INTRODUCTION

“Peculiar, elongated and sickle-shaped” cells in the blood of a 20-year-old dental student led Dr. James Herrick to describe the first case of sickle cell disease (SCD) in 1910 (1,2). The dental student, Walter Clement Noel, had repeated hospital admissions for episodes of pain accompanied by red cell hemolysis. Noel died when he was only 32 years old from a respiratory illness, likely acute chest syndrome (ACS) related to SCD. Unfortunately, frequent pain and early deaths still characterize the clinical course for many patients with SCD today.

Since publication of this index case, there have been great strides toward understanding the mechanistic underpinnings of SCD. In 1949, SCD was heralded as the first molecular disease after the discovery of sickle hemoglobin by Pauling et al. (3). The genetic basis for this abnormal hemoglobin was later found to be a missense mutation in the β-globin gene, resulting in the substitution of a valine for glutamic acid at position 6. This deoxy-sickle hemoglobin undergoes structural changes that promote its polymerization into long fibrils, distorting the red cell into a crescent or sickle shape. The sickle erythrocytes are dehydrated, rigid and prone to hemolysis. They occlude the microvasculature causing acute, and critically important, chronic tissue ischemia and injury. The two most common acute morbidities in patients with SCD, vaso-occlusive pain crises (VOC) and ACS, are due to sudden occlusion of small vessels in the bone marrow and lungs (4,5). On a chronic basis, vaso-occlusion may damage the lungs, kidneys or brain and ultimately may lead to end-organ dysfunction (6). These acute and chronic complications of vaso-occlusion account for most deaths in patients with SCD in the modern era (7).

Clinical care improvements for patients with SCD have lagged behind the science. During the two decades that followed the identification of the hemoglobinopathy, only 50% of afflicted children survived into adulthood (8). By the 1990s, however, widespread mandatory newborn screening and the routine administration of penicillin to prevent pneumococcal sepsis increased childhood survival to over 90% (9,10). Then in 1998,
hydroxyurea became the only U.S. Food and Drug Administration–approved therapy for SCD (11). First described as a potential therapy in 1984, hydroxyurea enhances the production of fetal hemoglobin in sickle erythrocytes (12). Clinical studies of hydroxyurea use have demonstrated a decreased rate of VOC, ACS and improved survival (11,13,14).

Although hydroxyurea has been a major therapeutic breakthrough for patients with SCD, additional treatments are surely needed. Up to 50% of patients will not benefit from hydroxyurea long term because of poor response, toxicity, nonaggressive therapists or noncompliance (15). Potentially related to suboptimal hydroxyurea use, life expectancy for patients with SCD remains around 50 years (7,16). The focus of this article will be on advances in SCD therapies in the hydroxyurea era, excluding further progress in antisickling approaches. Recent insights into the regulation of fetal hemoglobin will likely lead to new therapies in the near future, but a careful description of these developments is beyond the scope of this article (17). Since the approval of hydroxyurea, a deeper understanding of how sickle cells interact with and affect other blood cells, the vasculature and vital organs has uncovered new ways to treat SCD. There has also been much effort directed toward a cure. From these discoveries, therapies have emerged that target cell adhesion, inflammation and hemolysis, as well as innovations in curative approaches.

CELL ADHESION AND INFLAMMATION

The process of vaso-occlusion begins with the adhesion of sickle erythrocytes and neutrophils to activated endothelium (18,19). Aggregates of blood cells, including platelets, form on these adhered cells and occlude the microvasculature, ultimately causing acute and chronic tissue ischemia and injury. Integral to these cellular interactions are inflammatory mediators, which activate endothelial cells, leukocytes and platelets and attract additional leukocytes to the site of occlusion (20). After the resolution of the microvascular occlusion, ischemia-reperfusion injury may further amplify inflammation, creating a vicious cycle that sustains and propagates vaso-occlusion (21,22). New therapeutic targets have emerged as understanding of vaso-occlusion has progressed beyond the simple concept of a “log jam” of sickled erythrocytes in small vessels. Interruption of the process of vaso-occlusion downstream from erythrocyte sickling may either dampen the severity of an ongoing VOC or prevent one altogether, depending on the approach and efficacy of therapy.

