Sickle Cell Disease and Hematopoietic Stem Cell Transplantation

Emily Riehm Meier
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Preface

Sickle cell disease (SCD) was first described in the USA over 100 years ago [1]. Nearly six decades ago, SCD became known as the first molecular disease after Linus Pauling described the different electrophoretic movements of hemoglobin A compared to hemoglobin S [2]. In the 1970s, research funding for SCD significantly increased, and findings from those research studies allowed SCD to move from a nearly universally fatal disease of childhood to a chronic illness [3]. Today, there are nearly 100,000 children and adults in the USA alone with SCD and millions more worldwide [4]. Although hydroxyurea and blood transfusion therapy decrease many SCD-associated complications, they are often underutilized in developing countries and neither cure the disease. Patients with SCD experience multisystem complications including recurrent and painful vaso-occlusive crises, acute chest syndrome, stroke, splenic sequestration, and pulmonary hypertension. As a result, organ damage begins at a young age and is progressive, contributing to an average life expectancy of SCD patients that is half that of the general population [5, 6] . Even though life expectancy of patients has improved in the last several decades in the developed world, very high childhood mortality rates (50–90%) exist in underdeveloped countries of Africa and India where the vast majority of SCD patients are globally. SCD remains a highly morbid disease that causes chronic and progressive physical damage, as well as poorer overall quality of life for affected children and adults worldwide.

Currently, the only available cure for SCD is hematopoietic stem cell transplant (HSCT). Successful transplants in children with SCD additionally result in organ damage reversal or stabilization of CNS vasculopathy. When a matched sibling donor is available for SCD patients, HSCT has excellent overall survival (93%, 95% confidence interval 91.1–94.6%) and good event free survival (91%, 95% confidence interval 89.6–93.3%) [7]. HSCT, however, is still vastly underutilized, even for those with donors; in fact, just over 1000 transplants for SCD patients have been documented in the literature[7]. Since SCD affects nearly 100,000 children and adults in the USA alone, and millions more worldwide, considerably less than 1% of people effected by SCD have been cured. Causes for the low numbers of HSCT performed for SCD patients are multifactorial and

include concern about toxicities from conditioning regimens or graft-versus-host disease, lack of provider or family awareness of HSCT as a curative option for SCD, and, most importantly, lack of an available suitable donor. This has led to the field investigating less toxic conditioning regimens (reduced intensity), novel graft manipulation techniques and medications to prevent graft-versus-host disease, and donor options other than matched siblings referred to as alternative donors. While outcomes with alternative donor transplants still need to be optimized, encouraging progress with unrelated bone marrow, umbilical cord blood, and most recently half-HLA-matched (haploidentical) parents and siblings has led to the majority of patients having a transplant donor option. Probably the most exciting advance in the HSCT for the SCD field in the last decade has been the ability to transplant adults with SCD using very low intensity conditioning and HLA-matched donors [8, 9]. The success rate of approximately 88% with no graft-versus-host disease is remarkable especially in a patient group that for many decades were considered too sick to proceed to transplant. Although the concept that gene therapy may ameliorate human genetic diseases emerged in the 1970s, this field of medical research experienced many obstacles over the last three and a half decades which prolonged the translation of this genetic technology into clinical medicine. However, along with these obstacles came broadened knowledge of safe gene delivery and editing systems which carved the path to making the concept of gene therapy to alleviate human diseases such as SCD a reality. Gene therapy trials in SCD are now underway, and very preliminary results show promise [10]. Unlike HSCT, gene therapy offers the potential of curative therapy to virtually all people affected by SCD.

This book aims to provide a comprehensive, state-of-the art review of HSCT for SCD and serves as a valuable resource for clinicians and researchers with an interest in SCD as well as HSCT. The book reviews new data about risk prediction for severe SCD; presents unique challenges of HSCT for patients with SCD; profiles the supportive care guidelines for SCD patients who are undergoing HSCT; highlights our current understanding of the best transfusion support for SCD patients prior to, during, and after HSCT; and provides new perspectives about the ethics of HSCT for pediatric patients with SCD. Several landmark phase III trials that utilized matched unrelated and haploidentical donors for HSCT in SCD patients have been placed in context with respect to current management.

We hope that this book will serve as a useful resource for physicians and researchers interested in this challenging, yet exciting, curative therapy for SCD. We thank the authors who contributed chapters to this book. We dedicate this book to our spouses, children, parents, and other family members who have supported us throughout our careers. We also thank our mentors who inspired us to continue working to improve the care of children with SCD and their families: Drs. Naomi Luban, Jeffery L. Miller, Derek Persons, and Naynesh Kamani. We also dedicate this book to our patients who live with this debilitating disease and inspire our work with their strength, resilience, and fighting spirits.

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Part I
Sickle Cell Disease

Chapter 1
Clinical Manifestations of Sickle Cell Disease Across the Lifespan

Lydia H. Pecker and Jane Little

Abbreviations

ACE	Angiotensin-converting enzyme
ACS	Acute chest syndrome
ARB	Angiotensin receptor blocker
CBC	Complete blood count
CSSCD	Cooperative study of sickle cell disease
CXR	Chest X-ray
DVT	Deep vein thrombosis
ESRD	End-stage renal disease
Hb	Hemoglobin
HbS	Sickle hemoglobin
HbSβ^0	Hemoglobin Sβ^0 disease
HbSβ^+	Hemoglobin Sβ^+ disease
HbSC	Hemoglobin SC disease
HbSS	Hemoglobin SS disease
HU	Hydroxyurea
MCV	Mean corpuscular volume
MSOF	Multisystem organ failure
NHLBI	National Heart, Lung, and Blood Institute
NSAID	Nonsteroidal anti-inflammatory
PE	Pulmonary emboli
SCA	Sickle cell anemia

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SCD	Sickle cell disease
SCIC	Sickle cell intrahepatic cholestasis
TCD	Transcranial Doppler
UK	United Kingdom
VOC	Vaso-occlusive crisis
VTE	Venous thromboembolus
WBC	White blood count

Introduction

SCD is the quintessential chronic disease of childhood, manifesting significant clinical sequelae despite a defined molecular origin. Children and adults with SCD are anemic, make frequent emergency department visits, and are often hospitalized for pain and acute complications associated with progressive organ damage. Mortality for patients with SCD in high-income countries is shifting from infectious and cerebrovascular complications in childhood to progressive multisystem organ failure (MSOF) in adulthood [1]. An expanding population of adults with SCD, the happy result of decades of improved pediatric care, is facing the multifaceted consequences of decades of chronic and cumulative vascular damage. However, there is new hope for treating people with SCD. Hydroxyurea (HU) modulates many complications of SCD, and hematopoietic stem cell transplant (HSCT) is curative. Gene therapies and genome editing (of the gene itself or disease-modifying genes) are transforming prospects for curative therapy in single-gene disorders like SCD [2]. In this setting, clinicians, patients, and their families are increasingly challenged by the complicated therapeutic risk-benefit analyses for a disease that may be mild in children but can cause relentless morbidity and early mortality in adults [3, 4]. Figure 1.1 provides an overview of major milestones in SCD since its recognition as a pathobiologically distinct hemolytic anemia in the early decades of the last century.

Pathophysiology, Epidemiology, and Disease Modulators

SCD is a catch-all term for a group of hemoglobinopathies defined by the presence of a single sickle mutation in the beta-globin gene (chromosome 11: GAG → GTG in the sixth codon, substituting valine for glutamic acid). Homozygous HbSS arises from the inheritance of this mutation in both beta-globin genes. Intraerythrocyte polymerization of sickle hemoglobin (HbS) causes red cell deformity, fragility, and abnormal adhesion, leading to hemolysis, endothelial activation, hypercoagulability, vasculopathy, and multisystem organ injury. By convention, sickle cell anemia (SCA) refers to HbSS or hemoglobin $S\beta^0$ disease because in $HbS\beta^0$ disease, all detected hemoglobin is HbS.

Compound heterozygous SCD arises from co-inheritance of HbS and another abnormal beta-globin gene. The two most common mutations lead to hemoglobin C, also caused by a mutation in the sixth codon, substituting lysine for glutamic acid (common in Western Africa) and β^+-thalassemia, a quantitative deficiency in the normal beta-globin chain (common in Greece and India) [6]. Up to one-third of patients with SCD are compound heterozygotes with HbSC or HbSβ^+ [7, 8]. Symptoms of HbSC and HbSβ^+ are attributed to episodic sickling, modest hemolysis, and the cumulative effects of hyperviscosity and abnormal rheology. HbSC is uniquely associated with damage to red cell membrane channels that alter intracellular water and solute concentrations and lead to increased red cell density [9].

Pathophysiology

The presence of pathognomonic HbS initiates a cascade of biophysical and pathophysiologic effects. Intermolecular hemoglobin polymerization and deoxygenated hemoglobin cause physical and oxidative injury to red cells leading to abnormal red cell shape and rheology and hemolytic anemia associated with a significantly shortened red cell half-life (10–20 days instead of 120 days). The dramatic red cell changes of HbSS disease contrast with the more subtle effects of HbSC (Fig. 1.2). Hyperviscosity and intravascular hemolysis, with resultant inflammation and thrombophilia, contribute to widespread acute-on-chronic endothelial damage and vasculopathy. HbSC has a milder hemolytic phenotype, but abnormal red cell rheology contributes to the particular predisposition to thrombophilia, avascular necrosis, and retinopathy seen in this disease [10]. The pathophysiology of SCD is explored in depth in Chap. 2.

Epidemiology

The global incidence of SCD is 300,000 to 400,000 per year [11], with high childhood mortality in regions with developing economies, such as Africa and India. Sickle hemoglobin emerged independently in four or more sites worldwide. The geographic distribution of beta-globin gene mutations is the result of evolutionary pressures associated with endemic malarial infections. Both sickle cell trait (HbAS) and hemoglobin C trait (HbAC) protect against severe forms of cerebral malaria [12], without causing a significant hematologic syndrome. Inheritance of HbS or HbC trait allows children to reach childbearing age in malaria-endemic geographic areas, despite the significant "cost" to the population from increased prevalence of SCD. Because of the survival advantage of HbC compared to HbS, HbC is projected to ultimately overtake the more harmful HbS mutation in areas where they coexist (e.g., Western Africa) [11, 13, 14].

1910: Sickle morphology identified in the blood of a Grenadian dental student, Walter Clement Noel (1884 - 1916) by James Herrick and Edward Irons[1]
1924: Deoxygenated hemoglobin identified as trigger of sickling, vaso-occlusion & hemolysis[2]
1949: Pauling & Itano publish tertiary structure difference between HbS & HbA[3]
1958: Hunt and Ingram describe the single nucleotide differences that define the difference between HbS, HbC & HbA[4]
1970: NEJM & JAMA publish "SCA: High prevalence low priority" & "Health care priority & SCA" by RB. Scott[6]
1972: National Sickle Cell Control Act funds voluntary SCD screening, counseling, education & research[6]
1976: TheNational Heart & Lung institute adds blood, becomes the NHLBI[7]
1976: Prenatal SCD diagnosis using fetal DNA[9]
1978: The CSSCD starts -is the largest natural history study of SCD patients ever performed[8]
1984: First curative HSCT for patient with SCA & leukemia[10]
1986: PROPS I demonstrates penicillin prophylaxis for children with SCD < 3 years old reduces bacteremia/death by 84%. Intervention is rationale for newborn screening implementation[11]
1988: Conjugated H. influenza type b vaccine[12]
1992: TCD velocities predict stroke risk in children with SCD[13]
1994: STOP I - prophylactic transfusions prevent first stroke in children with abnormal TCD[14]
1995-1998: MSH study shows HU reduces pain, ACS, hospitalizations by 50% in adults with SCA. FDA approves HU for adults[16]
1996: First multicenter trial for myeloablative HSCT for SCD[15]
2000: Conjugated pneumococcal (PCV-7) vaccination[17]
2004: Sickle Cell Treatment Act renewed[18]
2006: NIH funding disparity identified: 4x more spent per patient with cystic fibrosis compared to SCD[19]
2008: Results of Ghanian newborn screening reported[20]
2009: First successful non-myeloablative HSCT series[21]
2010-2012: Reports highlight global pediatric survivor disparity –children in USA & Jamaican with near universal survival with high (80%) under-5 years mortality in sub-Saharan Africa[22]
2011: BABY-HUG shows HU reduces pain & ACS in children with sickle cell anemia[23]
2012: First series of haplo-identical HSCTfor SCD[24]
2013-2016: More newborn screening & hydroxyurea treaching (some) children in sub-saharan Africa[26]
2016: Selectin-inhibitors emerge as treatment & prevention of VOC[25]
2016: TWITCH - Children may be safely transitioned to HU after 1 year of chronic transfusions for abnormal TCD[27]
2017: Report of curative gene therapyfor the first molecular disease[28]

Fig. 1.1 Timeline of significant events in sickle cell disease. Significant advances in the first 110 years of SCD study have led to universal survival of children in high-income children [5]. [1]Herrick JB. Peculiar elongated and sickle-shaped red blood corpuscles in a case of severe anemia. 1910. [2]Wailoo, K. "A disease sui generis": the origins of sickle cell anemia and the emergence of modern clinical research, 1904–1924. [3]Pauling, L. & Itano, H. A. Sickle cell anemia, a molecular disease, 1949. [4]Hunt, J. A. & Ingram, V. M. Allelomorphism and the Chemical Differences of the Human Hemoglobins A, S and C, 1958. [5]Scott, R.B. Health care priority and sickle cell anemia, 1970; Scott, R. B. Sickle-cell anemia—high prevalence and low priority, 1970. [6]Wailoo, K. *Dying in the City of the Blues: Sickle Cell Disease and the Politics of Race and Health*, 2001. [7]"Important events in NHLBI History". https://www.nih.gov/about-nih/what-we-do/nih-almanac/national-heart-lung-blood-institute. [8]Gaston, M. et al. Recruitment in the Cooperative Study of Sickle Cell Disease (CSSCD), 1987. [9]Alter, B. P., Modell, C. B. & Fairweather, D. Prenatal diagnosis of hemoglobinopathies: A review of 15 cases, 1976. [10]Johnson, F. L. et al. Bone-marrow transplantation in a patient with sickle-cell anemia, 1984. [11]Gaston, M. H. et al. Prophylaxis with oral penicillin in children with sickle cell anemia. A randomized trial, 1986. [12]Rubin, L. G. Pneumococcal Vaccine, 2000. [13]Adams, R. et al. The use of transcranial ultrasonography to predict stroke in sickle cell disease, 1992. [14]Adams, R. J. et al. Prevention of a first stroke by transfusions in children with sickle cell anemia and abnormal results on transcranial Doppler ultrasonography, 1998. [15]Walters, M. C. et al. Bone marrow transplantation for sickle cell disease, 1996. [16]Charache, S. et al. Effect of hydroxyurea on the frequency of painful crises in sickle cell anemia. Investigators of the Multicenter Study of Hydroxyurea in Sickle Cell Anemia, 1995. [17]Peltola, H. Worldwide Haemophilus influenzae type b disease at the beginning of the twenty-first century: global analysis of the disease burden 25 years after the use of the polysaccharide vaccine and a decade after the advent of conjugates, 2000. [18]Serjeant, G. R. One hundred years of sickle cell disease, 2010. [19]Smith, L. A., Oyeku, S. O., Homer, C. & Zuckerman, B. Sickle cell disease: a question of equity and quality, 2006. [20]Ohene-Frempong, K., Oduro, J., Tetteh, H. & Nkrumah, F. Screening newborns for sickle cell disease in Ghana, 2008. [21]Hsieh, M. M. *et al.* Allogeneic Hematopoietic Stem-Cell Transplantation for Sickle Cell Disease, 2009. [22]Quinn, C. T. et al. Improved survival of children and adolescents with sickle cell disease, 2010; Grosse, S. D. et al. Sickle cell disease in Africa: a neglected cause of early childhood mortality, 2011; Aygun, B. & Odame, I. A global perspective on sickle cell disease, 2012. [23]Thornburg, C. D. et al. Impact of hydroxyurea on clinical events in the BABY HUG trial, 2012. [24]Bolaños-Meade, J. et al. HLA-haploidentical bone marrow transplantation with post-transplant cyclophosphamide expands the donor pool for patients with sickle cell disease, 2012. [25]Ataga, K. I. et al. Crizanlizumab for the Prevention of Pain Crises in Sickle Cell Disease, 2016. [26]McGann, P. T. et al. Hydroxyurea Therapy for Children With Sickle Cell Anemia in Sub-Saharan Africa: Rationale and Design of the REACH Trial, 2016; Tubman, V. N. et al. Newborn Screening for Sickle Cell Disease in Liberia: A Pilot Study, 2016. [27]Ware, R. E. et al. Hydroxycarbamide versus chronic transfusion for maintenance of transcranial Doppler flow velocities in children with sickle cell anaemia-TCD With Transfusions Changing to Hydroxyurea (TWiTCH): a multicentre, open-label, phase 3, non-inferiority trial, 2016. [28]Ribeil, J.-A. et al. Gene Therapy in a Patient with Sickle Cell Disease, 2017

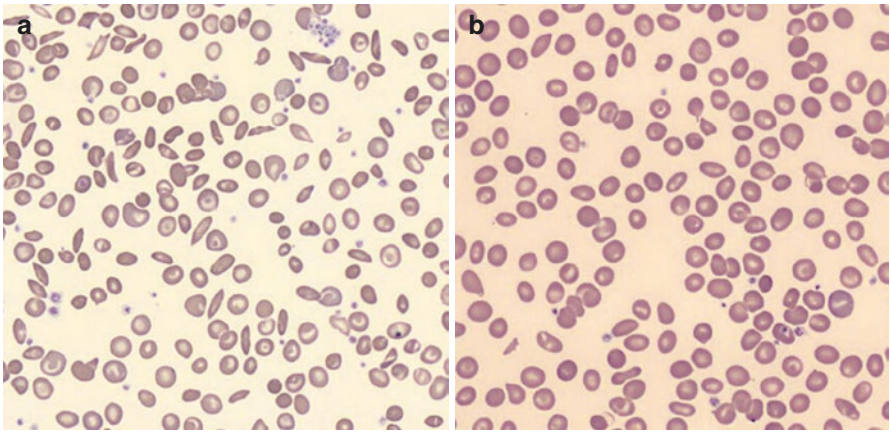

Fig. 1.2 Blood smears of HbSS, HbSS on HU, and HbSC. (**a**) Hemoglobin SS disease with many sickle forms. (**b**) Hemoglobin SC disease—target cells and hemoglobin C crystals are present, while sickle forms are rare

Genetic Modulators of Disease Severity

Inheritance of additional genetic mutations modifies SCD phenotype and clinical severity. The alpha-globin genes (α-thalassemia), UDP-glucuronosyltransferase gene, and genes that result in a hereditary persistence of fetal hemoglobin (HPFH) are established disease modifiers [15, 16]. Other genetic associations, for example, of Klotho in association with priapism, TGF-beta pathway signaling in SCD phenotype, and of APOL1 in renal disease, await full explication.

Hereditary persistence of fetal hemoglobin (HPFH): Inherited elevations in fetal hemoglobin (HbF) modulate SCD severity [17]. Because of the salubrious effects of an elevated HbF in SCD, the molecular causes of HPFH are of intense interest for researchers pursuing gene editing therapy [18]. Gene loci implicated in HPFH include the fetal gamma globin genes themselves (Xmn1-HBG2), the HBS1L-MYB intergenic region on chromosome 6q, and the fetal globin repressor BCL11a and its regulator KLF1 [19]. Gene therapy targets, such as the erythroid enhancer of the gamma chain repressor BCL11a or artificial chromatin modifiers of the fetal locus, are also being pursued [20–22].

Alpha-thalassemia: Approximately one in three African Americans carries a deletion of one or two of the identical alpha globin genes in the alpha-globin locus. These are characterized as "silent carrier" or "α-thalassemia trait," respectively, if the other chromosome is intact. Three to five percentage of African Americans have α-thalassemia trait [23]. Co-inheritance of α-thalassemia trait reduces intracellular hemoglobin concentration and mean corpuscular volume (MCV) in patients with SCA. α-Thalassemia trait is associated with decreased hemolysis [24] and

decreased risk of stroke, kidney disease, and gallstone formation. The moderating effect of α-thalassemia is seen in multi-gene analyses of risk, for renal disease [25] and for hyperbilirubinemia [16]. In patients with HbSC disease and α-thalassemia trait, the incidence of osteonecrosis, retinopathy, and painful crises is reduced, and the onset of gallbladder disease is delayed [10]. In concurrent α-thalassemia and SCA, MCV may not increase as might otherwise be anticipated during treatment with HU [26].

UGT1a polymorphism: Interest in the UGT1a gene in SCD arose because polymorphisms in TA repeats in the UGT1A promoter are associated with Gilbert's syndrome, a disorder of abnormal bilirubin metabolism and cholelithiasis [27]. The UGT1a gene encodes the UDP-glucuronosyltransferase, which transforms lipophilic molecules, such as bilirubin, into water-soluble, excretable metabolites. These polymorphisms also alter bilirubin metabolism in people with SCD and are associated with increased gallstone complications [28–30].

Prognosis

Life expectancy for children with SCD in high-income countries has markedly improved [31, 32]. Estimated median life expectancy for adults with SCA has been unchanged (at 40–50 years) for several decades [33–37], but recent studies report increases in mortality in adults and in adolescent-young adults, attributed respectively to lack of access to quality care [37, 38] and to vulnerability during transitions of care from pediatric to adult medicine [39]. However, predicting prognosis for individual patients with SCD remains challenging [40], complicating the timing of referrals for high-risk therapies [41].

Improved survival in SCD is closely tied to (1) **newborn screening** to identify affected infants, initiate penicillin prophylaxis, and educate parents and caregivers about fever management and spleen palpation; (2) **vaccinations** for encapsulated organisms *H. influenzae*, *N. meningitidis*, and *S. pneumoniae*; (3) **improved supportive, emergency, and intensive care**; (4) **screening for stroke risk** with transcranial Doppler (TCD) to identify and treat children with SCA who have elevated cerebrovascular velocities; and (5) **treatment** with blood transfusions and HU. The impact of more experimental therapeutic strategies, such as HSCT, on life expectancy, is not yet clear [34, 42–44].

In low-income regions with sizable SCD populations, such as sub-Saharan Africa, the Caribbean, India, and the Middle East, affected children do not universally receive early interventions, often due to a lack of newborn screening [45]. Eighty-five percent of children with SCD are born in Africa where newborn screening is not universal. Without the uniform ability to identify and optimally treat this population, SCD likely contributes significantly to overall mortality among children under 5 years of age in these regions [46].

Treatment

SCD treatments are addressed in detail elsewhere and are sketched here only to guide the reader's interpretation of discussions below. HU is the only FDA-approved treatment for SCA (Chap. 3). Simple red cell transfusions improve anemia, while exchange transfusions primarily decrease the concentration of sickled red cells (Chap. 5). HSCT is curative but not currently available for the majority of affected patients [47]. Notably, biomedical research appears closer than ever to an elusive gene therapy cure of SCD https://jhupbooks.press.jhu.edu/content/troubled-dream-genetic-medicine, via repair of the β-globin mutation or genetic enhancement of HbF expression [2, 48, 49], but significant challenges remain.

SCD treatments are changing the epidemiology of certain complications, for example, chronic transfusions decrease overt stroke and HU diminishes painful crises and acute chest syndrome. As expected, each promising disease-modifying therapy creates unique clinical challenges (adherence and acceptance of HU, alloimmunization and iron overload with transfusions, limited donor availability with transplant) [50].

Systemic Complications

Here we review the major effects of SCD by system and highlight the most important acute and chronic effects of SCD (Fig. 1.3), with the exception of the central nervous system which is reviewed in detail in Chap. 6. Acute vaso-occlusion and chronic vasculopathy are the striking complications of SCD, and most body systems are affected. Where possible, we identify genotypic distinctions in SCD complications. Figures 1.2 and 1.4 provide an overview of sickle cell disease complications across the lifespan and by system, respectively.

Anemia

SCD causes chronic hemolytic anemia. Baseline hemoglobin values vary by genotype, 7.8 ± 0.8 g/dL in SCA and 2–3 g/dL higher in compound heterozygote states [51]. The half-life of red cells in SCA is reduced compared to healthy red cells. Healthy red cells circulate for approximately 12 weeks, but in SCA, they last only 2–3 weeks. In the setting of brisk red cell turnover, steady state hemoglobin levels are only maintained by a robust reticulocytosis. Conditions that inhibit reticulocyte production or accelerate red cell destruction can cause acute worsening of baseline anemia.

In SCD patients found to have anemia below their baseline, reticulocyte count may direct the diagnostic differential.

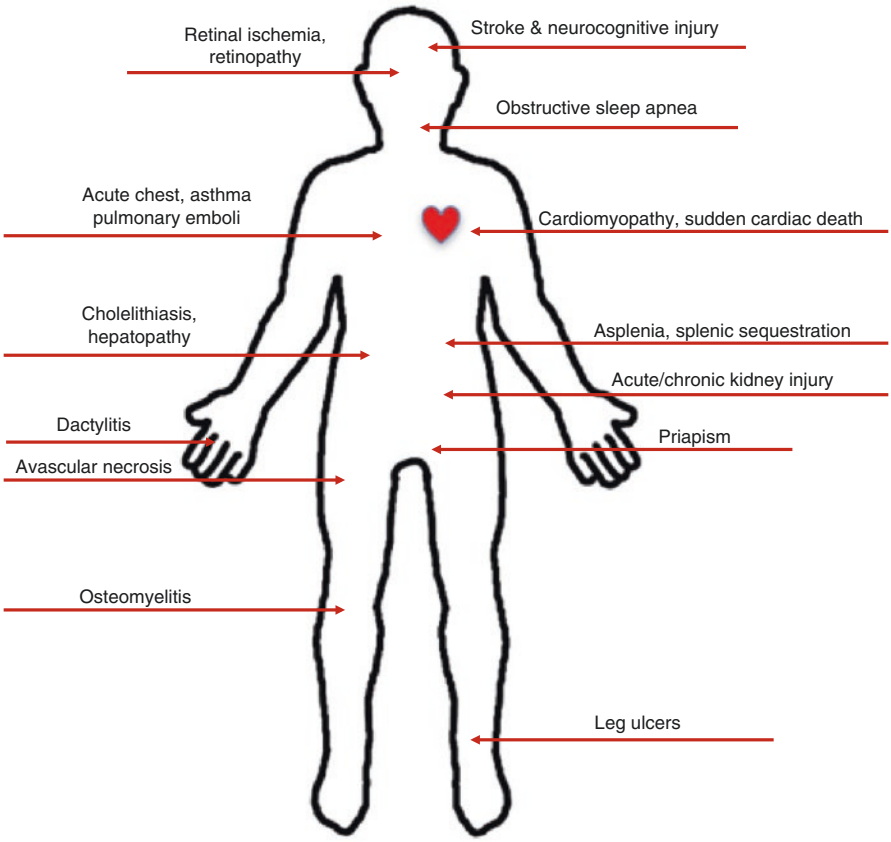

Fig. 1.3 Sickle cell disease causes diffuse bodily injury. Systemic injury is seen to most major organs because of anemia, endothelial activation and injury, inflammation, and vascular damage

Low reticulocyte anemia: Conditions that inhibit reticulocyte production or accelerate red cell destruction can derange the balance between red cell production and destruction, leading to acute exacerbations in anemia. An aplastic crisis, defined by severe anemia and inappropriately low reticulocyte count, may be due to infection or renal insufficiency. In children, infection is the most common cause of reticulocytopenic anemia. Classically, infection with parvovirus B19 causes reticulocytopenia, high fevers, and rash, but other infections may stimulate antibodies that impair reticulocyte production [52]. In adults, decreased reticulocyte counts may signal declining erythropoietin production in the setting of kidney disease [53, 54]. HU suppresses reticulocyte count, and treatment is held if reticulocytes are less than 80,000 *and* the total hgb is less than 9 g/dL. Reticulocytopenia in a recently transfused patient may indicate a delayed hemolytic transfusion reaction with alloantibodies causing bystander hemolysis of red cell progenitors [55–57].

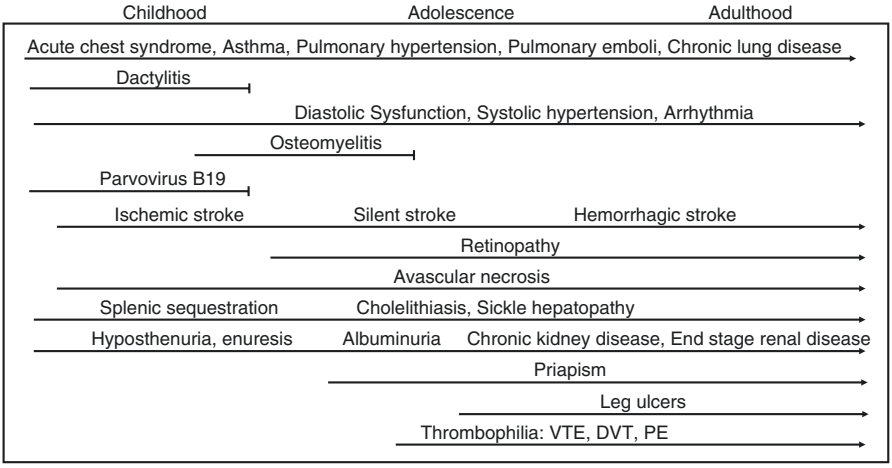

Fig. 1.4 SCD complications across the lifespan. In childhood, SCD is characterized by acute illness episodes which sometimes reveal susceptibility to overt organ injury but from which children usually recover. Complications such as dactylitis, aplastic anemia due to parvovirus B19, and acute chest syndrome secondary to pulmonary infections occur commonly. In adulthood, overt and insidious damage to endothelium causes chronic and multisystem damage. Chronic vascular damage and end-organ injury begin to manifest in adolescence when priapism, thromboembolic complications, and bone and chronic pain emerge as major challenges

Preserved reticulocytosis with anemia: When SCD patients present with worsened anemia, but intact or increased reticulocyte count, then hyperhemolysis—an above average, pathologic destruction of red cells—or sequestration of red cells in the liver, spleen, or both must be considered. Anemia with a preserved elevation in reticulocyte count may be seen in patients with acute chest syndrome or vaso-occlusive crisis. In auto- and alloimmune-mediated hemolysis, reticulocytosis or reticulocytopenia occur.

The clinical approach to severe anemia includes evaluation of the patient. Careful consideration of laboratory testing such as blood counts, reticulocytes, direct and indirect bilirubin, and antibody testing is important. Gastrointestinal and infectious symptoms, exam findings such as scleral icterus, hepatosplenomegaly, right upper quadrant tenderness, and transfusion history may guide evaluation and treatment. Red cell transfusion is not indicated for all anemia exacerbations in SCD patients [58]. The indications for red cell transfusion are reviewed in Chap. 5.

Painful Crises

Painful crises are the sine qua non of SCD, but sickle cell pain syndromes are diverse, and pain symptoms defy precise pathophysiologic explanations. A wide spectrum of pain phenotypes exists among SCD patients and which patients will be

severely affected is difficult to predict. Acute painful crises may occur with or without other disease complications.

Acute pain: Acute painful crisis is the leading cause of hospitalization for patients with SCD. These episodes are often called vaso-occlusive crises, referring to the presumed underlying pathophysiology, in which sickled red cells obstruct the microvasculature leading to local ischemia, inflammation, and severe pain. Patients are often able to identify their typical pain pattern, whether diffuse or localized, and pain in the chest, back, abdomen and extremities is common. The majority of pain episodes are managed in home settings; only a small fraction of painful crises require urgent or emergent medical care [59]. Painful crisis is a diagnosis of exclusion, and the conscientious clinician will treat their patient's pain while considering alternate explanations for pain rather than making an apriori diagnosis of painful crisis.

Several management principles guide the evaluation and treatment of patients with SCD and pain [60]. First, believe the patient [59]. Second, administer pain medications (including opiates, when needed) and intravenous fluids promptly; adjuvant therapy with nonsteroidal anti-inflammatories (NSAIDs) may be used in patients with adequate renal function. Patient-controlled analgesia may be used once the patient is old enough to understand how to use the device; young children may benefit from a PCA when appropriate assistance from a bedside nurse is available. Third, alternate sickle and non-sickle complications may precipitate a given painful crisis or be misinterpreted as a painful crisis. Finally, anticipating common side effects of pain medications, such as respiratory depression, constipation and pruritus (from opioids), and renal insufficiency (from NSAIDs), is clinically invaluable. Incentive spirometry has been shown to decrease the risk of pulmonary complications during crises and should be used uniformly [61].

HU and chronic red cell transfusions reduce the frequency of acute painful crisis. Non-opiate agents to treat acute and chronic pain are under investigation [62–65], however opiates remain the best treatment for acute pain and do not result in rates of opiate misuse greater than the general population [66].

Chronic pain: Unfortunately, many patients with SCD develop chronic pain that is challenging to treat and does not adhere to the pattern of a typical acute painful crisis pattern. Recent guidelines aid in diagnosis for these difficult symptoms [67]. Persistent pain may occur with avascular necrosis and bony infarcts; however, for many people with SCD and chronic pain, a precise pathophysiologic explanation is never identified. Chronic pain is often multifactorial and is influenced by altered pain sensitivity [68, 69], social circumstances [70], and comorbid mental health disorders, especially depression [71]. Some patients with SCD exhibit central sensitization, defined as nociceptive hyperexcitability on quantitative sensory testing. These patients have more pain and a greater likelihood of catastrophizing, low mood, and poor sleep [72]. Interdisciplinary teams of hematologists, psychiatrists, psychologists, social workers, and physical therapists are required to treat these patients.

Infections

Preventing and treating bacterial infections in patients with SCD is intimately tied to improved childhood survival [32]. Immune compromise in SCD is largely attributed to perturbed splenic function. The spleen's filtration system becomes clogged and fibrotic because of microvascular and ischemic damage in SCD, compromising the organ's essential immune function [73]. Loss of splenic function is an early complication of SCD and occurs in most children (>85%) with SCA before 12 months of age [74]. Compound heterozygotes may have preserved splenic function until adolescence [75], and hydroxyurea and chronic red cell transfusion are associated with splenic regeneration [76, 77]. Because of inadequate splenic function, patients are at dangerously increased risk for infection with encapsulated organisms [52] and consequently, fever management is conservative.

Fever and Bacteremia

The increased risk of bacteremia and sepsis means fever is always considered a life-threatening emergency in patients with SCD and requires urgent evaluation, blood cultures, and administration of a third-generation cephalosporin. Per consensus guidelines, fever is defined as a temperature greater than 101.5 °F (38.5 °C) [60]. These guidelines are not a substitute for consideration of patient condition. The duration of empiric antimicrobial therapy for patients with fever who are otherwise well is 24–48 h [78].

Infection Prevention

Penicillin prophylaxis: In the year the Mets last won the World Series (1986), analysis of the Penicillin Prophylaxis in Sickle Cell Disease trial (PROPS I) showed that twice daily pencillin led to an 84% reduction in *S. pneumoniae* infection in children under five years of age [79]. This provided the rationale for widespread implementation of newborn screening programs [80]. Follow-up studies showed penicillin prophylaxis did not significantly change sepsis rates in older children [81], and penicillin prescribing practices in this population vary [82]. All SCA patients are managed with similar infection prophylaxis and fever management [60]. Patients with HbSC and HbSβ^0 require similar fever management, but penicillin prophylaxis is not uniformly prescribed. The 2014 National Heart, Lung, and Blood Institute (NHLBI) Sickle Cell Disease Guidelines state that these compound heterozygotes may be managed without penicillin unless splenectomized (weak recommendation, low-quality evidence) [60].

Vaccinations: In high-income countries, children and adults with SCD benefit from access to standard childhood vaccinations to *H. influenzae, S. pneumoniae*, and *N. meningitidis*. The 23-valent pneumococcal vaccination boosts the routine

13-valent series and further contributes to decreased infection rates in children [83]. In low-income countries, vaccination availability and rates vary [11, 45]. Vaccination for people with SCD is covered in detail in Chap. 3.

Specific Infections

Malaria: SCT is protective against malaria, explaining the high prevalence of sickle trait in malaria-endemic regions of the world. HbSS is associated with increased vulnerability to severe malaria, and death is associated with secondary bacteremia in this setting [84]. These complications are caused in part by impaired splenic function; the spleen facilitates immunity to subsequent malaria infections. In this setting, malaria prophylaxis for children with SCD is attractive. Chemoprophylaxis reduces the need for blood transfusion in infected children and may reduce anemia, parasitemia, sickle-related events, and hospitalizations [52]. HU may prevent malaria-related morbidity, perhaps by improving splenic function in children; studies are ongoing [85].

Parvovirus B19: Prior to the identification of parvovirus, the characteristic severe anemia characterized by hemoglobin below baseline and reticulocytopenia was called an "aplastic crisis." Now children with these findings are tested for parvovirus, and pregnant caregivers (medical team and family members) are advised to avoid caring for children with suspected or confirmed infection because the red cell aplasia can cause hydrops fetalis. Parvovirus infection is usually acquired in childhood; thus, the transient red cell aplasia caused by this infection is typically diagnosed in children with SCD and is rare in adults [86]. Supportive care and, often, red cell transfusion are required.

Osteomyelitis (often salmonella): The combined effects of bone infarct, functional asplenia, and perhaps microvascular occlusion of the intestines, with subsequent bacterial translocation, are thought to predispose people with SCD to osteomyelitis with *Salmonella* and other gram-negative organisms. In children with *Salmonella* bacteremia, the incidence of osteomyelitis is as high as 77% [87]. Gram-positive *Staphylococcus* species may also be a cause. Although heightened suspicion of osteomyelitis is warranted in children with SCD, no specific evaluations or therapies differentiate treatment of children with SCD and osteomyelitis from those without SCD.

Central Nervous System

The central nervous system (CNS) is often affected by SCD (see Chap. 6). CNS injuries in childhood include silent cerebral infarct, overt stroke (usually ischemic), moyamoya disease, and an increased risk of aneurysms [88, 89]. While bony windows remain open, TCD screening identifies children at risk for stroke and facilitates intervention with red cell transfusion [90]. In adulthood, long-standing

cerebrovascular injury may evolve into hemorrhagic stroke. Headaches are common. Neurocognitive function is negatively affected by the insidious effects of chronic anemia, overt vascular remodeling, or perfusion injuries. In adults, cognitive ability is negatively associated with age and degree of anemia [91].

Cardiovascular

Cardiovascular complications are highly prevalent in the growing adult SCA population; these complications are not yet uniformly assessed or managed but likely play a significant role in morbidity and mortality in the adult SCD population. Heart disease in HbSC is less well studied. Adults with SCD, especially those with compound heterozygous disease, experience complications seen in the general population, such as hypertension and diabetes. The specific characteristics and prognosis of these overlap cardiac disease syndromes in SCD are not yet known.

Blood pressure: Systemic blood pressure in patients with SCA is lower than healthy African American controls or patients with HbSC or HbSβ^+ [92], a finding that eschews straightforward physiologic explanation. In the general population, systemic vascular pressures fall in anemic states and improve with correction of the anemia. In patients with SCA, normalization of hemoglobin does not fully correct blood pressure. This suggests that some combination of hemolysis, inflammation, and altered endothelial signaling likely contributes to low blood pressure in this population [93].

Low blood pressure predominates in people with SCD, but hypertension does occur. There is no consensus on the definition and management of high blood pressure in people with SCD, but hypertension is associated with silent and overt stroke, hypoxemia, and diastolic dysfunction [94–97]. Patients with high blood pressure and SCD should be evaluated for kidney disease, which is especially common in patients with SCA. In patients with diabetes and albuminuria, target blood pressure is <130/80 [98]. Absent definitive guidelines, this may be a reasonable goal in SCD and albuminuria. Diuretics and angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARB) should be used when tolerated [99]. Causes of hypertension unrelated to SCD should also be considered.

Intrinsic cardiac disease: As patients with SCD age, heart disease is emerging as a major comorbidity. In a cause-of-death analysis of autopsies and death certificates in people with SCD, "sudden death" was reported in a quarter or more of adults [100]. Structural remodeling because of chronically elevated cardiac output and microvascular insults rather than classic coronary arterial disease and atherosclerosis seems to drive this injury. Stable SCA patients at clinical baseline have increased cardiac output (of 10.9 L/min, standard deviation 0.6, range 7.6–13.7), attributed to increased stroke volume and heart rate, and decreased vascular resistance [101, 102]. Despite preserved systolic function, as SCD patients age, they develop cardiac chamber dilatation and diastolic dysfunction [103, 104]. Recent sensitive MRI examinations suggest an association between diffuse myocardial fibrosis and

diastolic dysfunction in SCA, as predicted by murine models [105, 106]. Notably, these models implicate the effects of anemia, local micro-occlusion, ischemia, and tissue damage in SCD cardiac disease. A SCA mouse model developed significant chaotic cardiomyocyte remodeling and experienced high rates of sudden death (40%) [106, 107]. Transfusional iron overload can impair cardiac function in chronically transfused adults with SCA, but is less common in patients being transfused to manage thalassemia [108–111].

