cells, but the graft failure rate in one study was fairly high (52%), and the overall survival was 94%.[141] In one trial utilizing a reduced-intensity conditioning regimen prior to transplantation with unrelated cord blood, a graft failure rate up to 63% was observed, leading the authors to conclude that donor engraftment needs to improve before unrelated cord blood transplants can be recommended.[142]

Related cord blood transplants are characterized by a significantly longer time to engraftment for neutrophils and platelets.[143-145] In one study with a median follow-up time of 70 months, disease-free survival at 6 years was reported to be 90%.[146] No grade IV GVHD or extensive chronic GVHD was seen, and the cumulative incidence of primary graft failure was low (9%). However, a limitation of this treatment modality is the inability to transplant large individuals or adults using cord blood as a source of donor cells because of insufficient numbers of nucleated or stem cells in the aliquots to be transplanted[147,148] and the slower engraftment of neutrophils and delays in immune reconstitution that may place the patient at increased risk of viral illness.[144]

Haploidentical transplants have been tried as well but have been reported to have a high rate of graft failure (43%).[149] To improve on this rate of engraftment failure, patients have been treated with cyclophosphamide posttransplantation.[150,151] Graft failure after one trial was still 43%, but no serious toxicities were seen.[150] Overall, the use of alternative donors (mismatched related or unrelated) has not resulted in the same measure of success. Graft failure rates of 38%-43% have been recorded, and long-term complications have included declines in renal, pulmonary, and cardiac function because of the transplantation procedure itself.[149,150]

In summary, transplantation is the optimal treatment for sickle cell disease, being the only curative approach. However, clarification is needed on who is an optimal candidate, and donor sources must be expanded to balance the lesser availability of donors among minorities.

Also, a clear relationship must be established between transplantation outcomes and improved quality of life, a relationship that to date has not been seen consistently or definitively. With regard to quality of life determinations, significant improvement may occur 1 year from a successful transplant, but the data are inconclusive.[152] The reluctance of primary providers to refer individuals for transplantation is a challenge to overcome as well because, as suggested in a retrospective study of hydroxyurea, patients treated with hydroxyurea may have had better survival than those treated with allogeneic stem cell transplantation.[153]

Gene Therapy

Because transplantation can be offered to relatively few individuals, hope for reaching more patients with a treatment of curative intent has focused on efforts to develop gene therapy. Recently, progress has been speeding along toward that goal. We now know that the most common single type of genetic variation in people is the single nucleotide polymorphism (SNP). Each SNP represents a nucleotide change in the DNA genome sequence and results in unique nucleotide change(s) in the genomic sequence of DNA. As a result, unique DNA patterns for each individual are produced. Capitalizing on this knowledge, investigators from several groups demonstrated that 3 SNPs are in the BCL11A and HBB gene regions that correlate with high hemoglobin F expression.[154-157] On the other hand, the gene MYB acted as a negative regulator of gamma globin expression. MYB was subsequently silenced by miR16 (microRNA R16) through binding of a 3'-untranslated region. Transfection of miR16 by Pounds and coinvestigators into human basophilic leukemia cell line KU812 cells in vitro resulted in gamma globin activation in a dose-dependent manner.[158] This work eventuated in genetic correction of the sickle cell mutation in human cells and ultimately in actual individuals. Genome editing systems, such as transcription activator–like effector nucleases (TALENs), zinc finger nucleases, and clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) have been developed that can target DNA sequences around sickle mutations in the beta globin gene.[158,159] These mutations are then cleaved in a site-specific manner, employing homologous donor templates to modify or replace altered DNA with the properly sequenced DNA. Gene modification of only 18% was sufficient to correct the sickle mutation and allow production of wild-type hemoglobin. On average, these efforts resulted in production of hemoglobin A, comprising 7.3% of total hemoglobin, with rates as high as 12.6%.[160] Effort has also been made to modify the gamma

globin gene because fetal hemoglobin is a more potent anti-sickling hemoglobin than adult or A hemoglobin.

Gene therapy has progressed to the point of human trial and was reported in 2017 in a patient having sickle cell disease.[160] Employing a lentiviral vector encoding the human HBB variant $\beta^{A\text{-}T87Q}$, researchers performed ex vivo gene transfer into the patient's own hematopoietic stem cells and then performed an autologous transplant utilizing these cells. The patient had undergone myeloablation with intravenous busulfan. After transduction of CD34+ cells, a steady rise in hemoglobin A^{T87Q} production was noted over time. The patient, previously transfusion-dependent, was able to discontinue red cell transfusions by day 88 posttransplant. The hemoglobin remained stable at levels of 10-12 grams% 6 months later. The hemoglobin percentage remained at 48% by posttransplant month 15, with a corresponding decrease in hemoglobin S levels. Despite concerns about off-target activity of CRISPR/Cas9 or similar nuclease or vector insertional error, no adverse effects were related to the lentiviral transduction of the stem cells, perhaps because lentiviruses tend to insert themselves randomly with a bias toward integration into areas of already expressed genes, thereby minimizing transactivation of nearby genes. This property acts to tamp down the potential for insertional oncogenesis. The patient had no replication-competent lentivirus extant. Most significantly, the patient had no sickle cell–related hospitalizations or other complications. Erythropoiesis progressively showed signs of normalization. No tendency towards clonal domination was detected. This case provides optimism that we are finally moving forward in the search for other curative therapies that can be offered to a wider array of patients than has ever been possible in the past.

CONCLUSION

These examples of new approaches to the treatment of patients with sickle cell disease sample some of the current attempts to moderate or cure the disorder. Interest in sickle cell research has blossomed and now can offer hope to the many individuals living with this disorder around the world. Many more clinical trials need to be initiated and subjected to more strenuous examination and analysis than have been used in the past. Efforts will have to be made to offer these therapies in less advanced countries where the majority of individuals with sickle cell disease live. These initiatives now appear more possible than ever before.

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