Adhesion: Selectin-Based Therapies

Several lines of evidence suggest that the selectin family of adhesion receptors may be a target for VOC therapies. Selectins are expressed on endothelial cells, platelets and leukocytes, as well as other cell types (23). P-selectin and E-selectin mediate rolling and tethering of blood cells to the endothelium, which may initiate vaso-occlusion in the postcapillary venules (24). Cellular and animal models of SCD demonstrate that interruption of selectin-mediated cellular adhesion decreases erythrocyte and leukocyte adhesion to the endothelium and improves blood flow (25–29). Several therapies that target selectins, including rivipansel (GMI-1070) and the anti-P-selectin monoclonal antibody SelG1, have been or are currently being studied in patients with SCD.

Rivipansel. Rivipansel is a synthetic pan-selectin inhibitor with effects predominantly mediated through E-selectin (25). After a loading dose, rivipansel is administered intravenously every 12 h, with the intent to diminish the severity of an ongoing VOC. A randomized, double-blind, placebo-controlled phase IIb trial of rivipansel (n = 76) was completed, although results have not yet been published. Communications from the company report that rivipansel decreased the duration of hospital stay and the amount of parenteral opioids used during VOC compared with placebo (30).

SelG1. SelG1 is a humanized monoclonal antibody directed against P-selectin. Investigators examined the pharmacokinetics and safety of monthly doses in a phase I study of SelG1 designed to prevent VOC. No results have yet been published. A phase 2 study is now planned in which patients will be randomized to high-dose SelG1, low-dose SelG1 or placebo and the effect on VOC will be measured (NCT01895361).

Inflammation: Invariant NKT (iNKT) Cell–Based Therapies

Reduction of inflammation constitutes another strategy to treat VOC. Studies of corticosteroids provided some of the first evidence that antiinflammatories may improve VOC in patients with SCD. When administered during VOC or ACS episodes, corticosteroids decreased length of hospital stay compared with placebo (31,32). Unfortunately, there was a high rate of readmission to the hospital because of rebound vaso-occlusion after the abrupt cessation of corticosteroids (31,32). A follow-up trial to evaluate the benefits of a tapering regimen of corticosteroids during ACS episodes was, unfortunately, stopped because of poor accrual (33).

iNKT cells. Therapies designed to target iNKT cells have recently been evaluated. iNKT cells comprise <1% of circulating lymphocytes in humans. Albeit small in number, iNKT cells possess potent characteristics of adaptive and innate immunity and “jumpstart” larger inflammatory responses (34). Similar to T cells that produce adaptive immune responses, iNKT cells express a T-cell receptor (TCR) that requires binding of an antigen presented on an antigen-presenting cell. Unlike T cells, which express a diverse TCR repertoire that recognizes different peptides, the iNKT cell receptor is invariant and recognizes only the pattern of a lipid antigen, akin to innate immune responses. Cytokines secreted from the antigen-presenting cell, in response to toll-like receptor activation, further enhance iNKT cell activation. Within hours, activated iNKT cells rap-
idly produce large quantities of cytokines (interferon-γ, tumor necrosis factor-α, interleukin [IL]-2 and IL-4), which may activate B cells, T cells, NK cells and dendritic cells (35). In addition, interferon-γ stimulates the production of the chemokines C-X-C motif chemokine 9 precursor (CXCL9), CXCL10 and CXCL11, potent chemotactic factors for CXCR3-expressing lymphocytes (36). In a mouse model of SCD, interruption of iNKT cell activation or depletion of iNKT cells reduces tissue injury (22).

**Regadenoson.** Administration of an adenosine A2A receptor agonist is one strategy used to block iNKT cell activation in a SCD mouse model (22,37). On the basis of that preclinical data, a phase I study of the A2A receptor agonist, regadenoson, was performed in patients (38). Twenty-seven SCD patients were administered infusional regadenoson. The target dose of 1.44 mg/kg/h was achieved without toxicity. During VOC, a 24-h infusion of regadenoson decreased the percentage of activated iNKT cells by a median of 50%. A multicenter, randomized, double-blind, placebo-controlled phase IIb trial is currently underway to examine the potential clinical benefits of regadenoson during VOC (clinicaltrials.gov, NCT01788631).