Abnormal functional tests: An elevated tricuspid jet velocity (TRV, ≥2.5 m/s) is present in up to one-third of adult patients with SCD, examined at clinical baseline. Elevated TRV is associated with an increased risk of long-term mortality, especially when TRV is ≥3.0 m/s, a finding that is more prevalent in adults. Elevated TRV is seen more in SCA than in compound heterozygous SCD [112–115]. A unifying underlying pathophysiology or single optimal management strategy for this finding is lacking. Approximately 5–10% of all SCD patients who undergo cardiac catheterization have pulmonary arterial or venous hypertension as an explanation for the elevated TRV [116–118]. The remainder may have hemodynamic or cardiac abnormalities that are reflected in a high TRV, and are associated with high-risk SCD, despite not being attributable to frank pulmonary vascular hypertension. Recent studies have implicated an SCD-associated cardiomyopathy in these clinical findings [105, 106]. Echocardiogram is indicated for symptomatic patients (practically speaking, after a careful history, this captures many adult patients with SCA) and, if an elevated TRV is found (≥ 3.0 m/s) is found, may be referred for cardiac catheterization [102].

Pulmonary

Pulmonary complications of SCD are common and perilous due to the role of hemoglobin desaturation in the underlying pathophysiology of sickle polymer formation. Hypoxic conditions exacerbate red cell sickling and should be diagnosed and managed aggressively (Fig. 1.5). Anemia alone seldom explains hemoglobin desaturation. Perfusion and oxygen diffusion may be compromised by chronic parenchymal lung disease, cardiopulmonary shunts, pulmonary edema, or lung infection. Ventilatory defects may be caused by central nervous system injury, opiates, pain, and/or sleep apnea. Clinical guidelines suggest maintaining oxygen saturation greater than 95% [60]. Attribution of hemoglobin desaturation to anemia should be a diagnosis of exclusion once other causes of low oxygen saturation have been explored and excluded.

Acute chest syndrome (ACS): ACS is a common pulmonary complication of SCD. Intrapulmonary sickling and fat emboli contribute to an infiltrate on chest X-ray that is indistinguishable from pneumonia. Infectious causes include viruses and typical or atypical bacteria [119]. Patients hospitalized for vaso-occlusive crisis are predisposed to develop ACS because of respiratory compromise from pain, atelectasis, and opiate therapy [120]. Many patients are admitted for an uncomplicated

- **Documentation of prior baseline saturations**
- **Known co-morbidities**
  - Asthma
  - Chronic obstructive pulmonary disease
  - Pulmonary emboli
  - Congenital cardiac anomalies (ASD, VSD and etc)
- **Current opiate medications?**
  - Respiratory rate
- **Current hemoglobin?**
- **Evaluations**
  - Complete blood count
  - Chest X-ray
  - Chest CT
  - V-Q scan
  - Venous/Arterial blood gas
  - Echocardiogram
  - Hemoglobin S fraction
- **Interventions**
  - Supplemental oxgen to maintain saturation > 95%
  - Treat underlying cause
    - Antibiotics
    - Anticoagulation
    - Bronchodilators, inhaled corticosteroids
    - Blood transfusion (severe anemia)
  - Supportive care
    - Incentive spirometry
    - Strict Ins and Outs (to avoid fluid overload)

Fig. 1.5 Causes of low oxygen saturation in patients with SCD. Low oxygen saturation in a patient with sickle cell disease is a diagnosis of exclusion. Avoid a priori assumptions that arteriovenous shunts or altered carrying capacity may contribute to this finding. Anemia does not usually cause low oxygen saturation until very severe (Hgb 2–5 g/dL). Alternative explanations for low oxygen saturation must be pursued

crisis and develop ACS 2–3 days into their hospitalization. Incentive spirometry can prevent ACS in patients hospitalized with pain [61]. Where formal incentive spirometry devices are unavailable or developmentally inappropriate, improvisation with balloons, harmonicas, bubbles, or pinwheels may be used.

ACS is defined as a new infiltrate on chest X-ray, often with hypoxemia and, more often in children, fever. Usually, infiltrates are in the middle or upper lobes in children and multi-lobar in adults. These findings may result from infection and/or from bone marrow fat emboli, intrapulmonary sickling, or atelectasis. Tachypnea, tachycardia, low oxygen saturation, chest pain, and acute drops in hemoglobin are common [120]. Hospitalization for patients with ACS is mandatory, and empiric treatment with an intravenous third-generation cephalosporin and a macrolide or quinolone to cover atypical infectious organisms is standard. If present, reactive airway disease should be treated. Close monitoring of intravenous fluid administration to avoid volume overload is critical.

Although red cell transfusion during crises may exacerbate alloimmunization [121], blood transfusions are often used to stabilize patients with ACS. Patients can rapidly deteriorate. Exchange transfusions, targeting HbS under 30%, are indicated in patients with serious symptoms or whose total hemoglobin is near 10 g/dL; the 2014 NHLBI Sickle Cell Disease Guidelines include criteria for exchange transfusion that include progressive hypoxia even with supplemental oxygen,

progressive pulmonary infiltrates, and/or declining hemoglobin even with simple transfusion [60]. Comorbid CNS complications occur more commonly in adults, and mortality can be high [120]. At a 9-year follow-up from the Multicenter Study of Hydroxyurea, ACS was associated with a 32% risk of death compared to 18% in patients without ACS ($p = 0.02$) [122]. Hydroxyurea decreases the risk of ACS in children and adults [122, 123].

Close surveillance of patients with severe VOC or ACS is necessary because they can develop MSOF syndrome. MSOF is a catastrophic outcome in which the lung, kidneys, liver, and brain worsen acutely. This is often linked to a sudden drop in both hemoglobin and platelets and a concomitant rise in LDH [124]. MSOF can affect both SCA and compound heterozygous patients and is conventionally treated with emergent exchange transfusion.

Chronic lung disease and asthma: Asthma is not more prevalent in children with SCD than in African American without SCD, but may be more deadly [125–127]. In adults with SCD, wheezing alone associates with morbidity (pain, risk of ACS, decreased lung function) and higher mortality. Screening pulmonary function tests are currently not recommended in SCD, but aggressive management of wheezing or asthma, and referral and testing in these symptomatic patients is appropriate [128].

Obstructive sleep apnea (OSA) and nocturnal hemoglobin desaturation: OSA is common in children and adults with SCD (40–50%), most of which is mild [129]. Small studies suggest that the clinical consequence of OSA in SCD may be significant. Nocturnal hemoglobin desaturation, often independent of OSA, is reported in up to 20% of adults with SCA and may contribute to nighttime symptoms [130]. Nocturnal hemoglobin desaturation is associated with impaired executive functioning on neurocognitive tests in children with SCD [131]. Sleep-disordered breathing and nocturnal hemoglobin desaturation are probably clinically very important in SCD, but their cause, consequence, and management remain somewhat obscure.

Renal

The kideney's acidemic and hypoxic environment makes it uniquely susceptible to injury in SCD because deoxygenated hemoglobin forms sickle polymers, and (through the Bohr effect) acidemia accelerates hemoglobin deoxygenation. Acute and chronic kidney injury are leading causes of morbidity and mortality in adults with SCD, and some form of kidney injury is seen in almost every person with SCD [132–134]. Renal damage occurs early in the life of these patients, and impairments in urine concentrating ability (and resultant enuresis), glomerular integrity, and hormone/cytokine regulation (erythropoietin, vitamin D) have all been described.

Remarkable pathologic studies performed in the 1970s showed progressively more blighted medullary vasa recta in HbAS and HbSS disease, compared with a normal hemoglobin controls [135]. Clinically, this is reflected in the nearly universal finding of a urinary concentrating defect that first manifests in childhood and which

may be ameliorated by hydroxyurea treatment in childhood [136]. Renal medullary damage is also evident in impairments of acid-base homeostasis in adults with HbSS (but not with HbSC). Serum bicarbonate is, on average, 1 mEq lower in patients with SCA than is seen in patients with HbSC or in the general population [137]. Both impaired urine concentrating ability and abnormal acidemia may exacerbate SCD, through dehydration and enhanced sickle hemoglobin formation, respectively.

Albuminuria, an indicator of glomerular damage, is common in SCD. Overall, albuminuria associates with other evidence of maladaptive endothelial changes. Albuminuria is pathophysiologically linked to intravascular hemolysis [138]. When biopsied, focal segmental glomerular sclerosis is commonly found, with a plausible corollary genetic link between renal disease and APOL1 mutations, as seen in African Americans without SCD [26]. Renal disease is associated with increased mortality in SCD [33]. Studies to mitigate the effects of hypertension and albuminuria on kidney disease progression in this population using ACE inhibitors or ARBs are ongoing [99]. Importantly, non-SCD-related glomerular injury should also be considered, especially in compound heterozygotes in which sickle cell-induced glomerular injury appears to be less common.

The endocrine role of the kidney, through production of erythropoietin and activation of vitamin D, is impaired with age and may further compromise sufficient red cell production and bone health, respectively. In some cases, renal insufficiency compromises treatment with HU, because of poor HU tolerance in patients with CKD and EPO-deficient anemia [54]. However erythropoietin replacement therapy must be initiated carefully in patients with SCD. Erythropoietin is usually given in tandem with hydroxyurea because when given alone erythropoietin can cause disease complications [139].

The Hepatobiliary and Gastrointestinal Systems

Abdominal complaints in SCD are common and present clinical challenges regarding diagnosis and optimal timing of surgical interventions. Hyperhemolytic and cholestatic manifestations are associated with increased morbidity in children and mortality in adults.

Gallbladder: The increased hemolytic rate of SCD increases bilirubin sludge and stones in the gallbladder, even in young patients. Cholelithiasis is detectable in 12% of young children with SCD, increases with age, and afflicts more than half of adult patients with SCD [140]. Cholelithiasis is more common in SCA [141] and may progress to symptomatic choledocholithiasis and cholecystitis [142]. However, acute cholecystitis develops in <10% of patients with SCD, despite the high rate of gallstones, and the standard of care has evolved from preventive to therapeutic cholecystectomy. When feasible, cholecystectomy can safely be performed laparoscopically in this population [60].

Liver: The liver is injured by chronic sickling and may be further damaged by SCD treatments and their sequelae (especially transfusion-related hemochromatosis).

Hepatic crises are challenging to manage and are associated with increased morbidity and mortality documented in adults [143, 144].

Sickle hepatopathy describes a spectrum of liver dysfunction associated with obstructive jaundice and abnormal liver function tests. Elevated aspartate aminotransferase (AST) is increased in hemolytic states, so alanine aminotransferase (ALT) is a more sensitive marker of hepatocellular injury in SCD. Sickle hepatopathy may be indolent, manifesting with severe hyperbilirubinemia with or without elevated ALT and AST [145] in an otherwise asymptomatic patient, while abrupt and potentially life-threatening manifestations include acute hepatic crisis, hepatic sequestration, or sickle cell intrahepatic cholestasis (SCIC) [146]. Acute hepatic crisis, hepatic sequestration, and intrahepatic cholestasis are overlapping syndromes, and an uptrending conjugated bilirubin should alert the clinician to these entities [147]. Interventions range from observation with intermittent laboratory monitoring to emergent exchange transfusion:

- **Acute hepatic crisis** is thought to be caused by hypoxic hepatocellular injury and is a self-limiting condition characterized by nausea, right upper quadrant pain, fevers, and jaundice. Total bilirubin is typically less than 15 mg/dL, and ALT is typically 300 mg/dL and rarely as high as 1000 mg/dL [146].
- **Hepatic sequestration** is caused by sinusoidal congestion and presents similarly to splenic sequestration: both the hemoglobin and platelet counts are reduced. Hepatic sequestration may occur in isolation, with splenic involvement, or in the setting of acute illness [148]. Small aliquots of red cells may be transfused to manage anemia, as in splenic sequestration. Resolution is suggested by improved thrombocytopenia and downtrending liver enzymes and bilirubin.
- **Intrahepatic cholestasis (SCIC)** is an uncommon but life-threatening syndrome, characterized by hepatomegaly, extreme conjugated hyperbilirubinemia with levels as high as 80 mg/dL, significantly elevated transaminases (>1000 mg/dL), coagulopathy, renal insufficiency, and acute liver failure in severe cases. SCIC is the most severe form of acute sickle hepatopathy, with an overall mortality rate up to 50% in adults and 30% in children due to uncontrolled bleeding and fulminant liver failure [146]. Treatment with exchange transfusions are supported by small retrospective reports [143, 149, 150]. Correction of coagulopathies with plasma, cryoprecipitate, and platelet transfusions is also often necessary.

Involving specialists from hematology, gastroenterology, and transfusion medicine is minimally required for severely ill patients with evidence of sickle hepatopathy. Patients with chronic liver disease have pathologic findings on liver biopsy [151], but there is no explicit indication for liver biopsy in patients with SCD [150]. Liver biopsy may be contraindicated in patients with SCD and an elevated prothrombin time and thrombocytopenia [152]. Non-sickle liver disease can mimic primary sickle hepatopathy. Clinicians should maintain a low threshold to evaluate patients for viral and autoimmune causes of hepatopathy. Liver injury, especially from iron overload, is a risk factor for worse HSCT outcomes, so a commitment by providers and patients to reduce liver iron concentration prior to transplant is essential [153].

Spleen: The spleen is uniquely susceptible to damage in congenital hemolytic anemias because of its valiant and futile efforts at "quality control" of an overwhelming number of malformed red cells. In SCD, microvascular damage exacerbates splenic dysfunction [154]. Progressive atrophy is characteristic of splenic disease in SCA. Hyposplenism is evident in early childhood [73] but may be mitigated by early initiation of transfusions or HU [155, 156].

The infectious risk conferred by loss of splenic function is considerable, greater in SCA than in compound heterozygous SCD, and is reviewed above (see Sect. *Stigma and the Patient Experience*). Splenic sequestration is characterized by abrupt painful splenic enlargement, often associated with an acute drop in hemoglobin (often 2 g/dL or more) and thrombocytopenia. Sequestration occurs in young children with SCA before the spleen fully atrophies and in adults with compound heterozygous SCD. This can be a life-threatening emergency and requires careful transfusion management to avoid a sudden rise in hematocrit as the spleen releases sequestered blood. Surgical therapy, if sequestration is severe or recurrent, is by convention delayed until the acute episode resolves and after vaccinations for encapsulated organisms. In young children with multiple hospitalizations for splenic sequestration, every effort is made to delay splenectomy until 2 years of age. Chronic hypersplenism (modest depressions in platelet counts and/or white blood cell counts) occurs in patients with compound heterozygous SCD and in some patients with SCA who are being treated with HU or chronic transfusion therapy [157, 158].

Gastrointestinal complications: Abdominal pain is common in patients with SCD. Gastroparesis may be secondary to vaso-occlusion or opiates. Constipation can be a serious problem and needs to be prevented whenever possible and treated when identified. Occasionally acute abdominal complaints requiring surgical intervention are overlooked because of overlapping symptomatology with SCD complications, in particular painful crises [159–162].

Bone Involvement

The bones of patients with SCD experience myriad insults pathophysiologically related to bone ischemia and marrow hyperplasia [163]. Patients typically present with pain and identifying a precise etiology can be difficult. Imaging does not always distinguish between infarct and infection, and inflammatory markers can be unreliable [164]. There is increasing recognition that patients with SCD are at risk for early-onset osteoporosis, osteopenia, and vitamin D deficiency [165].

Dactylitis: This classic pediatric complication of SCD is a unique vaso-occlusive event characterized by painful swelling of the fingers and toes and was considered a harbinger of worse disease [166], but in the modern era of newborn screening, penicillin prophylaxis, and annual TCD screening, dactylitis does not predict disease severity [167]. Infants and toddlers present with obviously swollen digits that

improve over days to weeks. Treatment is with hydration (IV or PO) nonsteroidal anti-inflammatories and sometimes opiates.

Avascular necrosis (AVN): Osteonecrosis occurs in joints—classically the hips and shoulders, but the jaw, spine, and ankles may also be affected. Inheritance of α-thalassemia trait is a risk factor for AVN in patients with HbSS but may be protective in HbSC [10]. AVN is a common complication of HbSC disease and is often bilateral. AVN presents with focal pain and should be suspected in patients with a specific site of recurrent or persistent pain. X-ray and MRI findings may lag behind clinical symptoms. Physical therapy, pain control, and finally surgical intervention may be required. The emphasis in caring for these patients is on functional outcomes. Early surgical intervention (core decompression) is not superior to physical therapy [168].

Osteomyelitis: see Sect. *Infections*.

Ocular Manifestations

Acute and chronic ocular manifestations of SCD are associated with vasculopathy and thromboembolism. Because eye involvement typically manifests after the first decade of life, annual ophthalmologic evaluation is indicated in all patients starting at age 10 years [60, 169].

Retinitis proliferans: The proliferative retinopathy of SCD is characterized by vascular overgrowth of the retinal vessels (in evocative "black sea fan," "sunburst," and "salmon patch" patterns [170]), leading to vitreous hemorrhage loss, retinal detachment, and blindness. Retinitis proliferans occurs in 30–70% of patients with HbSC disease compared to 3% of those with SCA [171]. Co-inheritance of α-thalassemia trait is associated with decreased rates of retinopathy in HbSC [10]. One explanation for this difference in disease manifestation is that the hyperviscosity of HbSC allows adequate oxygen delivery for neovascularization, whereas obstructed retinal vasculature causes overt retinal ischemia in SCA [9]. Ophthalmologists treat retinitis proliferans using transpupillary laser photocoagulation or cryotherapy [172].

Nonproliferative eye injury: Infarctive injury to the eye damages the conjunctiva, anterior segment, choroid, retina, or optic nerve and can present with acute vision loss that may or may not be painful. Attempts to restore and preserve vision are performed with urgent exchange transfusion [173].

Leg Ulcers

Leg ulcers are a chronic and debilitating complication that occurs almost exclusively in adults with SCA and susceptibility varies with genotype and geography. The pathophysiology of leg ulcer development associates with male gender and age,

while HbF, α-thalassemia, and total Hgb levels are protective [174–176]. Non-globin gene associations such as TGF-β and Klotho have been described [177]. Geographic differences are seen: in Jamaica where up to 70% of people with SCD have leg ulcers, compared with 5–10% of patients reported in the USA. Infections and local trauma are anecdotally associated. In West Africa, the incidence of leg ulcers is low, but this may be attributable to regional increases in childhood mortality for SCD.

Leg ulcers occur most commonly over the lateral malleoli and are vexing to treat. Once present, leg ulcers heal slowly and recur often, and no specific regimen promotes rapid resolution. Mainstays of treatment are analgesia, topical hydrocolloid dressings to protect newly forming epithelium, hygienic wound care to prevent infections, and pressure stockings to improve venous stasis. Organisms most commonly identified in infected leg ulcers are *S. aureus*, *Pseudomonas*, *Streptococci*, and *Bacteroides* [52], but colonization rather than infection is more typical clinically. Whether topical or systemic antibiotics are useful is unknown. Chronic transfusions have not been conclusively shown to improve healing, but chronic transfusions may promote healing among patients undergoing surgical debridement, skin grafts, or muscle flaps [178]. Patients with ulcers are at risk for school or work failure and depression. Addressing the psychosocial aspects of ulceration is critical and may facilitate improved wound care and healing.

Thrombosis

Venous thromboembolic (VTE) complications of SCD are common in adults with SCD and associated with worse outcomes. Deep vein thrombosis (DVT) and pulmonary embolus (PE) occur in 10–15% of affected patients by 40 years of age [179]. VTE events are associated with increased risk of death [180]. Among patients ≥15 years of age, the cumulative incidence of VTE was 11.3% [180]. Patients with HbSC have a higher incidence of VTE (7.6 events/1,000 person years, 95% CI 5.3 – 10.6), and patients with other sickle cell variants are also at increased risk. In a single-center study of patients with HbSC and HbSβ$^+$, older age and higher hemoglobin were significantly associated with VTE events, and PE was more common than DVT [179]. Increased mortality was also seen in patients with SCD and a history of VTE [181].

The unique coagulation profile of SCD patients needs consideration in assessing treatment response. SCD is a prothrombotic condition with endothelium and platelet activation, and derangements in many clotting factor levels occur [182]. Additional thrombophilic risk factors should also be investigated after a VTE event. Antiphospholipid antibodies may be more common in patients with SCD [183–185]. The manifestations of thromboses in patients with SCD are as in other populations: tenderness and swelling in lower extremities or chest pain with oxygen

desaturation. In patients with SCD, complaints of pain may be mistakenly attributed to painful crisis or ACS, and the possibility of VTE/PE should be considered, as isolated PE without antecedent DVT is seen in SCD. Normal D-dimer levels may be reassuring at follow-up, but screening D-dimer measurements are not useful in patients with SCD since these are commonly elevated at baseline and with crisis, even absent VTE [186].

There are important empiric implications of thrombosis risk in patients with SCD for care providers. First, hospitalized patients with SCD require appropriate DVT prophylaxis and recommendations for high-risk populations can be followed [60, 187]. Second, in patients with chest pain, a low threshold to evaluate for PE using electrocardiogram and spiral CT scan should be maintained. V/Q scans can be used in patients with renal insufficiency, but chronic lung disease in SCD complicates their interpretation.

Priapism

Priapism is a painful, unwanted erection lasting more than 4 hours. Priapism occurs in over one-third of males with SCD some time during their life and may be stuttering (lasting <4 h, but recurrent) or major (lasting >4 h). The latter may be preceded by the former and is associated with subsequent erectile dysfunction [188, 189]. The average age at first priapism attack is 15 years old but can occur in younger children. Men with mild SCA or with compound heterozygous SCD sometimes have priapism as a presenting symptom of disease, especially if they are born in countries without newborn screening. Acute management to induce detumescence includes hydration, analgesia, sympathomimetics (such as pseudoephedrine), and penile aspiration. Red cell transfusions during an episode are not recommended due to case reports of significant neurologic sequelae in this setting [60]. However, chronic red cell exchange transfusions, to maintain HbS level <30%, may reduce the frequency of priapism events [189, 190].

Treatment Toxicity

Treatment toxicities are increasingly managed as SCD complications. Acute HU toxicity is uncommon, even in accidental overdose [191]. Chronic use may cause cytopenias, nail bed discoloration, nausea commonly relieved by taking doses prior to bed, weight gain, and mild hair loss. Chronic transfusions may lead to systemic iron overload [108, 192] and alloimmunization [121, 193–195]. The infectious and thrombotic complications of central lines placed for transfusion therapy are a common clinical concern.

Other Major Life Events Complicated by SCD

Growth and development, reproductive health and hospital interactions are complicated by SCD. Here we briefly list these and provide references to direct the interested reader:

- **Growth and development**:
  - **Growth**: Children with SCD in the CSSCD were modestly, but significantly, shorter and weighed less than age-matched African American controls; those with SCA were smaller than peers with HbSC or HbSβ$^+$ [196]. HU and chronic transfusions are associated with improved growth [197, 198], and obesity is an emerging challenge [199, 200]. Cardiovascular and cardiopulmonary risks related to obesity (hypertension, type 2 diabetes, obstructive sleep apnea) are likely to emerge as significant complicating comorbidities in SCD in the next decades.
  - **Sexual maturity**: Puberty in adolescents with SCD is delayed approximately two years [196], but normal sexual maturation is anticipated [201]. Over 50% of young women with SCD report cyclical non-menstrual cramp pain associated with their menses which may be improved with progesterone-only contraception ([202–204].
- **Reproductive health**:
  - **Contraception**: Progesterone-only contraception (delivered via pill, intramuscular injection, or intrauterine device forms) is indicated for women with SCD who desire birth control [179, 180]. The baseline risk of unplanned pregnancies is high [205], and hormonal contraception may relieve dysmenorrhea and cyclic sickle pain associated with menstruation.
  - **Fertility**: A quarter of affected men with SCA experience infertility attributed to baseline hypogonadism, erectile dysfunction secondary to priapism, and sperm abnormalities [206]. Azoospermia and infertility may be further exacerbated by HU therapy. Whether SCD affects female fertility is unknown [201].
  - **Pregnancy**: Women with SCD, especially those with SCA, experience increased pregnancy-associated mortality and morbidity, and infants are also at risk for complications and death [207–209]. Co-management of these patients with high-risk obstetricians is recommended.
  - **Genetic heritability of SCD**: SCD is an autosomal recessive condition. Genetic testing, preimplantation genetic diagnosis, and prenatal testing may help patients, partners and offspring [210–212].
- **Medical care**:
  - **Surgery**: Patients with SCD require specific pre- and postoperative care to reduce SCD-related morbidity [213, 214], usually from pulmonary complications. For patients with SCA undergoing non-minor surgical procedures,

preoperative care includes simple red cell transfusion with a goal hemoglobin of 10 g/dL [60].

– **Transition from pediatric to adult care**: Patients with SCD are at increased risk for morbidity and mortality during the transition from pediatric to adult medical care [39]. Understanding the extent to which morbidity and mortality at transition reflects disease progression, inadequate access to medical care, or the unique developmental vulnerabilities of young adults is an area of intense interest for patients, providers, and the health-care community [215–218].

Clinical Challenges

Structural and behavioral challenges significantly influence clinical care for patients with SCD and are important to the global optimization of care for people with SCD.

Newborn Screening: Identifying Patients for Early Clinical Care

In places where penicillin prophylaxis is available, newborn screening fundamentally changes the prognosis of SCD by identifying and treating children who are at risk from deadly infectious complications. In malaria-endemic regions of Africa, where SCD has the highest prevalence in the world, newborn screening efforts are emerging, increasing the number of infants in whom life-saving penicillin prophylaxis and vaccinations may be administered [219]. Rapid diagnostic tests to aid in this endeavor are also in development [220–222].

Risk Stratification

Approaches and challenges to risk-based therapy are the subject of Chap. 4. The heterogeneity of SCD even within a genotype and the lack of well-validated biomarkers or clinical scores complicate attempts to dictate early and aggressive curative or even disease-modifying interventions for many patients [223, 224].

Stigma and the Patient Experience

Complex societal pressures on people with SCD are poorly recognized and inadequately managed. People with SCD rely on fully equipped hospitals and sophisticated interdisciplinary care to maintain quality of life and to diminish morbidity and

mortality. Patients with SCD may be stigmatized [40, 41]. They are disproportionately people of color, often socioeconomically disadvantaged, and patients and parents perceive disease-specific differences in care [225–229]. SCD causes pain that is often treated with opiates, which poses complex challenges and can lead to additional discrimination. Further, health-care disparities extend to research. SCD receives less research funding than other, less common, conditions [230–232].

Knowledge Gaps

SCD is heralded as the first molecular disease, and studies in hemoglobinopathies served as a springboard to launch decades of biomedical research in genetics and red cell biology, yet precision-medicine-worthy recommendations for clinical care remain exceptional in the care of affected patients [232]. Providers are hobbled by data that combines or excludes certain genotypes [233, 234]. With few notable exceptions in Spain and Greece [235, 236], robust patient registries that accurately capture causes of morbidity and mortality are lacking. New efforts to establish criteria for excellence in the care for children with SCD in the USA [237] must be coupled with efforts to address the clinical needs of a growing adult SCD population, here and abroad [208, 223, 238, 239].

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Chapter 2
Pathobiology of Sickle Cell Disease Vaso-occlusion and Targeted Therapies

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Abbreviations

HbS	Sickle hemoglobin
RBC	Red blood cell
SCD	Sickle cell disease
VOC	Vaso-occlusive crisis

Sickle cell disease (SCD) originates from a single base pair change in the β-globin subunit, yet the complex manifestations that result are manifold. The abnormal HbS is insoluble when deoxygenated, leading to polymer formation. These cells are less deformable and are prone to hemolysis. Wide-field digital interferometry demonstrates that sickle red blood cells (RBCs) are stiffer than those with normal adult hemoglobin [1]. Adhesion of low-density sickle RBCs and reticulocytes in postcapillary venules leads to trapping of the older, more dense, and misshapen SS-RBCs and results in reduced blood flow, hence contributing to vaso-occlusive crisis (VOC) [2]. The sickle RBC is only one reason for the systemic multi-organ damage in this disease. Many cells that are not affected by the β-globin mutation play a role in this lifelong debilitating illness. The interactions between the sickle RBCs, endothelium, leukocytes, platelets, cytokines, and inflammatory mediators are all responsible for a chronic inflammatory state and cumulative organ injury [3, 4]. Sickle RBCs easily dehydrate, leading to HbS polymerization and subsequently to altered shape and

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surface cell properties. This leads to hemolysis and triggers activation of coagulation factors, platelets, white blood cells, endothelium, and intracellular signaling pathways [5]. This chapter will highlight the underlying pathophysiology of the ischemia reperfusion injuries, the abnormal interactions between the red cell and its surrounding environment (particularly the endothelium, neutrophils, and platelets), the prothrombotic milieu, and the novel therapies that are being investigated to treat this disease.

Adhesion Pathways

Adhesive Interactions of Red Cells and Leukocytes with the Endothelium

The mutated HbS causes deformation of RBC membranes by polymer formation, RBC membrane damage via iron-mediated generation of oxidants [6], and altered lipid properties [7]. The endothelium in SCD has an activated phenotype, demonstrated by the upregulation of adhesion molecules such as vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), and the selectin family [8, 9]. Selectins regulate leukocyte adhesion to the endothelium, and their expression is enhanced by inflammatory cytokines such as tumor necrosis factor (TNF-α) or interleukin-1 (IL-1) [10]. Two of the selectins (P-selectin and E-selectin) are expressed on the endothelium and can markedly slow down the rolling of the white blood cells with the additional interactions of cytokines and inflammatory markers [8, 11]. Activated $\alpha_M\beta_2$ (macrophage-1 antigen [MAC-1]) on adherent neutrophils captures sickle RBCs leading to decreased blood flow [12]. Sickle RBCs can also adhere via numerous adhesive partners to the endothelium directly (e.g., VCAM-1), with or without intervening bridging molecules (thrombospondin, von Willebrand factor [VWF]) or with subendothelial matrix proteins (laminin, VWF) [13, 14]. Activation of NF-κB upregulates expression of these adhesion molecules (E-selectin, VCAM-1, and ICAM-1) on the surface of the endothelium [2, 15]. Activated circulating endothelial cells and increased levels of plasma sVCAM-1, P-selectin, and E-selectin have all been implicated in participating in VOC [11, 16, 17]. Specific erythrocyte ligands also play a role in adhesion such as Lutheran blood group antigen [18], VLA-4 [19, 20], CD 36, and sulfated glycolipids [21]. Basal cell adhesion molecule/Lutheran blood group (BCAM/LU) and ICAM-4 can both be activated by epinephrine [2, 22]. Activated ICAM-4 by epinephrine leads to VOC and increased leukocyte adhesion to the endothelium via endothelial $\alpha\nu\beta3$ integrin [23, 24].

Neutrophil-RBC Interactions

Neutrophils participate in the pathogenesis of SCD, and *in vitro* studies demonstrate that sickle RBCs directly bind to neutrophils [25]. This is supported by *in vivo* studies in Berkeley SCD mice where the dynamics of circulating blood cells are

analyzed in the cremasteric microcirculation using intravital microscopy [11, 25]. Clinically, patients with more severe symptoms have higher neutrophil counts than racially matched controls [26, 27]. Patients that have required GCSF or GMCSF for treatment of other comorbidities such as neutropenia or stem cell harvest have had severe or fatal VOC [28–32].

Therapeutic Interventions Targeting Adhesion Molecules

Inhibition of these adhesion molecules, or their downstream targets, has been the focus for novel therapeutic targets. Administration of an anti-P-selectin aptamer in SCD mice resulted in a decreased adhesion of sickle RBCs by 80–90%, increased microvascular flow velocities, and reduced adhesion of the leukocyte to the endothelium [16]. In a recent publication in the *New England Journal of Medicine*, Ataga et al. describe the results of a double-blind, randomized, placebo-controlled, phase 2 trial of crizanlizumab for treating pain crises. Crizanlizumab is a humanized monoclonal antibody against the adhesion molecule P-selectin, and adult sickle cell patients were prophylactically administered with the medication over 52 weeks. In the high-dose crizanlizumab arm, there was a significantly lower rate of sickle cell-related pain crises per year than placebo (1.63 vs. 2.98) and a low incidence of adverse events [17]. Rivapansel (GMI1070) a pan-selectin inhibitor (particularly against E-selectin) has been shown in sickle cell mice to improve sickle RBC-leukocyte interactions leading to improved microcirculatory blood flow and reduced VOCs [33]. The phase 2 clinical studies have shown the drug to be a safe intervention with a markedly reduced use of opioids during hospitalization (83% reduction compared to placebo) and a trend toward a faster resolution of VOC (41 h versus 63 h) [34]. Currently a phase 3 study of rivapansel (NCT02187003) in adults is ongoing.

Two groups have demonstrated the efficacy of RNA aptamers to inhibit P-selectin-mediated RBC adhesion to endothelial cells [16, 35] in preclinical models, lending further support to this adhesive target, but currently there are no open clinical trials for these agents.

In a multicenter phase 3 study, poloxamer, a surfactant that inhibits cell adhesion, did not meet its primary efficacy endpoint of reduction in the mean duration of VOC (82 h in the vepoloxamer group compared to 78 h in the placebo group in the intent-to-treat population ($p = 0.09$). There were also no statistically significant differences between treatment groups in the intent-to-treat population across the two secondary efficacy endpoints, rate of rehospitalization for VOC, and the occurrence of acute chest syndrome [36].

Intravenous γ-globulin (IVIG) inhibits leukocyte activation and adhesion by decreasing leukocyte-erythrocyte interaction and improving microcirculation [37]. Fcγ receptors are on many different hematopoietic cells including neutrophils and macrophages and can be of the activating or inhibitory subtype. Engagement of FcγRIII receptors (activating receptor subtype) on neutrophils triggers phagocytosis, reactive oxygen production, and release of inflammatory cytokines [37]. Surprisingly, IVIG binds the FcγRIII receptor reducing Mac-1 activity and

mediates these interactions by recruitment of Src homology 2 (SH2)-containing tyrosine phosphatase-1 (SHP-1) that inhibits downstream Src kinase [37]. In SCD mice, IVIG reverses VOC by inhibiting neutrophil adhesion to the endothelium and modulating the interactions between leukocytes and circulating red blood cells [38, 39]. In a phase 1 trial in pediatric and adult SCD patients with acute VOC, IVIG also decreased human neutrophil Mac-1 function and was safe and well tolerated [40]. Currently a phase 2 trial of IVIG is recruiting patients (NCT01757418). While this section highlighted a selected few therapeutic interventions, Table 2.1 summarizes the studies discussed above as well as additional agents targeting cell adhesion.

Table 2.1 Novel agents in clinical trials targeting adhesion

Study title	Intervention	Clinical trials/phase	Status
Selectin inhibitors			
Study of GMI-1070 for the Treatment of Sickle Cell Pain Crisis	GMI-1070 (rivapansel) [34]	NCT01119833 Phase 2	Complete
Efficacy and Safety of Rivipansel (GMI-1070) in the Treatment of Vaso-Occlusive Crisis in Hospitalized Subjects with Sickle Cell Disease	GMI-1070 (rivapansel)	NCT02187003 Phase 3	Ongoing
Study to Assess Safety and Impact of SelG1 with or Without Hydroxyurea Therapy in Sickle Cell Disease Patients with Pain Crises	SelG1 [17]	NCT01895361 Phase 2	Complete
Sevuparin Infusion for the Management of Acute VOC in Subjects With SCD	Sevuparin	NCT02515838 Phase 2	Ongoing
Phase 1–2 Trial of Gamunex (Intravenous Gammaglobulin) for Sickle Cell Acute Pain	IVIG	NCT01757418 Phase 1 [40]/2	Ongoing
β-Blockers			
Study of Propranolol as Anti-adhesive Therapy in Sickle Cell Disease (SCD)	Propranolol [41]	NCT01077921 Phase 2	Complete
Propranolol and Red Cell Adhesion in Non-asthmatic Children with Sickle Cell Disease	Propranolol	NCT02012777 Phase 1	Ongoing
Other inhibitors of adhesion			
Phase III Randomized Study of Poloxamer 188 for Vaso-Occlusive Crisis of Sickle Cell Disease	Poloxamer [36]	NCT00004408 Phase 3	Complete
Evaluation of Purified Poloxamer 188 in Vaso-Occlusive Crisis of Sickle Cell Disease (EPIC)	Poloxamer	NCT01737814 Phase 3	Complete
A Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics Study of PF-04447943, Coadministered With and Without Hydroxyurea, In Subjects with Stable Sickle Cell Disease	PDE9 inhibitor 1	NCT02114203 Phase 1	Complete

Inflammatory Pathways

Adenosine and Invariant Natural Killer T (iNKT) Cells

Invariant natural killer T (iNKT) cells are increased in number and activity in SCD [42–44] and promote the inflammatory cascade. Adenosine A2A receptors (A2AR) are expressed on iNKT cells, and activation of these receptors downregulates the activity of iNKT cells [43, 45, 46]. Regadenoson is a selective A2AR agonist that is approved for radionuclide myocardial imaging. A side effect of this drug can be decreased blood pressure and reflexive tachycardia. Low-dose infused regadenoson was postulated to have the selective binding of A2AR receptor and to have less cardiac toxicity. Animal models showed that an A2AR agonist led to reversal of pulmonary dysfunction in mice [43], and a phase 1 study in adults with SCD demonstrated that low-dose regadenoson infusion decreased activation of iNKT cells during a VOC without significant toxicity [47]. A phase 2 trial during VOC in pediatric and adult patients is currently ongoing (NCT01788631).

Leukotrienes

Leukotrienes are proinflammatory lipid molecules produced by all leukocytes in response to various stimuli. Leukotrienes LTC_4, LTD_4, and LTE_4 are produced by mast cells and macrophages and as a group are classified as cysteinyl LTs (CysLTs). In the lung, CysLTs cause airway edema, smooth muscle proliferation, and fibrotic tissue formation [48]. In the endothelium they cause vasoconstriction, upregulation of adhesion molecules, and recruitment of inflammatory cells such as eosinophils, monocytes, and T cells [49–53]. Secretory phospholipase A2 (sPLA2) which releases arachidonic acid, the precursor of leukotrienes, is increased in individuals with acute chest syndrome [54]. LTE_4 is elevated in adults and children with SCD at baseline and increases during pain crisis [55–58]. Montelukast is a CysLT inhibitor and an FDA-approved drug for asthma. Currently an 8-week phase 2 study of the addition of montelukast is being conducted (NCT01960413) in individuals on hydroxyurea with outcomes looking at tissue injury, lung function, and microvascular blood flow. Another FDA-approved drug for asthma, zileuton, is being examined in a phase 1 trial (NCT01136941) in children and adults. Zileuton inhibits 5-lipoxygenase a key leukotriene synthetic enzyme. In a mouse model, zileuton attenuated the amount of activated neutrophils and decreased sickle RBC adherence in the lung [59]. Corticosteroids are potent antileukotrienes by inhibiting the release of arachidonic acid. The Inhaled Mometasone to Reduce Painful Episodes in Patients With Sickle Cell Disease (IMPROVE) trial (NCT02061202) is a phase 2 trial ongoing currently to investigate if individuals without asthma could have decreased VOCs with inhaled corticosteroids [60].

Oxidative Stress and Impaired Nitric Oxide Biology

Plasma hemoglobin released from hemolyzed sickle erythrocytes consumes nitric oxide (NO) [61] 1000-fold faster than intraerythrocytic hemoglobin [62, 63]. NO has multiple vascular effects including vasodilation, anti-adhesive, antithrombotic, and antioxidant [64]. Reduced endothelial NO bioavailability in SCD impairs downstream vascular functions of NO, like vasodilation. Decreased NO also results in increased expression of cell adhesion molecules, VCAM-1, ICAM-1, P-selectin, and E-selectin [64]. Decreased NO bioavailability occurs in SCD at baseline and is associated with VOCs and acute chest syndrome [65, 66]. Statins modulate NO production through upregulation of endothelial nitric oxide synthase and hence are protective against endothelial injury [67, 68]. Children with SCD were treated with simvastatin for 21 days and had decreased IL-6 levels and CRP with increased NO metabolites (NOx) [69].

L-arginine is an obligate substrate for NO and is relatively deficient in SCD due to high levels of plasma arginase released from hemolyzed erythrocytes. L-arginine supplementation improves erythrocyte integrity [70], and inhibition of arginase in sickle cell mice reverses endothelial dysfunction and vascular stiffness [71]. Exogenous supplementation of L-arginine (100 mg/kg three times a day) was administered during VOC for 5 days in a double-blind, randomized controlled trial in children. The treatment was well tolerated and had significant reduction in opioid use and lower pain scores [72].

Omega-3 fatty acids have been demonstrated in preclinical models to also mitigate vasculopathy. This is achieved by various mechanisms such as favorable changes in the red cell membrane lipid composition, modulation of inflammation and coagulation, and production of nitric oxide [73]. In two single-center studies, omega-3 fatty acids have shown to decrease the frequency and severity of VOC episodes in adults and children [74, 75]; multicenter studies are ongoing NCT02973360. Table 2.2 highlights other treatments that are being investigated in modulating oxidative stress and decreasing inflammatory markers to improve outcomes in SCD.

Table 2.2 Interventions targeting inflammation

Study title	Intervention	Clinical trials/phase	Status
Adenosine and invariant NKT cells			
Adenosine 2A Agonist Lexiscan in Children and Adults With Sickle Cell Disease	Regadenoson [76]	NCT01085201 Phase 1	Complete
A Phase II Trial of Regadenoson in Sickle Cell Anemia	Regadenoson	NCT01788631 Phase 2	Ongoing
Safety, Pharmacokinetic, and Pharmacodynamic Study of NKTT120 in Adult Patients with Stable Sickle Cell Disease (SCD)	NKTT120 [76]	NCT01783691 Phase 1	Complete

Table 2.2 (continued)

Study title	Intervention	Clinical trials/phase	Status
Leukotrienes			
Phase 2 Study of Montelukast for the Treatment of Sickle Cell Anemia	Montelukast	NCT01960413 Phase 2	Ongoing
Trial of Zileuton CR in Children and Adults with Sickle Cell Disease	Zileuton	NCT01136941 Phase 1	Complete
Inhaled Mometasone to Reduce Painful Episodes in Patients with Sickle Cell Disease (IMPROVE)	Mometasone	NCT02061202 Phase 2	Ongoing
Other anti-inflammatory reagents			
Effect of Simvastatin Treatment on Vaso-occlusive Pain in Sickle Cell Disease	Simvastatin [69]	NCT01702246 Phase 2	Complete
Atorvastatin Therapy to Improve Endothelial Function in Sickle Cell Disease	Atorvastatin	NCT00072826 Phase 1	Complete
The Effect of Factor Xa Inhibition, with Rivaroxaban, on the Pathology of Sickle Cell Disease	Rivaroxaban	NCT02072668 Phase 2	Ongoing
Antioxidants			
A Phase III, Prospective, Randomized, Double-Blind Placebo-Controlled, Parallel-Group, Multicenter Study of L-Glutamine for Sickle Cell Anemia and Sβ0-Thalassemia	L-glutamine [77]	NCT01179217 Phase 3	Complete
A Phase 2, Randomized, Double-Blind, Placebo-Controlled, Parallel-Group, Dose-Finding Study of SC411 in Children with Sickle Cell Disease	Omega-3 fatty acids [78]	NCT02973360 Phase 2	Ongoing
A Phase 1 Study of Continuous Intravenous L-Citrulline During Sickle Cell Pain Crisis or Acute Chest Syndrome	L-citrulline	NCT02697240 Phase 1	Ongoing
N-Acetylcysteine in Patients with Sickle Cell Disease: Reducing the Incidence of Daily Life Pain	N-acetyl cysteine	NCT01849016 Phase 3	Complete
A Pilot Study of N-Acetylcysteine in Patients with Sickle Cell Disease	N-acetyl [79] cysteine	NCT01800526 Phase 1/2	Ongoing
Physiological Effect of Sulforaphane Obtained from Broccoli Sprouts Homogenates (BSH) on the HbF and Anti-oxidative Capacity of Human Sickle RBC	Broccoli sprouts [80]	NCT01715480 Phase 1	Complete
Arginine Supplementation in Sickle Cell Anemia: Physiological and Prophylactic Effects	L-arginine [72]	NCT00513617 Phase 2	Complete

NRF2 nuclear factor (erythroid-derived 2)-like 2, *NAD* nicotinamide adenosine dinucleotide, *NO* nitric oxide, *RBC* red blood cell

Role of Fetal Hemoglobin

Hemoglobin F interferes with polymerization of HbS [81]. Individuals with hereditary persistent fetal hemoglobin are clinically very different since the elevated fetal hemoglobin ameliorates SCD severity by preventing polymerization of HbS. Hydroxyurea is currently the only FDA-approved drug for SCD that has been shown to elevate fetal hemoglobin production [81–83], but many other medications are currently being investigated in clinical trials. Intravenous sodium butyrate infusion was effective in increasing fetal hemoglobin from 7% to 22% in a small adult study [84]. A randomized placebo-controlled trial of HQK-1001 had to be halted after a planned interim analysis showed no significant increase in fetal hemoglobin in the HQK-1001 group [85]. Gene therapy rather than pharmacologic therapy may be the answer to solving this dilemma. The locus control region (LCR) is the major transcriptional enhancer of the β-globin gene. Blobel and colleagues successfully redirected globin synthesis from the adult β-globin promoter to the fetal γ-globin promoter by custom-designed zinc finger-Ldb1 fusion proteins (ZF-Ldb1) that redirected binding of the long-range enhancer [86, 87]. ZF-Ldb1 sickle-treated hematopoietic cells from individuals with SCD showed more than twice the increase of HbF (45%) and a concomitant decrease in HbS (50%) compared to various pharmacologic treatments [86]. *In vivo* work needs to be performed to further determine the feasibility of this in clinical practice.

Anti-sickling Agents

Common themes of agents that target sickling focus on ways to prevent polymerization of the HbS. By shifting the oxyhemoglobin dissociation curve to the left, improved oxygenation of hemoglobin will decrease sickling. AES-103 (5-hydroxymethyl furfural) is a compound made of a five-carbon-ring aromatic aldehyde that exists naturally in coffee, honey, and dried fruits. *In vitro* assays and sickle mice data both show decreased sickling and polymer formation with improved red cell survival [88]. Compounds that shift the oxyhemoglobin dissociation curve to the left (AES-103 and GBT440) [89, 90] have been well tolerated in adult patients with SCD in early phase 1 studies [91]. AES-103 was renamed Bax 555 when Baxalta was acquired by Shire and the phase 2 trial was terminated. GBT440 is a small molecule that increases HbS affinity for oxygen, delays *in vitro* HbS polymerization, and prevents sickling of RBCs. In a mouse model, GBT440 extends the half-life of RBCs, reduces reticulocyte counts, and prevents ex vivo RBC sickling [89]. A phase 3 trial for GBT440 is registered and recruiting (NCT03036813). Carbon monoxide (CO) attaches to hemoglobin and acts as an anti-sickling agent by preventing HbS polymerization. Sanguinate is a pegylated hemoglobin product that delivers CO to HbS and has been shown to be safe in a phase 1 trial [92, 93]. It also

acts as an oxygen transfer agent and has anti-inflammatory properties [94]. A phase 2 study of sanguinate for VOC in adults (NCT02411708) is currently recruiting patients in an adult ambulatory setting. SCD-101 a botanical-derived drug is currently in a phase 1 trial (NCT02380079) in adults with SCD. While *in vivo* and *in vitro* studies show anti-sickling activity, the underlying mechanism is unknown, but has been well tolerated in an adult cohort [95]. Table 2.3 highlights the various treatments that are being investigated to target fetal hemoglobin induction and enhance anti-sickling.

Table 2.3 Therapeutics targeting hemoglobin F induction and anti-sickling

Study title	Intervention	Clinical trials/phase	Status
Hemoglobin F induction			
Phase 1 Placebo-Controlled Study of the Safety, Activity and Pharmacokinetics of HQK-1001 in Healthy Subjects	Oral sodium butyrate (HQK-101)	NCT00717262 Phase 1	Complete
Phase 1/2 Study to Evaluate the Safety, Tolerability and Pharmacokinetics of HQK-1001 Administered Daily in Patients with Sickle Cell Disease	Oral sodium butyrate [96] (HQK-101)	NCT00842088 Phase 1/2	Complete
A Study of HQK-1001 in Patients with Sickle Cell Disease	Oral sodium butyrate [97] (HQK-101)	NCT01322269 Phase 2	Complete
A Study of HQK-1001 in Patients with Sickle Cell Disease	Oral sodium butyrate [85] (HQK-101)	NCT01601340 Phase 2	Terminated
Study of Panobinostat (LBH589) in Patients with Sickle Cell Disease (LBH589)	Panobinostat	NCT01245179 Phase 1	Ongoing
Study of Decitabine and Tetrahydrouridine (THU) in Patients With Sickle Cell Disease	Decitabine-THU	NCT01685515 Phase 1	Ongoing
Study to Determine the Maximum Tolerated Dose, Safety and Effectiveness of Pomalidomide for Patients with Sickle Cell Disease	Pomalidomide [98]	NCT01522547 Phase 1	Complete
Use of Metformin as a Fetal Hemoglobin Inducer in Patients with Hemoglobinopathies	Metformin [99]	NCT02981329 Phase 1	Ongoing
Anti-sickling agents			
Dose-Escalation Study of SCD-101 in Sickle Cell Disease	SCD-101 [95]	NCT02380079 Phase 1	Ongoing
Study of SANGUINATE™ Versus Hydroxyurea in Sickle Cell Disease (SCD) Patients	Sanguinate [92]	NCT01848925 Phase 1	Complete

(continued)

Table 2.3 (continued)

Study title	Intervention	Clinical trials/phase	Status
Study of SANGUINATE™ In the Treatment of Sickle Cell Disease Patients with Vaso-Occlusive Crisis	Sanguinate	NCT02411708 Phase 2	Ongoing
Safety Study of MP4CO in Adult Sickle Cell Patients	MP4CO [100]	NCT01356485 Phase 1	Complete
A Study of the Efficacy and Safety of ICA-17043 (With or Without Hydroxyurea) in Patients with Sickle Cell Anemia	Senicapoc (ICA-17043) [101, 102]	NCT00040677 Phase 2	Complete
A Stratified Sickle Event Randomized Trial (ASSERT)	Senicapoc (ICA-17043) [101, 102]	NCT00102791 Phase 3	Terminated
A Study Evaluating the Long-Term Safety of ICA-17043 in Sickle Cell Disease Patients With or Without Hydroxyurea Therapy	Senicapoc (ICA-17043) [101, 102]	NCT00294541 Phase 3	Terminated
A Single Dose Study of the Safety, Blood Levels and Biological Effects of Aes-103 Compared with Placebo in Subjects with Stable Sickle Cell Disease	Aes-103 (Bax 55)	NCT01597401 Phase 1	Complete
Evaluation of Different Dose Regimens of Aes-103 Given for 28 Days to Subjects with Stable Sickle Cell Disease	Aes-103 (Bax 55)	NCT01987908 Phase 2	Terminated
A Study of the Safety, Blood Levels and Biological Effects of GBT440 in Healthy Subjects and Subjects with Sickle Cell Disease	GBT440	NCT02285088 Phase 1	Ongoing
A Study of the Safety, Blood Levels and Biological Effects of GBT440 in Healthy Subjects and Subjects with Sickle Cell Disease	GBT440 [91]	NCT02285088 Phase 1	Complete
Study to Evaluate the Effect of GBT440 Administered Orally to Patients with Sickle Cell Disease (GBT_HOPE)	GBT440	NCT03036813 Phase 3	Ongoing

Chronic Pain in Sickle Cell Disease

Frequent and persistent pain is common in SCD, particularly in the adult population. According to a recent study, patients report sickle cell pain characteristics that are consistent with both nociceptive and neuropathic pain, contrary to prior belief that all SCD pain is nociceptive [103]. Trifluoperazine, a potent Ca/calmodulin protein kinase IIa inhibitor commonly used to treat neuropathic pain, has been recently studied in adult SCD patients. Half of the patients in this phase 1 trial reported a

Table 2.4 Therapeutic targets investigating chronic pain

Study title	Intervention	Clinical trials/phase	Status
Clinical Trial to Study the Safety and Tolerability of Memantin Mepha® in Sickle Cell Disease Patients	Memantine hydrochloride	NCT02615847 Phase 2	Ongoing
Pain Management of Vaso-Occlusive Crisis in Children and Young Adults with Sickle Cell Disease	Gabapentin	NCT01954927 Phase 2	Ongoing
Cannabinoid-Based Therapy and Approaches to Quantify Pain in Sickle Cell Disease	Vaporized cannabis	NCT01771731 Phase 1	Ongoing

50% reduction in their chronic pain, suggesting a role for neuropathy in the pathogenesis of SCD pain [104]. SCD mice also exhibit altered sensitivity to pain as demonstrated by musculoskeletal and cutaneous hyperalgesia [105]. Nociceptive neurons in the spinal cord of BERK sickle mice have increased phosphorylation of mitogen-activated protein kinases (MAPKs) that are known to contribute to neuronal hyperexcitability, including c-Jun N-terminal kinase (JNK), p44/p42 extracellular signal-regulated kinase (ERK), and p38, which suggests that central sensitization contributes to the pain phenotype [106]. In SCD mice, activators of neuropathic and inflammatory pain (p38 mitogen-activated protein kinase, STAT3, and mitogen-activated protein kinase/extracellular signal-regulated kinase) are increased in the spinal cord in addition to neurochemical changes in the peripheral nerves [107]. Mast cells in murine models promote neurogenic inflammation and nociceptor activation through the release of substance P in the skin and dorsal root ganglion [108]. Targeting mast cells in sickle cell mice by small molecule inhibitors or by stabilizing mast cell degranulation ameliorates hyperalgesia [108, 109]. Although treatments for neuropathic pain appear to be promising as novel therapeutics for chronic pain in SCD, further investigations are urgently needed. Table 2.4 summarizes the treatments that are being investigated in chronic pain.

Role of Activated Coagulation in SCD

Venous thromboembolism (VTE) has been an underappreciated complication of HbSS, although the increased incidence and recurrence of thrombosis in SCD patients suggest a chronic hypercoagulable state [110]. It has been reported that up to 25% of adults with SCD have developed VTE, with the median age of first VTE being considerably younger than in the general population [111] and comparable to the age observed in families with high-risk thrombophilia [112]. The risk of VTE in SCD is heightened by recurrent hospitalizations, prolonged episodes of immobility, frequent use of central venous catheters, and infection [113]. Increased mortality was observed in adults with SCD and thrombosis [111, 114]. There is an increased prevalence of pulmonary embolism found in SCD patients at autopsy, especially

those with sudden death [115, 116], and it has been suggested that pulmonary embolism may underlie development of some cases of pulmonary hypertension. Additionally, both retrospective and prospective analyses of patients with acute chest syndrome report increased pulmonary embolism [115–118]. Use of administrative discharge databases further corroborates the increased incidence of pulmonary embolism in adults with SCD when compared to age- and race-matched controls [118, 119]. In the pediatric SCD population, central venous catheter placement increases the risk of DVT [120, 121]. Pregnancy-related VTE is also increased in women with SCD [122, 123].

Nearly every component of coagulation, including platelets, is affected by SCD. Tissue factor (TF) is an essential component of the factor VIIA-TF complex enzyme, the initiator of blood coagulation in vivo. TF is expressed by endothelial cells and monocytes, and increased levels are reported in SCD [124–127]. The number of circulating TF-laden cells and microparticles increases during painful crises, as compared to steady state [124, 127, 128]. In general, increased numbers of TF-expressing endothelial cells, monocytes, red blood cells, and their associated microparticles influence the coagulation cascade [129]. In accordance with this, there is an association between increased markers of hemolysis in SCD and whole blood TF procoagulant activity [130].

An overall increased state of thrombin generation in SCD is evidenced by chronic elevation of procoagulant proteins such as thrombin-antithrombin (TAT) complexes, prothrombin fragments (F1.2) and D-dimers, and other markers of thrombin generation [131]. Moderately decreased levels of the anticoagulant proteins C and S are observed in patients with SCD in steady state, and these may be further decreased during acute pain episodes [132–135]. Decreased levels of factor V have also been reported, suggesting chronic consumption of procoagulant factors due to the increase in tissue factor expression and thrombin generation [110, 136].

Von Willebrand factor (VWF) has also been implicated in the thrombophilic state of SCD [137]. Extracellular hemoglobin binds with high affinity to VWF, thus preventing VWF from being cleaved by ADAMTS-13. This could be considered a form of acquired ADAMTS-13 deficiency [138]. The inability to proteolize VWF leads to accumulation of ultra-large, extremely adhesive VWF multimers in circulation and on the endothelium [138]. Plasma free heme also induces exocytosis of VWF from Weibel-Palade bodies [139], and total activity of VWF has been shown to directly correlate with hemolysis [140]. This pathophysiology is demonstrated clinically by the description of a thrombotic thrombocytopenic purpura-like syndrome in SCD patients [141, 142].

Overall the balance of the coagulation system in SCD is tipped toward thrombosis (Fig. 2.1). This system is a potential target for disease-modifying interventions with anticoagulants. For example, the use of low-dose warfarin was shown to significantly decrease D-dimer during crisis in a small group of patients with SCD [143]. Multiple studies targeting coagulation in SCD are ongoing (Table 2.5).

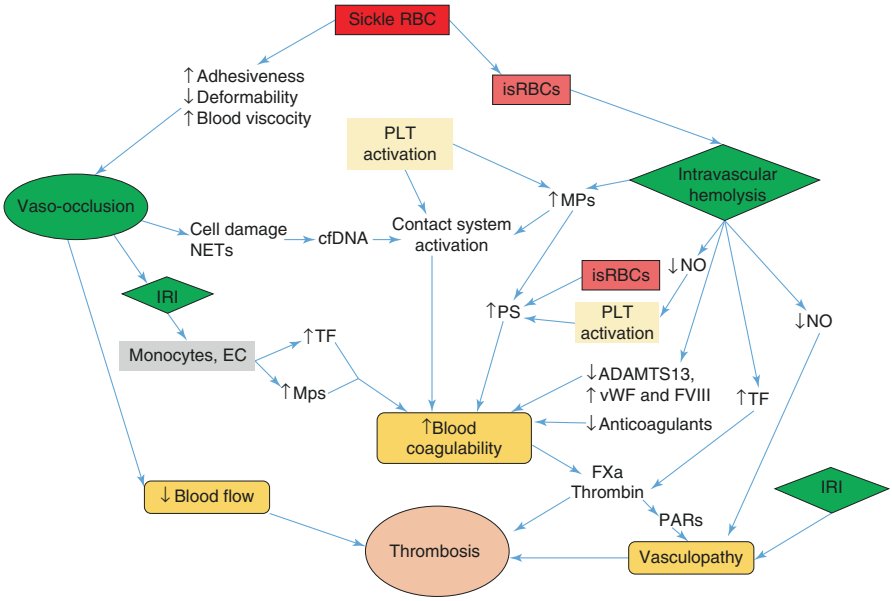

Fig. 2.1 Pathogenesis of thrombosis in sickle cell disease. *RBC* red blood cells, *isRBC* irreversibly sickled red blood cells, *PLT* platelets, *MP* microparticles, *cfDNA* cell-free DNA, *NETs* neutrophil extracellular traps, *NO* nitric oxide, *IRI* ischemic reperfusion injury, *TF* tissue factor, *PARs* protease-activated receptors, *PS* phosphatidylserine, *EC* endothelial cell, *VWF* von Willebrand factor, *FVIII* factor VIII, *FXa* activated factor X. Adapted from Ref. [124]

Table 2.5 Studies involving anticoagulants

Study title	Intervention	Clinical trials/phase	Status
An Exploratory Study of Anticoagulation for Pulmonary Hypertension in Sickle Cell Disease	Warfarin	NCT01036802 Phase 2	Terminated
Apixaban in Patients with Sickle cell Disease	Apixaban	NCT02179177 Phase 3	Ongoing
Treatment of Sickle Cell Patients Hospitalized in Pain Crisis with Prophylactic Dose Low-Molecular-Weight Heparin (LMWH) vs. Placebo	Dalteparin [144]	NCT01419977 Phase 2	Complete
The Effect of Rivaroxaban in Sickle Cell Disease	Rivaroxaban	NCT02072668 Phase 2	Ongoing
Feasibility Study or Unfractionated Heparin in Acute Chest Syndrome	Unfractionated heparin	NCT02098993Phase 2	Ongoing

Targeting Coagulation in SCD

Heparin

Trials of heparins have shown efficacy in treating painful crises. The anti-adhesive effect of heparins mediated via blockade of P-selectin is an additional mechanism of these agents. NCT01419977 studied prophylactic dosing of dalteparin on change in D-dimer, change in pain score, and change in the thrombin generation assay during VOC [144]. Results showed that prophylactic dosing did not significantly affect markers of coagulation; however there was a greater decrease in pain scores at days 1 and 3 in patients treated with dalteparin. A single-center, randomized, double-blind clinical trial showed reduction in the severity and duration of acute VOC when using tinzaparin vs. placebo [145]. A study of the effects of unfractionated heparin in acute chest syndrome in SCD (NCT02098993) is ongoing, with the primary outcome being time to hospital discharge.

Direct Thrombin and Factor X Inhibitors

Current studies of new oral anticoagulants and their potential role in SCD are ongoing. NCT02179177 studies the effect of prophylactic dosing of apixaban on daily pain scores and NCT02072668 the effect of rivaroxaban on sVCAM and IL-6.

Vitamin K Antagonists

NCT01036802 studied anticoagulation with warfarin for pulmonary hypertension, but was terminated due to poor accrual.

Role of Platelets in SCD

Platelets have been shown to circulate in SCD patients in an activated state in both "steady state" and during painful crisis. This is evidenced by elevated platelet expression of CD62, CD63, PAC, P-selectin, activated glycoprotein IIb/IIIa, plasma soluble factor 4, and β-thromboglobulin [124, 131, 146–150]. It is also proposed that platelets contribute to the inflammatory milieu of SCD via manufacture and release of pro- and anti-inflammatory molecules upon activation [151, 152]. Increasing cytokine levels are associated with increased platelet number in SCD [153]. Platelets are well known to form aggregates in SCD by binding erythrocytes, monocytes, and neutrophils [3, 148, 150, 154]. At the molecular level, the

neutrophil serine/threonine kinase isoform AKT2 plays a critical role in both neutrophil recruitment and neutrophil-platelet interactions resulting in vascular inflammation and lung damage [12]. Inhibition of AKT2 diminishes neutrophil adhesion and neutrophil-platelet interactions, leading to improved blood flow [155] and prolonged survival when coadministered with hydroxyurea [156] in SCD mice. Overall, evidence suggests that the chronic activation of platelets in SCD contributes to the vasculopathy and thrombo-inflammatory state described in SCD. Accordingly, these alterations have been targeted by antiplatelet therapies with the goal of ameliorating the SCD phenotype (Table 2.6).

Table 2.6 Studies involving antiplatelet agents

Study title	Intervention	Clinical trial #/phase	Status
A Phase I/II Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Safety of Eptifibatide as Treatment for Acute Pain Episodes in Sickle Cell Disease	Eptifibatide [157]	NCT00834899 Phase 1, 2	Terminated
An Open-Label, Dose-Ranging Study of Prasugrel in Pediatric Patients with Sickle Cell Disease	Prasugrel [158]	NCT01476696 Phase 2	Complete
A Phase 3, Double-Blind, Randomized, Efficacy and Safety Comparison of Prasugrel and Placebo in Pediatric Patients with Sickle Cell Disease	Prasugrel [159]	NCT01794000 Phase 3	Terminated
A Pharmacokinetic and Pharmacodynamic Assessment of Prasugrel in Healthy Adults and Adults with Sickle Cell Disease	Prasugrel	NCT01178099 Phase 1,2	Complete
Prasugrel Versus Placebo in Adult Sickle Cell Disease	Prasugrel [160]	NCT01167023 Phase 2	Complete
Aspirin Prophylaxis in Sickle Cell Disease	Aspirin	NCT00178464 Phase 1, 2	Complete
Abciximab (ReoPro) as a Therapeutic Intervention for Sickle cell Vaso-Occlusive Pain Crisis	Abciximab	NCT01932554 Phase 2	Withdrawn
Dipyridamole/Magnesium to Improve Sickle Cell Hydration	Dipyridamole and magnesium	NCT00276146 Phase 2	Withdrawn
A Pharmacokinetic (PK) and Pharmacodynamic (PD) Dose-Ranging Phase II Study of Ticagrelor Followed by a 4-Week Extension Phase in Pediatric Patients With Sickle Cell Disease	Ticagrelor	NCT02214121 Phase 2	Complete
A Study to Evaluate the Effect of Ticagrelor in Reducing the Number of Days with Pain in Patients with Sickle Cell Disease (Hestia2)	Ticagrelor	NCT02482298 Phase 2	Complete

Antiplatelet Agents

The effect of aspirin on hemoglobin level and frequency of painful crises have been evaluated in several clinical trials [161–164]. While one study showed an effect on hemoglobin level [163], none showed a significant effect on frequency of painful crises. In a single-site randomized trial, the glycoprotein IIb/IIIa inhibitor, eptifibatide (NCT00834899), was shown to be safe in SCD. However treatment with eptifibatide did not improve the time to crisis resolution or hospital discharge [157]. A multicenter phase 2 trial of the P2Y12 inhibitor, prasugrel (NCT01167023), showed a decrease in platelet activation biomarkers and a trend toward decreased pain that was nonsignificant [160]. A phase 3 randomized, double-blind, placebo-controlled study of prasugrel (NCT01794000) for prevention of VOC also demonstrated a nonsignificant trend toward fewer painful crises in the treatment versus placebo arm [165]. A phase 2 study using ticagrelor (NCT02482298) to determine whether the P2Y12 inhibitor can reduce the number of days of pain, pain intensity, and analgesic use has recently been completed, and results are not yet available. Thus, while platelets have been implicated in the pathophysiology of SCD vaso-occlusion and painful crises, antiplatelet agents have not proven to be effective in targeting that specific clinical outcome. However, given the correlation between hemolysis and activation of the hemostatic system, and the cross talk between coagulation and inflammation, it is possible that different aspects of SCD pathophysiology may be positively affected by antiplatelet therapy.

Summary

Improved understanding of the pathophysiology of sickle cell VOC has led to new targeted therapeutics as well as emerging gene therapies. Given the complexity of the sickle RBC interactions with the endothelium, platelets, and neutrophils, it is likely a multimodal approach will be necessary for optimal results. It is crucial that clinicians, scientists, and patients continue to collaborate together and participate in multi-institutional and international trials for investigating novel treatments in this highly variable disease.

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Chapter 3
Current Non-HSCT Treatments for SCD

Claire L. Anderson and Deepika S. Darbari

Sickle cell disease (SCD) is characterized by episodes of acute complications and ongoing chronic organ disease which has been associated with significant morbidity and premature death. Advances in health care along with public health measures have led to significant reduction in mortality for children with SCD, at least in developed countries. Universal newborn screening, penicillin prophylaxis, vaccinations, and hydroxyurea (HU) therapy, along with transcranial Doppler for stroke screening and use of chronic transfusion, have successfully changed SCD from a life-threatening disease to a chronic condition. The majority of patients now survive into adulthood requiring lifelong comprehensive care.

Management of SCD has been largely supportive until recently. The use of disease-modifying therapies such as HU and chronic blood transfusions is expanding, and hematopoietic stem cell transplant (HSCT) is also becoming increasing available as a potential curative therapy for SCD. This chapter will primarily focus on HU in addition to reviewing supportive care for SCD and emerging therapies. Chronic transfusions and HSCT are discussed briefly as these topics are being reviewed in chapters focused on these specific areas.

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Disease-Modifying Therapies

Hydroxyurea

Hydroxyurea (hydroxycarbamide, HU) is a FDA-approved disease-modifying therapy for SCD. Until recently, HU was primarily prescribed to individuals with severe disease who experienced complications such as recurrent vaso-occlusive painful episodes and acute chest syndrome. However recent National Heart, Lung, and Blood Institute (NHLBI) practice guidelines strongly endorse HU and suggest offering it to patients with sickle cell anemia (SCA) from 9 months of age regardless of clinical severity [1]. While the majority of evidence supporting HU use comes from SCA, HU is also considered in patients with HbSC and HbSβ^+ thalassemia who have severe disease as indicated by frequent pain or other complications of SCD [2, 3].

HU is an old drug first synthesized in 1869 by Dresler and Stein, although its clinical application was not until a century later for treatment of various myeloproliferative disorders. Clinical observations from the initial cohort studies had documented the beneficial effects of fetal hemoglobin (HbF) in SCD prompting investigation of the induction of HbF in patients with SCD [4]. In the 1980s the effect of HU as an HbF-inducing agent in SCA was published [5]. Subsequently, studies conducted to determine if the laboratory effects of HU would translate into clinical benefits showed that increased HbF was associated with improved outcomes in SCA. It is however becoming clear that induction of HbF is not the only mechanism that underlies the beneficial effects of HU in SCD.

Several cell cycle-specific agents are known to have the ability to induce HbF production, some of which have been tried in SCD [6]. Given other convenient properties such as rapid absorption, high bioavailability, oral formulation, and once-daily dosing, the clinical application of HU in SCD became of interest, and Platt et al. pioneered its use in SCD [5]. HU, a monohydroxyl-substituted urea (hydroxycarbamate) antimetabolite, selectively inhibits ribonucleotide diphosphate reductase, an enzyme required to convert ribonucleoside diphosphates into deoxyribonucleoside diphosphates, thereby preventing cells from leaving the G1/S phase of the cell cycle [7]. Reversible inhibition of ribonucleotide reductase and resulting inhibition of progression of cellular division and temporary arrest of hematopoiesis lead to altered erythroid kinetics by recruitment of early erythroid progenitors that maintain their HbF-producing capability [8]. Another mechanism postulated for increased HbF suggests that HU may act directly on the late erythroid precursors to produce HbF or may alter the transcription factors that modulate globin gene enhancers [9, 10]. Nitric oxide-dependent activation of soluble guanylyl cyclase has also been proposed to play a role in HU-induced HbF production [11]. Increased HbF concentration leads to reduction in polymerization of sickle hemoglobin (HbS) and sickling of red blood cells. Red blood cells with high HbF are larger, less dense, and more deformable, thus exhibiting improved rheology [12]. HU also affects myelopoiesis leading to dose-dependent leukopenia, neutropenia,

and thrombocytopenia. Additionally, its use has been shown to be associated with reduction in chronic inflammation and increased levels of nitric oxide that may improve vascular tone and reduce expression of surface molecules that adhere to endothelium. [11, 13, 14].

The multicenter study of HU (MSH) was a double-blind placebo-controlled trial in adults with SCA which was designed to test the efficacy of HU in reducing the frequency of painful crises. A report of three or more pain crises in a year was needed to meet the study entry criteria. The study showed that HU reduced the frequency of hospitalization for pain, acute chest syndrome, and need for blood transfusion [15]. The trial was stopped prior to its planned 2-year duration due to the observed beneficial effects of HU. These clinical benefits of HU were also associated with beneficial laboratory findings which included increase in hemoglobin concentration and HbF levels and reductions in neutrophil count and markers of hemolysis. In addition, a later follow-up study of MSH participants showed prolonged survival in patients treated with HU [16]. HU was approved for use in symptomatic SCD by the FDA in 1998. Although the approval did not extend to pediatric patients, comparable studies in children have confirmed that HU is safe, efficacious, and well tolerated [17–19]. Due to the favorable effects of HU observed in adults and children with SCD, it was hypothesized that early institution of HU could reduce or prevent damage to organs especially the brain, spleen, and kidneys. This hypothesis led to the phase III randomized double-blind placebo-controlled trial (BABY HUG) conducted in 9–18-month-old children with SCA. Unlike the MSH trial, meeting of disease severity criteria was not needed for the study entry. At the end of the study, while the primary end point of preventing organ damage was not met, HU was effective in reducing the number of episodes of dactylitis and other vaso-occlusive episodes, number of hospitalizations, and need for transfusions [20]. Benefits of HU extend beyond the outcomes evaluated on initial studies. Data is emerging suggesting efficacy of HU in preventing end-organ damage. HU has been shown to be effective in children with conditional transcranial Doppler velocities, as an alternative to transfusion in non-transfusable patients, and to delay the onset of end-stage renal disease in patients with nephropathy [21–23]. Additionally, long-term use of HU may improve survival in patients with SCD [16, 24].

Stroke is a devastating complication of SCD. Until recently blood transfusion was the only treatment available for prevention of stroke in this population. Many recent studies have evaluated the effect on HU on SCA-associated stroke. The Stroke with Transfusions Changing to HU (SWiTCH) trial was a multicenter phase III randomized open-label, non-inferiority trial designed to compare standard treatment (transfusions and chelation) to alternative treatment (HU at maximum tolerated dose and monthly phlebotomy) for reduction of secondary stroke and management of iron overload [25]. The study population included children with SCA and previous stroke who had been on chronic transfusions for 18 months or more with iron overload. The composite primary end point of the study included recurrence of stroke and iron overload. Results of interim analysis showed no recurrence of strokes in subjects on transfusions/chelation arm compared to 7 (10%) on HU/phlebotomy arm. Although this difference in stroke recurrence was still in the non-inferiority

range (12%), liver iron content was equivalent in the groups. Given these results the study was closed early. It was concluded that in children with SCA, stroke, and iron overload, current approach of transfusions and chelation is superior to HU and phlebotomy for secondary stroke prevention [25]. Recently the outcomes for TCD with transfusions changing to HU (TWiTCH) trial have been published [26]. This was a multicenter, phase III, randomized, open-label, non-inferiority trial that enrolled 121 participants aged 4–16 years with SCA on chronic transfusions with abnormal TCD $\geq$ 200 cm/s but no severe vasculopathy who had received at least 12 months of red cell transfusions. Patients were randomly assigned to continue transfusions (standard group $n = 61$) or transition to HU (alternative group $n = 60$). The study was terminated early as the first interim analysis showed non-inferiority of HU to ongoing transfusions. It was concluded that children with SCA with abnormal TCD velocities on chronic transfusions for a least 1 year with no MRA-defined severe vasculopathy can be switched to HU for primary stroke prevention [26].

HU is a myelosuppressive agent so it is recommended that a protocol for monitoring should be followed to ensure patients are receiving an adequate dose without evidence of myelotoxicity. The NHLBI published evidence-based guidelines providing a consensus treatment protocol for the implementation of HU therapy [1]. The effect of HU is dose dependent, and studies indicate that reaching maximum tolerated dose (MTD) may be beneficial in SCD [19, 21]. Prior to starting HU, baseline studies should be obtained including complete blood count (CBC) with differential, reticulocyte count; platelet count; RBC mean corpuscular volume (MCV); quantitative measurement of HbF if available; comprehensive metabolic profile, including renal and liver function tests; and pregnancy test if appropriate. These parameters should be followed periodically as described in the NHLBI guidelines and summarized below [1]. During monitoring visits, providers should elicit symptoms of toxicity and reiterate adherence, advising patients not to take extra doses if a dose is missed and to continue to take HU when sick or hospitalized unless instructed by a physician. Contraceptive counseling prior to HU initiation and at follow-up visits should also be provided to patients of both genders.

The usual starting dose for infants and children is 20 mg/kg/day and a lower dose of 15 mg/kg/day for adults with SCD. For patients with concomitant chronic renal disease, starting dose should be lowered to 5–10 mg/kg/day. Patients should be monitored with CBC with differential and reticulocyte counts every 4 weeks during dose escalation which is typically done in increments of 5 mg/kg/day every 8 weeks to a maximum dose of 35 mg/kg/day. Adult dose typically is 1500–2000 mg daily. The goal absolute neutrophil count (ANC) is >2000 μL^{-1}; however children with lower baseline ANC may safely tolerate counts down to 1250 μL^{-1}. HU is held for significant cytopenias of ANC < 1000 μL^{-1}, platelet count <80,000 μL^{-1}, or reticulocyte count <100 K/μL (unless hemoglobin >8.0 g/dL). CBC with differential and reticulocyte counts is monitored weekly till count recovery following which HU can be resumed at a dose 5 mg/kg/day lower than prior to the onset of cytopenias. Once a patient is on a stable dose, CBC with differential and reticulocyte count can be monitored every 2–3 months, and HU is continued long term. Liver and kidney function tests as well as a hemoglobin electrophoresis should be obtained every

3–6 months [2]. RBC MCV and HbF levels provide evidence of consistent or progressive laboratory response; however a lack of increase in MCV and/or HbF is not an indication to discontinue therapy. A clinical response to treatment with HU can take 3–6 months. Therefore, a 6-month trial of HU on the maximum tolerated dose is recommended prior to considering discontinuation of HU. Although poor adherence is the most common cause of treatment failure, a proportion of patients are biologically resistant to HU [27].

HU is generally well tolerated with very few reversible side effects. The most common and anticipated short-term side effect is reversible myelosuppression, leading to neutropenia, reticulocytopenia, and thrombocytopenia [27, 28]. Patients should be counseled regarding integumentary effects of skin or mucosal hyperpigmentation, melanonychia (darkening of nails), and less commonly hair thinning/alopecia [28, 29]. Mild gastrointestinal upset can also occur [28]. HU is renally excreted and small elevations in creatinine may be observed [27]. Long-term side effects are rare but may become pertinent with improved survival in SCD. Despite initial concern about effect on growth based on murine studies, no significant change in growth velocity has been reported in children taking HU [17, 20, 29–31]. There is a theoretical increased risk of malignancy with HU; however available data does not support an association between HU use in SCD and leukemia [19, 20, 29, 32]. The negative impact of HU on reproductive health is unconfirmed. A transgenic sickle cell mouse model revealed that HU treatment exacerbated SCD-induced hypogonadism to gonadal failure [33]. Small retrospective cohort studies have revealed sperm abnormalities in males with SCD which are exacerbated by HU [34, 35]. While reproductive issues and HU are an area of future research, current recommendations suggest that patients should be counseled on the risks of HU. Sexually active couples are recommended to use contraception if one person is on HU and individuals trying to conceive should stop HU 3–6 months before discontinuing contraception [27]. Nonetheless, literature documents males and females taking HU who have given birth to healthy offspring. During the MSH study, despite mandated use of contraception for study inclusion, several successful pregnancies occurred in women taking HU and in the partners of male participants [36]. A number of cohort studies and case reports also document healthy pregnancy outcomes in women taking HU [29, 37, 38]. It is known that HU crosses the placenta and is excreted in breast milk [39]. It is recommended that HU be discontinued in pregnant and lactating women due to a lack of human data on its potential teratogenic effects [3] and with animal studies documenting reproductive toxicities of HU [40–43].

Despite the substantial body of evidence in favor of HU, this medication remains underutilized [28]. Barriers to use are many and exist at the provider, patient/parent, and systems levels [44]. Provider surveys have uncovered a lack of awareness and varying interpretation of the risk versus benefits of HU [45] and perceived patient apprehension about adverse effects, treatment adherence, and compliance with contraception [46, 47], and burdens of laboratory monitoring [47] contribute to underutilization of HU. Parental survey revealed factors such as that HU was not always being offered by hematologists and also concerns for side effects related to HU use in particular efficacy, long-term safety, and off-label use affect HU use [48].

System-related barriers including issues surrounding health disparities also play a role. For patients on HU, ongoing medical monitoring for toxicities is vital. The need for frequent visits may present monetary challenges for the families belonging to disadvantaged populations that may have poorer access to transportation, coordinated health care, and health insurance which in turn may lead to underutilization of HU [44]. Nonetheless, HU has been shown to result in significantly lower overall estimated medical care costs. Data from the BABY HUG study compared cost of medical care in children taking HU to those on placebo. The study revealed that while estimated outpatient expenses were increased in the HU group, the number of hospitalizations was lower resulting in overall lower cost for SCD children on HU. The total annual estimated cost was 21% lower in the HU group ($11,072) compared to those on placebo ($13, 962: $P = 0.038$) [49].