**NKTT 120.** NKTT 120 is a humanized monoclonal antibody directed against a unique epitope on the invariant T-cell receptor of iNKT cells (39). Preliminary results of an ongoing phase I study (clinicaltrials.gov, NCT01783691) demonstrate that NKTT 120 is safe in doses up to 0.01 mg/kg and rapidly depletes iNKT cells in a dose-dependent fashion (40). The return of iNKT cells to circulation is also dose-dependent and inversely related to the concentration of iNKT cells. Further studies will be needed to determine if long-term depletion of iNKT cells with NKTT 120 decreases VOC rate and chronic morbidity.

**Limitations of selectin- and iNKT cell-based therapies.** Even though rivipansel and regadenoson may improve outcomes during VOC, they are unlikely to completely abrogate the associated risks of VOC, including the risk of death. Monoclonal antibodies, such as SelG1 or NKTT 120, offer the potential to prevent VOC, a much superior approach. Long-term inhibition of P-selectin or iNKT cells in patients with SCD may, however, carry concomitant risks. Both play important roles in immunity, and their chronic inhibition may increase susceptibility to infection in an already infection-prone patient population.

**HEMOLYSIS**

Recent studies have suggested an adverse impact of red cell hemolysis on SCD beyond that of anemia. Sickled red cells have a shortened lifespan of 10–20 d, and irreversibly sickled cells may be removed in hours (41). This rapid clearance of sickle erythrocytes may be due to engulfment by monocytes or macrophages, complement deposition or entrapment in the microvasculature (42). Rapid hemolysis releases red cell contents, including hemoglobin, free heme and arginase, into the circulation with a myriad of downstream effects that ultimately affect the pathogenesis of SCD (43).

**Nitric Oxide and Free Heme**

Detrimental effects of hemolysis are mediated in part by a reduction in NO bioavailability and the actions of free heme (44–47). When hemoglobin is digested into the circulation, heme iron reacts with and depletes nitric oxide (NO) to form nitrate (NO3 -). Intraerythrocyte arginase is also released and metabolizes l-arginine, a key substrate for NO synthesis. The actions of NO, including vasodilation, platelet inhibition and decreased inflammation, oppose many of the pathogenic mechanisms that contribute to vaso-occlusion. Therefore, a disease model emerges whereby release of cell-free hemoglobin during hemolysis and depletion of NO may lead to vasoconstriction and a prothrombotic, proinflammatory environment that promotes vaso-occlusion (48). Whether cell-free hemoglobin and arginase are released in amounts sufficient to deplete NO and induce VOC in most patients is a matter of dispute (49).

Heme groups liberated from cell-free hemoglobin during hemolysis may also contribute to the toxicity associated with hemolysis (45–47). In mouse models of SCD, free heme has been shown to activate endothelial cells through toll-like receptor 4 signaling, inducing a proadhesive endothelial cell phenotype that promotes the red cell, white cell and platelet interactions that underlie vaso-occlusion (46,47). These negative effects of heme can be blocked by inhibition of toll-like receptor 4 or the administration of the heme-binding protein hemopexin (46,47).

**NO and Pulmonary Hypertension**

Pulmonary hypertension is the most notable complication of SCD thought to be, in part, secondary to hemolysis and reduced NO bioavailability. In a prospective cohort study of 195 patients with SCD, 30% had evidence of pulmonary hypertension, defined as a peak tricuspid regurgitant jet velocity (TRJV) ≥2.5 m/s on echocardiogram (50). Individuals with pulmonary hypertension had a 10-fold higher risk of death than those without. The pulmonary hypertension described in this study was mild compared with the definitions used for the general population, raising questions as to whether the pulmonary hypertension was actually a contributing cause of death or just a marker of severe SCD.

More controversy surrounding the role of pulmonary hypertension arose when a prospective cohort study of 398 adult patients with SCD, 96 of whom underwent right heart catheterization, was published (51). Although 27% of the cohort had TRJV ≥2.5 m/s, only 6% had evidence of pulmonary hypertension that was confirmed on right heart catheterization, defined as a mean pulmonary artery systolic pressure >25 mmHg. Of those patients with confirmed pulmonary hypertension, there was mix of pulmonary arterial hypertension, consistent with an NO depletion model, and
pulmonary venous hypertension, more consistent with left-sided heart disease. A TRJV ≥2.5 m/s had a positive predictive value of only 25% for pulmonary hypertension, confirmed on right heart catheterization; however, the positive predictive value increased to 64% when a TRJV cutoff of 3.0 m/s was used. The group with TRJV ≥3.0 m/s was older and had worse exercise capacity and a higher brain naturetic peptide level (consistent with symptomatic, physiologically relevant disease).