SCD is a global disease due to the fact that HbS trait bestows a survival advantage from malaria [50]. The burden of SCD is increasing particularly in Africa; it was estimated in 2010 that 79% of children with SCD were born in sub-Saharan Africa, projected to increase to 88% by 2050 [51]. Africa and other low-income countries worldwide are resource-limited with immense disparities in management and health outcomes in SCD compared to high-resource countries [52]. Due to its efficacy, low cost, and ease of administration, HU is an ideal drug for low-resource countries. HU is safe and of proven benefit in resource-rich settings; whether this translates to low-income settings remains to be established [53, 54]. Studies evaluating the safety, sustainability, and efficacy in these low-resource settings challenged with comorbidities such as malaria, HIV, and severe malnutrition are imperative [28]. Several studies are ongoing to bridge this knowledge gap. The Realizing Effectiveness Across Continents with HU (REACH, ClinicalTrials.gov NCT01966731) trial is a multicenter prospective phase I/II open-label, dose escalation study of HU. The study plans to recruit a total of 600 children age 1–10 years with SCA in sub-Saharan Africa. The study will evaluate feasibility, safety, and benefits of HU. The Novel use of HU in an African Region with Malaria (NOHARM, NCT01976416) is a prospective randomized, placebo-controlled, double-blind phase III trial that seeks to identify hematologic toxicities, adverse events, and risk of malaria with low fixed-dose oral HU versus placebo. It will also establish correlations between HU treatment and fetal hemoglobin, soluble intracellular adhesion molecule-1, and nitric oxide levels and between levels of these biomarkers and risk of malaria. This study aims to recruit 200 Ugandan children aged 1.00–3.99 years with SCA [53]. Primary stroke prevention is a priority in the care of patients with sickle cell. The Primary Stroke Prevention in Nigerian Children (SPIN, NCT01801423) study is a NIH-sponsored SCD clinical trial in sub-Saharan Africa [55]. This is a single-site, single-arm pilot trial in which children ages 5–12 years with elevated TCD measurements >200 cm/s in the middle cerebral artery will receive low fixed-dose HU (approximately 20 mg/kg) and will be monitored for adherence and adverse events. Preliminary data is promising with 10 out of 11 patients within 3 months showing reduced middle cerebral artery velocities below 200 cm/s [55]. If these studies show HU is safe and beneficial in resource-limited settings, this could transform the care of children with SCA in Africa.

Blood Transfusions

Red blood cell transfusions are a mainstay treatment for SCD. Transfusions are often used to treat or prevent some of the complications of SCD. Donor red cells contain hemoglobin A; thus transfusion of these cells lowers the percent of sickle hemoglobin-containing red blood cells and lowers the rate of SCD-related complications. Side effects include allergic reaction, delayed hemolytic transfusion reaction, alloimmunization, and iron overload. All individuals with SCD should be given leukocyte-reduced sickle-negative blood. To prevent alloimmunization, many institutions match the units for minor Rh and Kell antigens per NHLBI guidelines [1]. The most common indications for acute transfusions include complications such as stroke or acute neurological deficit, acute exacerbation of anemia, acute chest syndrome, and preoperative management. The most common indication for chronic blood transfusion includes primary or secondary prevention of stroke in SCD [56]. Details of transfusion therapy including indications and complications are reviewed in Chap. 5 (Transfusion support for SCD patients).

Hematopoietic Stem Cell Transplantation

Allogeneic hematopoietic stem cell transplantation (HSCT) is currently the only available therapy with curative potential for SCD. Its availability has been increasing but still remains limited due to various reasons. Goals of HSCT include establishing stable donor engraftment and long-term donor-derived erythropoiesis [57]. Decision to transplant is generally weighed based on risk-benefit ratio depending on patient's status and donor availability. Details of HSCT in SCD are reviewed in the chapters dedicated to this subject in this book.

Supportive Therapies

Analgesics

Analgesics are used to treat pain associated with SCD. When possible, non-pharmacological measures should also be utilized as an adjuvant therapy for managing pain. It is important to remember that pain management is guided by patient report, and there are no biomarkers or imaging studies to assess pain at this time. Safe and effective management of pain requires ongoing assessment and individualization of therapy with combined use of pharmacologic and non-pharmacologic approaches. For appropriate age-based dosing, please refer to drug-specific package inserts and formulary resources.

Non-opioid Analgesics

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the first-line agents for managing pain of mild to moderate intensity. NSAIDs are available in oral or parenteral preparations and are also used as an adjuvant to opioids for painful episodes of moderate to severe intensity. NSAIDs work by blocking the action of cyclooxygenase (COX) which is responsible for converting arachidonic acid to prostaglandins. While aspirin is not recommended for children due to association with Reye's syndrome, NSAIDs such as ibuprofen are commonly used [58]. Parenteral preparations of NSAIDs such as ketorolac tromethamine are often used during inpatient management of severe painful episode. The evidence supports use of NSAID in reducing pain and decreasing length of hospital stay associated with acute vaso-occlusive pain [59]. Gastric, renal, and platelet toxicities should be considered when using NSAIDs.

Acetaminophen and other para-aminophenol derivatives have analgesic and antipyretic activity but only moderate anti-inflammatory activity. These agents typically do not inhibit platelet aggregation. Care should be taken however with its use in patients with preexisting liver disease as well as those who receive large dose of acetaminophen (>4000 mg/day), and they can experience severe hepatotoxicity. Many preparations of acetaminophen are available in combination with opioids such as codeine and oxycodone. Care should be taken when using combinations as the risk of toxicity from acetaminophen may increase if dose is escalated to increase the opioid effect.

Opioid Analgesics

Opioids remain the mainstay of acute pain management in SCD. Opioids are available in oral, parenteral, rectal, subcutaneous, and transdermal preparations and may be available as short- or long-acting compounds. Morphine and other opioid agonists bind to opioid receptors and produce analgesia. Several RCTs and observational studies support using opioid therapy for treating acute pain associated with SCD [60–63]. Studies also support the use of around-the-clock dosing of analgesics versus intermittent administration when treating vaso-occlusive crises (VOCs) [64, 65]. Patients and parents should be counseled about the side effects including potential for dependence, abuse, and diversion.

The role of opioids in treating chronic pain associated with SCD has not been investigated. While reports from the American Pain Society (APS) suggest opioids are not effective in treating chronic non-cancer pain [66, 67], it is important to recognize that SCD patients can experience acute episode of pain on the background of chronic pain and opioids are indicated for such acute on chronic pain [68]. Patients with chronic pain or high rates of recurrent pain often require higher and more frequent dosing of opioids. These patients may benefit from working with a multidisciplinary pain management team which may include a hematologist, pain physician, and psychologist. These patients should have a pain management plan which includes a detailed written pain plan that provides guidance for dosing of analgesic agents for baseline and/or acute episodes of pain. These plans are typically based on an individual's pain and analgesic history.

Commonly used compounds in this category include codeine, morphine, oxycodone, hydromorphone, fentanyl, and methadone with morphine being the prototype of opioid analgesics. Relative potencies vary between various opioids and should be considered in the decision of opioid rotation and dose selection. Side effects include constipation, nausea, vomiting to sedation, and respiratory depression. It has been proposed that opioid-induced hyperalgesia can contribute to development of chronic pain [69]. Metabolism of opioids can be impacted by pharmacogenomics as well as the state of organ dysfunction in SCD [70, 71]. Recently FDA issued US boxed warning against using codeine in children due to the potential risk of life-threatening or fatal respiratory depression in children and adolescents [72]. This complication is related to pharmacogenomics of codeine. Codeine is primarily metabolized in the liver by the enzymes UGT2B7, CYP3A4, and CYP2D6. Ultrarapid metabolizer patients who carry two or more copies of the variant CYP2D6*2 allele have increased conversion of codeine to morphine and thus are at increased risk for opioid-mediated side effects [73]. Opioid therapy also carries the risk of tolerance, dependence, and potential for abuse; therefore patient counseling should be part of the therapy. If possible one provider should be assigned to prescribe opioids, and a treatment agreement may be considered for patients on long-term opioid therapy.

Other Adjuvant Therapies for Pain

In addition to frequently used analgesic medications above, many drugs are used to improve pain control in SCD. Most of these medications have not been tested in SCD in randomized controlled trial. Medications in this category include ketamine, dexmedetomidine, antidepressants such as amitriptyline or duloxetine, and anticonvulsants such as gabapentin and pregabalin. Ketamine is a *N*-methyl-D-aspartate (NMDA) receptor blocker and can modulate opioid tolerance and opioid-induced hyperalgesia and may be effective in managing pain in SCD [74]. Results of ketamine therapy in SCD have been mixed and are mostly based on single case report, case series, and retrospective studies data review [75, 76]. Gabapentin is often prescribed for chronic neuropathic pain and is currently being studied in SCD in a phase II study (clincialtrials.gov NCT01954927). Dexmedetomidine, an α2-adrenoreceptor agonist with sedative and analgesic properties, is used in the perioperative and intensive care settings. Dexmedetomidine has been shown to reduce opioid requirement and to facilitate opioid weaning, and its use has been reported in case series in SCD [77, 78].

Antibiotics and Immunizations

While prophylactic antibiotics and pneumococcal vaccination have reduced the incidence of invasive pneumococcal infection significantly, fever in a SCD child is still considered an emergency. Patients with SCD are at very high risk for serious

infections including bacteremia and meningitis. Due to functional hyposplenia in SCD, patients are highly susceptible to encapsulated bacteria such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Neisseria meningitidis* [79, 80]. Frequent systemic infections with enteric organisms such as *Salmonella species* and *Staphylococcus aureus* are also seen [81]. Patients are also at risk for diverse group of organisms such as *Mycoplasma* in acute chest syndrome. Gram-negative enteric infection can involve the urinary tract, bones, and hepatobiliary system. In addition to functional asplenia, deficits of immunologic function have been reported in SCD. Innate immune system abnormalities include neutrophil dysfunction with decreased chemotaxis, migration, phagocytosis, and bactericidal properties [81] and inability to utilize the alternative pathway for C3 fixation causing defects of opsophagocytosis [82, 83]. The adaptive immune system is also impaired with a deficiency of specific circulating antibodies [83], dysfunctional IgG and IgM antibody response [84], and reduced CD4+ and CD8+ subsets in SCD patients with hyposplenia [85].

Children with SCD presenting with a fever ≥101.3 °F or 38.5 °C should be promptly evaluated in a medical facility to receive parenteral antibiotics to provide coverage for *Streptococcus pneumoniae* and other potential pathogens. Complete blood count and blood and urine culture (if urinary tract infection is suspected) should be collected. While policies on how to manage febrile children with SCD may vary between institutions, in general after initial parenteral antibiotics, patients can subsequently be managed with or without oral antibiotics in children who are 1 year or older, clinically appear well, and can be followed reliably.

Penicillin Prophylaxis

Young children with SCD are at the greatest risk for bacteremia and sepsis resulting from absent or diminished splenic function which can start as early as the first year of life [86, 87]. Risk is especially high in very young children who may lack humoral immunity against *Pneumococcus*. The risk of infection in recent years has decreased significantly due to initiation of penicillin prophylaxis early after a child is diagnosed with SCD on newborn screening and immunization against *Pneumococcus* and other capsulated organisms [88]. The dose of penicillin is 125 mg PO BID which is then increased to 250 mg PO BID after 3 years of age. Erythromycin can be used in children allergic to penicillin. These measures along with parental education about the importance of fever and need for seeking urgent medical attention have contributed to improved outcomes. Current guidelines suggest that penicillin prophylaxis can be discontinued in children with SCD at age 5 years unless they have had a splenectomy or invasive pneumococcal infection [89]. However it is important to remember that compared to general population, older children and adults with SCD are at greater risk for invasive bacterial infection and should receive urgent medical attention in case of fever or any other concern for infection.

Immunizations

Various vaccine schedules have been adopted in different countries based on available resources, local epidemiology, cost-effectiveness, and legal issues [90]. All children should be immunized in accordance with their standard national immunization program unless medically contraindicated [90]. Additional vaccines to cover for *Pneumococcus* and *Meningococcus* are recommended for children with SCD. ***The Advisory Committee on Immunization Practices (ACIP) updates the immunization schedule annually; therefore readers should refer to their latest recommendations and also guidance for catchup vaccines in children with SCD***.

Children with SCD are at greater risk of pneumococcal infection [91]. Therefore, in the USA, according to the Advisory Committee on Immunization Practices (ACIP 2016), available at https://www.cdc.gov/vaccines/schedules, four injections of pneumococcal conjugate vaccine (PCV13) should be administered before 24 months of age (starting from >6 weeks of age; generally given at 2, 4, 6, and 12–15 months). PPSV23 (23-valent pneumococcal polysaccharide vaccine) is given at 24 months of age (at least 8 weeks after the last dose of PCV), and a second PPSV23 dose is given 5 years after the first dose with a third dose at age ≥65. It should be noted that practice of PPSV23 may vary among the intuitions and some institution may elect to revaccinate the adults with SCD. Providers however should be aware and counsel patients that SCD patients may be at higher risk for developing severe inflammatory reaction at the vaccination site and severe painful episode requiring hospitalization that can occur following PPSV23 vaccination [92]. SCD children particularly before 3 years of age are at higher risk of meningitis or sepsis compared to the general population [93]. It is recommended that children with SCD should be immunized against meningitis. The licensed meningococcal conjugate ACWY vaccines include MenHibrix, Menveo, and Menactra. MenHibrix (meningococcal groups C and Y and haemophilus b), a combination vaccine, is licensed for use in infants >6 weeks old. Children should receive four doses of the meningococcal vaccine at 6 weeks to 2, 4, 6, and 12–15 months. Menveo can be used in children >8 weeks and doses should be administered at 2, 4, 6, and 12 months of age. Menactra is given to children 24 months or older who have not received a complete series. Two doses of Menactra are administered at least 8 weeks apart and at least 4 weeks after completion of all PCV13 doses for children 24 months and older. Meningococcal B vaccines (Bexsero and Trumenba) are now available vaccine and should be given at 10 years to children who have not received a complete series. Children can receive a two-dose series of Bexsero, at least 1 month apart, or a three-dose series of Trumenba, with the second dose at least 2 months after the first and the third dose at least 6 months after the first. It is important to note that the two meningococcal B vaccines are not interchangeable. The same vaccine product must be used for all doses, or available guidelines should be utilized when combining two different vaccine products. The influenza vaccine should also be provided annually as viral influenza may cause severe morbidity in individuals with SCD [94]. The inhaled flu is not recommended. It is important to note that vaccine responses in SCD may be impaired with documented suboptimal responses to the influenza [95], pneumococcal [96, 97], and other vaccines [98, 99].

Fluids

Patients with SCD often receive additional fluids, regardless of hydration status, as fluids are perceived as beneficial although there is a paucity of data to support this established practice [100]. It is unclear which route (enteral vs. intravenous), amount, and type of fluid administration are optimal [100]. Water content of RBCs is a determinant of intracellular hemoglobin concentration. Even a modest increase in HbS concentration caused by RBC dehydration can precipitate HbS polymerization leading to loss of cell deformability because of the strong concentration dependence of the sickling process [101, 102]. Therefore it has been extrapolated that fluid administration may be beneficial in SCD. A common and early manifestation in SCD is hyposthenuria, an inability to concentrate urine due to progressive infarction of the vasa recta in the renal medulla; therefore patients are prone to dehydration especially in the setting of acute illness [103–105]. Historically, hyperhydration with IV fluids at 1.5 times maintenance has been routinely practiced for patients with VOC [106]. However patients receiving parenteral fluids should be monitored closely for iatrogenic congestive heart failure and electrolyte imbalance [107]. In acute chest syndrome, patients are given fluids at maintenance rate [108] as zealous hypotonic parenteral fluids predispose to pulmonary edema [109]. Furthermore overhydration (like dehydration) of RBCs leads to decreased cell deformability, and there appears to be a water content RBC range where RBCs exhibit optimal rheologic behaviors [110]. In a consensus statement, the NHLBI currently endorses that in euvolemic adults and children with SCD and VOC who are unable to drink fluids, intravenous hydration should be provided at maintenance rate to avoid overhydration. Evidence on the type of parenteral fluid to use in SCD is similarly lacking. As hyposthenuria may cause impaired renal excretion of the sodium load in normal saline, hypotonic solutions have been recommended [111]. However, the induction of hyponatremia hasn't been proven to be of benefit in the management of painful crises [112]. In a recent microfluidic model study of the human capillary system, normal saline was associated with stiffening of RBCs and prolonged transit times [113]. In summary, carefully conducted RCTs are required to elucidate optimal fluid management in patients with SCD.

Evolving Therapies

In recent year many mechanisms and pathways of sickle cell pathobiology have been explored. Understanding of the pathophysiology of SCD has evolved from a simplistic view of chronic hemolysis, vaso-occlusion, and ischemia. SCD is now recognized as an elaborately complex disease state involving several interacting vasculopathic processes consisting of ischemia-reperfusion injury, inflammation,

hemolysis, a procoagulant state, oxidative stress, deficiency of nitric oxide (NO), activation of endothelium, and altered vascular reactivity [114]. A burgeoning area of drug development is targeting these pathways directly to prevent or treat the complications associated with SCD. Some of these drugs under investigation are novel, while others have been used previously for similar or different indications in other diseases. A full account of these therapies is beyond the scope of this chapter and can be found in referenced review articles [115–117]. Some of the promising drugs categorized by their mechanism of actions are listed below. Refer to Chap. 2 for further discussion of many of these pathways being targeted toward drug development.

Drugs Targeting Adhesion

Sickle red cells and leukocytes adhere to endothelium in the microcirculation and contribute to vaso-occlusion [118, 119]. Endothelial selectins (P- and E-selectins) play a critical role in these adhesive interactions. In SCD, inflammatory cytokines upregulate the expression of endothelial E-selectin which along with P-selectin increases adhesion of leukocytes and capture of sickle red cells by leukocytes. The drugs targeting this pathway are attractive therapeutic options for vaso-occlusion. Several selectin inhibitors are currently under investigation. Some of the drugs in this category include IVIG, heparin (tinzaparin), propranolol, and others. Two drugs in this category (GMI-1070 and SelG1) are discussed below [117].

GMI-1070

Also known as rivipansel is a pan-selectin inhibitor initially shown to be effective in sickle mice in improving vaso-occlusion and survival [120]. A randomized, double-blind, placebo-controlled, phase II study of GMI-1070 recruited 12–60-year-old patients with SCD requiring hospitalization for the treatment of VOC. The primary end point of the study was time to resolution of VOC defined as one of the following: decrease in pain score 1.5 cm on visual analog scale from baseline, transition to oral analgesia, or discharge or readiness for hospital discharge. While the primary end point of resolution of vaso-occlusive pain crises was not statistically different between the treatment groups, it showed clinically meaningful reductions in mean and median times to VOC resolution in the GMI-1070 treatment group compared to the placebo group. GMI-1070 was safe without any differences in adverse events between the groups. Additionally mean cumulative IV opioid analgesic use reduced by 83% in patients treated with GMI-1070 [121]. Currently a phase III, multicenter, randomized, double-blind, placebo-controlled study of GMI-1070 is recruiting (clincialtrials.gov NCT02187003).

Crizanlizumab (SelG1)

Crizanlizumab is a humanized monoclonal antibody which binds to P-selectin and results in blockage of its interaction with P-selectin glycoprotein ligand 1 (PSGL-1). A recently completed clinical trial assessed safety and efficacy of crizanlizumab (SelG1) in a phase II, multicenter, randomized, placebo-controlled, double-blind, 12-month study in patients with SCD with or without HU and sickle cell-related pain [122]. Study participants were 16–65 years old with SCD who had 2–10 sickle cell-related pain crises in 12 months before the recruitment in the trial. Patients were assigned to low-dose crizanlizumab (2.5 mg/kg), high-dose crizanlizumab (5 mg/kg), or placebo. The primary goal of the study was to determine the effect of high-dose crizanlizumab on the rate of sickle cell-related crises during 52 weeks of treatment. Patients received two intravenous doses of crizanlizumab or placebo (loading doses) 2 weeks apart and then a maintenance dose every 4 weeks. At the end of the treatment phase, crizanlizumab therapy resulted in a significantly lower rate of sickle cell-related pain crises per year than placebo (1.63 with crizanlizumab vs. 2.98 with placebo). Crizanlizumab was well tolerated and was associated with a 10% rate of adverse events which included arthralgia, diarrhea, pruritus, vomiting, and chest pain [122]. None of the patients developed detectable antibody response during the trial; however long-term follow-up will be needed to detect emergence of late neutralizing antibodies which could limit long-term use of crizanlizumab.

Drugs Targeting Hemoglobin Polymerization

The hallmark of SCD is the polymerization of sickle hemoglobin which results in formation of rigid and sickled erythrocyte which leads to impaired transit of cells and vaso-occlusion. Sickle hemoglobin (HbS) has significantly reduced oxygen affinity as compared to normal hemoglobin (HbA) [123, 124]. Pharmacologic agents that can stabilize the higher oxygen affinity relaxed state (R-state) and/or destabilize the lower oxygen affinity T-state of hemoglobin have the potential to delay the sickling of circulating red cells and may be of clinical benefit. Several compounds with this capability have been described, one of which (GBT400) is being reviewed [125].

GBT440

GBT440 binds to the N-terminal of α-chain of hemoglobin and increases oxygen affinity which leads to reduced polymerization and sickling. In the murine model, this molecule given once daily orally was effective in increasing half-life of erythrocytes, reducing reticulocyte count and sickling (ex vivo) [126]. Currently patients are being recruited on a placebo-controlled study of GBT440 (clincialtrials.gov NCT03036813).

Other Mechanism-Based Therapies Under Investigation

Induction of hemoglobin F has been a long-standing disease-modifying strategy for SCD. Beneficial effects of hemoglobin F on the pathophysiology of SCD are well known, and this approach has been confirmed by the effects of HU therapy in SCD. Besides HU other drugs which augment hemoglobin F production have been in various stages of development and include agents such as decitabine, sodium dimethylbutyrate, vorinostat, and pomalidomide [127–130].

SCD is known to be an inflammatory state. Patients with SCD have chronically elevated white cell counts as well as pro-inflammatory cytokines including TNF, IL-1, and IL-8. In addition to damaged erythrocytes, other cells also contribute to a pro-inflammatory environment. Intravascular hemolysis and release of cell-free hemoglobin and hemin contribute to inflammation and so does increased production of placental growth factor which increases reactive oxygen species. Furthermore role-activated invariant natural killer T (iNKT) cell in SCD pathophysiology has been recently demonstrated and has led to development of a new group of targeted therapies. Drugs being tested in this general category include adenosine 2A receptor antagonist regadenoson; ADP receptor antagonist; ticlopidine, Anagregal; prasugrel; omega-3 acid ethyl esters; a-lipoic acid; *N*-acetyl cysteine; NKTT120; and others [131]. Prasugrel, a thienopyridine P2Y12 ADP receptor antagonist, inhibits ADP-mediated platelet activation and aggregation. Recently a phase II randomized, double-blind, placebo-controlled trial tested its efficacy in children 2 through 17 years of age with SCA who were randomly assigned to receive oral prasugrel or placebo for 9–24 months. The primary end point was rate of vaso-occlusive events which was found to be not significantly lower in the prasugrel compared to placebo [132]. Other agents in this category being tested include omega-3 fatty acid. Omega-3 fatty acid may play a role in reducing inflammation and is being tested for reducing the frequency of vaso-occlusive pain episodes [133]. IVIG has been proposed to inhibit adhesion and activation of leukocytes, and its effect on vaso-occlusive episodes is currently being evaluated in a phase I–II study [134].

Poloxamer-188, a nonionic surfactant with beneficial rheological and antithrombotic properties, was evaluated in a placebo-controlled trial to determine its impact on reducing the duration of the painful episodes. The study showed a significant but small difference between the groups with a 9-hour reduction of pain crisis in the treatment group ($P = 0.04$) [135]. The beneficial effect was more significant in children and in patients receiving HU leading to a recently completed phase III trial in children with SCD. L-arginine is a semi-essential amino acid and NO donor. NO has been implicated in SCD pathology which has been considered a NO-deficient state. Studies in transgenic mice demonstrated that inhibition of arginase improved NO bioavailability and attenuated systemic and pulmonary vascular endothelial dysfunction [136]. Currently, human studies evaluating effect of arginine on pain and other complication of SCD are in various stages of development (clinicaltrials.gov NCT02447874, NCT00513617, NCT01142219, NCT02536170, NCT00004412, NCT01796678, NCT00029731, NCT00056433).

In conclusion, recent advances in the management of SCD have led to its evolution from a life-threatening illness to a chronic disease. Interventions such as universal newborn screening, penicillin prophylaxis, vaccinations along with disease-modifying therapies such as HU and transfusions, and curative HSCT have improved outcomes dramatically for patients living with SCD in developed countries. FDA-approved disease-modifying drug HU has a favorable risk-to-benefit profile in reducing the acute and chronic manifestations of SCD and has a potential to be utilized to improve outcomes in low- and mid-income countries. Improved understanding of the pathophysiology of SCD in recent years has identified new pathways which are being targeted to prevent and treat complications of SCD. Novel therapeutic drugs targeting specific pathophysiologic processes are set to offer an era of mechanistic therapies in SCD.

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Chapter 4
Risk-Based Therapies for Sickle Cell Disease

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Abbreviations

ACS	Acute chest syndrome
ARC	Absolute reticulocyte count
CAR	Central African Republic
CSSCD	Cooperative Study of Sickle Cell Disease
G6PD	Glucose-6-phosphate dehydrogenase
GVHD	Graft-versus-host disease
HbF	Fetal hemoglobin
HbS	Sickle hemoglobin
HSCT	Hematopoietic stem cell transplant
SCA	Sickle cell anemia (HbSS or HbSβ^0thalassemia)
SCD	Sickle cell disease
SCI	Silent cerebral infarct
SITT	Silent infarct transfusion trial
SNP	Single-nucleotide polymorphism
STOP	Stroke prevention trial in sickle cell anemia
TCD	Transcranial Doppler
TNF-A	Tumor necrosis factor-alpha
VOC	Vaso-occlusive crisis

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Introduction

Sickle cell disease (SCD) describes a group of diseases where sickle hemoglobin (HbS) is the predominant adult-type hemoglobin. HbS is caused by a single-point mutation in the beta globin gene substituting a hydrophobic valine amino acid for glutamic acid at position 6 of the beta chain, making HbS molecules much more likely to polymerize in states of dehydration or acidosis. HbS polymerization causes the characteristic sickle shape change and downstream effects of sickling. Two-thirds of people with SCD produce only HbS, because they are either homozygous for the HbS mutation (HbSS) or compound heterozygous for the HbS and β^0thalassemia mutations (HbSβ^0thalassemia), and have sickle cell anemia (SCA) [1]. The remaining third of patients with SCD have other compound heterozygous β globin chain mutations, the two most common being HbSC and HbSβ^+thalassemia [1, 2]. Because SCA is typically more severe than other forms of SCD, the majority of the research that has been performed to identify risk markers has involved only people with SCA. Additionally, people with SCA are generally the only eligible participants for the experimental unrelated donor hematopoietic stem cell transplant (HSCT) where disease severity prediction is most needed because of the increased risk of graft-versus-host disease (GVHD) [3]. Hence, the remainder of this chapter will focus on disease severity predictors for people with SCA.

Sickle hemoglobinopathies are the most commonly identified serious disease via newborn screening in the United States, affecting an estimated 100,000 Americans [1]. In 1970, the median survival of a person with SCA was 20 years [4]. Universal newborn screening for SCD and improved supportive care like penicillin prophylaxis have almost eliminated childhood mortality from SCD today [5], but overall survival for people with SCA remains almost half that of the average American [6]. While some people with SCA will have few disease-related complications and have a normal lifespan, many will unfortunately suffer significant SCA complications like stroke, painful vaso-occlusive crisis (VOC), acute chest syndrome (ACS), and splenic sequestration and die as young adults. Identifying the infants and toddlers at highest risk for future SCA complications is an important gap in the care of pediatric SCA patients because if severity of SCA could be predicted early in life, disease-modifying therapies like HSCT could be instituted in high-risk patients prior to the onset of organ damage, while those with milder disease could be spared from potentially toxic therapies.

Importance of Severity Prediction for Children with Sickle Cell Disease

SCA is an inherited hemolytic anemia that leads to end-organ damage and shortened life expectancy [6, 7]. While the genotype of SCA is straightforward and well-defined, the clinical severity varies and is currently impossible to predict early in

life. Prediction of clinical severity and long-term outcome of SCA early in life is a significant clinical issue because early intervention using treatments like HSCT has been shown to prevent end-organ damage before serious complications occur [8, 9]. In fact, matched sibling HSCT in asymptomatic pediatric SCA patients is becoming more commonplace. However, clinical equipoise still exists regarding the ethics of HSCT for those children who have only minimal symptoms to date but who may be at risk for future severe complications (so-called "less severe" SCA) because HSCT places children at risk of significant complications like graft-versus-host disease (GVHD) and death [10].

Early trials of HSCT in SCA patients revealed that SCA patients have higher complication rates than other patients undergoing HSCT for nonmalignant hematologic disorders. Vascular occlusion and vasculopathy cause organ damage which is the most likely cause of discrepant mortality rates between children and adults with SCA who have HSCT [11]. One of the great ironies of SCA is that by the time a patient with SCA meets criteria for HSCT, he or she typically has experienced serious sequelae of the disease which increases the likelihood of morbidity and mortality during the HSCT. In a recent study of matched unrelated donor, reduced intensity HSCT for children and adolescents with SCA, GVHD, and mortality rates was directly associated with age [3]. Hence, the earlier a child can be identified as eligible for HSCT, the risk of HSCT-associated complications decreases. If a reliable predictive model for severity of SCA existed, HSCT could be done on those with greatest risk of poor outcomes, and prior to the onset of organ damage, which would result in improved survival rates. An additional challenge is the lack of a validated definition of severe SCA, though the use of eligibility for unrelated HSCT trials is commonly used as defining criteria for severe SCA [11]. Most studies discussed in this chapter selected stroke or death, number of VOCs or episodes of ACS, and abnormal or conditional TCD as outcome measures that were the proxies for severe SCA. Tables 4.1, 4.2, 4.3 and 4.4 list all of the predictors and outcomes studied.

Disease Severity Predictor or Association?

The definition of a severity predictor is important as we review the literature published to date. A true disease severity *predictor* would identify high-risk children prior to the onset of disease sequelae. Many published studies have looked for *associations* between laboratory variables, clinical exam findings or radiologic exam findings, and SCA outcome variables. These laboratory and clinical variables were usually obtained just prior to or at the time the individual presented with the outcome variable. In many cases, it is difficult to know what the presumed predictor's value was prior to the time of the event, since hemoglobin, reticulocyte count, and/or clinical exam can vary with time and often change dramatically with acute SCA complications. The presence of genetic polymorphisms could be considered a severity predictor since DNA is present at birth and doesn't change over time. The difficulty with genetic polymorphisms, however, is the cost of obtaining results and the

Table 4.1 Severity predictors studied in historical cohorts (assembled in the 1970s–1980s)

First author, year	Predictor	Predicts SCA complication	Does not predict SCA complication
Clinical and laboratory factors			
Odenheimer DJ, 1987 [12]	• HbF	Hospitalization, transfusion	VOC
Lande WM, 1988 [13]	• Red cell deformability (in patients older than 8 years of age) when adjusted for alpha globin gene number and HbF level	Higher red cell deformability associated with more VOC	–
	• Hemoglobin when adjusted for alpha globin gene number and HbF level	Higher hemoglobin associated with higher rate of VOC	–
Kinney TR, 1999 [14]	• Hemoglobin • Alpha thalassemia • Beta globin gene haplotype • WBC count • Pocked red cell count • AST • Reticulocyte percentage • Total bilirubin • Blood Pressure • Growth parameters • Number of pain crises • Dactylitis • History of seizures	Patients at increased risk of SCI if they have: • Low VOC rate • History of seizure • WBC of at least 11.8 K/μL • SEN Beta globin gene haplotype	• Dactylitis was not associated with an increased risk of SCI • No association of SCI and BP (systolic or diastolic), height, weight, or growth velocity • No association between SCI and platelet count, HbF level, retic percentage, serum AST, total bilirubin, or presence of alpha thalassemia
Miller ST, 2000 [15]	• Increased WBC • Dactylitis before age 1 year • Anemia	Associated with: • 2 or more VOC annually • 1 or more episodes of ACS annually • Stroke • Death	–
Boyd JH, 2006 [16]	Asthma	• Twice as many ACS episodes and more frequent VOC in children who had SCA + asthma (and younger at time of ACS)	–
Uong EC, 2006 [17]	Pulse oximetry	–	No association with number of VOC and ACS episodes and pulse oximetry levels

Table 4.1 (continued)

First author, year	Predictor	Predicts SCA complication	Does not predict SCA complication
Field JJ, 2008 [18]	Sibling history of asthma	• Sibling history of asthma was associated with a greater rate of VOC	Parental history of asthma not associated with increased rate of VOC
Meier ER, 2014 [19]	ARC and hemoglobin between 2 and 6 months of age	• Stroke • Death	–
	WBC and dactylitis between 2 and 6 months of age	–	• Stroke • Death
Genetic polymorphisms			
Hoppe C, 2004 [20]	Polymorphisms in vascular disease genes	• *IL4R* 503, *TNF-A*(−308) and *ADRB2* 27 alleles were associated with increase large vessel stroke • Variants in *VCAM1*(−1594) and *LDLR Ncol* genes were associated with small vessel stroke • Combination of *TNF-A*(−308)GG homozygosity and the *IL4R* 503P variant was associated with a particularly strong predisposition to large vessel stroke	–
Carpenter SL, 2008 [21]	*UGT1A1* polymorphisms [wild type: 6 tandem repeats (6); variant alleles have 5, 7, or 8 repeats (5), (7) (8); homozygosity for 7 repeats is found in Gilbert syndrome (7/7)]	• Genotypes 6/8, 7/7, and 7/8 have the most hyperbilirubinemia that increases with age • Heterozygous 6/7 genotype leads to an intermediate level of hyperbilirubinemia • Genotypes 7/7 and 6/7 had an increased odds of developing gall bladder disease compared with wild type	–

ACS acute chest syndrome, *ARC* absolute reticulocyte count, *AST* aspartate aminotransferase, *BP* blood pressure, *HbF* fetal hemoglobin, *SCI* silent cerebral infarction, *UGT1A1* UDP glucuronosyltransferase 1 family, *VOC* vaso-occlusive crisis, *WBC* white blood cell count

Table 4.2 Predictors studied in modern cohorts (universal newborn screening and TCD screening routinely performed, penicillin prophylaxis administered)

First author, year	Predictor	Predicts SCA complication	Does not predict SCA complication
Clinical and laboratory factors			
Rees DC, 2008 [22]	Age AST Creatinine Hemoglobin LDH WBC count	Abnormal or conditional TCD TAMMV = 220 + (8^a hemoglobin) – (1.4^a age) + (0.4^a AST)	–
Quinn CT, 2009 [23]	SpO2	Lower SpO2 associated with abnormal and conditional TCD	–
Bernaudin F, 2011 [24]	Reticulocyte count and LDH	Baseline reticulocyte count and LDH levels were independent predictive factors for all cerebral vasculopathy (strokes, abnormal TCD, stenosis, silent strokes)	–
DeBaun MR, 2012 [25]	Hemoglobin Systolic BP Gender HbF Diastolic BP WBC O2 sat Pain rate Frequent headaches ACS rate	Increased risk of SCI associated with: • Baseline hemoglobin less than 7.6 g/dL • Baseline systolic BP 113 mmHg or greater • Male gender	–
Meier ER, 2013 [26]	ARC between 2 and 6 months of age	Increased ARC associated with hospitalization for splenic sequestration or VOC before 3 years of age	Increased ARC was not associated with hospitalization for ACS before 3 years of age
	Hemoglobin and WBC between 2 and 6 months of age	–	Low hemoglobin and high WBC not associated with hospitalization for splenic sequestration, ACS, or VOC before 3 years of age
DeBaun MR, 2014 [27]	Asthma risk factors	Wheezing causing SOB associated with future risk of ACS. Obstructive lung disease when tested with spirometry also associated with future ACS Two or more positive skin tests also predicts future ACS	–
	History of ACS early in life	ACS episode before 4 years of age was associated with future ACS	
Vance LD, 2015 [28]	Early ACS (between the ages of 1 and 4 years)	Early ACS is associated rehospitalization for VOC within 2 years	–

Table 4.2 (continued)

First author, year	Predictor	Predicts SCA complication	Does not predict SCA complication
Genetic polymorphisms			
Belisario AR, 2010 [29]	Alpha thalassemia	Risk of cerebrovascular disease 3.9 times higher in those four alpha globin genes than in those with one or two alpha globin gene deletions	–
Filho IL, 2011 [30]	Alpha thalassemia	–	Did not predict cerebrovascular disease
	Beta globin gene haplotype	Bantu/atypical βS-globin gene haplotype had 15 times higher chance of cerebrovascular disease (defined as stroke, TIA, abnormal TCD) than other beta globin haplotypes	–
	MTHFR Factor V Leiden Prothrombin Gene Mutation	–	Did not predict cerebrovascular disease
Flanagan JM, 2011 [31]	Alpha thalassemia	Associated with decreased stroke risk	–
	Candidate polymorphisms	SNPs in the *ANXA2*, *TGFBR3*, and *TEK* genes were associated with increased stroke risk *ADCY9* SNPs associated with decreased stroke risk	–
	G6PD A-variant deficiency	–	Not linked to stroke risk
	Beta globin gene haplotype	–	Not associated with stroke risk
Bean CJ, 2012 [32]	Heme oxygenase 1 (*HMOX1*) allele length	–	No associations between repeat length OR *HMOX1* genotype and rate of VOC identified
Thangarajh M, 2012 [33]	Number of alpha globin genes	–	Not associated with SCI
	G6PD deficiency	Associated with CNS vasculopathy, which was associated with SCI	–
Bean CJ, 2013 [34]	Beta globin locus haplotype[a]	H3 (increased HbF) had significantly lower rates of hospitalizations for ACS after correcting for asthma, gender, alpha thalassemia, and *HMOX1* promoter repeat class. H2 and H1 groups had similar levels of hospitalization for ACS	–

(continued)

Table 4.2 (continued)

First author, year	Predictor	Predicts SCA complication	Does not predict SCA complication
Sheehan VA, 2013 [35]	G6PD deficiency	Fewer VOC	Dactylitis or number of ACS
	Number of alpha globin genes	Alpha thalassemia trait trend toward increased VOC	–
	BCL11A polymorphisms associated with higher HbF	Fewer VOC	–
	Variant *UGT1A1* alleles	More gallbladder sludge and stones	–
	Hemoglobin, HbF	–	Low hemoglobin and HbF levels associated with increased risk of stroke, abnormal or conditional TCD

ACS acute chest syndrome, *ARC* absolute reticulocyte count, *AST* aspartate aminotransferase, *AUC* area under the curve, *BP* blood pressure, *CNS* central nervous system, *G6PD* glucose-6-phosphate dehydrogenase, *HbF* fetal hemoglobin, *LDH* lactate dehydrogenase, *MTHFR* methylenetetrahydrofolate reductase, *SCI* silent cerebral infarction, *sNPs* single-nucleotide polymorphisms, *sPO2* oxygen saturation, *SOB* shortness of breath, *TAMMV* time-averaged mean maximum velocity, *TCD* transcranial Doppler, *TIA* transient ischemic attack, *UGT1A1* UDP glucuronosyltransferase 1 family, *VOC* vaso-occlusive crisis, *WBC* white blood cell count

[a]H1 is the most frequent haplotype and served as the reference. Carrying one or two H2 alleles was associated with lowest HbF levels, while carrying one or two H3 alleles was associated with highest HbF levels. Carrying one or two H1 alleles was associated with average HbF

Table 4.3 Combination of pre- and post-penicillin prophylaxis, TCD screening, and newborn screening

First author, year	Predictor	Predicts SCA severity	Does not predict SCA severity
Clinical and laboratory factors			
Adams R, 1992 [36]	TCD velocity	Higher TCD velocities are associated with higher rate of overt stroke	–
Adams RJ, 1997 [37]	Age Hematocrit Reticulocyte count WBC Abnormal TCD	Age and hematocrit negatively correlated with TCD velocity Higher WBC and reticulocyte counts were associated with higher TCD velocities 40% of patients with abnormal TCD had stroke, compared to 2% of patients with conditional or normal TCD	–
Tam DA, 1997 [38]	Protein C and S activity levels	Levels decreased in children with stroke	–
Adams RJ, 1998 [39]	Abnormal TCD	Higher TCD velocities are associated with higher rate of overt stroke	–

Table 4.3 (continued)

First author, year	Predictor	Predicts SCA severity	Does not predict SCA severity
Adams RJ, 2004 [40]	TCD velocity	Risk of stroke with abnormal TCD was higher than normal and conditional TCD Stroke risk with conditional but not inadequate TCD is higher than with normal Higher TCD velocities indicate both higher risk and more proximate risk for stroke	–
Kwiatkowski J, 2006 [41]	ACA velocities 170 cm/s or greater	ACA velocities 170 cm/sec or greater associated with increased risk of stroke after adjusting for ICA/MCA classification	–
Quinn CT, 2008 [42]	SpO2	Lower SpO2 associated with higher risk of stroke	–
Bhatnagar P, 2013 [43]	HbF	Meta-analysis revealed that increased HbF levels are associated with decreased risk of VOC	–
	Hematocrit	Higher hematocrit strongly associated with VOC in SITT and CSSCD cohorts	–
	Age	Older age associated with VOC in SITT and CSSCD cohorts	–
	Gender	–	Not associated with VOC
Genetic polymorphisms			
Adams RJ, 1994 [44]	Alpha globin gene number	Absence of alpha thalassemia associated with increased stroke risk when controlling for age and HbF levels	–
Styles LA, 2000 [45]	HLA alleles	DRB1*0301 and *0302 were both associated with an increased risk of both overt stroke and SCI Protective associations were found in the DR2 group, where DRB1*1501 was protective for both overt stroke and SCI	–
Hoppe C, 2001 [46]	60 polymorphisms in 34 vascular disease genes	*CBS* 278thr variant allele showed protection from stroke *apoE3* allele showed trend toward increased risk of stroke as did *TNF-A*(−308)A, *CETP*(−628)A, *apoCIII*(−641)A	–
Sarnaik SA, 2001 [47]	Number of alpha globin genes	Three quarters of patients with stroke did not have alpha thalassemia, and none had two alpha globin gene deletions	–
	Beta globin gene haplotype	Stroke is most prevalent in Ben/CAR haplotype	–

(continued)

Table 4.3 (continued)

First author, year	Predictor	Predicts SCA severity	Does not predict SCA severity
Hsu LL, 2003 [48]	Number of alpha globin genes	Alpha thal-2 (α-/αα or α-/α-) protective against abnormal TCD	–
Hoppe C, 2007 [49]	104 polymorphisms among 65 vascular disease genes	*TNF*(−308)GG genotype associated with more than threefold increased risk of large vessel disease *TC4S*(−444)A/C variant also associated with large vessel stroke risk	–
Hyacinth HI, 2012 [50]	BDNF	Mean BDNF significantly higher in SCA patients with abnormal TCD	–
	PDGF-AA and PDGF-AB/BB	PDGF-AA higher in SCA patients who developed stroke	–
Joly P, 2015 [51]	Alpha globin gene number	Alpha thalassemia trait protects against cerebral vasculopathy	–
	G6PD deficiency	G6PD deficiency more common in group with stroke, SCI, or abnormal TCD	–

ACA anterior cerebral artery, *BDNF* brain derived neurotropic factor, *CSSCD* cooperative study of sickle cell disease, *CVA* cerebrovascular accident, *G6PD* glucose-6-phosphate dehydrogenase, *HbF* fetal hemoglobin, *HLA* human leukocyte antigen, *ICA* internal carotid artery, *MCA* middle cerebral artery, *PDGF-AA* and *PDGF-AB/BB* platelet-derived growth factors-AA and platelet-derived growth factors-AB/BB, *SCI* silent cerebral infarct, *SITT* silent infarct transfusion trial, *sPO2* oxygen saturation, *TCD* transcranial Doppler, *abnormal TCD* MCA or ICA velocities of ≥200 cm/s, *TNF* tumor necrosis factor, *VOC* vaso-occlusive crisis, *WBC* white blood cell count

Table 4.4 Unknown TCD screening, penicillin prophylaxis, and newborn screening

First author, year	Predictor	Predicts SCA complication	Does not predict SCA complication
Clinical and laboratory factors			
Stevens MC, 1981 [52]	HbF tertiles • Low: less than 21% • Medium: 21–27.5% • High: greater than 27.5%	HbF levels significantly lower in patients who had dactylitis, early splenomegaly, acute splenic sequestration, and death	–
Bailey K, 1992 [53]	HbF level at age 5 years	Higher HbF levels associated with a lower frequency and later onset of ACS, dactylitis, VOC, and acute splenic sequestration	–
Thomas PW, 1997 [54]	Number of alpha globin genes	Patients with a normal complement of alpha globin genes were less likely to have frequent VOC dactylitis and bone necrosis	–
	HbF	Patients with higher HbF levels more likely to have benign disease	–

Table 4.4 (continued)

First author, year	Predictor	Predicts SCA complication	Does not predict SCA complication
Foucan L, 2006 [55]	Dactylitis	Dactylitis before 6 months of age associated with increased risk of severe SCA complications	–
Silva CM, 2011 [56]	Reticulocyte count	Higher reticulocyte count is associated with higher rates of stroke and abnormal or conditional TCD	–
Al-Saqladi AWM, 2012 [57]	Serum transferrin receptor	–	No difference in mean serum transferrin receptor levels between those with mild and severe disease
	C-reactive protein, serum amyloid A	–	Not associated with severe SCA
Lagunju IO, 2014 [58]	Hemoglobin Age sPO2	Low hemoglobin, young age, and low sPO2 (less than 95%) predicted increased TCD velocities	–
Genetic polymorphisms			
Bernaudin F, 2008 [59]	Alpha gene number Beta globin gene haplotype G6PD deficiency LDH MCV Reticulocyte count WBC count	G6PD deficiency, normal number of alpha globin genes, lower hemoglobin, and higher LDH levels remained as independent predictors of high TCD velocities in multivariate logistic regression analysis	Beta globin gene haplotype, WBC, and HbF levels had no effect on risk for high TCD velocities
Belisário AR, 2010 [60]	Beta globin gene haplotype	–	β^S-haplotypes CAR and Ben were not associated with rates of ACS, abnormal or conditional TCD, acute splenic sequestration, or number of blood transfusions
Cajado C, 2011 [61]	$alpha_2$-$thal^{3.7kb}$ *TNFα*-308G > A and *IL8*-251A > T polymorphisms	*TNFα*-308G > A polymorphism and the presence of $alpha_2$-$thal^{3.7kb}$ associated with increased risk of splenic sequestration *IL8*-251A > T polymorphism protective for splenomegaly	*TNFα*-308G > A and *IL8*-251A > T polymorphisms were not associated with rate of VOC, ACS, or stroke
Redha NA, 2014 [62]	*VEGF* polymorphism	Enrichment of -583 T/T genotypes (associated with decreased VEGF levels) in patients with ACS	–

(continued)

Table 4.4 (continued)

First author, year	Predictor	Predicts SCA complication	Does not predict SCA complication
Badr AK, 2015 [63]	HbF	HbF level negatively correlated with frequency of VOC and ACS. HbF positively correlated with splenomegaly	–
Silva IV, 2015 [64]	WBC greater than 15 K/μL	Associated with a higher number of hospitalizations and chronic SCA complications	–
	Dactylitis in the first year of life	Associated with a higher number of hospitalizations	–
	Presence of alpha thalassemia trait	Associated with a lower number of chronic complications	–
	Hemoglobin, HbF	–	Not associated with hospitalizations or chronic complications

ACS acute chest syndrome, *G6PD* glucose-6-phosphate dehydrogenase, *HbF* fetal hemoglobin, *IL8* interleukin 8, *LDH* lactate dehydrogenase, *LV* left ventricle, *MCV* mean corpuscular volume, *PMN* polysegmented mononuclear cells, *sPO2* oxygen saturation, *TCD* transcranial Doppler, *TNFα* tumor necrosis factor-alpha, *VOC* vaso-occlusive crisis, *WBC* white blood cell count

varying level of willingness for insurance companies to cover the costs. The lack of a single predictor of overall SCA severity can be applied to genetic polymorphisms as well since certain single-nucleotide polymorphisms (SNPs) are "more likely" to be associated with different disease complications but do not, in general, predict overall disease severity.

The ideal scenario for children with SCA would be one similar to childhood acute lymphoblastic leukemia risk stratification. Although pediatric oncologists are unable to predict which children will ultimately develop leukemia, those who unfortunately do are put into risk categories, and treatment intensity varies with risk level: those children with low-risk disease receive less intense chemotherapy regimens, while the highest-risk patients receive the most intense chemotherapy regimens and are watched more closely for relapse and complications. This has led to improved survival and decreased late effects of therapy in the low-risk and standard-risk groups [65]. As the number of therapies for SCA increases, pediatric hematologists should strive to have the same ability to personalize treatment for children with SCA as we do children with oncologic diseases.

Historical Attempts to Identify Severity Predictors

The sickle mutation arose in order to protect against malarial infection. Haplotype studies of β globin have revealed that the sickle mutation likely developed independently in five distinct geographic regions where malaria is endemic—4 in Africa and

1 in India. These haplotype studies began because of the heterogeneity in both HbF levels and clinical phenotype noted by clinicians over three decades ago [66]. Researchers identified unique β globin haplotypes from four geographical regions of Africa, Benin, Bantu/Central African Republic (CAR), Senegal, and Cameroon, and India from which HbS mutations arose [67]. Despite the initial hypotheses that SCA severity and β globin haplotype were associated with HbF variation, subsequent studies have not found this to be the case [67, 68]. Further, clinical severity does not differ between the four African β globin haplotypes [67, 68]. Migration patterns because of slavery and other reasons explain the diversity of haplotypes now seen throughout the world (Fig. 4.1) [67]. Because of a lack of clear association with severe clinical manifestations and the blending of these haplotypes globally, β globin haplotypes alone are not useful in disease severity prediction for people with SCA.

The search for an explanation of the varying severity among patients with SCA began in the United States in the 1970s when President Richard Nixon signed the National Sickle Cell Anemia Control Act into law and funding became available for more research [69]. The Cooperative Study of Sickle Cell Disease (CSSCD) was funded through this legislation and initiated by the National Heart, Lung, and Blood Institute in the late 1970s. The goals of the CSSCD were to describe the natural history of SCA, identify risk factors for complications, and understand the factors that impact the clinical variability [70].

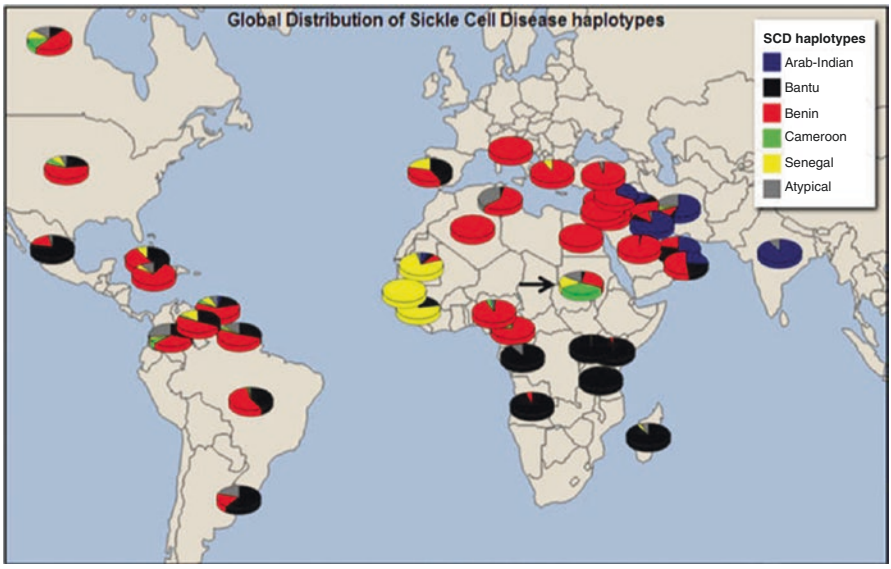

Fig. 4.1 Global distribution of haplotypes among various world SCD populations. Previously reported data allow drawing a global haplotype distribution that was consistent with known historical migrations of people of Africa. Among Indigenous African populations published data from Sudan (*arrow*) showed a distinctly unusual pattern; all four classical haplotypes were reported, with an exceptionally high proportion of the Senegal and Cameroon haplotypes. Permissions acquired from OMICS. A Journal of Integrative Biology via Copyright Clearance Center

From 1978 through 1988, 380 newborns with SCA were enrolled in the CSSCD before age 6 months. Dactylitis before 1 year of age, baseline hemoglobin <7 g/dL in the second year of life, and baseline leukocytosis in the second year of life were found to be predictive of death, stroke, frequent painful crises, and recurrent acute chest syndrome [15]. Increased reticulocytosis (absolute reticulocyte count [ARC]) and decreased hemoglobin, but not white blood cell count or dactylitis, between 2 and 6 months of age, were associated with higher rates of stroke and death in the CSSCD newborn cohort. In multivariate analysis, only ARC was independently associated with stroke or death ($p = 0.011$) [19]. Most deaths in this newborn cohort were due to infection or stroke, complications that have become increasingly rarer with improvements in supportive care of children with SCA.

In another study from the CSSCD cohort, pediatric patients who had asthma as a comorbid condition had twice as many episodes of ACS and more frequent VOC as those children who did not have asthma [16]. Sibling history of asthma was also found to be associated with a higher pain rate in the CSSCD newborn cohort [18]. Pulse oximetry levels, however, were not associated with the number of VOC or ACS in the newborn cohort of the CSSCD [17]. Polymorphisms of bilirubin UDP glucuronosyltransferase 1A1 (*UGT1A1*) and genes associated with vascular disease were performed on banked samples from patients enrolled in the CSSCD. Specific *UGT1A1* polymorphisms were associated with more hepatobiliary disease [21]. SNPs in inflammatory and adhesion pathways were associated with both large and small vessel strokes, respectively [20] (see Table 4.1 for specific SNPs).

Two studies were performed in cohorts assembled in a single academic institution during the years that the CSSCD was being conducted [12, 13]. One of these studies found that lower HbF levels were associated with higher rates of transfusion and hospitalization for all causes but not rate of painful crises [12]. The second correlated red cell deformability during steady state with incidence of VOC for a mean follow-up period of 6.8 years. Enrolled patients who had more deformable erythrocytes at steady state had higher rates of VOC in the future [13].

Risk Prediction in the Modern Era

Fifteen studies have been performed after universal newborn screening, penicillin prophylaxis, and routine TCD screening were implemented. These three interventions decreased SCA mortality and lead to the current expectation that over 95% of children who are born with SCA today will survive into adulthood [5]. Nearly half (7/15) of these studies evaluated genetic predictors for SCA severity with mixed results [29–35]. Alpha globin gene number was not associated with increased risk of cerebrovascular disease in three studies [30, 33, 56], while having one or two alpha globin genes deleted was protective against cerebrovascular disease in two other cohorts of patients [29, 31]. Similarly, glucose-6-phosphate dehydrogenase

(G6PD) deficiency was associated with cerebrovascular disease in one study [33] but not in another [31]. See Table 4.2 for other genetic risk factors that were studied.

The remaining eight studies in this category evaluated different laboratory markers as well as personal and family medical history risk factors [22–28, 71]. Family or personal history of asthma consistently was associated with an increased risk of ACS and/or VOC [23, 27, 28]. Steady-state ARC above 200 K/μL between 2 and 6 months of age also was consistently associated with more severe SCA as defined by early hospitalization or cerebrovascular disease [24, 26, 56].

Risk Stratification When Universal Newborn Screening, Penicillin Prophylaxis, and Routine TCD Screening Utilization Were Inconsistent or Unknown

Sixteen studies were performed during the time when not all states had implemented newborn screening and routine penicillin prophylaxis, or before routine TCD was applied for stroke screening [36–51] (Table 4.3). The 13 studies in the "unknown" category for these supportive care initiatives were all performed in cohorts outside of the United States [52–64] (Table 4.4). Of the 16 studies performed in the time before TCD screening was implemented, 6 (38%) were performed in the Stroke Prevention Trial in Sickle Cell Anemia (STOP) study cohort [36, 37, 39–41, 48]. Five of these six studies involved TCD, the only validated screening test that identifies pediatric patients at the highest risk of stroke [36, 37, 39–41]. The presence of four alpha globin genes was associated with an increased risk of stroke in a case control study prior to the STOP study [44] which was validated by Hsu et al. [48] in the STOP cohort and more recently in a single-center cohort [48, 51]. A meta-analysis of the CSSCD and SITT cohorts confirmed the protective effect of increasing HbF levels in preventing VOC [43]. Analyses of genetic polymorphisms and their effects on stroke risk were also evaluated in this time frame. A tumor necrosis factor-alpha (*TNF-A*) SNP was associated with an increased risk of stroke, along with other genes known to be associated with vascular disease [46, 49, 50].

The studies with unknown preventive care programs were performed in the Caribbean, South America, Europe, Asia, and Africa. Three of these 13 studies were performed in Jamaica. Nearly one-third (4/13) evaluated HbF levels; 75% of these found increased HbF to be protective against severe SCA complications including VOC, ACS, and death [52–54]. One of the four did not find an association between higher HbF levels and fewer hospitalizations or chronic complications (retinopathy, leg ulcers, left ventricular dilatation, cholelithiasis, enuresis, delayed puberty) [64]. Alpha globin gene number was evaluated in this group of studies, and the results were again mixed. Thomas et al. reported that patients with a full complement of alpha globin genes were more likely to have a benign disease course than patients with one or two alpha globin gene deletions [54]. Alternatively, alpha thalassemia

trait was found to be protective against abnormal TCD velocities and other chronic sickle-related complications [56, 59, 61]. Increasing reticulocytosis, low hemoglobin, and low oxygen saturation were associated with increased TCD velocities [56, 58]. Beta globin gene haplotype was not associated with the number of ACS, blood transfusions, episodes of splenic sequestration, strokes, or TCD velocities [60].

Summary of the Predictive Value of the Three Most Commonly Studied Predictive Variables

HbF as a Predictor of SCA Complications

HbF interferes with HbS polymerization, thus preventing erythrocyte sickling, hemolysis, and vaso-occlusion. High HbF levels have been associated with lower mortality levels in adults with SCA and fewer clinical complications [7]. Infants with SCA do not typically experience SCA symptomatology until HbF begins to decrease after 6 months of age. Initiation of HU during late infancy, when HbF levels are typically above 20%, is associated with fewer clinical complications of SCA [8]. Practicing hematologists around the globe agree that HbF protects against SCA-related complications [68]. Thus, it is not surprising that one-fifth of all studies included in this chapter evaluated the association of HbF levels with SCA complications [12, 14, 25, 35, 43, 52–54, 59, 63, 64]. Over half (6/11) of the studies were performed outside of the United States and did not clearly state if the newborn screening, penicillin prophylaxis, and TCD screening were being used as supportive care measures [52–54, 59, 63, 64]. Five studies, both retrospective and prospective, found that higher HbF levels were associated with fewer clinical complications like ACS, dactylitis, VOC, and splenic sequestration as expected [43, 52–54, 63]. All but one study [12] reported that high HbF protected against VOC [35, 43, 52, 53, 63]. Each study utilized a different definition of high HbF, but clinical and laboratory studies have found that HbF levels above 20% significantly decrease the rate of HbS polymerization, and thus a HbF of at least 20% should be the therapy goal utilized by treating clinicians [52, 72–74].

Surprisingly, four of the eleven studies did not find a protective benefit for higher HbF levels [14, 25, 59, 64]; three of these four negative studies involved CNS complications [14, 25, 59]. These studies that conflict with what is widely held as truth in the pediatric hematology community are when the distinction between association and severity prediction becomes very important. Since age is an important factor in HbF levels, standardizing the age at which HbF levels are measured and compared to outcomes is important. Additionally, standardized follow-up for outcome measures is also important so that a narrow window exists to help researchers pinpoint when the outcome occurred and its relationship to the predictor. These variables were not standardized in the studies that did not find a protective effect for high HbF levels. Three of the studies evaluated HbF levels at varying ages (2–6 years of age in one [14] and an even wider range in two others [25, 59]), and the outcome

variable for all three of these studies was silent cerebral infarct (SCI). Since SCIs are known to occur very early in life, with over 10% of 1 year olds who were screened with brain MRI/MRAs in the BABYHUG study having SCI [75], it is difficult to know when the SCI occurred and what the HbF level was at that time. In the silent infarct transfusion (SIT) trial, HbF levels obtained after 3 years of age in the children who did not have SCI were slightly higher, though not statistically significant, than the children who suffered SCI (mean HbF level in non-SCI group 12.5 ± 9.6% vs. 11.1 vs. 9.9% in the SCI group, $p = 0.055$) [25]. Therefore, it is difficult to conclusively say that lower HbF levels are not associated with SCI since the time of the SCI and the level of HbF preceding the SCI are not known based on the designs of these studies. The third negative study [64] utilized the Kleihauer-Betke method to measure HbF levels which is considered a semiquantitative, highly operator-dependent method and is well-known to provide highly variable results, with reported interobserver F-cell quantitation agreement as low as 46% [76].

Another negative study found that the presence of known HbF genetic modifiers (like *BCL11A*) was not associated with TCD velocities. The presence of genetic modifiers for HbF levels was, however, associated with fewer episodes of VOC. Genetic analysis would eliminate the age-related variability of HbF levels, but since *BCL11A* is only responsible for 20–50% of the variability in HbF levels in humans, not all of the variability can be explained by the presence or absence of this SNP [77]. Results were also mixed in a single-center study of 140 patients that reported that higher HbF levels protected against hospitalization (13.8 ± 5.3% in those not hospitalized vs. 8.7 ± 3.8% in those requiring hospitalization, $p < 0.001$) and transfusion (11.0 ± 4.4% in those who needed a transfusion vs. 7.4 ± 3.4% in those who did not require transfusion, $p = 0.01$) but not VOC (11.4 ± 7.3% in those who did not have VOC vs. 9.1 ± 3.8% in those who had VOC, $p = 0.17$) [12]. This study highlights two consistent problems with disease severity prediction studies. First, the laboratory values studied may find statistically significant differences, but those differences may be very difficult to apply clinically when seeing a patient and making treatment decisions, given the wide standard deviation of the HbF values reported in this study. Secondly, the lack of a formal definition of severe SCA makes rigorous study for predictors challenging; the methodology utilized in this study relied upon participants' parents' report if their child had been hospitalized and transfused or had VOC lasting more than 24 h instead of a chart review looking for these complications. Hospitalization and transfusion were most likely easier to remember than the number of VOCs which families may have tried to manage at home.

Alpha Globin Gene Number as a Predictor of SCA-Related Complications

Fourteen studies evaluated the association between the number of alpha globin genes and clinical complications, primarily cerebrovascular disease [14, 29–31, 33, 35, 44, 47, 48, 51, 59, 61, 64]. Five of the fourteen studies were performed in children who benefited from universal newborn screening, early penicillin prophylaxis,

and TCD screening [30, 31, 33, 35, 60]. Ten of the fourteen (71.4%) reported protective effects of two or three alpha globin genes instead of the full complement of four alpha globin genes [29, 31, 44, 47, 48, 51, 54, 59, 61, 64]. Seven of these studies revealed that one or two gene deletion alpha thalassemia is protective from stroke, abnormal TCD, or SCI [29, 31, 44, 47, 48, 51, 59]. Three studies, however, found that alpha thalassemia offered no protection against cerebrovascular disease [14, 30, 33]. The presence of alpha thalassemia on non-cerebrovascular complications of SCA such as VOC and splenic sequestration was more consistent, with three of the four studies reporting that children missing one or two alpha globin genes had more SCA-related complications [35, 54, 61]. A small single-center retrospective study reported that alpha thalassemia trait was protective against chronic complications of SCA that are not commonly thought of as markers for severe SCA in children (cholelithiasis, enuresis, delayed puberty, cardiac ejection fraction <55%, obstructive lung disease) [64].

The majority of studies evaluating the association between the number of alpha globin genes and cerebrovascular disease found that one or two alpha globin gene deletions were protective against large vessel cerebrovascular disease (overt stroke and abnormal TCD) but did not protect against SCI. One reason for this could be the different pathophysiology of SCI and overt stroke. Since SCI is considered a disease of the small cerebral vasculature and overt stroke and abnormal TCD are usually due to large vessel vasculopathy, one could make the argument that alpha thalassemia (either one or two gene deletions) is protective against large vessel cerebral disease, but its protective effect on small vessel disease (e.g., SCI) still remains unknown.

Alpha thalassemia trait does seem to increase the risk of VOC and other non-cerebrovascular complications of SCA. In all but one study evaluating the number of alpha globin genes, one or two missing alpha globin genes were associated with a higher rate of VOC and splenic sequestration. The lone outlier study was a small, retrospective evaluation of patients from a single center, which was also limited by the choice of outcome variables (cholelithiasis, enuresis, delayed puberty, cardiac ejection fraction <55%, obstructive lung disease) not routinely used as proxies for severe SCA because the etiology of these complications is commonly multifactorial [64]. For example, obstructive lung disease could be due to SCA but also could be a result of asthma which is a common comorbidity in children with SCA [78]. Because of the numerous limitations of this one outlier study, alpha thalassemia may be viewed as a predictor for VOC in children with SCA given that two studies in large cohorts of children with SCA support this finding.

Reticulocyte Count as a Predictor of SCA Severity

Six studies have evaluated reticulocyte count as a predictor of SCA severity [14, 19, 24, 26, 44, 56]. Two studies included children who received universal newborn screening, early penicillin prophylaxis, and routine TCD screening [26, 59], and

two studies utilized data from children enrolled in the CSSCD [14, 19]. Half of the studies of reticulocyte count utilized a retrospective design [26, 37, 56]. Only one study found that increased reticulocyte percentage was not associated with the outcome variable of interest, in this case SCI [14]. Two other studies utilized reticulocyte percentage, however, and found an association with increasing reticulocytosis and higher TCD velocities and stroke [44, 56]. The remaining three studies utilized the ARC as a marker for SCA severity and found that higher ARC was associated with cerebral vasculopathy (including stroke), early hospitalization for VOC, and splenic sequestration but not ACS and death [19, 24, 26]. Two of these utilized ARC between 2 and 6 months of age which is prior to the onset of SCA complications, allowing them to truly utilize ARC as a predictor for SCA severity [19, 26]. The other four studies of reticulocyte percentage or ARC used the steady-state value either immediately prior to the TCD or a baseline value at an age where SCA complications (like the first VOC) may have already occurred.

Increasing reticulocytosis was associated with increased risk of hospitalizations, early death or stroke, and elevated TCD velocities in five of the six studies. Only one study did not find reticulocytosis to be associated with the outcome of interest, in this case SCI [14]. One important limitation of this negative study was the utilization of the reticulocyte percentage, which is not a quantitative measure of the total number of circulating reticulocytes since its calculation is dependent on the total number of erythrocytes in circulation. In children with hemolytic anemias like SCA, therefore, the reticulocyte percentage can be disproportionately increased. The ARC is a more accurate way to enumerate the number of circulating reticulocytes since it is not dependent on the patient's hemoglobin level. Additionally, ARC during the physiologic nadir period (2–6 months of age) was the only predictor studied *before* the development of any sickle cell manifestations, allowing it to function as a true predictor of disease severity rather than an associated factor in SCA complications that have already occurred. ARC above 200 K/μL between 2 and 6 months of age was consistently associated with more SCA complications, with a stronger association noted as ARC increased over 200 KuL.

Where Do We Go from Here?

Early predictors of SCA severity are important for directing therapy in pediatric patients with SCA. The only predictive tests that have consistently been associated with a severe SCA outcome are elevated TCD velocities, increased ARC, and alpha globin gene number for some SCA complications. HbF is viewed by most (if not all) hematologists as having a protective effect against most SCA-related complications; high HbF levels have negatively correlated with mortality in adults with SCA [7]. Additionally, infants less than 6 months of age with high HbF levels do not typically experience SCA symptoms. Surprisingly, several of the studies cited in this chapter found evidence contrary to this. The number of alpha globin genes, while frequently studied, also has varied results, depending on outcome variables and

sample size. ARC above 200 K/μL between the ages of 2 and 6 months of age is associated with triple the risk for hospitalization for SCA-related clinical events within the first three years of life and has also been associated with higher rates of death and stroke in a historical cohort of SCA infants. A strong relationship between early reticulocytosis and future abnormal or conditional cerebral flow as measured by TCD, the only validated predictor of a severe outcome for SCA patients, has been described. Additionally, ARC above 200 K/μL during the physiologic nadir period (2–6 months of age) was the only predictor studied *before* pediatric patients had any SCA-related symptomatology, allowing it to function as a true predictor of disease severity rather than an associated factor in SCA complications that have already occurred.

Since the included studies span over 30 years of work and include the decades of work that has led to improved survival due to early identification of affected infants and early institution of penicillin prophylaxis as well as TCD screening which has dramatically decreased the stroke rate in this patient population, it is difficult to critically review them en masse. As survival has improved throughout childhood and adolescence and stroke rate has decreased, the classification of severe SCA has become more challenging [5]. The lack of consensus about SCA severity criteria impedes the research focused on identifying useful predictors. Because many of the included studies were retrospective in nature, data collection may have been limited. Many (if not all) of the SNP association studies have not been replicated or validated in independent studies which limit their utility [79].

Currently, TCD is the only predictive test utilized as a severity predictor, but it can only predict a patients' risk of stroke. Abnormal TCD is an inclusion criterion for unrelated donor HSCT trials. The recently published TWiTCH (TCD with Transfusions Changing to Hydroxyurea) study revealed that after 1 year of chronic transfusions, children who have abnormal TCDs may be transitioned to HU if they do not have severe cerebral vasculopathy [80]. It remains to be seen how the results from the TWiTCH trial will affect the use of abnormal TCD as an inclusion criteria for HSCT.

Conclusions

In summary, only TCD velocities and increased reticulocytosis have been shown in multiple studies to be predictive of serious complications of SCA. HbF and the number of alpha globin genes may also be useful in a subset of SCA patients, though it is difficult to make definitive conclusions due to conflicting reports. Much work is still needed to identify early predictors in asymptomatic pediatric patients with SCA to guide treatment decisions. As the number of treatment options for patients with SCA increase, it will be important for clinicians to have these tools to best counsel patients and families. The decision to pursue HSCT is a highly personalized one and should be made in consultation with the patient's Hematologist, HSCT physician, and family. Consideration of all known risk factors for pursuing as well as forgoing HSCT should weigh heavily into the decision.

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Chapter 5
Transfusion Support of the Patient with Sickle Cell Disease Undergoing Transplantation

Jeanne E. Hendrickson and Ross M. Fasano

Abbreviations

ACS	Acute chest syndrome
AIHA	Autoimmune hemolytic anemia
DHTR	Delayed hemolytic transfusion reaction
DRE	Delayed RBC engraftment (DRE)
HB	Hemoglobin
HSC	Hematopoietic stem cell (HSC)
HSCT	Hematopoietic stem cell transplantation
IVIg	Intravenous immunoglobulin
LIC	Liver iron content
PME	Partial manual exchange
PRCA	Pure red cell aplasia
RBC	Red blood cells
RIC	Reduced intensity conditioning
SCD	Sickle cell disease

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Introduction

In the most recent National Blood Collection and Utilization Survey report from the US Department of Health and Human Services (HHS), red blood cell (RBC) transfusion was the most common procedure listed on hospital discharge summaries [1]. RBC transfusion therapy is a key component of the comprehensive management of patients with sickle cell disease (SCD), increasing oxygen carrying capacity and decreasing the percentage of hemoglobin S (HbS) containing erythrocytes. By adulthood, most SCD patients will have received at least one RBC transfusion, and many will have received more than a hundred [2].

Transfusions can be lifesaving in the treatment of acute complications of SCD, in the prevention of chronic complications, and as supportive care during hematopoietic stem cell transplantation (HSCT). However, transfusions are not risk free. This chapter will provide an overview of general transfusion support for patients with SCD, highlighting some of the NHLBI expert panel guidelines [3, 4] as well as potential transfusion-associated complications. With an increasing number of children and adults with SCD receiving HSCTs, peri-transplant transfusion support has recently come into the spotlight. This chapter will also review transfusion support for patients undergoing HSCT, with an emphasis on issues that may be particularly relevant to patients with SCD but where formal guidelines are currently lacking.

Types of RBC Transfusion Therapy for Patients with SCD

RBC transfusions can be given as a one-time therapy for an acute illness (such as a parvovirus-induced aplastic crisis) or can be given as chronic transfusion therapy (such as primary or secondary stroke prevention). These transfusions can be given as simple transfusions, also termed “top-off” transfusions, whereby 10–20 mL/kg (for children) or 1–3 units (for adults) are simply infused into a patient without removal of whole blood from the patient. Alternatively, exchange transfusion techniques are commonly employed to minimize transfusional iron burden, to more efficiently decrease the proportion of HbS, and/or to minimize the potential risks associated with alterations in blood viscosity associated with simple transfusion. Exchange transfusion can be performed manually (e.g., partial manual exchange, PME) but is often preferably completed by automated erythrocytapheresis (also termed automated RBC exchange). Erythrocytapheresis requires a high volume of RBCs (a minimum of 6–8 RBC units are needed for a routine exchange transfusion on an average-sized adult) compared to simple transfusion and PME but simultaneously removes the patient’s RBCs, allowing the HbS level to be efficiently modulated independently of the individual’s hematocrit (Hct). Alternatively, PME

involves phlebotomy of 5–10 mL/kg of whole blood (depending on baseline Hb and hemodynamic status) immediately before transfusion and can be utilized for patients with a higher Hb (>8.5 g/dL) [5].

The decision to use simple versus exchange transfusion depends on specific clinical needs, availability of resources including apheresis equipment and technical support, adequate supply of antigen-negative RBC units, and the potential requirement for central venous access [5]. Advantages of simple transfusion include the ease of such a transfusion, including IV access and the relatively small number of RBC units needed. The main disadvantage of simple transfusion therapy includes a high iron load per transfusion, which eventually leads to iron overload. Erythrocytapheresis offers the advantage of minimizing iron accumulation and can negate the need for iron chelation therapy in some patients on chronic transfusion therapy [6, 7]. It also rapidly reduces HbS independent of the patient's Hct, allowing emergent intervention during acute sickle complications (e.g., acute ischemic stroke) while eliminating risks associated with alterations in blood viscosity. Apart from the availability of specialized apheresis equipment and technical support, the main two limitations of erythrocytapheresis include the potential need for the dual lumen high-flow central venous catheters/ports in many patients due to inadequate peripheral venous access, as well as the requirement for a relatively large number of antigen matched RBC units.

Indications for RBC Transfusions for Patients with SCD

Indications for RBC transfusion for patients with SCD are well described and referenced in the 2014 NHLBI "Evidence-Based Management of Sickle Cell Disease" guidelines [3], which is an expert panel consensus document. These indications have also been recently reviewed by Chou and Fasano [5] and are summarized in Table 5.1. There exist no evidence-based criteria for performing exchange transfusions over simple transfusions for particular indications, with the exception of acute ischemic stroke. In these instances, emergent automated RBC exchange transfusion is recommended since it has been shown that children with SCD who receive only simple transfusion(s) have a fivefold greater risk of suffering a second stroke than those who receive an exchange transfusion during an overt stroke [8]. Although little literature exists for the optimal treatment of hemorrhagic strokes [9, 10], transfusion is typically completed to decrease the HbS once the immediate threat of ongoing bleeding has passed.

For severely critical SCD patients, such as those suffering from severe acute chest syndrome (ACS), intrahepatic cholestasis, or multi-system organ failure, the decision toward performing (multiple) simple transfusions versus an exchange transfusion often includes the patient's existing vascular access, initial Hb, and RBC

Table 5.1 Transfusion indications

Transfusion indication	Transfusion method
Generally accepted indications for transfusion	
Acute ischemic stroke	Exchange transfusion preferred
Primary stroke prevention	Chronic simple or exchange transfusion[a]
Secondary stroke prevention	Chronic simple or exchange transfusion[a]
Acute chest syndrome (acute)	Simple or exchange transfusion[a]
Acute splenic sequestration	Simple transfusion
Acute splenic sequestration, recurrence	Chronic simple transfusion (prior to splenectomy)[b]
Preoperative (when general anesthesia required)	Simple transfusion
Transient aplastic crisis	Simple transfusion
Acute multi-system organ failure	Simple or exchange transfusion[c]
Acute hepatic sequestration	Simple or exchange transfusion[±]
Acute intrahepatic cholestasis	Simple or exchange transfusion[±]
Acute sickle or obstetrical complications during pregnancy	Simple or exchange transfusion[±]
Controversial indications for transfusion	
Acute chest syndrome (recurrent)	Chronic simple or exchange transfusion[±]
Vaso-occlusive painful episode (recurrent)	Chronic simple or exchange transfusion[±]
Pulmonary hypertension	Chronic simple or exchange transfusion[±]
Transfusion generally not indicated	
Uncomplicated vaso-occlusive painful episode	NA
Priapism	NA
Uncomplicated pregnancy	NA
Leg ulcers	NA
Non-surgically managed avascular necrosis (AVN)	NA

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[a]Exchange transfusion may be preferred in a rapidly deteriorating patient when emergent HbS reduction is needed or when there are concerns for posttransfusion hyperviscosity due to a high pre-transfusion hemoglobin (i.e., >9 g/dL)

[b]Chronic transfusion may be used to delay but not prevent the need for splenectomy in very young children (i.e., <2 years) who are at increased risk for invasive pneumococcal infections

[c]Exchange transfusion may be preferred in patients with iron overload. *NA* not applicable

alloantibody status (and thus the ability of the blood bank to obtain several multi-antigen-negative units emergently). Additionally, the ability of the patient to tolerate volume shifts associated with and citrate required for exchange transfusion must be taken into consideration.

Transfusion is not indicated to treat acute uncomplicated vaso-occlusive crisis (VOC) pain. However, studies designed with other primary outcome measures have documented lower incidence rates of VOC in children undergoing chronic RBC transfusion for CNS indications [11, 12]. These same studies have also documented lower incidence rates of ACS in children undergoing chronic transfusion therapy

[11, 12], leading some providers to consider a short-term trial of chronic transfusion therapy for patients with recurrent and/or severe ACS.

Complications of Transfusion Therapy in Patients with SCD

Transfusion of all blood products carries the possibility of infectious or noninfectious complications, many of which are thoroughly reviewed elsewhere [13, 14]. However, the main transfusion-related ill effects which impact the care of patients with SCD and possibly HSCT outcomes are RBC alloimmunization and iron overload.

RBC Alloimmunization

RBC alloimmunization is the most significant complication associated with transfusions in SCD, with prevalence ranging between 20 and 50% (rate: 1.7–3.9 antibodies/100 units transfused) when patients receive ABO/Rh(D)-compatible-only transfusions [15]. Based on multiple reports demonstrating that up-front phenotype matching for Rh (C/c, E/e) and K antigens can reduce alloimmunization rates to under 15% (0.26–0.50 antibodies/100 units transfused), the NHLBI expert panel recommends for all patients with SCD to receive prophylactic C-, E-, and K-matched RBCs [4]. However, this has not been universally implemented due in part to a lack of awareness of these recommendations and in part to the increased cost of antigen-negative units. Alloimmunization rates can likely be further reduced by preemptive extended RBC antigen matching beyond C, E, and K antigens (e.g., Duffy, Kidd, +/− S antigen) [16]; however, this is often not feasible, due to a lack of availability of multiple antigen-negative donors. Based on the concept that lower alloimmunization rates are seen in SCD patients in countries with ethnically similar donor and recipient populations, many sickle cell centers in collaboration with blood suppliers have developed transfusion programs aimed at providing RBC units from ethnically similar donors to minimize other non-Rh, non-K donor-recipient RBC antigen discrepancies [17–20].

Despite receiving Rh phenotypically matched RBCs, many SCD patients still produce anti-Rh antibodies. Historically considered autoantibodies because the patient's own RBCs serologically type positive for the corresponding Rh antigen, it is being increasingly appreciated that these antibodies are indeed alloantibodies based on genotyping results in these patients demonstrating *RHD* and *RHCE* variant alleles encoding partial D, C, and/or e antigens. Many of these antibodies have been demonstrated to result in decreased survival of transfused RBCs and/or delayed hemolytic transfusion reactions (DHTRs) [21, 22]. Therefore, future transfusions should be either antigen negative for the putative Rh (D, C, or e) antigen or *RH* genotype matched, which can present significant challenges to transfusion services in providing compatible RBCs to these patients.

RBC Alloimmunization and HSCT

In addition to being problematic in a transfusion setting, RBC alloantibodies may potentially lead to complications for HSCT, described in more detail toward the end of this chapter. Considerations of alloimmunized patients being evaluated for HSCT include an ability to provide an adequate number of RBC units to safely transfuse in the peri-transplant period. An additional consideration includes HSC donor selection (and choice of conditioning regimen) in the face of multiple recipient alloantibodies. Historic studies suggest that RBC transfusion burden is a risk factor for graft rejection during transplantation in patients with nonmalignant hematologic disorders, but it is not clear whether this remains a risk factor in the current era. For example, independent of alloantibody status, heavily transfused patients with severe aplastic anemia have historically been shown to have higher rates of graft rejection and worse outcomes after HSCT [23, 24]. At least one study of 67 patients with SCD in 30 countries also suggested that high transfusion burden may be associated with adverse transplant outcomes [25].

Transfusions have been shown in animal models to be capable of inducing cellular mediated transplant rejection [26–28]. Additional mechanistic studies completed in a murine model of transplant rejection have suggested that residual leukocytes or platelets may be responsible for the recipient CD8+ T cell response to minor histocompatibility antigens in the transfused product, rather than the RBCs themselves [26, 29]. Taken together, these data suggest that pretransplant transfusion burden (even of stringently leukoreduced RBC units) in SCD patients may have the capability of inducing a cellular immune response to minor histocompatibility antigens, which may at least theoretically impact engraftment of HSCs during subsequent transplantation under reduced intensity conditioning (RIC). Notably, this recipient cellular immune response is independent of a humoral immune response, with no study to date having identified recipient RBC alloimmunization as a risk factor for graft rejection [30].