Sildenafil for Pulmonary Hypertension
Extending these findings to a therapeutic trial, investigators sought to determine whether treating pulmonary hypertension with sildenafil in adults with SCD would improve exercise capacity (52). Sildenafil is a phosphodiesterase-5 inhibitor that increases levels of cyclic guanosine monophosphate (cGMP), which mediates the vasodilation effects of NO. Unfortunately, the trial was stopped because a significantly high percentage of patients in the group receiving sildenafil required hospitalization for pain. The most likely explanation for the increased rate of pain in the sildenafil group is cGMP-related effects on pain signaling as opposed to worsened vaso-occlusion (53).

Limitations of Hemolysis- and Pulmonary Hypertension-Based Therapies
The contribution of pulmonary hypertension to morbidity and mortality of patients with SCD remains an important concern whether the hypertension is produced by NO depletion or other mechanisms such as pulmonary arterial thrombosis (54, 55). There is a growing concern in the field that even mild pulmonary hypertension, defined by a TRJV ≥2.5 m/s, might contribute to SCD mortality, perhaps because of exacerbations of pulmonary pressures during acute VOC. Whether treatment of patients with SCD and pulmonary hypertension reduces morbidity or mortality remains an open question. In the absence of definitive data, an American Thoracic Society consensus group recently recommended treating patients with TRJV ≥2.5 m/s with aggressive SCD-based therapy, either hydroxyurea or chronic transfusions, while reserving pulmonary hypertension therapies (for example, endothelin-1 receptor antagonists) for those with right heart catheterization-proven pulmonary arterial hypertension (56). The use of chronic transfusion with its attendant risks for patients with TRJV ≥2.5 m/s will be disputed in many quarters.

HEMATOPOIETIC STEM CELL TRANSPLANT/GENE THERAPY

Hematopoietic Stem Cell Transplantation
Hematopoietic stem cell transplantation (HSCT) offers the potential for a cure from SCD. Largely as an extension of the thalassemia experience, HSCT has been investigated in patients with SCD for the past 30 years. In 1984, there was an initial report of a successful HSCT in a child with SCD who developed acute myeloid leukemia (57). Thereafter, a larger study (n = 22) using matched-sibling donors and high-intensity preparatory chemotherapy demonstrated 90% survival and 70% disease-free survival, along with stabilization of end-organ dysfunction (58). Despite these promising results, high mortality rates in patients older than 16 years and a paucity of suitable HLA-identical donors have limited the widespread implementation of HSCT in this patient population (59). Overcoming these obstacles to allow more patients with SCD to undergo HSCT is the focus of current investigations.

Reduced-intensity HSCT. Recent data suggest that reduced-intensity regimens before HSCT decrease toxicity in adults with SCD. Preparatory therapy eliminates the recipient hematopoietic cells and creates space in the bone marrow, allowing for donor engraftment and hematopoiesis. Reduced-intensity regimens do not completely ablate the bone marrow, but do make enough space for donor hematopoiesis, often creating a state of chimerism between the recipient and donor. Thus, the patient still produces sickle erythrocytes, albeit at a lower percentage of the circulating cells that may reverse the SCD phenotype. Using a reduced-intensity regimen, 10 adults with SCD, ages 16–45 years, underwent HSCT from matched-sibling donors (60). No deaths occurred, and there was no graft versus host disease, which has complicated other lower-intensity regimens (61). Stable engraftment was achieved in 9 of 10 subjects. In these nine engrafted subjects, the percentage of sickle hemoglobin was similar to donors, five of whom had sickle cell trait. Similar to studies in children, end-organ disease stabilized and pain symptoms improved. Recently, this group published findings from a larger cohort of 30 adults with SCD who underwent an HSCT after reduced-intensity conditioning. Consistent with the prior study, the regimen was well tolerated with a high rate of stable engraftment (87%) and low rate of graft versus host disease (62). This approach addressed the problem of toxicity in adults. Identification of suitable donors remained a problem, however, since only 20% of patients screened as part of the original study had an HLA-identical donor (60). HSCT with alternative donors. The use of haploidentical donors has the potential to increase the number of patients with SCD able to undergo HSCT. Nearly all patients will have a haploidentical donor, who is half-matched at HLA antigens (for example, a parent). The downside of this approach is a graft failure rate of nearly 50%. Fourteen patients underwent HSCT from a haploidentical donor after a low-intensity preparatory regimen (63). Although six patients rejected the graft (43%), there were also five patients who achieved full donor chimerism. Those who rejected their grafts reverted back to SCD phenotype. There were no deaths.