Delayed Hemolytic Transfusion Reactions (DHTRs)

DHTRs are the most life-threatening consequence of alloimmunization in SCD, because of the potential for bystander hemolysis of the patient's erythrocytes [31, 32]. RBC alloantibodies may develop after a single transfusion or after repeat transfusions and may remain detectable for life or may evanesce and fall below the level of detection by traditional blood banking methodology [33]. The phenomenon of RBC alloantibody evanescence, in combination with multisite transfusion and a lack of a universal medical record system in the United States, makes RBC alloantibodies especially problematic. One institution may be unaware of an antibody detected at another hospital, and the patient may be transfused with an RBC

unit that appears safe because the pre-transfusion antibody screen is negative and the unit is crossmatch compatible. However, if the RBC unit(s) expresses antigens to which the patient has previously developed alloantibodies, an anamnestic response is likely to occur, and this response may result in a DHTR. In addition, the lack of standard recommendations for obtaining follow-up antibody screens at set intervals after every episodic transfusion results in some antibodies going unrecognized with associated future risk of DHTR upon reexposure from additional transfusion [34].

DHTRs are often under-recognized due their clinical presentation, which can resemble a VOC. Diagnosis of a DHTR is imperative, as further transfusions should be modified based on new antibody findings or avoided altogether. Identifying a DHTR often requires a high index of suspicion, with a DHTR being considered when any patient that has been recently transfused presents with VOC, fever, or a drop in hemoglobin level. Specific features of DHTRs in SCD patients may include involvement of more than one alloantibody, the co-occurrence of autoantibodies (or autoantibodies in isolation), or the most perplexing circumstance when no antibody can be detected at all (termed: antibody-negative DHTR), which can account for approximately 30% of cases [35]. Development of RBC autoantibodies concurrently with alloantibodies can further complicate the clinical picture and potentially contribute to hyperhemolysis [31, 36–38]. Although RBC autoantibodies are only encountered in a minority of transfused SCD patients and are often thought to be clinically insignificant, their development during DHTRs can resemble a clinical picture of autoimmune hemolytic anemia (AIHA), with severe life-threatening anemia, profound reticulocytopenia from bystander hemolysis of reticulocytes, and a panagglutination pattern on antibody evaluations, making the identification of compatible blood and the characterization of underlying alloantibodies challenging [39]. In addition to potentially contributing to the development of AIHA, it can be difficult to distinguish autoantibodies from alloantibodies to high-prevalence antigens, notably those in the Rh system which may arise when an SCD patient expressing a variant *RH* antigen is exposed to RBCs expressing conventional Rh antigens. In these cases, RBC genotyping is often necessary to discriminate autoantibodies from alloantibodies [40].

The management of DHTR in SCD remains quite challenging because there are few effective treatments, and those tried may be deleterious. For example, corticosteroids and erythropoiesis-stimulating agents may lead to a rebound of SCD-related symptoms [41]. Currently, no optimal treatment of DHTR has been defined, except for the avoidance of additional RBCs during an acute hemolytic event unless significant hemodynamic compromise exists since further transfusions often exacerbate the hemolysis [42]. Potential therapies that have been utilized include erythropoiesis-stimulating agents with iron, corticosteroids, and intravenous immunoglobulin (IVIg) [43]. B cell depletion with rituximab has been tried in some patients with a history of severe/recurrent DHTRs [44], though plasma cells are not immediately impacted by rituximab and its efficacy in DHTR prevention or

treatment remains under investigation. Eculizumab, an anti-C5 monoclonal antibody, has also been utilized as a treatment for severe DHTRs and has been reported to have a beneficial effect on hemolysis in some cases [43, 45, 46].

Iron Overload

Another complication of transfusion therapy that affects many multiply transfused patients with SCD is iron overload. Assuming that each mL of erythrocytes contains approximately 1 mg of iron and that a unit of RBCs has a hematocrit of 65%, approximately 0.75 mg Fe is accumulated per mL transfused (e.g., 7.5 mg Fe/kg from a 10 mL/kg RBC transfusion) [5]. The average body iron excretion in most healthy men and women is limited to 1–2 mg/day. Consequently, iron overload occurs after just 1–2 years of chronic transfusion therapy (or after 10–20 cumulative RBC units; 120 mL/kg), [12], and can impact the heart, liver, and endocrine system [7, 47]. Patients receiving chronic (simple) transfusions accumulate an average of 0.25–0.42 mg of Fe per kg per day compared to approximately 0.05–0.08 mg of Fe per kg per day if maintained on chronic erythrocytapheresis [48]. Despite chelation therapy, children with SCD on chronic transfusion therapy receiving simple transfusions typically become iron overloaded with serum ferritin levels greater than 2000 ng/mL within 2 years [12]. This fact is likely related to the relatively high doses of iron chelator needed to maintain negative iron balance [>20 mg/kg per day of deferasirox (Exjade); (>14 mg/kg per day of deferasirox (Jadenu)] and less than ideal long-term chelation therapy adherence [49–51].

Iron Overload and HSCT

Iron overload may impact HSCT outcomes in patients who are at the highest risk for transfusion-related iron overload. Past reports have demonstrated that iron overload and related liver fibrosis portended a higher risk of morbidity and late complications in thalassemia major patients undergoing myeloablative HSCT [52, 53]. In fact, investigators in Italy developed potential risk factors for poor transplant outcome in thalassemia major patients which included poor quality of previous iron chelation therapy, marked hepatomegaly, and portal fibrosis. Using these criteria, patients were categorized into three classes of risk for adverse outcome following BMT (termed Pesaro class) [53]. As illustrated in Fig. 5.1, patients with all three adverse criteria (class III) demonstrated lower probabilities for overall survival and event-free survival and higher rejection and non-rejection mortality as patients with none (class I) or one to two (class II) of the criteria following allogeneic BMT from an HLA-identical family member. The impact of iron overload on HSCT outcomes in the modern era is less clear as reduced intensity approaches are becoming more common to reduce the organ toxicities. Further, the effect of iron overload on HSCT in SCD patients has not been extensively evaluated.

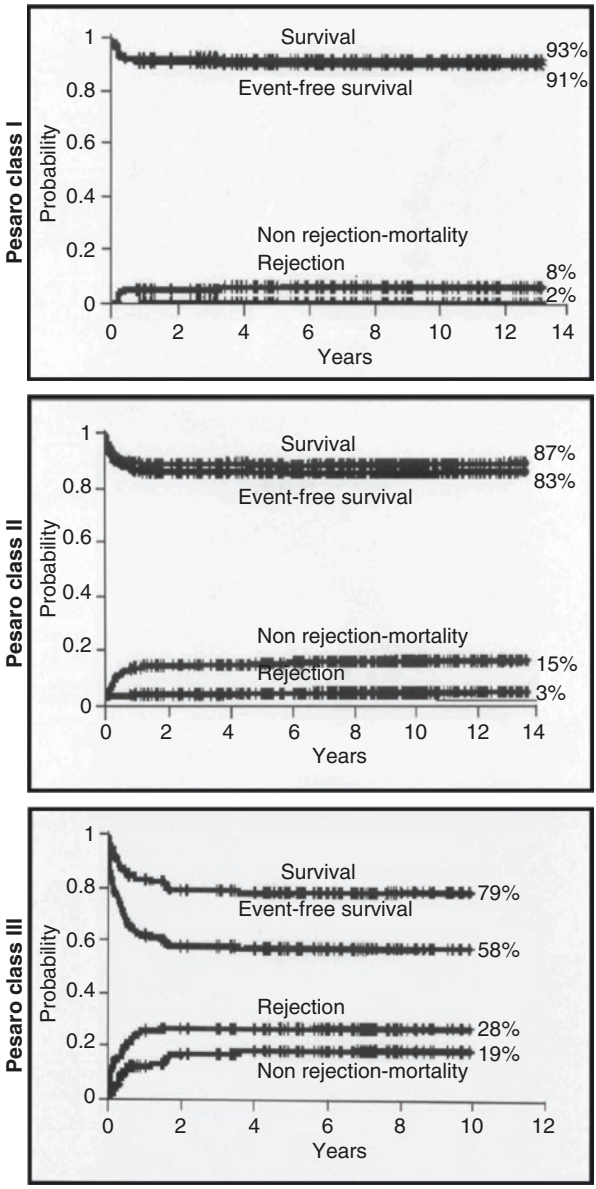

Fig. 5.1 Probabilities of survival, event-free survival, rejection, and non-rejection mortality in thalassemia major patients undergoing allogeneic bone marrow transplantation from an HLA-identical family member donor based on Pesaro class. Criteria for Pesaro classification include hepatomegaly, liver fibrosis, and noncompliance with iron chelation therapy. Class I, none of the criteria present; Class II, one or two criteria present; class III, all three criteria present. Modified from Lucarelli G, Galimberti M, Giardini C, Polchi P, Angelucci E, Baronciani D, et al. Bone marrow transplantation in thalassemia. The experience of Pesaro. Ann N Y Acad Sci. 1998;850:270–5, with permission from Wiley

Prevention of Complications of RBC Transfusion Therapy

Prevention of RBC Alloimmunization

The most effective way to minimize transfusion complications is to avoid transfusion altogether. However, such avoidance is not possible in many patients. The NHLBI recommends extended RBC antigen typing for patients with SCD aged

more than 6 months and transfusion with prophylactic C-, E-, and K-matched RBCs [3] since such phenotypic matching has been shown to decrease alloimmunization in some but not all studies [5]. Some sites provide RBCs that are further matched at antigens including those in the Duffy (Fy), Kidd (Jk), and S families. Most institutions obtain extended RBC phenotypes on patients with SCD within the first year of life, with DNA-based RBC typing methodologies being increasingly used because of the reliability, cost-efficiency, and ability to overcome certain limitations of traditional hemagglutination assays. For example, RBC genotyping can be used to determine predicted RBC antigen expression in patients recently transfused or with interfering allo- or autoantibodies, to resolve discrepant serologic typing, and/or when typing antisera are not readily available (e.g., $Do^{a/b}$, $Js^{a/b}$, $Kp^{a/b}$, V/VS) [40]. An additional benefit is the ability to detect the GATA site mutation in the Duffy antigen/chemokine receptor (DARC) gene, which is common in individuals of African descent and prevents expression of the Fyb antigen only on erythrocytes while permitting expression in nonerythroid cells. These patients can receive Fy(b+) RBCs because they are not at risk of forming anti-Fyb alloantibodies despite typing as Fy(b−) [54]. Currently, there is one Food and Drug Administration-approved RBC genotyping assay for molecular typing as a "test of record," meaning no confirmatory typing with antisera is required.

Patients of African descent frequently have *RH* variants, resulting in partial D, C, and e expression on their RBCs. Patients with SCD with these *RH* variants may lack high-prevalence Rh epitopes (e.g., hrB, hrS) and can form alloantibodies to these Rh epitopes when exposed to conventional Rh antigens through transfusion. These alloantibodies often appear to be autoantibodies since their serologic typing for putative Rh antigen is positive; RBC genotyping can discriminate a potential alloantibody from an apparent autoantibody [40]. One common example is that a fraction of all SCD patients that serologically type as C antigen positive may actually express a variant (partial) C antigen due to the expression of hybrid *RHD*DIIIa-CE* [4–7]-*D* gene. These individuals are at risk of forming an anti-C alloantibody upon exposure to RBCs from donor RBCs expressing the conventional C antigen [55]. Other SCD patients who phenotype as Rh(D) positive or e antigen positive may express variant antigens and thus be at risk of forming anti-D or anti-e alloantibodies upon exposure to Rh(D) or e-positive RBCs, respectively [21]. At the present time, there is no mandate or standard to routinely genotype the RBC antigens in patients with SCD. However, up-front RBC genotyping may prevent long-term transfusion complications, and genotyping for patients with unexpected allo- or autoantibodies is often necessary to resolve cases where serologic evaluation is limiting [40].

Although data suggest that the risk of alloimmunization increases with successive transfusions, patients at risk of becoming alloimmunized may generate alloantibodies upon their initial exposure to the antigen in question [34, 56]. It is generally accepted that certain transfusion recipients ("responders") are more likely to form RBC alloantibodies, while others ("nonresponders") are unlikely to form alloantibodies regardless of transfusion exposure [57]. However, the ability to identify a responder versus a nonresponder transfusion recipient before transfusion exposure

currently does not exist [58]. Further, the inflammatory status of a transfusion recipient at the time of RBC exposure has been shown to increase the likelihood of alloantibody development in animal models [59] and in humans [60, 61]. One retrospective study involving a cohort of alloimmunized pediatric patients with SCD demonstrated that transfusion during pro-inflammatory states, most notably ACS and simple VOC, resulted in a significantly higher likelihood of alloimmunization compared to patients receiving chronic transfusions at steady state [61]. These findings demonstrate that, in addition to minimizing donor/recipient antigenic differences by transfusing RBCs matched for Rh and Kell antigens, judicious use of RBC transfusion therapy is necessary to minimize exposure in circumstances where there is not a clear benefit from transfusion (e.g., uncomplicated VOC).

Strategies to prevent other transfusion reactions, including febrile reactions, involve the provision of RBCs that have been leukoreduced. Leukoreduction not only decreases febrile reactions but also decreases HLA alloimmunization and the transmission of infectious diseases carried in white blood cells [13]. Some retrospective human [62] and animal studies [63] suggest that leukoreduction may decrease the risk of RBC alloimmunization, but other studies suggest that leukoreduction may not impact RBC alloimmunization [64].

Prevention of Iron Overload

Monitoring for iron overload, adjusting the method of transfusion if necessary, and initiating chelation when indicated are important to long-term health of patients with SCD. Patients on chronic transfusion therapy should have serum ferritin levels monitored every 1–3 months and liver iron content (LIC) (preferably by R2 MRI techniques which correlate well with LIC determined by liver biopsy) every 1–2 years if serum ferritin remains greater than 1000 ng/mL [5, 65]. Cardiac iron overload occurs in a small proportion of patients with SCD with exceptionally poor control of iron status [66]. In such patients, cardiac T2* MRI should be obtained to predict the risk of developing heart disease and prompt intensification of chelation therapy [67]. Iron chelation therapy should be initiated within 1–2 years of instituting chronic transfusions, after 10–20 cumulative RBC units (120 mL/kg), or when the serum ferritin is greater than 1000 ng/mL on two separate occasions. Two oral formulations of deferasirox (Exjade® 20–40 mg/kg/day; Jadenu® 14–28 mg/kg/day) are licensed and the most commonly used chelators for the treatment of iron overload in SCD. Deferoxamine (Desferal®) is less commonly used due poor adherence due to its route of administration (25–40 mg/kg/day, 5 days per week by subcutaneous infusion over 8–10 h). Deferiprone (Ferriprox® 75–100 mg/kg/day by mouth, three times daily dosing) has been available for thalassemia major patients in the United States since 2011; however, experience in SCD is limited [5, 48]. A clinical trial (ClinicalTrials.gov Identifier: NCT02041299) investigating the efficacy and safety of deferiprone in patients with SCD is currently ongoing.

Patients with severe iron overload who need continued chronic transfusion therapy may benefit from erythrocytapheresis combined with chelation and from adjusting automated RBC exchange parameters to achieve minimal iron loading [6, 7]. Of note, erythrocytapheresis has also recently been suggested to be associated with lower rates of alloimmunization than simple transfusions, despite the larger blood product exposure [6, 68–70]. The immunologic reasons behind these findings are not yet well understood but may involve the degree of RBC antigen exposure or the removal of platelets or plasma components during the exchange transfusion procedure.

Considerations for Transfusion Therapy for Patients with SCD Undergoing HSCT

Much has been written about transfusion support and product processing considerations for adults undergoing HSCT. However, the emphasis of most of this literature has been on adult transplant recipients with oncologic diagnoses, with little written about transfusion support and product processing considerations for pediatric transplant recipients with SCD. In the following sections, we will briefly review considerations for transfusion support in different phases of transplantation, with an emphasis on issues that may be particularly relevant to patients with SCD undergoing transplantation.

Donor Selection

Discussed in more detail elsewhere in this book, donor selection is one of the most important steps in the transplant process. Close coordination between the transfusion and transplantation services is recommended early in the donor selection process so RBC or HLA alloantibody issues are brought to the forefront.

The most closely matched HLA donor will typically be selected for transplantation. However, if multiple siblings are fully HLA matched, then other issues such as ABO or minor RBC antigen compatibility between donor and recipient may be taken into consideration. For example, if two donors are equally HLA matched and eligible, then selecting the donor that avoids a major ABO mismatch is typically preferred. Standard blood bank processes include performing a major and minor crossmatch between the patient and potential HSCT donor(s). If the patient has anti-A, -B, or -AB or is expressing antibodies to minor RBC antigens from previous alloimmunization, then the major crossmatch may be positive (donor RBCs incompatible with patient's serum). Further, if a recipient with SCD has multiple RBC alloantibodies, then phenotyping (or genotyping) RBC antigens of the potential donor(s) may further assist in donor selection. A donor expressing

minor RBC antigens to which a recipient is alloimmunized can still be chosen, just as an ABO-incompatible donor can be selected; however, knowing the antigen/antibody incompatibilities of potential donor/recipient pairs up front may prove beneficial in terms of planning the graft processing and assessing the risk of delayed RBC engraftment (DRE) and/or developing pure RBC aplasia (PRCA) posttransplant.

Also of relevance is consideration of the HLA alloantibody status of the recipient. HLA alloimmunization has been shown to be relatively common in multiple transfused nulliparous children with SCD receiving solely leukoreduced RBC units, with the presence of HLA antibodies correlating with RBC immunologic "responder" status [71–73]. Thus, any transfused patient with SCD is potentially at risk of HLA alloimmunization. The presence of HLA antibodies directed against donor HLA antigens (termed donor-specific antibodies or DSAs) may impact donor selection for transplants that do not involve fully HLA-matched donors. HLA antibodies may have serious adverse consequences in allogeneic HSCT, resulting in rejection of HLA-nonidentical grafts [74–78] and platelet transfusion refractoriness during the pre-engraftment period given the expression of HLA class I antigens on platelets [79]. HLA antibody desensitization methods have been tried in the weeks prior to the initiation of the transplant conditioning regimen with some success; these methods include plasmapheresis alone or in combination with immunomodulatory agents such as IVIG, tacrolimus, mycophenolate mofetil, rituximab, or bortezomib [80, 81].

Given that patients with SCD may have complex RBC or HLA alloantibody issues, it is recommended that consultation with the transfusion medicine service occurs as far in advance of a potential transplant as possible. Despite the current lack of widely accepted guidelines, such a consultation may help to optimize donor selection, stem cell product processing, and transfusion support in the pre- and posttransplant period (Fig. 5.2).

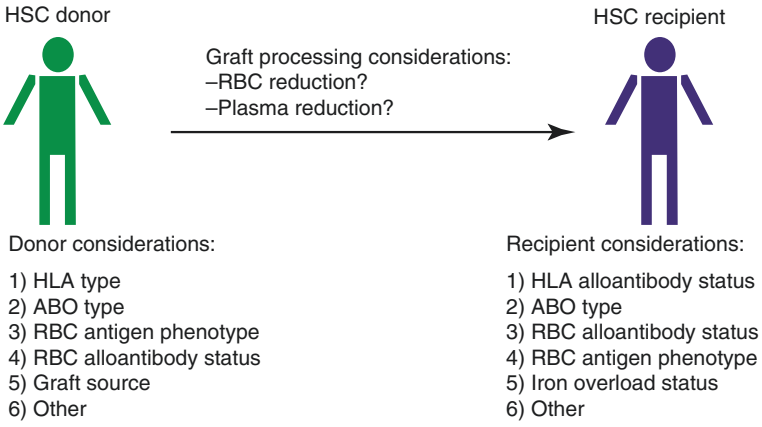

Fig. 5.2 Considerations for the donor, graft, and recipient

Pre-HSCT Recipient Considerations

One of the primary pretransplant clinical considerations for patients with SCD is when, how, and to what level to decrease their HbS. Pre-HSCT reduction of HbS to less than 30% has historically been recommended and accomplished through chronic simple or exchange transfusions in the months leading up to the transplant or by a single automated RBC exchange immediately prior to initiating the conditioning regimen. A large portion of patients with SCD who are eligible for HSCT are already on chronic transfusion therapy for primary or secondary stroke prevention [82]. In these patients, the HbS is most often maintained at less than 30%, and therefore no particular changes in transfusion management are necessary prior to transplantation (Table 5.2). With a lack of published studies or trials to guide practice, many centers target the HbS to be below 30% at the time of transplantation; however, many transplant trials allow for a higher pre-HCT HbS (e.g., <45%) for patients with multiple and/or challenging RBC alloantibodies. Such situations must be evaluated on a case by case basis in consultation with the transfusion medicine service.

Table 5.2 Considerations for the pre- and post-HSCT periods

Pre-HSCT considerations	Post-HSCT (pre-engraftment) considerations	Post-HSCT (post-engraftment) considerations
RBC transfusion threshold Target HbS (<30%) – Chronic transfusion vs. RBC exchange transfusion × 1	*RBC transfusion threshold* Higher Hb threshold (9–11 g/dL) Degree of RBC phenotypic matching	*RBC transfusion* Phenotypically matched RBCs if transfusions are needed post-engraftment
Donor/recipient RBC incompatibilities ABO and minor RBC antigens – Graft manipulation – Donor selection	*Donor/recipient RBC incompatibilities* Risk of immediate and/or delayed hemolysis Risk of DRE/PRCA	*Donor/recipient RBC incompatibilities* Timing of conversion to donor blood type Treatment of PRCA (if present)
Recipient HLA antibodies Donor-specific antibodies (DSAs) Impact on donor selection	*Recipient HLA antibodies* Risk of graft rejection due to DSAs Platelet transfusion refractoriness	*Recipient HLA antibodies* Optimal reevaluation (if present)
Iron overload Optimal evaluation (MRI vs. biopsy) Conditioning regimen selection related to liver fibrosis	*Iron overload* Risk for drug-induced hepatotoxicity	*Iron overload* Optimal monitoring Optimal treatment (phlebotomy vs. chelation)
	Platelet transfusion threshold Higher platelet threshold ($50{,}000\ \mu L^{-1}$)	

In addition to the pretransplant HbS target, other questions include the ideal timing of the pretransplant transfusion and the blood type selected for transfusion. Immune changes are known to occur in response to transfusion [83], and thus the timing of the pretransplant transfusion may at least theoretically be important to study. Currently, there is no evidence to support that transfusions many weeks (or months) prior to the transplant are any less or more immunogenic than those administered immediately prior to the transplant. It must be considered that any transfusion reaction may complicate the transplant course.

The blood type of RBCs selected for a pretransplant simple or exchange transfusion is also something to consider. In cases where pretransplant transfusion is necessary and there is a minor ABO mismatch HSCT anticipated (e.g., blood type A recipient being transplanted with a blood type O donor), RBCs compatible with the donor in addition to recipient (which would be type O in this case) are advantageous to minimize delayed hemolysis due to passenger lymphocyte syndrome [84]. Although case series exist of pretransplant therapeutic plasma exchange for major ABO-mismatched transplants (e.g., blood type O recipient being transplanted with a blood type A donor) to decrease the risk of immediate hemolysis of the donor HSC product and posttransplant pure red blood cell aplasia (PRCA) [85, 86], this is not typically recommended because standard RBC reduction procedures of the HSC graft exist to minimize hemolysis upon infusion. Furthermore, therapeutic plasma exchange typically has little effect on longer-term complications of major ABO-mismatched transplants because recipient isohemagglutinin-producing plasma cells, which are responsible for posttransplant DRE/PRCA, are unaffected by this procedure [87].

Despite data from the Pesaro group illustrating that transplant outcomes are adversely affected by transfusional hemosiderosis in thalassemia major patients, the impact of iron overload on SCD HSCT outcomes is not yet defined. Regardless, SCD patients often undergo pretransplant liver biopsies to assess the presence for and extent of liver fibrosis so that the most appropriate conditioning regimen can be chosen (liver toxic myeloablative versus reduced intensity conditioning) similar to thalassemia major-HSCTs [79]. However, liver biopsy is invasive and thus poses additional risks (including those from preoperative transfusion) immediately prior to undergoing HSCT. An MRI-based liver iron quantification associated with minimal or absent liver fibrosis has been determined to be adequate for patients with thalassemia major. Thus determination of MRI-based LIC which both correlates with the degree of liver fibrosis and predicts HSCT outcomes for SCD patients similar to thalassemia major would be desirable.

HSC Graft Processing Considerations

Blood group mismatches may occur when the recipient or donor has preformed isohemagglutinins antibodies (e.g., anti-A, anti-B, anti-AB) against the blood type of the other. These ABO mismatches can result in immediate hemolysis due to infusion of incompatible RBCs, DRE, and/or PRCA in major ABO mismatch settings;

delayed hemolytic reactions (5–15 days post graft infusion) due to passenger lymphocyte syndrome can occur in minor ABO mismatch settings [88]. These mismatches remain a significant concern in SCD patients undergoing HSCT because of the increasing use of reduced intensity conditioning (RIC) regimens, which carry an increased risk of DRE/PRCA due to prolonged persistence of recipient antibody-producing plasma cells [87].

Standard operating procedures primarily focused on the ABO and Rh(D) status of the stem cell donor and the transplant recipient are well established in stem cell processing laboratories [89]. For example, grafts from donors for a major ABO-incompatible transplant are typically RBC reduced if their volume of incompatible RBCs exceeds that accepted by a particular stem cell laboratory's standard operating procedure (which is usually 50 mL for adults, 0.3–0.4 mL/kg or maximum of 20–30 mL for children) [79, 90]. However, the management of major ABO-mismatched HSC grafts varies considerably due to the lack of strong evidence-based guidelines in regard to the threshold for residual incompatible RBC volume in the product [91]. Although most institutions use the above criteria, the "safe" residual volume of incompatible RBCs for smaller pediatric patients is not clear, especially in those with SCD [92]. RBC depletion of the graft can be accomplished using automatic cell processors, hydroxyethyl starch sedimentation, or Ficoll-Paque density gradient separation which is quite labor intensive but can achieve a much lower residual RBC volume (<0.3 ml/kg) than other techniques. At least one report describes an SCD patient who developed significant hemolysis-induced renal impairment after receiving less than 1 mL/kg of incompatible RBCs in the infused HSC product despite premedication, hyperhydration, and relatively slow infusion rates, suggesting that special HSC processing considerations may be needed for patients with SCD undergoing major ABO-mismatched BMTs [93]. In addition, there is the potential for non-ABO RBC antigen mismatches in patients with SCD which may impact donor selection, HSC processing, and peri-transplant management. Accordingly, special attention should be paid to the recipient alloantibody status for patients with SCD. Preemptive donor RBC phenotyping (or genotyping) and graft RBC reduction may be recommended if the donor expresses cognate antigens against which the recipient is alloimmunized or if the major crossmatch is incompatible despite the absence of an ABO mismatch. However, there are currently no evidence-based guidelines for the management of these situations, and each case should be evaluated in consultation with the transfusion medicine service.

Minor ABO-incompatible grafts (e.g., blood type A recipient; blood type O donor) are typically plasma reduced to minimize infusion-related hemolysis. Similar to major ABO-incompatible grafts, there are no standard guidelines for the goal residual plasma volumes; however, most institutions reduce the plasma to less than 300 mL or 5 mL/kg [79]. Likewise, the graft should be plasma reduced in cases in which the donor is alloimmunized against recipient RBC antigens or in cases in which the minor crossmatch is incompatible despite the absence of an ABO mismatch. Patients with SCD may have existing warm autoantibodies which may cause the major and minor crossmatch to be incompatible. In such situations,

collaboration between the transplant and transfusion medicine services is warranted to determine optimal HSC product manipulation to minimize risks of hemolytic reactions while preventing excessive HSC content loss of the product.

Post-HSC Infusion Transfusion Considerations

Transfusion Thresholds

Transfusion thresholds have not been studied in the SCD transplant population in a randomized controlled manner given the relatively small numbers of patients transplanted to date [94]. Historically, SCD patients' Hb is maintained between 9 and 11 g/dL, and the threshold for platelet transfusion is 50,000 μL^{-1} [95]. This practice is largely based on early clinical results on the SCD national collaborative study where four of the first seven patients experienced serious neurologic complications post-myeloablative HSCT [96], which prompted multiple prophylactic measures to minimize these adverse events going forward. In addition to the more conservative changes in transfusion triggers, anticonvulsant prophylaxis (initiated during the administration of busulfan and continued for 6 months), aggressive management of hypertension and electrolyte derangements were adopted, which minimized the occurrence of neurologic events during the post-HSCT course [95]; please refer to Chap. 6. With these adopted transfusion thresholds, the number of transfused products that an individual may receive in the peri-transplant period could be quite large [97]. Of note, some centers now use Hb thresholds as low as 8 g/dL and platelet thresholds as low as 30,000 μL^{-1} in SCD patients in the peri-transplant period.

RBC Product Selection

RBC products selected for transfusion for SCD patients undergoing transplantation are similar to those selected for any patient undergoing transplantation, in that they are ABO and Rh compatible with donor and recipient as per standard guidelines and irradiated to prevent transfusion-associated graft-versus-host disease [98]. The RBCs should also be from sickle-negative donors, and some centers select RBCs that are relatively fresh (often stored for fewer than 14 days) to increase the likelihood of a longer circulatory half-life. In addition, the RBCs selected for transfusion may be phenotypically matched to the recipient at C/c, E/e, and K/k, similar to transfusions for SCD patients in the absence of HSCT. Little data exists to support or refute such prophylactic phenotypic matching in the peri-transplant period. Although the incidence of de novo RBC alloimmunization occurring after transplant is relatively low (rate of 2–9%), these estimates are predominately from patients with malignancies with low

alloimmunization rates overall compared to SCD patients. A study recently presented at the 2016 AABB Annual Meeting, involving 61 patients with SCD that had undergone transplant, documented the formation of 11 alloantibodies in six patients after non-myeloablative/RIC transplants [99]. These antibodies could represent the reemergence of previously generated antibodies that had evanesced to the point of being undetectable pretransplant, or they could represent de novo antibodies. Of note, one center's experience suggests that such prophylactic phenotype matching in the pre- and posttransplant period may potentially decrease RBC transfusion requirements [97].

Special RBC Considerations

Autoimmune hemolytic anemia (AIHA), DRE, and PRCA are all associated with prolonged RBC transfusion requirements posttransplant. Known risk factors for these include donor/recipient ABO major mismatch, use of RIC or non-myeloablative regimens, matched unrelated donor transplants, and older age of the recipient [100], all HSCT-related features which are being considered and/or expanded for SCD patients in the future.

AIHA has been reported to occur in 2.4–6% of pediatric patients following allogeneic HSCTs and is caused by donor antibodies directed against donor RBCs [101, 102]. This complication is often attributed to immune dysregulation and is thought to be partly attributed to a narrowed T cell repertoire from delayed clonal T cell reconstitution which allows for subsequent dysregulation of B cells and outgrowth of autoreactive B cell clones [100, 103]. As such, there is a higher incidence of AIHA following T cell-depleted HSCTs, in patients with nonmalignant diseases and in recipients of mismatched cord blood transplants. The use of RIC regimens, especially those aiming for the achievement of mixed hematopoietic chimerism, in combination with the increased use of peri-transplant immunomodulatory therapies (e.g., fludarabine), may place patients at increased risk for posttransplant AIHA.

The production of isohemagglutinins is well known to result in PRCA in major mismatched transplants in general, with slow platelet engraftment or even pancytopenia reported in some studies [104]. Plasma cells are known to be long lived and may produce antibodies for many months posttransplant. In severe PRCA, transplant recipients may require transfusion support for at least 6 months posttransplant [105]. RIC regimens, such as those increasingly being used for SCD transplants, may result in longer-term persistence of recipient plasma cells and an increased risk of developing DRE and PRCA [106]. Whereas rates of PRCA range from 0–16% after myeloablative conditioning, the risk has been reported to increase to 8–38% after reduced intensity transplants [107]. In one study of patients with myelodysplasia and AML, conditioning with fludarabine and busulfan was a particular risk factor for PRCA development posttransplant [107].

In addition to isohemagglutinin production, plasma cells may continue to produce RBC alloantibodies for many months posttransplant. At least one study has

described a SCD patient with a preexisting anti-Jka alloantibody that required continued transfusion support for 18 months after a RIC transplant from a Jka + HSC donor, due to continued anti-Jka production from recipient plasma cells [30]. Therefore, in addition to donor/recipient ABO compatibility, recipient RBC alloantibody status in the context of various conditioning regimens and engraftment measures are variables that warrant continued study in the SCD patient population. Furthermore, as HSCT options expand to include older SCD patients, RIC regimens, and alternative donors, it may become necessary to develop and evaluate potential strategies to specifically target recipient B lymphocytes and/or plasma cells in order mitigate risks of AIHA and DRE/PRCA due to ABO and/or minor RBC antigen incompatibilities.

Platelet Transfusions and Transfusion Refractoriness

High platelet transfusion burdens have been observed in SCD patients in the posttransplant period [97]. At the present time, it is not known whether this high burden is simply due to higher platelet count thresholds (e.g., >50,000 μL^{-1}) or whether recipient platelet transfusion refractoriness is contributory. Crossmatch-compatible or HLA-matched platelets are recommended when antiplatelet (platelet glycoprotein or HLA) alloantibodies are detected and platelet refractoriness is confirmed by documentation of two 1-h posttransfusion corrected count increments (CCI) of less than 5000 μL^{-1}, whereby the CCI is calculated as [(platelet increment per μL) × (body surface area in m^2)]/[(number of single donor platelets units transfused) × (3×10^{11})] [108].

HLA alloimmune-mediated platelet refractoriness requiring HLA-matched (and/or crossmatched) platelets has been demonstrated in a few SCD patients undergoing myeloablative and RIC regimens [96, 109]. There currently is no standard guideline for pretransplant evaluation for HLA antibodies. However, a high index of suspicion for HLA alloimmunization should be kept in mind if lower than expected posttransfusion platelet counts are observed in SCD patients in the posttransplant setting. Given that HLA antibodies may persist for many months following RIC transplants [110], SCD patients with delayed engraftment following RIC transplants may present significant transfusion challenges to continue to maintain high platelet transfusion thresholds using ongoing specialized platelet products.

Long-Term Post-HSCT Considerations

Few studies have evaluated the immune systems of SCD patients in the long-term posttransplant period, to determine whether they remain at high risk for transfusion complications such as RBC and HLA alloimmunization or to evaluate when in the transplant course recipient RBC or HLA alloantibodies become undetectable.

Most patients become transfusion independent after engraftment, and blood product selection posttransfusion is rarely an issue in such cases. However, for those who continue to require blood products for the long-term posttransplant, the selection of RBCs phenotypically matched at C/c, E/e, and K/k of the donor seems logical; optimal transfusion thresholds for patients requiring long-term blood product support have not been established. Most transfusion services have protocols to switch the transplant recipient to the ABO blood group of the donor as soon as the recipient demonstrates repeatedly that their erythrocytes express donor antigens only and as long as no recipient isohemagglutinins remain. It may take many months posttransplant for donor-specific isohemagglutinins to be produced, however, and the formation of those hemagglutinins may not be a rate-limiting step for switching the blood type [98].

Post-HSCT iron overload has been associated with long-term complications and therefore warrants assessment and treatment. However, the timing and method of assessment, as well as the method for treatment are not defined. Similar to thalassemia major patients, SCD patients are likely to have significant transfusional hemosiderosis, especially if maintained on chronic transfusion therapy for years prior to HSCT. Serum ferritin levels have traditionally been used to detect and monitor response to therapy for iron overload in the post-HSCT period, but liver R2 MRI with/without cardiac T2* MRI may be necessary in some patients with severe hemosiderosis. Therapeutic phlebotomy is most often used post-HSCT to remove iron. However, this practice varies widely based on frequency of treatments and volume of blood removed and requires multiple treatments in the clinic over time. Therefore, the use of iron chelation therapy may be justified in this setting. However, it remains to be determined if the replacement of therapeutic phlebotomy with iron chelation therapy is safe and as efficacious and if it would result in higher patient satisfaction. Further, it is unclear whether the two treatments could be safely combined to enhance iron removal efficiency in the posttransplant setting.

Conclusions

HSCT remains the only curative therapy to date for patients with SCD. Although transfusion therapy can be lifesaving in the pre- and peri-transplant period, it is not without risk. The risk profile for transfusion therapy for patients with SCD is skewed toward the production of alloantibodies (both RBC alloantibodies and HLA alloantibodies), for reasons that likely involve blood donor as well as recipient factors. Transfusion sequelae may complicate donor selection and graft processing, as well as transfusion supportive care in the peri-transplant period. Close collaboration between transplant physicians, hematologists, and transfusion medicine physicians and continued research of best practices are necessary for optimal HSCT outcomes.

Conflicts of Interest The authors declare no conflicts of interest related to this manuscript.

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Chapter 6
Neurological Manifestations of Sickle Cell Disease and Their Impact on Allogeneic Hematopoietic Stem Cell Transplantation

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Abbreviations

AED	Antiepileptic drug
ASCIEs	Acute silent cerebral ischemic events
CNS	Central nervous system
CSSCD	Cooperative Study of Sickle Cell Disease
CSVT	Cerebral sinovenous thrombosis
CT	Computed tomography
EDAS	Encephaloduroarteriosynangiosis
EEG	Electroencephalogram
FLAIR	Fluid-attenuated inversion recovery
GVHD	Graft-versus-host disease
Hb	Hemoglobin
HbS	Hemoglobin S
HRQL	Health-related quality of life
HSCT	Hematopoietic stem cell transplantation
ICH	Intracranial hemorrhage
IQ	Intelligence quotient
MRA	Magnetic resonance angiogram
MRI	Magnetic resonance imaging
PRBC	Packed red blood cell
PRES	Posterior reversible encephalopathy syndrome

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PSOM	Pediatric Stroke Outcome Measure
SCA	Sickle cell anemia—includes HbSS and HbS-β^0 thalassemia
SCD	Sickle cell disease—includes all sickle hemoglobinopathies
SCI	Silent cerebral infarction
SCURT	Sickle Cell Unrelated Transplant Trial
SIT	Silent Infarct Transfusion Trial
SWiTCH	Stroke With Transfusions Changing to Hydroxyurea
TCD	Transcranial Doppler
TWiTCH	TCD With Transfusions Changing to Hydroxyurea
US	United States

Spectrum of Neurological Disease in SCD

Children and adults with sickle cell disease (SCD) experience a broad array f neurological complications. Overt strokes are the most visible and have long been considered a clear indication for hematopoietic stem cell transplant (HSCT). Children with sickle cell anemia (SCA), encompassing those with homozygous HbSS disease or compound heterozygosity for HbS and β^0-thalassemia trait, are at greatest risk; patients with other types of SCD have lower risks. Overt ischemic strokes have a bimodal distribution in the sickle cell population with a first peak in childhood, especially 5–10 years of age, and a second peak in mid-adulthood (30–50 years). In contrast, hemorrhagic strokes have a single peak in older teens and young adults [1]. The significance and impact of more subtle forms of brain injury, including cerebral vasculopathy, silent infarctions, and cognitive deficits, are increasingly acknowledged, but the role of HSCT is less established for patients with these conditions.

Overt Strokes

Clinically apparent, or "overt," strokes were first recognized as a complication of SCA starting in the 1930s [2, 3]. Individuals with overt strokes often present with classic stroke symptoms such as hemiparesis, aphasia, or seizures. Early reports of postmortem examination of the brain and cerebral arteries described infarctions in multiple vascular territories of varying chronicity [4, 5]. In 1998, the Cooperative Study of Sickle Cell Disease (CSSCD) documented an 11% prevalence of overt strokes by age 20 years [1]. Risk factors for ischemic strokes included severe baseline anemia, leukocytosis, early dactylitis, and high rate of acute chest syndrome episodes [1, 6]. A minority of overt strokes occur during concurrent illness such as acute chest syndrome, fever, or acute exacerbation of chronic anemia [7]. In fact, patients with strokes precipitated by an acute illness have a lower risk of stroke recurrence while receiving blood transfusion therapy [7]. During the years spanned

by the CSSCD, no specific treatment was uniformly recommended for primary or secondary stroke prevention, and recurrent ischemic strokes occurred in over two-thirds of patients [8].

In the 1970s and 1980s, blood transfusion therapy was reported to reduce the risk of overt stroke recurrence [8, 9]. In 2002, a study of 137 children receiving chronic blood transfusion therapy for secondary stroke prevention found that the risk of death in early adulthood remained high despite chronic transfusion therapy. This was often due to complications of transfusion therapy itself such as infection or hepatic failure from iron overload [7]. In an attempt to address the long-term mortality risks, the Stroke With Transfusions Changing to Hydroxyurea (SWiTCH) trial randomized children receiving chronic transfusion therapy for secondary stroke prophylaxis to "switch" to hydroxyurea plus serial phlebotomy or to continue transfusion therapy plus iron chelation medications. The experimental (hydroxyurea plus phlebotomy) arm of SWiTCH did not have enough benefit in iron burden reduction to justify the greater stroke recurrence rate compared with the transfusion arm [10]. The long-term risk-benefit assessment of chronic transfusions is further complicated by the experience of young adults transitioning care to adult care providers who frequently discontinue the transfusion therapy [11, 12]. In the United States, there is an increase in mortality in the early third decade of life coinciding with the transition to adult care [13], commonly due to acute SCD complications such as recurrent strokes or acute chest syndrome [12]. Thus, the 2014 National Heart, Lung, and Blood Institute's Evidence-Based Management of Sickle Cell Disease recommended that children and young adults with overt strokes and SCD should continue transfusion therapy indefinitely [14].

Because of the need for lifelong transfusion therapy, with its inherent risks of iron overload, transfusion-transmitted infections, and alloimmunization, children with SCA and strokes were considered candidates for curative treatment in the first studies of matched sibling donor HSCT for SCD [15, 16].

Elevated Transcranial Doppler Ultrasonography Velocities and Cerebral Vasculopathy

Given the high prevalence of overt strokes, and known associations with severe anemia, Adams et al. hypothesized that the noninvasive and relatively inexpensive transcranial Doppler (TCD) ultrasonography technique could be used to screen for high risk of strokes in children with SCA. After this strategy was validated as identifying high-risk children [17], the Stroke Prevention (STOP) Trial randomized high-risk children to receive chronic transfusion therapy or observation for primary stroke prevention. The trial was halted early due to a 90% reduction of first overt strokes in the transfusion therapy arm [18]. After TCD screening was implemented as a clinical tool, approximately 10% of children with SCA initiated chronic transfusion therapy for primary stroke prevention. The success of TCD screening and primary stroke prevention has been demonstrated in clinical populations with a

reduction in stroke incidence from approximately 1 stroke/100 patient-years to 0.2 strokes/100 patient-years [19, 20].

While primary stroke prevention was successful, a number-needed-to-treat analysis of the STOP trial results showed that seven children would receive transfusion therapy to prevent one stroke [18]. Recognizing the long-term risks of transfusion therapy, two subsequent studies tested whether transfusion therapy could be discontinued safely. The Optimizing Primary Stroke Prevention in Sickle Cell Anemia (STOP 2) trial randomized children with normalization of TCD velocities after at least 30 months of chronic transfusion therapy to continue or discontinue transfusions. It too was halted early in 2004 due to a high rate of strokes or recurrence of abnormal velocities in the discontinuation arm [21]. At that point, transfusion therapy was recommended indefinitely for primary stroke prevention. In 2016, however, the TCD With Transfusions Changing to Hydroxyurea (TWiTCH) study demonstrated that with careful management, children without severe cerebral vasculopathy could transition to hydroxyurea therapy without reverting to high-risk status [22]. The study had a short follow-up duration (24 months), and the authors noted that longitudinal follow-up is needed to fully define the risk/benefit ratio of hydroxyurea for primary stroke prevention. Whether hydroxyurea's benefit is due primarily to amelioration of chronic anemia, reduction of cerebral artery injury by sickled red blood cells, or a combination of both is unknown. Furthermore, hydroxyurea is most effective with daily administration at maximum tolerated dose, so medication adherence becomes critically important. Transfusion therapy requires more frequent clinic visits, but adherence is easier to monitor. Close monitoring of hydroxyurea, including adherence, is strongly recommended (see Chap. 3) [14].

Among 24 children who underwent matched sibling donor HSCT in the prospective Créteil newborn cohort, successful HSCT was more effective at normalizing TCD velocities than transfusion therapy [23]. On the other hand, the TWiTCH results suggest many children with abnormal TCDs can transition from transfusion therapy to hydroxyurea. Thus, there is less consensus on the appropriateness of HSCT for children with SCA and abnormal TCD velocities than for children with overt strokes. For this group of patients, an analysis of the risks and benefits for HSCT vs. hydroxyurea may be more appropriate. When families and clinicians are deciding between the therapeutic options of HSCT and hydroxyurea, the calculation should include the curative nature of HSCT. While HSCT carries a greater short-term mortality risk, for individual patients with a tendency toward non-adherence and/or reluctance to take daily hydroxyurea over a lifetime, HSCT may be more beneficial over the long term.

A small percentage of children with SCA develop severe cerebral vasculopathy, comprised of arterial stenosis or occlusion of the internal carotid, middle cerebral, and/or anterior cerebral arteries. Some may progress to moyamoya syndrome [24, 25]. TCD screening is intended to identify children with vasculopathy prior to overt infarction so that preventive transfusion therapy can be initiated [26]. To date, though, there is no way to predict which children with abnormal TCDs will develop severe arterial stenosis. Specifically, there are no large-scale imaging studies to determine the prevalence of severe cerebral vasculopathy among all children with SCA or among children with SCA and abnormal TCD.

Severe cerebral vasculopathy is a risk factor for transient ischemic attacks and infarction despite chronic transfusion therapy [24, 27]. For patients with moyamoya syndrome, cerebral revascularization surgery may be offered as a surgical treatment. Although randomized studies have not been performed, centers skilled in the procedure can often perform an indirect revascularization procedure (e.g., pial synangiosis, encephaloduroarteriosynangiosis (EDAS), or burr holes) resulting in long-term reduction of stroke risk [28–31]. These patients are also candidates for HSCT since they continue chronic transfusion therapy after revascularization [28–31].

Children with severe cerebral vasculopathy (defined as moderate stenosis in two or more intracerebral arterial segments or severe stenosis in one or more intracerebral arterial segments on time-of-flight MRA) were excluded from TWiTCH, and indefinite chronic transfusion therapy continues to be recommended for these children [22]. For that reason, HSCT is more likely to be recommended for this group of patients.

Silent Cerebral Infarctions (SCI)

While not clinically apparent, the so-called "silent" strokes (or silent cerebral infarctions, SCIs) affect many more children with SCA than both overt strokes and abnormal TCD velocities. SCIs are diagnosed by magnetic resonance imaging (MRI) and are defined as FLAIR/T2 hyperintensities at least 3 mm in greatest dimension and seen on two planes [32]. Children most commonly present with changes in cognition or school performance, but often the SCIs are found incidentally as part of an evaluation for an unrelated complaint such as headache. In 1996, the CSSCD reported that approximately 30% of children develop SCIs by adolescence [33]; however, as observation time has been extended in several small cohorts, a steady trend of higher prevalence has been identified, with up to 50% of adults with SCD demonstrating SCIs [34–36].

Baseline anemia, relative hypertension, male gender, and lower pulse oximetry have been identified as risk factors for SCIs [37]. Because SCIs are clinically silent, identifying clinical events that may provoke SCIs is difficult. Acute silent cerebral ischemic events (ASCIEs) have been found during acute anemic episodes (defined as hemoglobin concentration of $\leq$5.5 g/dL, regardless of etiology, with at least a 30% decrease from the patient's clinically established baseline) in children with and without SCD [38]. In the prospective Créteil cohort study in which all children underwent screening MRI, the relative risk of SCIs was 2.27 for each acute anemic event (defined as Hb concentration $\leq$6 g/dL) per year [36]. SCIs have also been described during or after episodes of severe acute chest syndrome [39].

The prognostic significance and optimal treatment of SCIs are less clear than that of overt strokes or abnormal TCD velocities. In the CSSCD's newborn cohort, the risk of an overt stroke after silent stroke detection was 3% over 35 months compared with 18% over 35 months for a child with an abnormal TCD in STOP [40]. The Silent Infarct Transfusion (SIT) trial randomized children with SCIs but without overt strokes or abnormal TCD velocities to observation or chronic transfusion therapy.

This study found a reduction in the combined endpoint of new overt plus silent strokes in the children randomized to transfusion therapy; however, 13 children would require chronic transfusion therapy to prevent one stroke [32]. Furthermore, the impact of hydroxyurea on SCI prevalence and recurrence is unknown. Given the high prevalence of SCIs and the modest effect of transfusion therapy seen in SIT, a randomized controlled non-inferiority trial of transfusion therapy vs. hydroxyurea for SCI prevention would require enrolling more than 1000 subjects [41]. Given this prohibitively large number, a definitive answer as to the best long-term treatment of SCIs is unlikely to be provided by a formal study. Some authors have proposed HSCT for those with SCIs based on the known risks of long-term transfusion therapy [41, 42].

Cognitive Impairment

Children with SCD and overt or silent strokes have cognitive deficits [43, 44], and the severity of deficit is correlated with amount or severity of brain infarction on MRI [45]. Even among very young children with normal TCD velocities and no SCIs, there is a risk for lower cognitive function [46]. Adults with SCD have high rates of unemployment or disability in the US, potentially due in part to cognitive deficits [34, 35].

Biological factors, such as anemia severity and thrombocytosis, are associated with cognitive deficits [47]. Chronic anemia leads to lower oxygen content of blood, necessitating increased cerebral blood flow and oxygen extraction fraction to maintain normal brain metabolism [48]. Additionally, socioeconomic factors, including parent/guardian level of education and maternal depression, influence cognitive impairment in SCD [46]. To date, the impacts of disease-modifying therapies including chronic transfusion therapy, hydroxyurea, and HSCT on cognition have not been adequately studied.

Finally, pre-existing cognitive deficits may affect outcomes of HSCT. Executive function deficits are common in children and adults with SCA [49–52], potentially resulting in difficulty with adherence to the complicated medication regimen after HSCT. For children undergoing HSCT, parents provide medication oversight, but adolescents and adults may need more assistance with medication adherence. Medication adherence, during and after HSCT, is dependent on many variables [53]. Children with SCA should be screened for factors that may impact adherence, including cognitive deficits, family support, and physical health [54–56].

Neurological Complications of HSCT

There are a variety of potential neurological complications for SCA patients undergoing HSCT (Table 6.1). Many factors contribute to the risk of neurological complications, including stem cell donor source, patient age, conditioning regimen,

Table 6.1 Differential diagnosis and suggested evaluation of neurological symptoms following HSCT for SCD

Symptom	Differential diagnosis	Diagnostic tests
Seizure	Medication side effect Ischemic stroke Hemorrhagic stroke PRES Infection	Head CT Brain MRI/MRA/MRV Lumbar puncture Electroencephalogram Check plasma calcineurin inhibitor levels Review medications
Altered mental status	Medication side effect Subclinical seizure PRES Infection Sinovenous thrombosis GVHD of the CNS	Head CT Brain MRI/MRA/MRV Lumbar puncture Electroencephalogram Check plasma calcineurin inhibitor levels Review medications
Focal neurological deficit	Ischemic stroke Fungal cerebral abscess	Head CT Brain MRI/MRA/MRV Biopsy of abscess, if present
Severe headache	PRES Hemorrhagic stroke Sinovenous thrombosis	Head CT Brain MRI/MRA/MRV
Visual disturbance	PRES Sinovenous thrombosis Medication side effect	Head CT Brain MRI/MRA/MRV Review medications

CT computed tomogram, *MRI* magnetic resonance imaging, *MRA* magnetic resonance angiogram, *MRV* magnetic resonance venogram, *PRES* posterior reversible encephalopathy syndrome, *GVHD* graft-versus-host disease

and pre-existing SCA complications. Most published reports of HSCT in SCA have utilized matched sibling donors; the complication rates of HSCT may be higher when alternative stem cell donor sources are used. A patient's pretransplant morbidities may impact the risk of developing certain neurological complications, such as ischemic stroke, while others, such as posterior reversible encephalopathy syndrome (PRES) and seizures, are related to the acute course, without a clear link to pretransplant neurological disease. Neurological complications are most likely to occur in the first 12 months after transplant [57]. Adverse events can be reduced by targeted supportive care. Table 6.1 delineates common presentations of HSCT complications in children with SCA and suggested diagnostic evaluation.

Seizures

Seizure is probably the most common neurological complication during and after HSCT [16, 58]. Seizures can occur due to a variety of risk factors (Table 6.1) [59]. Seizures can be the presenting symptom of an acute CNS insult (e.g., PRES, stroke), an adverse effect of a medication (e.g., busulfan, cyclosporine), or electrolyte

Table 6.2 Modifiable risk factors for seizures during and after HSCT

Include prophylactic antiepileptic drug (phenytoin or levetiracetam) in care plan
Avoid higher dosing of specific medications (busulfan, calcineurin inhibitors)
Rigorous management of electrolytes including magnesium
Avoidance of hypertension[a]

[a]Except for patients with severe vasculopathy who are reliant on higher blood pressure to maintain cerebral perfusion

disturbance (e.g., hypomagnesemia, hyponatremia). Seizures can also occur as sequelae of a prior ischemic stroke. The elevated seizure risk begins during conditioning and continues through the first year after HSCT [15, 16, 60]. As a result, SCA HSCT protocols now include a prophylactic antiepileptic medication (AED) such as phenytoin or levetiracetam. Typically, an AED is started prior to the initiation of busulfan and continued for 6 months to a year depending on the other medications provided during that time. For children with SCA undergoing HSCT without busulfan, antiepileptics are still part of the standard supportive care regimen, although this strategy has not been tested in a randomized controlled fashion [61–63]. Table 6.2 lists suggested strategies for reducing seizure risk.

Posterior Reversible Encephalopathy Syndrome (PRES)

PRES is relatively common following HSCT [64]. It is a disorder of cerebral autoregulation that occurs in the setting of hypertension, exposure to specific medications (e.g., calcineurin inhibitors), or both. The pathophysiology of PRES is unclear but may result from elevated cerebral blood flow and failure of cerebral autoregulation, leading to increased vascular permeability and cerebral edema. It has been reported in SCD patients during acute illnesses, especially acute chest syndrome [39, 65]. Children undergoing HSCT for hemoglobinopathies, including SCA, have higher reported occurrence of PRES than those undergoing HSCT for other reasons [63]. In a recent cohort of children with SCA undergoing sibling donor HSCT, 6% (3/52) of children developed PRES [61], whereas approximately 30% of children with hemoglobinopathies receiving unrelated donor HSCT developed PRES in two published studies [62, 66]. The higher prevalence in the unrelated donor cohort may reflect a greater need for corticosteroids to control graft-versus-host disease (GVHD) [62, 63].

Patients with PRES typically present with acute onset of headache, encephalopathy, seizures, and/or visual disturbance [67]. There is a characteristic radiographic appearance that can be seen on head computed tomography (CT) but is best appreciated by brain MRI. The characteristic pattern is T2 lesions that tend to be bilateral and posterior predominant with involvement of the gray and white matter but no diffusion restriction. PRES can be complicated by intracranial hemorrhage, ranging from microhemorrhages to subarachnoid bleeding and rarely intracerebral hematoma [68]. Symptoms typically resolve with treatment of the hypertension and/or adjustment of medications. While most patients recover completely, chronic

neurological deficits and persistent lesions, including SCIs, have been reported in patients with SCD and PRES [30, 39, 65]. Figure 6.1 shows the progression over time of one patient with PRES after HSCT. PRES can complicate the management of GVHD, since calcineurin inhibitors are frequently discontinued after PRES onset [62]. Because GVHD is a major cause of morbidity and mortality after HSCT, discontinuing calcineurin inhibitors could potentially increase these risks [62]. This challenge has led to the development of GVHD prophylaxis regimens that use sirolimus instead of cyclosporine or tacrolimus [69].

Overt and Silent Ischemic Strokes

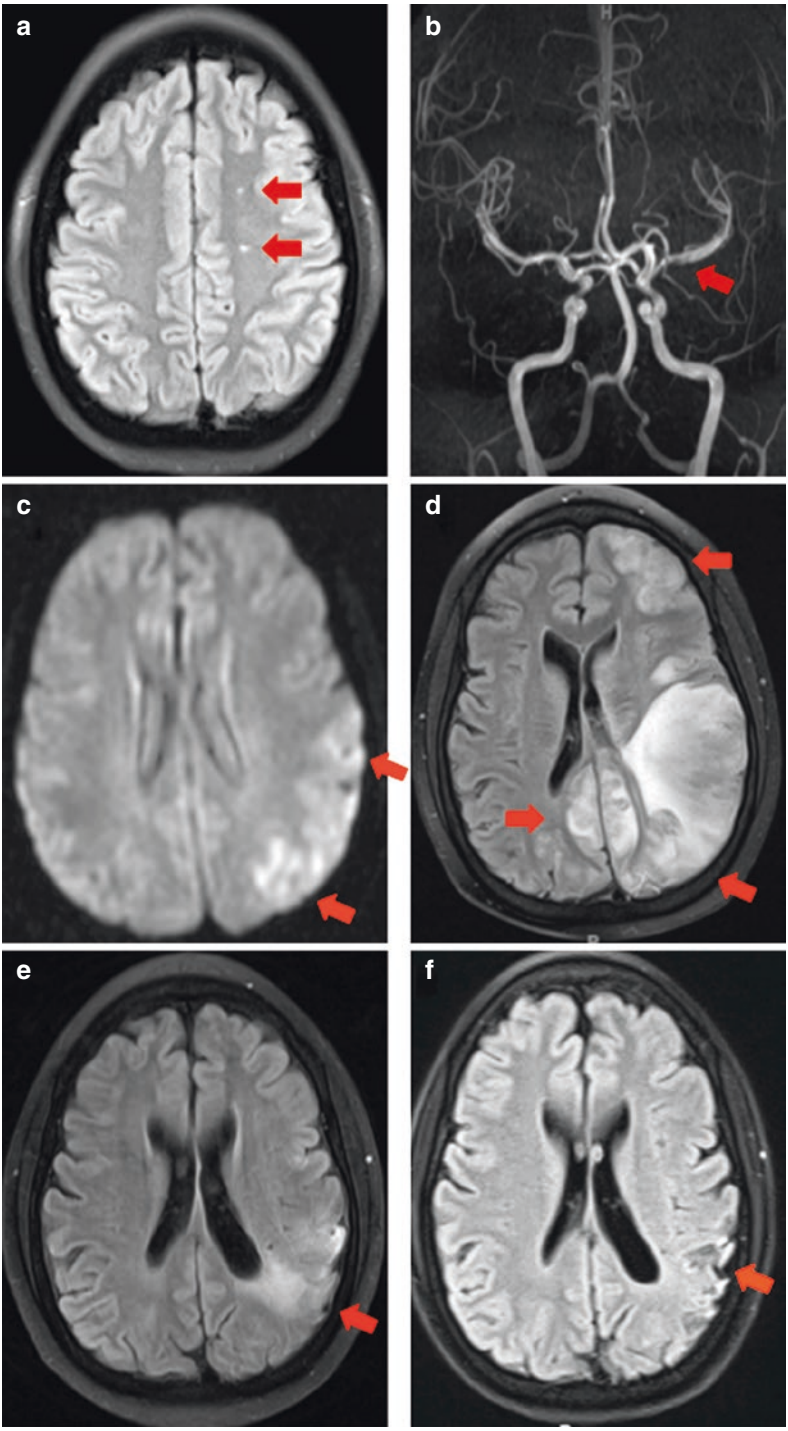
The risk for ischemic strokes during and after HSCT, while low, is not absent. Infarction may occur due to abnormal cerebral hemodynamics from a patient's pre-existing vasculopathy or as an indirect complication of HSCT with or without an underlying vasculopathy. Acute complications during HSCT, such as infections, anemia, and GVHD, may cause hemodynamic instability and relative hypotension, jeopardizing cerebral blood flow for patients with vasculopathy and putting them at increased stroke risk. A cohort study identified two teens with severe cerebral vasculopathy who had new cerebral infarctions during the posttransplant period, both in the context of chronic GVHD, anemia, and continued transfusion dependence [30].

Intracranial Hemorrhage (ICH)

An interim report from the Multicenter Study of Bone Marrow Transplantation for Sickle Cell Disease noted a higher risk of ICH for HSCT patients with prior stroke. Specifically, 38% (3 of 8) of patients with prior overt strokes experienced ICH compared with 0 of 13 patients without prior overt stroke [60]. Patients who experienced ICH also were hypertensive, thrombocytopenic, or had GVHD [60]. A French cohort reported 1 of 87 patients undergoing HSCT experienced ICH; that child had a pre-existing moyamoya syndrome which likely increased his risk for hemorrhage [58]. While less common than PRES, ICH is more likely to be fatal. A recent reduction in fatal ICH events has been attributed to more aggressive control of blood pressure and maintaining platelets >50,000/μL [61, 62].

Cerebral Sinovenous Thrombosis (CSVT)

Thrombosis of the intracranial venous sinuses is an uncommon, but described, complication of SCD [70] and of HSCT [71]. Presence of a central venous line can be a risk factor for thrombosis originating in the jugular veins and extending into the intracranial sinuses. Common symptoms include headache, seizure, and altered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mental status [70]. Diagnosis is made with MR venogram or CT venogram. CSVT can lead to venous congestion of the brain parenchyma. Secondary venous infarctions can occur, and venous infarctions have a tendency for hemorrhagic conversion. When CSVT is extensive or involves multiple cerebral sinuses, increased intracranial pressure can develop resulting in visual disturbances from pressure on the optic nerves, cranial nerve six palsies, or both. Treatment consists of anticoagulation, which can be challenging in a patient with or at risk for thrombocytopenia [70, 72].

Central Nervous System Infection

Infections of the central nervous system following HSCT have the potential for high morbidity and mortality [73]. Patients with SCA are at high risk of infection with encapsulated bacteria, including pneumococcal and meningococcal bacteremia and meningitis, due to functional or surgical asplenia [74]. Furthermore, after the immunosuppressive transplant condition regimen, patients lack protective antibody titers against these organisms until they complete post-HSCT re-immunization. Patients with fever and encephalopathy or seizure should undergo prompt evaluation for bacterial meningitis and empiric treatment until bacterial meningitis is ruled out. Patients should also continue prophylactic antibiotics to prevent invasive pneumococcal disease during and after HSCT.

During periods of immune suppression, patients are also at risk for opportunistic infections of the CNS, including viral and fungal pathogens [75, 76]. Patients presenting with seizures or encephalopathy should be evaluated for bacterial, fungal, and viral infections of the CNS. Herpesviruses (cytomegalovirus, Epstein-Barr virus, herpes simplex virus 1 and 2, human herpesvirus-6, varicella-zoster virus) are common and often treatable with antiviral drugs such as acyclovir, ganciclovir, or foscarnet. Adenovirus infection is more difficult to control and has a high mortality rate; treatment requires cidofovir, which has significant renal toxicity. JC virus, the

Fig. 6.1 Posterior reversible encephalopathy syndrome (PRES) complicating HSCT. This 15-year-old with a past history of silent cerebral infarctions in the left centrum semiovale (**a**, *arrows*) and left middle cerebral artery stenosis (**b**, *arrow*) underwent matched unrelated donor HSCT. Approximately 3 months after HSCT, she was receiving corticosteroids and calcineurin inhibitors for acute graft-versus-host disease and presented with a right-sided seizure. Emergent MRI demonstrated diffusion restriction in the left posterior parietal watershed region (**c**, *arrows*) consistent with acute ischemic stroke. Five months after that event, she had acute onset of right-sided weakness and aphasia. Emergent MRI demonstrated patchy T2 enhancement in all lobes but with a posterior predominance (**d**, *arrows*), consistent with PRES. She had persistent right-sided weakness for several months, requiring neurorehabilitation. Follow-up brain MRI 2 months later demonstrated evolving white matter hyperintensities indicating chronic infarctions (**e**, *arrow*). Two years later, she has normal speech and very mild right-sided weakness, and her MRI shows atrophy of the left hemisphere, especially in the parietal lobe, with cortical laminar necrosis (**f**, *arrow*)

cause of progressive multifocal leukoencephalopathy (PML), has been reported rarely after HSCT [77]. Polymerase chain reaction testing of spinal fluid is the most common diagnostic modality for viral detection.

Fungal CNS infections typically present with focal neurological findings rather than encephalopathy. The most commonly reported CNS fungal infections are abscesses due to aspergillus and invasive mucormycoses. Fungal abscesses are typically diagnosed by brain imaging with MRI or CT, followed by biopsy. Surgical resection may be needed for control of the fungal infection [78]. Even with aggressive intervention, fungal CNS infections carry high mortality rates [79].

Graft-Versus-Host Disease of the Nervous System

Evidence from murine studies supports graft-versus-host disease (GVHD) occurring in the nervous system [80]. Peripheral nervous system manifestations include myositis, immune-mediated neuropathy, and myasthenia gravis; central nervous system manifestations include encephalopathy, demyelination, and vasculitis [81]. These entities may be difficult to diagnose, due to symptoms that overlap with other HSCT complications (e.g., infection, drug toxicity) and due to difficulty in obtaining a biopsy of the CNS. While immune suppression is the appropriate treatment for GVHD, it can worsen opportunistic infections, so typically immune suppression is pursued after reasonable attempts to exclude infectious causes.

Muscle weakness from GVHD myositis can be clinically difficult to distinguish from steroid-induced myopathy, which has a characteristic pattern on muscle biopsy. Treatment is based on biopsy results, with steroid-induced myopathy treated by discontinuing steroids or changing to intermittent rather than daily administration, while GVHD myositis is treated with immune suppression [81].

Neurological Supportive Care During HSCT

Transfusions with Red Blood Cells and Platelets

Packed red blood cell (PRBC) transfusions are a critical component of HSCT care. PRBC transfusions suppress endogenous HbS synthesis and thus protect against vaso-occlusion as well as complications of anemia. Transfusion thresholds have not been compared in prospective studies, but the early trials of HSCT without PRBC transfusion parameters had high rates of strokes and other neurological complications [60, 82]. The transfusion parameters used for chronic transfusion therapy (e.g., HbS <30% and Hb concentration >9 g/dL but ≤11 g/dL) have been adopted for the HSCT setting, along with other supportive care measures resulting in a decline of

treatment-related adverse events [61, 62]. PRBC transfusions should begin before the preparative regimen and continue until stable engraftment, when the patient maintains normal hemoglobin without transfusions. (See Chap. 5 for more information on transfusion support during HSCT.)

In early SCA HSCT cohorts, platelet transfusions were given for platelet counts <10,000/μL based on prior experience with HSCT for malignant conditions. Intracranial hemorrhage was a significant complication, occurring in 3 out of 21 recipients in the Multicenter Study of Bone Marrow Transplantation for Sickle Cell Disease [60]. As a result, more recent studies recommended maintaining platelet counts >50,000/μL with a noticeable reduction in intracranial hemorrhages [61, 62]. Other factors associated with ICH in these studies were active GVHD and hypertension [60].

Electrolyte Supplementation

Hypomagnesemia occurs in almost all patients receiving calcineurin inhibitors for GVHD prophylaxis [83]. Oral or intravenous magnesium supplementation is used routinely in HSCT recipients with hypomagnesemia to decrease seizure risk.

Blood Pressure Management

Control of hypertension is a critical part of PRES and ICH prevention. Calcineurin inhibitors and corticosteroids are regularly used for both GVHD prophylaxis and treatment, but these medications can increase blood pressures significantly. In addition, children with SCD may have pre-existing subclinical renal disease [84], predisposing them to hypertension during and after transplant. Supportive care guidelines recommend use of a calcium channel blocker as initial management to counteract the effects of calcineurin inhibitors on renal blood flow [62]. Additional antihypertensives may be needed, especially for patients with renal disease. Blood pressure goals are to maintain blood pressure within two standard deviations of the mean for age and sex of individuals with SCD [84].

On the other hand, hypotension and hypovolemia should be avoided. Patients with SCA have baseline cerebral blood flow elevation but reduced ability to increase cerebral blood flow and oxygen delivery during hemodynamic stress [85]. This inability to increase tissue oxygen delivery acutely puts patients at risk of ischemic strokes [48]. Also, patients with severe cerebral vasculopathy require higher blood pressures to maintain cerebral perfusion. For these patients it is critical to maintain blood pressures at their own pre-HSCT baseline to prevent relative hypotension that could increase their stroke risk.

Recommended Neurological Evaluations for SCA Patients Undergoing HSCT

Pre-HSCT

- Children with SCA who will undergo HSCT should have a comprehensive neurological evaluation, including complete neurological exam by a child neurologist with documentation using a validated examination tool such as the Pediatric Stroke Outcome Measure (PSOM) [86]. Use of a validated instrument for a pre-HSCT evaluation allows for comparison of subsequent exams to the patient's pre-HSCT baseline.
- Prior to HSCT, children should have a brain MRI and MRA to document baseline infarct burden and cerebral vasculopathy since presence of either of these may influence the likelihood of neurological complications. If time-of-flight MRA suggests severe cerebral vasculopathy or moyamoya, a conventional cerebral angiogram should be performed for a more accurate evaluation of arterial abnormalities.
- A comprehensive neuropsychological battery should be administered, both to document pre-HSCT deficits and to identify cognitive issues that may impact the supports the patient will need for post-HSCT care, such as assistance with medication administration and transportation to appointments.
- If children planning to undergo HSCT have severe cerebral vasculopathy or moyamoya syndrome, consideration should be given to cerebral revascularization surgery prior to transplant. Pial synangiosis, the most common approach to revascularization in children, improves blood supply to the brain parenchyma by ingrowth of new collaterals from an unaffected arterial segment that is placed directly on the brain surface [28]. This may provide protection against cerebral hemodynamic fluctuations during and after HSCT when patients are likely to experience hemodynamic stresses such as fevers, blood pressure changes, and GVHD. Many clinical centers perform revascularization surgery at least 6 months prior to HSCT, to allow for healing and neovascularization to occur.

During HSCT and Within the First Year Post-HSCT

Beginning at the start of the preparative regimen and through the first year post-HSCT, evaluations should be guided by symptoms.

- A thorough neurological examination should be documented any time acute neurological symptoms occur.
- Emergent neuroimaging is warranted with any severe or abrupt onset headache, seizure or potential seizure, or alteration in mental status. CT is the best imaging modality to identify ICH, and can be performed quickly and without sedation.

For these reasons, CT is typically the first imaging study for evaluation of an acute neurological change.

- If a CT is performed due to acute neurological symptoms, an MRI, MRA, and contrast-enhanced MRV should be performed as soon as feasible and safe, both to confirm CT findings and evaluate for parenchymal injury and/or venous thrombosis.
- A lumbar puncture should be considered for any patient presenting with seizures or encephalopathy because opportunistic pathogens, especially viruses, can infect the CNS of an immunosuppressed patient. An opening pressure should be performed to assess for increased intracranial pressure.
- Electroencephalogram (EEG) should be performed when seizures are suspected, and continuous EEG monitoring should be considered in patients with encephalopathy.
- For patients presenting with neurological changes, medications should be reviewed to identify any potential agents that can cause or exacerbate neurological symptoms. Table 6.3 lists medications commonly used during and after HSCT that can cause neurotoxicity.

Table 6.3 Medications with potential neurotoxicity commonly used during HSCT

Medication	Neurological symptom	Neurological disorder/toxicity
Antiepileptic		
Fosphenytoin	Ataxia	Acute and chronic neurotoxicity[a]
	Nystagmus	
	Confusion	
Alkylating agent		
Busulfan	Seizures	Acute neurotoxicity[a]
Calcineurin inhibitors		
Cyclosporine	Tremor	
	Seizures	PRES
	Visual disturbance	Pseudotumor
	Encephalopathy	
Tacrolimus	Weakness	
	Seizures	Neuropathy, myasthenia
	Encephalopathy	PRES
Corticosteroids		
Methylprednisolone	Insomnia	
Prednisone	Mania	Myopathy
	Weakness	Neuropathy
	Paresthesias	
Purine antimetabolite		
Fludarabine	Paresthesias	PML
	Focal weakness	Demyelination[a]
	Encephalopathy	
Monoclonal antibodies		

(continued)

Table 6.3 (continued)

Medication	Neurological symptom	Neurological disorder/toxicity
Alemtuzumab (anti-CD 52)	Headache	
	Weakness	CIDP
	Paresthesias	
Rituximab (anti-CD 20)	Focal weakness	PML
	Seizure	PRES
	Encephalopathy	
Antifungal agent		
Voriconazole	Encephalopathy	Toxic encephalopathy[a]
	Delirium	
	Hallucinations	

PRES posterior reversible encephalopathy syndrome, *CIDP* chronic inflammatory demyelinating polyradiculoneuropathy, *PML* progressive multifocal leukoencephalopathy
[a]Dose dependent

Long-Term Follow-Up After HSCT

- The PSOM or other validated stroke outcome measure should be repeated 1–2 years after HSCT.
- A brain MRI/MRA should be repeated 1–2 years after HSCT in patients who have stable exams and no new symptoms. For patients with extensive pretransplant infarctions, cerebral vasculopathy, or other CNS pathology, consideration should be given to monitoring with MRI/MRA every 1–2 years, since clinically silent progression has been reported even after HSCT [30, 57].
- Neuropsychological testing should be repeated 1–2 years after HSCT to determine whether the patient has any persisting or new cognitive deficits that require educational or vocational accommodations.

Neurological Outcomes Following HSCT

Recurrent Overt and Silent strokes

The risk of ischemic stroke is low after successful HSCT. With sustained engraftment and in the absence of severe chronic GVHD, patients typically remain free of overt strokes. In the Multicenter Study of Bone Marrow Transplantation for Sickle Cell Disease, 2 of 57 patients had recurrent overt strokes following graft rejection [16]. In a case series of nine children from St. Jude Children's Research Hospital, none of the patients had new overt strokes, but five children with pre-HSCT MRAs showing stenosis, occlusion, or moyamoya collaterals had new subclinical infarctions within the first 18 months posttransplant. Subsequent imaging ranging from 2

to 7.5 years after HSCT did not show continued progression of infarctions; however, two patients were noted to have progression of cerebral vasculopathy 6 years after HSCT [57].

Cerebral Vasculopathy

The progression of cerebral vasculopathy may be ameliorated by HSCT as well. Most patients have stabilization or improvement of vasculopathy post-HSCT [87]. A review of 196 patients from four cohort studies reported post-HSCT vasculopathy progression in 16% of children with pre-HSCT vasculopathy [88]. The true prevalence of vasculopathy progression remains unclear, but if there is a risk for progression, it is thought to be highest in the first year after HSCT [57, 58, 88]. In comparison, progression of cerebral vasculopathy was demonstrated in 38% of children in a cohort receiving chronic blood transfusion therapy for secondary stroke prevention [24].

Interpretation of the data can be complicated by variances in imaging techniques. Time-of-flight MRA in children with anemia may have the artifactual appearance of stenosis due to turbulent blood flow, especially in the internal carotid arteries [89]. In patients with cerebral artery irregularity on time-of-flight MRA before HSCT, apparent normalization of MRA after HSCT may be due to reduction in turbulent blood flow with normalization of hemoglobin concentration. Cerebral vasculopathy progression is also possible following graft rejection, accompanied by increased risk of infarction (Fig. 6.2).

Cognitive Outcomes and Health-Related Quality of Life

The impact of HSCT on cognition has not been fully characterized in children with SCA. In a cohort of nine children who underwent matched sibling donor HSCT and received busulfan as part of their preparative regimen, full-scale intelligence quotient (IQ), math achievement scores, and reading achievement scores were not significantly different after HSCT [57]. While cognitive impairment is correlated with severity of anemia in children with SCA [47], it is not known whether normalizing hemoglobin after HSCT reverses cognitive deficits. Children who have experienced neurological complications during transplant, such as PRES or ICH, may have new cognitive deficits. Busulfan-containing myeloablative conditioning regimens have also been implicated in post-HSCT cognitive impairment [90]. One goal of non-myeloablative conditioning regimens is to reduce the potential cognitive cost of HSCT.

The longitudinal impact of HSCT on health-related quality of life (HRQL) has been reported in two recent patient cohorts. The prospective Sickle Cell Unrelated Transplant (SCURT) study reported that at 1 year after HSCT, patients and their

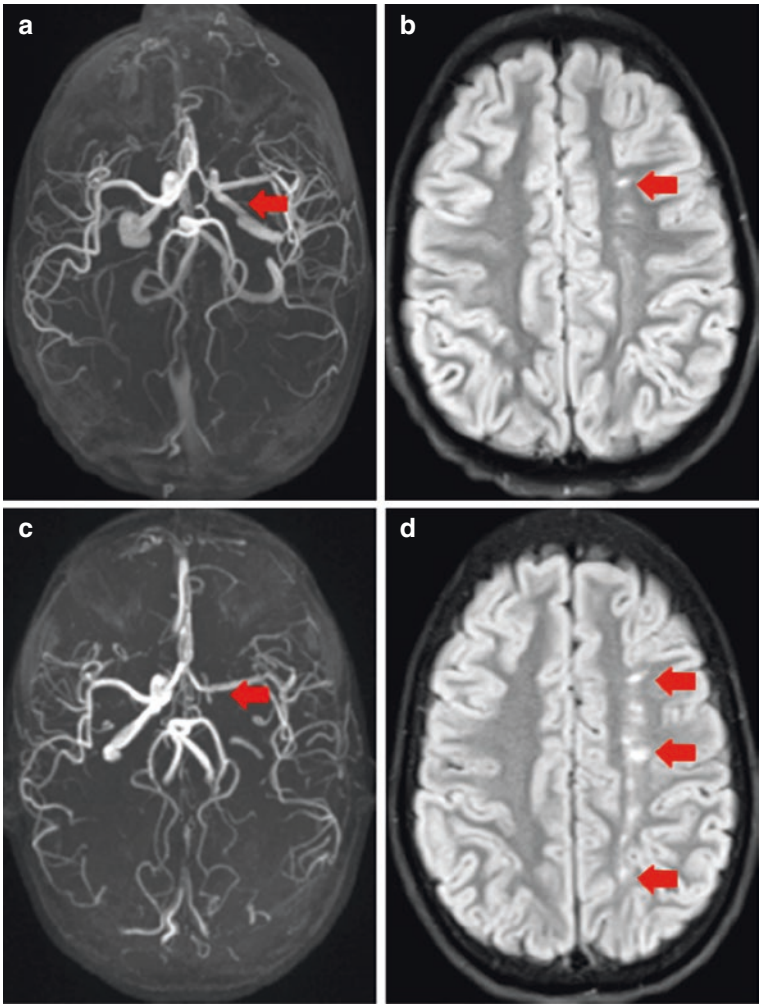

Fig. 6.2 Progressive cerebral vasculopathy and recurrent cerebral infarction after rejection of matched sibling donor HSCT. This 8-year-old girl with HbSS received chronic blood transfusion therapy due to an abnormal transcranial Doppler study, an MRI/MRA demonstrating reduced flow in the left internal carotid artery (**a**, *arrow*), and left periventricular white matter silent cerebral infarcts (**b**, *arrow*). She presented with increasing memory deficits 2 years after matched sibling donor HSCT. At that time, she was anemic with 20% donor engraftment, indicating graft rejection. Her MRI at that time showed absence of blood flow in the left internal carotid artery (**c**, *arrow*) and new chronic silent infarctions (**d**, *arrows*). She resumed chronic blood transfusion therapy and underwent cerebral revascularization surgery, with a second HSCT from the same donor planned for 9–12 months after revascularization

parents reported improvement in health, physical functioning, behavior, and self-esteem as measured by the Child Health Questionnaire, a validated measure of HRQL [62]. In a single-institution study of 18 children with SCD, parents of

children with little or no cerebral infarct burden before HSCT reported significantly higher overall HRQL (as measured with the PedsQL validated measure) at a mean 3 years after HSCT, but the children themselves did not report a significant improvement [91]. Neither children with more severe pre-HSCT infarct burden nor their parents reported HRQL change [91]. This suggests that HRQL may not be normalized by simply curing SCA. Further delineating the impact of HSCT on HRQL using the same validated instruments in larger groups of HSCT recipients is important future work.

Conclusions

Neurological disease in children with SCA is stabilized and even improved following HSCT, but the transplant process itself carries significant neurological risks. Advancements in GVHD prophylaxis and supportive care measures have reduced the risk of PRES and ICH, making HSCT a safer and more realistic option for more patients with SCA. More work remains to be done to understand and minimize the risks of HSCT so that it can be safely offered to more patients as a definitive cure for SCA and its associated neurological complications. Areas of active investigation, including cerebral hemodynamics, cognition, and HRQL, will help clarify the neurological outcomes of HSCT.