Based on these data, HSCT is recommended for those children with an HLA-identical sibling donor. HSCT is not yet standard for adults with SCD, although a
50% chance for a cure with a haploidentical transplant may be a viable treatment option in the future, since the risk of mortality appears to be low.

**Gene Therapy**

Correction of the β-globin gene may be the ideal approach to cure SCD in the future. In mouse models, strategies to transplant genetically modified hematopoietic stem cells (HSCs), expressing normal β-globin gene or an antisickling globin, have been investigated (64–67). One approach relies on a lentivirus vector to integrate β-globin or antisickling globin genes into the genome of mouse HSCs, which are then transplanted into irradiated mice (68). Insertional mutagenesis is a theoretical and factual concern. If the lentivirus disrupts a tumor suppressor gene or activates an oncogene, leukemia may result. Despite these concerns, there are trials ongoing to evaluate this approach in patients with β-thalassemia major (clinicaltrials.gov, NCT01639690) and SCD (clinicaltrials.gov, NCT02186418). Homologous recombination, a strategy to edit DNA that may avoid insertional mutagenesis, is also being studied. This technique has the potential to change a βs-globin (sickle) gene to a normal β-globin gene in a site-specific manner. In mice with SCD, investigators have used homologous recombination to correct a β-globin gene in fibroblast-derived induced pluripotent stem (iPS) cells (69). Corrected iPS clones are then differentiated to HSCs and transplanted into irradiated mice, reversing the SCD phenotype. Genetic modification of human iPS cells could cure patients with SCD; however, there are many challenges to overcome before such clinical trials become a reality (70).

**CONCLUSION**

With several drugs in the investigational pipeline and new approaches to gene therapy under development, the prospect of new therapies for patients with SCD has never been better. The goal of any of these therapies is to help patients with SCD and to ease suffering. To this end, some of the approaches discussed are theoretically better than others. A guiding principle when considering the potential benefit of SCD therapies is: the further upstream the target, the better. In SCD, a single gene defect causes red cell sickling, which has widespread downstream effects on nearly every organ. If the gene is fixed, red cell sickling does not occur, and all of the downstream pathologies are prevented. Approaches that address mechanisms downstream of the mutation and erythrocyte sickling (for example, adhesion, inflammation and hemolysis) attempt to minimize the damage, concealing that some damage from erythrocyte sickling is probably inevitable. Potentially, the greatest value from these downstream therapies is in combination with other therapies, such as an antisickling therapy. Short of a cure, multiple drugs to target multiple mechanisms, similar to chemotherapy regimens in cancer, may be the optimal approach for SCD.

Finally, health care delivery markedly influences the potential benefits of new therapies for patients with SCD. Much of the care provided for patients with SCD occurs in the primary care setting, outside of specialized centers. Because primary care physicians have not been trained to treat patients with SCD, new treatments may not be prescribed, even if they are highly effective. Bear in mind that nearly two decades after the approval of hydroxyurea, most patients with SCD are suboptimally treated with it, or not treated at all. Any of the new therapies discussed here may be similarly underused, which may be the most difficult problem of all.

**ACKNOWLEDGMENT**

This paper is dedicated to Dr. Anthony Cerami, founder of the journal Molecular Medicine and longtime friend and colleague.

**DISCLOSURE**

DG Nathan and JJ Field receive research support from NKT Therapeutics and Astellas. DG Nathan and JJ Field are the PIs of the phase 2 regadenoson study supported by NIH.

**REFERENCES**