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Part II
Hematopoietic Stem Cell Transplantation

Chapter 7
Overview of Hematopoietic Stem Cell Transplantation for Nonmalignant Diseases

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Abbreviations

ATG	Antithymocyte globulin
BMF	Bone marrow failure
Bu/Flu	Busulfan/fludarabine
CMV	Cytomegalovirus
CNS	Central nervous system
Css	Concentration at steady state
DLI	Donor lymphocyte infusion
EBV	Epstein-Barr virus
EFS	Event-free survival
GVHD	Graft-versus-host disease
GVLE	Graft-versus-leukemia effect
HLA	Human leukocyte antigen
HLH	Hemophagocytic lymphohistiocytosis
HSCT	Hematopoietic stem cell transplant
IMD	Inherited disorders of metabolism
IST	Immunosuppressive therapy
MSD	Matched sibling donor
MUD	Matched unrelated donor
NMD(s)	Nonmalignant disorder(s)
OS	Overall survival
PTLD	Post-transplant lymphoproliferative disease

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RIC	Reduced intensity conditioning
SAA	Severe aplastic anemia
SCD	Sickle cell disease
SCID	Severe combined immunodeficiency syndrome
SOS	Sinusoidal obstruction syndrome
TRM	Transplant-related mortality
UCB	Umbilical cord blood
URD	Unrelated donor
VST	Virus-specific T-cell therapy

Introduction

The first successful curative stem cell transplant was done in 1968 using a human leukocyte antigen (HLA)-matched sibling donor for a child with severe combined immunodeficiency syndrome (SCID) [1]. Since then, the field of hematopoietic stem cell transplant (HSCT) has rapidly evolved. Marked improvement in HSCT outcomes has occurred due to several critical advances in HLA matching, management of infections, graft-versus-host disease (GVHD) prevention, and the use of less intensive conditioning. Svenberg et al. compared outcomes in patients undergoing HSCT from 1992–2002 with those from 2003–2013. The 3-year overall survival (OS) for patients undergoing HSCT for nonmalignant disorders (NMDs) improved from 77% to 87%. They also noted a higher rate of transplantations being performed for nonmalignant indications [2]. According to the Center for International Blood and Marrow Transplant, nonmalignant conditions accounted for 27.5% of the 11,875 HSCT done in patients 20 years and younger from 2004–2014 [3].

Indications for NMD Transplant and Overview of Transplant Experiences by Disease Group

The ideal nonmalignant conditions treated with HSCT are those that have life-threatening defects restricted to lymphoid and/or hematologic manifestations without major somatic defects and those metabolic diseases arising from enzyme deficiencies where hematopoietic stem cells are able to produce sufficient enzyme levels, preventing disease progression (e.g., Hurler syndrome). Historically, only severe disease manifestations were considered for NMD HSCT. With improved supportive care, HSCT is being performed for NMDs with moderate disease severity. Table 7.1 lists common indications for HSCT in NMDs.

Table 7.1 Common indications for HSCT in NMDs

Common nonmalignant indications for hematopoietic stem cell transplant	
Hemoglobinopathies and other disorders of RBCs	Sickle cell disease Beta/alpha thalassemia major Congenital sideroblastic anemia Pyruvate kinase deficiency
Primary immunodeficiency syndromes	Severe combined immunodeficiency syndrome Wiskott-Aldrich syndrome X-linked hyper-IgM syndrome (CD40L deficiency) T-cell immunodeficiency syndromes Phagocyte disorders (chronic granulomatous disease)
Acquired bone marrow failure syndromes	Idiopathic severe aplastic anemia
Inherited bone marrow failure syndromes	Fanconi anemia Congenital amegakaryocytic thrombocytopenia Kostmann's syndrome Diamond-Blackfan anemia Dyskeratosis congenita Nijmegen breakage syndrome
Inherited metabolic/genetic disorders	Hurler's disease (MPS IH) Krabbe disease X-linked adrenoleukodystrophy (childhood onset) Metachromatic leukodystrophy Infantile osteopetrosis Epidermolysis bullosa
Refractory autoimmune disorders	Immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome Early-onset inflammatory bowel disease with immune defects

Primary Immunodeficiency Syndromes

Since the first successful allogeneic HSCT for SCID, the paradigm for SCID management has changed, largely due to the implementation of a newborn screening program in the majority of states in the USA. The development of newborn screening for SCID was prompted by studies showing that transplantation early in life, prior to infectious complications, resulted in improved outcomes [4–8]. With the majority of newborns in the USA currently screened for SCID, the standard-of-care treatment has become the elective HSCT of otherwise asymptomatic infants. Numerous studies have confirmed that OS rates for HSCT in SCID using a matched related donor under optimal conditions approaches 90% or greater [4, 6, 9–11]. Unique to SCID, due to the lack of functional T-cells which are responsible for graft rejection, HLA-identical HSCTs lack an absolute requirement for pre-transplant conditioning and are rarely associated with the development of either acute or chronic GVHD [7, 12, 13]. The main exception to this statement is NK+ SCID patients; these patients require conditioning as host NK cells have been found to

compete with donor T-cells resulting in graft failure [14]. The treatment of choice for young patients with typical SCID without prior opportunistic infections remains an unmodified (i.e., non-T-cell depleted) HSCT from a matched sibling donor (MSD) without the use of pre-transplant conditioning. However, increasing evidence has shown better long-term T- and B-cell reconstitution is achieved with the use of pre-HSCT conditioning, regardless of donor type. In 2014, Pai and colleagues reported a 5-year survival and immune reconstitution analysis in a cohort study of 240 patients who underwent HSCT for typical SCID from 2000–2009. This study showed good OS rates regardless of donor source used—94% for patients transplanted at 3.5 months or younger, 90% among infants older than 3.5 months without prior infection, and 82% in older infants with a history of infection that had resolved. The largest factors impacting survival were age at HSCT and infection status [11].

For SCID patients, there is a presumed survival advantage for donor T-cell engraftment, and repleting the lymphoid compartment alone with donor cells can result in disease phenotype correction. However, in many other immunodeficiency syndromes, the defect lies in both lymphoid and myeloid components, hence a robust lymphoid and myeloid chimerism is essential for optimal HSCT outcome. For example, in addition to lymphocytes, Wiskott-Aldrich syndrome patients have defective platelets, CD40 ligand deficiency has neutrophil and monocytic defects, and chronic granulomatous disease results from neutrophil dysfunction. For these immunodeficiency syndromes, robust stem cell/myeloid engraftment is critical in order to correct both the lymphoid and myeloid compartments and thereby achieve long-term disease cure. Conventionally pre-HSCT conditioning with both immunosuppressive and myeloablative regimens has been used [15]. Traditional myeloablative regimens include busulfan and cyclophosphamide with reported long-term survival rates of about 70–80% [16–18]. Reduced toxicity regimens based on fludarabine with either busulfan, treosulfan, or melphalan are being increasingly used with better outcomes [19–22].

The scope of immunodeficiency syndromes undergoing HSCT continues to expand and now includes immune dysregulation indications such as immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome and early-onset inflammatory bowel disease with immune dysfunction. Table 7.2 details the variety of immunodeficiency syndromes that have been cured via HSCT. Currently, the use of HSCT for refractory autoimmune disorders such as systemic lupus erythematosus, scleroderma, and rheumatoid arthritis remains experimental.

Hemoglobinopathies and Disorders of Red Blood Cells

Hemoglobinopathies including thalassemia and sickle cell disease (SCD) are the most common genetic disorders worldwide. However, unlike rarer inherited disorders which rapidly progress to serious disease manifestations and early death, supportive care measures allow patients with hemoglobinopathies to live into adulthood. Initially, HSCT for non-thalassemia hemoglobinopathies was limited to severe manifestations such as clinical stroke and recurrent acute chest syndrome in

Table 7.2 Selected examples of primary immunodeficiency syndromes that have been cured by HSCT

Primary immunodeficiency syndromes that have been cured by HSCT	
SCID	Classical SCID Leaky SCID Reticular dysgenesis
T-cell deficiencies and defective T-cell activation syndromes	X-linked hyper-IgM (CD40 ligand deficiency) Purine nucleoside phosphorylase deficiency MHC II deficiency
Phagocytic disorders	Severe congenital neutropenia Chronic granulomatous disease Leukocyte adhesion deficiency
Combined immune defects	Wiskott-Aldrich syndrome Cartilage-hair hypoplasia DOCK8 deficiency
Hyperinflammatory disorders	Familial hemophagocytic lymphohistiocytosis Griscelli syndrome Chediak-Higashi syndrome

SCD [23]. More recently, focus on quality of life and poor long-term disease outcome has brought HSCT to the forefront of therapy in SCD. The first successful HSCT demonstrating cure for hemoglobinopathies was in β-thalassemia major [24]. Due to concerns regarding increased risk of HSCT in hypertransfused patients from HLA sensitization and iron overload, these first transplants were performed in young patients who had received minimal transfusions. Multiple studies have confirmed a negative impact of iron overload and its associated tissue damage on outcomes of HSCT for transfusion-dependent thalassemia and SCD. Specific risk factors for poor outcomes in transfusion-dependent thalassemia include hepatomegaly more than 2 cm, the presence of liver fibrosis, and irregular iron chelation in the 18 months prior to transplant [25, 26]. In patients with 0–2 of these risk factors, OS using MSD exceeds 90%. Current outcomes using HLA-identical siblings for HSCT in SCD report disease-free survival estimates at 90–95% using conventional myeloablative conditioning [27, 28]. These results advocate for the consideration of HSCT using MSD in hemoglobinopathies prior to severe disease manifestations.

The main problems in high-risk hemoglobinopathy patients are a heightened rate of graft rejection and increased regimen-related toxicity. Factors such as marrow hyperactivity, alloimmunization from frequent transfusions, and an immunocompetent host all contribute to increased graft failure. In addition, underlying vasculopathy and potential cardiopulmonary complications often present in SCD can complicate the HSCT process, making preparative regimens difficult to tolerate. Increased immunosuppression in the pre-transplant regimen has led to decreased rates of graft rejection. This has been accomplished via the addition of pre-transplant hydroxyurea, azathioprine, thiotepa, or antithymocyte globulin (ATG) [29–32]. Reduced intensity conditioning (RIC) protocols have also led to improvement in the toxicity profile, but the challenge of maintaining adequate engraftment is amplified

[33, 34]. Unique to the hemoglobinopathies, stable mixed chimerisms in the presence of high red cell chimerism can result in disease-free survival. Acceptance of stable mixed chimerisms is key to the application of RIC regimens. Better success in RIC regimens has been achieved via the addition of alternative immunoablation methods such as alemtuzumab or post-engraftment sirolimus [35].

Perhaps the most pressing issue and primary barrier to the use of HSCT in hemoglobinopathies, and especially SCD, is the lack of available donors. Approximately only 14% of patients with SCD will have a MSD, and only 19% will have a matched unrelated donor (MUD) in the registry [36, 37]. A recent phase II study of MUD for SCD resulted in OS of 79% at 2 years; however, outcomes were complicated by extensive chronic GVHD in almost 40% of patients [38]. Due to constraints on donor availability, haploidentical transplants are being explored in phase I and II trials [39]. Newer developments in the use of alternative donors are needed to expand the utility of HSCT for hemoglobinopathies [40].

Inherited Disorders of Metabolism

Transplantation for inherited disorders of metabolism (IMD) is aimed at disorders associated with an enzyme deficiency or transporter mutations. While there are several small experimental studies using HSCT for a variety of IMD, HSCT has become standard of care mostly for a few of the mucopolysaccharidoses and leukodystrophies. These defects lead to the buildup of toxic metabolic products with subsequent development of cardiopulmonary complications, skeletal deformities, and progressive neurologic disease. The rationale for using HSCT as treatment for IMD stems from the ability of the transplanted cells to produce the deficient or absent enzyme and "cross-correct" neighboring cells. This cross-correction provides continuous enzyme-replacement therapy that unlike traditional enzyme-replacement therapy is capable of traversing the blood-brain barrier and improving cognitive and CNS disease [41]. The first HSCT for IMD was performed for Hurler's syndrome using a MSD in 1981 [42]. Early efforts at HSCT for IMD utilized bone marrow as the primary graft source and focused on matched related donors. Matched umbilical cord blood (UCB) has since become a preferred graft source. The use of UCB in HSCT for Hurler's syndrome has been associated with higher rates of full donor chimerism and normal enzyme levels in comparison with bone marrow or peripheral blood grafts. These results are partly related to the ability of UCB cells to differentiate into non-hematopoietic cells [43, 44]. In addition, the use of sibling donors who are heterozygous carriers of the enzyme defect has resulted in inferior outcomes thought to be due to the delivery of only half the enzyme "dose" compared to noncarrier donors [45]. The increased HLA permissiveness afforded by UCB grafts and readily available donor banks are associated with a shorter interval between diagnosis and transplant, which also correlates with improved survival outcomes [46]. A phase II trial investigated the use of unrelated UCB in 69 patients undergoing HSCT for IMD during 1999–2004 using a conditioning regimen consisting of busulfan,

cyclophosphamide, and ATG. This study found a 1-year OS of 72%, similar to results reported in other studies [47, 48]. The cumulative incidence of primary neutrophil engraftment (defined as ≥90% documented donor cells) by day 42 was 78%, with the median time to primary neutrophil engraftment occurring at day 25 [47]. In more recent studies, OS rates approach 95%. Mismatched donors and older age are predictive of acute GVHD and worsened event-free survival (EFS) [49].

There are many limitations regarding transplantation for IMD. HSCT often does not correct all disease manifestations. For example, the skeletal deformities associated with Hurler's syndrome remain unaffected by HSCT. Some IMD, such as Sanfilippo syndrome, entail the potential for progressive neurodevelopmental decline despite HSCT. The variability in clinical phenotype associated with many of these diseases makes accurate prediction of disease severity at diagnosis challenging. Underlying genetic defects can also present insidiously with a slow disease course. In these disorders, the indication for transplantation is less clear. In other disorders, rapid progression early in life precludes the use of transplant [50]. It is critical to perform HSCT for IMD at an early age, while intellectual and neurocognitive development are generally maintained. In the largest reported study analyzing long-term outcomes after transplant in Hurler's patients, 217 patients were assessed at a median of approximately 9 years post-HSCT. The majority of patients had residual disease burden. Significant predictors of poor prognosis included age > 12 months at the time of transplant, low leukocyte enzyme levels post-transplant, and lower baseline DQ/IQ scores pre-transplant. This data highlights the need for early diagnosis, the preferential use of unrelated UCB grafts, and the goal of obtaining full donor chimerism and normal enzyme levels post-transplant [51].

Acquired Aplastic Anemia and Bone Marrow Failure Syndromes

Aplastic anemia represents a group of disorders characterized primarily by pancytopenia with a hypocellular bone marrow. Aplastic anemia is further subdivided into either idiopathic acquired aplastic anemia or inherited bone marrow failure (BMF) syndromes. Approximately 70% of aplastic anemia cases are idiopathic. HSCT with a MSD is considered first-line therapy in patients younger than 40 years old with severe idiopathic aplastic anemia (SAA), and survival rates range from 85 to 95% [52–54]. Patients without a MSD are treated with immunosuppressive therapy (IST), and those refractory to this regimen are subsequently referred for alternative donor HSCT [55]. In an analysis of 1448 patients receiving a MUD or MSD HSCT for SAA from 2005–2009, Bacigalupo noted the best predictors for survival are age younger than 20 years old, use of ATG in the conditioning regimen, less than 180 days between diagnosis and HSCT, and the use of bone marrow as a graft source. Of note, there was no statistical difference in OS between patients receiving MSD or MUD grafts [56]. Approximately 10–15% of patients treated with IST will develop clonal evolution with cytogenetic abnormalities that increase the risk of myelodysplastic syndrome or acute myelogenous leukemia. Given this risk, along with a 30–40% risk of relapse after IST, the use of MUD HSCT as first-line therapy for young patients without a

MSD is being explored. A United Kingdom study assessed the feasibility of unrelated donor (URD) HSCT instead of IST in pediatric patients with SAA and compared this to historical controls. Outcomes for upfront URD HSCT were similar to MSD HSCT and superior to IST and URD HSCT post-IST failure [57]. While recent efforts support the upfront use of an URD HSCT in younger patients provided a good match can be rapidly identified, this strategy currently remains experimental [58].

In many of the inherited BMF syndromes, HSCT remains difficult given their underlying disease manifestations which impact organ function and tolerance of chemotherapy. For example, HSCT outcomes for dyskeratosis congenita have been marred due to the often fatal development of pulmonary and hepatic fibrosis [59]. BMF syndromes associated with chromosomal instability have increased sensitivity to alkylating agents and radiation [60]. The initial attempts at HSCT for Fanconi anemia involved cyclophosphamide 200 mg/m^2 and total body irradiation (TBI) and resulted in poor survival rates due to excessive regimen-related toxicity and severe acute GVHD. Another complication especially poignant for inherited BMF syndromes has been the high incidence of secondary malignancies. The addition of fludarabine and use of reduced-dose cyclophosphamide and busulfan have resulted in improved outcomes [61]. Additional reports using fludarabine-based conditioning and T-cell depletion have had positive results with good OS and EFS using MSD [62–66]. Prospective multicenter and international trials are enrolling patients to further improve outcomes and decrease conditioning regimen-related toxicity [67–69]. Interestingly, approaches with alkylator or irradiation-free HSCT conditioning regimens with immunosuppression only, using campath and fludarabine, have shown promising results in dyskeratosis congenita patients presenting with BMF [70].

Transplantation Process for Nonmalignant Diseases

Transplantation for NMDs is unique in many ways, some of which are highlighted in Table 7.3. Detailed discussion on every aspect of NMD HSCT is beyond the scope of this chapter; we have attempted to address important components and considerations in NMD HSCT.

Table 7.3 Comparison of the transplantation for NMDs versus malignant disorders

	HSCT for malignancy	HSCT for nonmalignant disorder
Conditioning	In general, myeloablative conditioning involving high dose busulfan or TBI is the preferred approach	Reduced toxicity regimes, preferably with decreased dose of either busulfan, melphalan, or treosulfan
GVHD	Some degree of GVHD tolerated to facilitate graft-versus-leukemia effects	No role for tolerating GVHD, aggressive GVHD prevention regimen advocated
Chimerism	Goal is to achieve complete donor chimerism	Stable mixed donor chimerism is associated with symptom resolution in many diseases

Conditioning Regimen in NMD HSCT

Various conditioning regimens have been used in the HSCT for NMDs. Conditioning regimens commonly used in the transplantation of NMDs are outlined in Table 7.4. For some NMDs, intensive immunosuppression without myeloablation is sufficient to ensure engraftment. For other disorders, myeloablation is needed to create the bone marrow niche space necessary for donor stem cell engraftment. In general, high-dose TBI has been avoided due to late effects on the developing brain as well as the risk of secondary malignancy. The combination of cyclophosphamide, busulfan, and anti-T-cell serotherapy has been a successful regimen for many NMDs. However, busulfan comes with an increased risk of sinusoidal obstruction syndrome (SOS) and late effects including infertility, pulmonary fibrosis, and endocrine abnormalities. Busulfan drug level monitoring and pharmacokinetic-based dosing have enabled the transplant physician to target different busulfan exposure levels depending on the underlying disease. Both busulfan and cyclophosphamide rely on hepatic glutathione S-transferase for drug metabolism. Using these medications in combination can lead to depletion of glutathione stores resulting in the subsequent buildup of toxic metabolites. Fludarabine does not rely on glutathione for detoxification. Substituting fludarabine for cyclophosphamide has been utilized to decrease the adverse effects of busulfan such as SOS. In a small cohort study using a myeloablative busulfan/fludarabine (Bu/Flu) preparative regimen with serotherapy, Bartelink et al. showed similar survival rates and decreased toxicities including pulmonary disease, SOS, and chronic GVHD in comparison with a busulfan/cyclophosphamide regimen [71].

Table 7.4 Examples of common conditioning regimens

Myeloablative		
Cy/TBI	Cyclophosphamide	120 mg/kg
	Total body irradiation	12–14 Gy
Bu/Cy	Busulfan	16 mg/kg[a]
	Cyclophosphamide	120 mg/kg
Reduced intensity		
Flu/Bu	Fludarabine	150–180 mg/m^2
	Busulfan	8–10 mg/kg[a]
Flu/Mel	Fludarabine	150–180 mg/m^2
	Melphalan	140 mg/m^2
Flu/Bu/TT	Fludarabine	150–180 mg/m^2
	Busulfan	8 mg/kg[a]
	Thiotepa	5 mg/m^2
Minimal intensity		
Flu/TBI	Fludarabine	90 mg/m^2
	Total body irradiation	2 Gy
TBI	Total body irradiation	1–2 Gy

[a]For institutions where PK monitoring is available, desired busulfan target Css for myeloablative regimens is 900 ng/ml and 600–700 ng/ml for reduced toxicity regimens

As standard myeloablative preparative regimens come with a high rate of HSCT-related toxicities, especially in patients with underlying comorbidities, myeloablative conditioning is being replaced by the use of reduced intensity conditioning. RIC protocols are associated with fewer regimen-related toxicities however entail a higher rate of mixed chimerisms and an increased risk of graft rejection. It has been noted that conditioning regimens achieving busulfan concentration at steady state (Css) of less than 600 ng/ml are associated with high rates of graft failure and mixed chimerisms [72]. However, busulfan Css ranging from 600 to 700 ng/ml has achieved robust engraftment without these high graft failure rates [73, 74]. A multicenter prospective trial of 56 patients undergoing MUD or MSD transplants for chronic granulomatous disease utilized reduced toxicity busulfan (busulfan Css in most patients was in 550–700 ng/ml) with fludarabine and serotherapy. At 21-month follow-up, OS was 93% with EFS of 89%. Graft failure occurred in 5% of patients. Interestingly, the patients with primary graft failure had achieved a busulfan Css of <600 ng/ml. This study confirms that RIC Bu/Flu can be used successfully and safely as a conditioning regimen for NMDs [20].

Treosulfan is a prodrug of an alkylating agent that is not metabolized by the liver, has predictable pharmacokinetics, and causes potent immunosuppression and myeloablation. It has been used in combination with fludarabine and cyclophosphamide in an effort to decrease regimen-related toxicities. Several retrospective studies and a small prospective study of 31 patients receiving MSD or MUD HSCTs for a variety of NMDs report the safety and efficacy of a preparative regimen containing treosulfan and fludarabine with and without serotherapy [22, 75, 76]. Patients with SCD and hemophagocytic lymphohistiocytosis were more likely than patients with other NMDs to have difficulty engrafting with this regimen [19]. The addition of thiotepa to fludarabine and treosulfan has been proposed as a means of intensifying the regimen without adding excessive toxicity [33, 77–79].

Recently a combination of alemtuzumab, fludarabine, and melphalan has been used for RIC in patients undergoing HSCT for NMDs. A retrospective review of 206 patients with this regimen found good OS (84% at 3 years for MSD grafts) with a rate of mixed chimerisms of 46%. Patients with BMF syndromes and SCID had a low risk of mixed chimerisms. Patients receiving UCB grafts had an increased incidence of mixed chimerisms, and the authors suggest avoiding this regimen when UCB is the donor source [21]. A cohort study of 43 children followed for over 2-years post-HSCT for NMDs using this regimen found a decreased prevalence of regimen-related toxicities at long-term follow-up [80].

Studies using a variety of RIC regimens have proven that adequate engraftment is possible with decreased incidence of transplant-related mortality (TRM). Therefore, a RIC regimen should be considered when performing HSCT for NMDs. The precise protocol will be dictated by the underlying disease and its associated comorbidities, the type of donor, and the degree of mismatch.

Several experimental non-genotoxic targeted conditioning approaches are being actively explored. Use of anti-CD45 antibody and its radioimmunoconjugate as a conditioning agent have shown very encouraging results [81, 82]. In a phase I/II trial, Straathof et al. reported the use of anti-CD45 antibody as an alternative

approach to myelosuppression in combination with alemtuzumab immunosupression in 16 patients with primary immunodeficiency syndromes (i.e., SCID, combined immunodeficiency, dyskeratosis congenita, and hemophagocytic lymphohistiocytosis) undergoing matched related or unrelated HSCT in whom other myeloablative or reduced intensity conditioning was contraindicated based on age or preexisting organ toxicity. Eleven of the sixteen patients (69%) achieved full or high-level mixed chimerism in both lymphoid and myeloid lineages, and three achieved engraftment in the T-lymphoid lineage only. The median time to normal CD3+ counts was 10 months, to CD4+ counts 12 months, and to normal CD19+ counts 12 months. Thus, anti-CD45 monoclonal antibody when used as a conditioning regimen in patients with NMDs and preexisting organ toxicity or DNA repair defects was well tolerated and resulted in donor engraftment [81]. Other antibodies that preferentially target hematopoietic stem and progenitor cells are currently under evaluation. Antibody blockade of c-KIT has shown promising results as a conditioning agent in immune-deficient and Fanconi anemia mouse models [83–85]. Drugs enhancing the antibody efficacy and also the conjugation of a cellular toxin to c-KIT and anti-CD45 antibody have also shown encouraging results in immune-competent murine HSCT models [86]. A phase I clinical trial of human c-KIT antibody is currently awaiting patient enrolment (ClinicalTrials.gov Identifier: NCT02963064).

Graft-Versus-Host Disease Prevention

Donor-derived T-cells aid in establishing hematopoietic engraftment, transferring pathogen-specific immunity, and, in patients receiving HSCT for malignancies, creating a graft-versus-leukemia effect. In NMDs donor T-cells that recognize host major histocompatibility complex (MHC) antigens are primarily responsible for the development of GVHD, a major cause of transplant-associated morbidity and mortality. Standard-of-care GVHD prophylaxis consists of combined cyclosporine and short-course methotrexate [87]. Post-HSCT cyclophosphamide has been used for GVHD prevention by depleting the alloreactive donor T-lymphocytes responsible for graft rejection and GVHD while preserving the non-alloreactive memory T-cells needed for adoptive immunity and successful engraftment [88]. Post-HSCT cyclophosphamide has largely been used in haploidentical transplants for hematologic malignancies where it has led to successful engraftment with rates of GVHD and TRM similar to transplants using MSDs [89, 90]. Encouraging results have been seen using post-HSCT cyclophosphamide with haploidentical HSCT for SCD [39]. Newer studies reveal the potential for this protocol in alternative donor transplants for NMDs [91–93]. In a recent report, post-HSCT cyclophosphamide was used in 11 patients with a variety of NMDs who underwent HSCT using alternative donors with RIC. At a median follow-up of 25-months, OS was 100% with low rates of GVHD and enough engraftment to allow elimination of the primary disease manifestations [94]. Although limited by size as well as by heterogeneity in patient

disorders and conditioning, these results suggest that further development of post-HSCT cyclophosphamide protocols holds promise for alternative donor transplants in NMDs.

T-cell depletion is often also employed in order to prevent GVHD. Pan T-cell depletion can be accomplished through a variety of in vitro graft manipulation methods or various in vivo anti-T-cell therapies (aka serotherapy), such as ATG or alemtuzumab. Initially, the use of alemtuzumab was associated with increased rates of graft failure; more recent studies show equivalence in graft rejection when alemtuzumab is limited to pre-HSCT treatment [95]. While broad-spectrum T-cell depletion is effective in decreasing the incidence of GVHD, it is associated with delayed immune reconstitution and increased infections. Therefore targeted T-cell blockade techniques are being sought to prevent GVHD without causing broad T-cell depletion.

One of the in vivo methods targeting suppression of T-cell activation involves blocking T-cell co-stimulation pathways such as the CD28–80/86 interactions which synergize with stimulatory T-cell receptor signals to cause T-cell activation. Abatacept was developed to specifically block T-cell co-stimulation mediated through this pathway and has been shown in early studies to reduce CD4+ T-cell activation and achieve improvement in acute GVHD prevention without delayed immune reconstitution [96]. Selective depletion of T-cell subtypes that mediate GVHD has also resulted in promising results. Graft manipulation approaches such as TCR α/β T-cells or CD45RA naïve T-cell depletion are currently two important approaches employed to decrease GVHD risk [97–101]. α/β T-cells are the primary drivers of GVHD, and they represent 90–100% of the circulating T-cell population. Moreover, depletion of α/β T-cells has been associated with spontaneous increases in γ/δ T-cells which provide early T-cell recovery and immune reconstitution. γ/δ T-cells, which represent only 1–10% of circulating T-cells, function as effector cells in the innate immune system, rapidly reacting with antigens without the need for MHC stimulation or additional processing. They have been shown in animal studies not to cause GVHD and are proposed to facilitate engraftment [102, 103]. Small, single-institution studies and case reports using depletion of α/β+ T-cells and CD19+ B-cells have documented early immune recovery with limited GVHD after alternative donor transplantation [99–101]. Further studies are working on in vitro γ/δ T-cell expansion to be used either in combination with α/β T-cell depletion or as adoptive therapy in the early post-transplant course.

Management of Post-HSCT Infection Risks

Opportunistic infections (OI) are one of the most common complications of HSCT [104]. Viral infections are of particular concern, as they are often not adequately treated by current antiviral medications and lead to increased morbidity and mortality. The main risk factors for serious viral infections are high viral load and delayed T-cell recovery/prolonged T-cell suppression. Donor T-lymphocytes are primarily

responsible for the development of GVHD but also are important in protecting against viral infections. Umbilical cord blood transplant recipients are at risk of serious viral infections due to delayed T-cell recovery [105]. HSCT from sources with higher degrees of mismatch which require stronger immunosuppression are at increased risk of viral-induced OI. Finally, GVHD itself, especially if requiring treatment with steroids, predisposes to serious OI [106, 107]. Active viral infections impart an increased risk of graft rejection and GVHD. The most problematic viral infections in the post-HSCT period are those attributed to the double-stranded DNA viruses that are able to cause persistent infections subject to reactivation: adenovirus, cytomegalovirus (CMV), and Epstein-Barr virus (EBV) [108–113].

Unfortunately, antiviral medications have shown little efficacy in treating serious adenovirus infections or in preventing EBV-associated post-transplant lymphoproliferative disorder (PTLD) [114–116]. The introduction of ganciclovir prophylaxis has successfully decreased serious early CMV infections. Early ganciclovir resistance has been observed in children transplanted for primary immunodeficiency syndromes as well as those receiving T-cell depleted grafts [115, 117]. Rituximab, an anti-CD20 monoclonal antibody, is a treatment option for PTLD; however, this medication causes profound B-cell depletion that can persist for up to 6 months [115, 118–120].

Newer efforts at controlling serious viral infections have focused on novel treatments and adoptive therapies, which aim to restore T-cell immunity. These include donor lymphocyte infusions (DLI), infusions of unmanipulated T-cells, and virus-specific T-cell therapy (VST). In 1990, Hans Kolb et al. began using DLI to treat relapsed malignant diseases after HSCT [121]. DLI continues to primarily be used for post-HSCT relapse; however, its role in infection treatment has also been recognized. In 1994, Papadopoulos et al. utilized infusions of unirradiated donor lymphocytes to treat EBV-associated PTLD with resultant complete response in 5/5 patients [122]. More recently, a 3-year-old with refractory EBV-PTLD after haploidentical HSCT for β-thalassemia was successfully treated with three-monthly infusions of unmanipulated CD3+ cells [123]. In addition to EBV-PTLD, there have been case reports documenting the successful use of DLI in the setting of other viral illnesses such as life-threatening adenovirus disease [124, 125]. Overall, the application of DLI in the treatment of viral infections is limited mostly by its lack of specificity and also by the underlying risk of GVHD. Various techniques have been employed to modify the T-cell population in the DLI infusion product to decrease GVHD risk such as depletion of alloreactive T-cells and also the introduction of suicide genes for inducible apoptosis should severe GVHD occur [126, 127].

Over the past two decades, many advances have been made in the development of antigen-specific T-cell therapy in order to treat viral infections in the post-HSCT setting [128]. CMV was the first virus targeted. In the early 1990s, Riddell et al. generated CMV-specific CD8+ T-cells and infused them to patients undergoing HSCT. After infusion, there were no cases of CMV reactivation or disease, no increase in GVHD, and no additional side effects. This landmark study was the first to demonstrate the potential use of VST in reconstituting cellular immunity [129]. A multitude of advancements in the development of these products using a variety

of antigen-presenting cells and antigen sources has since confirmed excellent response rates of CMV-specific T-cell therapy [128, 130–134]. The successful use of DLI in the treatment of EBV-PTLD spawned the development of EBV-specific T-cell therapy [135]. In a retrospective review of 114 patients who received EBV-VST for either treatment or prevention of EBV-PTLD, Heslop et al. demonstrated 100% efficacy in prophylactic administration with none of the 101 high-risk patients developing PTLD compared with 11.5% of controls. There was an 80% response rate in the 13 patients receiving EBV-VST for treatment of established PTLD. The main toxicity was inflammation at the site of bulky disease with no patients developing de novo GVHD [136]. The successes with both EBV- and CMV-specific T-cell therapy sparked interest in developing T-cell lines specific to multiple viruses and in expanding the range of antigens detected which currently includes adenovirus, BK virus, influenza, varicella-zoster virus, and human herpesvirus 6. Early studies using multivirus-specific T-cells have thus far shown excellent response rates [137–142].

While the clinical results using VST have shown this to be a safe and effective treatment modality, the widespread use of these products has been constrained by the need for complex, timely, and expensive manufacturing. Research efforts are focusing on rapid production methods which include short ex vivo culture techniques and methods of immediate selection using multimers or interferon-gamma capture. None of these methods are possible when the donor is seronegative for the virus of interest or with UCB grafts which are inherently naïve [128, 131]. A method proposed to expedite VST production that also has application in virus-naïve patients is the generation of third-party VST banks. These products are available for urgent use, choosing the most closely HLA-matched product for the patient. Potential risks to using third-party VSTs relate to the partial mismatch that is likely to occur and include the possibility of curtailed product activity and alloreactivity against recipient antigens leading to GVHD and/or stem cell graft rejection. Overall, the response rates using banked VSTs are slightly lower than observed with donor-derived VSTs, ranging from 50–70% [143]. These banked products have had a shorter persistence than donor-derived cells, mandating the use of multiple infusions. There has been no observed increase in alloreactivity despite theoretical concerns. Questions remain regarding optimal selection criteria, reasons underlying failure to respond, and factors contributing to the duration of VST activity [128, 131].

Late Effects

The majority of information regarding late effects after HSCT stems from studies of patients with acute leukemia. In these studies, risk factors for late effects include younger age, use of TBI, and chronic GVHD [144]. Patients receiving HSCT due to NMDs often have differing pre-transplant treatment modalities, comorbidities, and baseline disease-specific risk factors that can influence the complications associated with HSCT. Despite the increasing number of HSCTs for NMDs, there is limited

knowledge regarding the late effects of transplant in this unique patient population. Small studies have thus far shown complications in survivors of HSCT for hemoglobinopathies, BMF syndromes, and immune deficiencies that include infertility, endocrine dysfunction, immunologic abnormalities, and adverse effects associated with GVHD [32, 51, 145]. A retrospective study at Duke University examined 102 patients aged less than 2 years old who survived at least 5 years after UCB HSCT using busulfan conditioning. Approximately 80% were transplanted for NMDs. Primary late complications included abnormal dentition, short stature, pulmonary dysfunction, pubertal delay, and cognitive deficits. At least one late effect was present in 98% of all patients [146]. The 2011 and 2015 international consensus conferences on late effects after pediatric hematopoietic cell transplantation brought to light the need for NMD-specific late effect screening guidelines [144, 147–149]. NMD-specific recommendations for long-term follow-up and late effects screening are forthcoming.

Future Directions

MSD HSCTs can be safely and successfully performed for most nonmalignant diseases affecting the hematopoietic and immune systems. The outcomes for unrelated and haploidentical HSCT for NMDs are also improving with MUD having achieved survival rates approaching that of MSD. Thus, the questions of feasibility, safety, and efficacy have been answered. With these positive results, the application of HSCT for NMDs will continue to broaden and include more diverse disease settings. Largely due to the diversity of NMDs treated via HSCT, early successes have led to more questions regarding selection criteria, transplant regimens, and outcomes. Long-term follow-up data and monitoring for late effects are critical to understand the risks and benefits of HSCT for NMDs, allowing its judicial application to diseases proven to be curable. Standardized protocols and collaborative efforts are needed to advance the use of HSCT for these rare disorders. As we learn more about the molecular and genetic basis of many of these illnesses, gene therapy or gene editing may have a greater role to play.

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Chapter 8
Unique Challenges of Hematopoietic Stem Cell Transplant for Sickle Cell Disease

Pooja Khandelwal and Michael Grimley

Abbreviations

CSA	Cyclosporine
Cy	Cyclophosphamide
DNA	Deoxy nucleic acid
HLA	Human leukocyte antigen
HSCT	Hematopoietic stem cell transplant
LIC	Liver iron content
MAC	Myeloablative
MMF	Mycophenolate mofetil
MP	Methylprednisolone
MTX	Methotrexate
NMA	Non-myeloablative
RIC	Reduced intensity
Tacro	Tacrolimus
TA-TMA	Transplant-associated thrombotic microangiopathy

Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is a curative option for children and adults with sickle cell disease (SCD) [1, 2]. Several risks are common for all patients regardless of underlying diagnosis including risks associated with chemotherapy such as alopecia, anorexia, nausea, and emesis; regimen-related

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toxicities such as sinusoidal obstructive syndrome, infectious complications, and acute and chronic graft-versus-host disease; and long-term adverse effects such as infertility, endocrinopathies, cardiovascular complications, and secondary malignancies. HSCT physicians should be aware of particular challenges that are unique to patients with SCD as they counsel them for a HSCT. These are outlined in the following seven subheadings.

Donor Availability in Sickle Cell Disease

Allogeneic HSCT in SCD, while curative, is often limited due to a lack of suitable donors; less than 20% of people with SCD have an HLA-matched sibling. Unrelated matched donors are limited in large part because of a relative lack of non-Caucasian donors in bone marrow donor registries. The National Marrow Donor Program and Center for International Blood and Marrow Transplant Research have used human leukocyte antigen (HLA) data from their donor registries to predict the likelihood of identifying a matched unrelated donor within the worldwide registries [3]. The likelihood of finding a donor varies considerably and is based on ethnic and racial groups. The probability of finding a fully matched unrelated HLA donor is the highest in Caucasians of European decent (75%) and the lowest among blacks of South and Central American ancestry (16%). Patients of Middle Eastern or North African descent have a probability of 46%, African-Americans have a probability of 19%, and patients of African descent have an 18% probability of finding a fully matched unrelated donor. Additionally, there are limitations to insurance access, lack of awareness and health-care access, socioeconomic barriers, and historical mistrust in the health-care system which fuels this problem.

While several other stem cell sources such as mismatched unrelated bone marrow, umbilical cord blood, and haploidentical stem cells from a parent or sibling are potential alternative options for HSCT in SCD patients, they come with the price of increased risk of graft rejection and/or graft-versus-host disease [4, 5]. Until strategies for unrelated mismatched donor transplant, umbilical cord blood transplant, or haploidentical transplant are improved, allogeneic HSCTs as a curative option will remain limited only to a select number of children with SCD with matched sibling or matched unrelated donors.

Iron Overload in Sickle Cell Anemia Patients on Chronic Transfusions and Its Management Pre- and Post-hematopoietic Stem Cell Transplant

In patients with SCD who are on chronic transfusions and are preparing for an allogeneic HSCT, a pre-transplant evaluation should comprise assessment of iron overload in the liver and the myocardium. Transplant outcomes are poorer in

beta-thalassemia patients with evidence of iron overload. Luceralli et al. developed the Pesaro classification system which divides patients into three groups (Classes I, II, and III) based on the poor quality of previous iron chelation, marked hepatolomegaly and the presence of portal fibrosis. For patients undergoing transplant from an HLA-matched sibling using a myeloablative preparative regimen, the overall survival and event-free survival were 95% and 90% for Class I patients, 87% and 84% for Class 2 patients, and 89% and 64% for Class III patients [6, 7]. A similar classification for risk assessment for sickle cell patients does not exist, nor is there a standard approach for SCD patients with severe iron overload being considered for HSCT. Evaluation of the degree of iron overload can be determined by T2 MRI as assessment of the burden of iron overload in the heart and liver. Our approach in patients with liver iron concentration (LIC) > 7 mg iron/g dry weight or myocardial iron deposition observed by MRI screening is to delay allogeneic HSCT to allow for adequate chelation therapy. Other practices may include obtaining liver biopsy for patients with LIC > 7 mg iron/g dry weight, with consideration of delaying the HSCT and/or altering conditioning regimen if bridging fibrosis is present. Severe iron overload is often partly attributed to nonadherence and factors including inadequate family support and home infrastructure, all of which must also be addressed.

The goal of iron chelation therapy is to reduce the burden of iron in plasma and body tissues, thereby minimizing the production of reactive oxygen species and reducing damage to the liver, heart, and endocrine organs. The approach to chelation therapy in SCD is managed similarly to patients who are chronically transfused (e.g., beta-thalassemia major). It is important to note that the clinical interaction of SCD pathophysiology and iron overload alters iron deposition and tissue injury in SCD, so aggressive chelation recommendations for beta-thalassemia major may not be generalizable for SCD until additional risk/benefit studies are completed.

Chelation therapy is typically started after 1–2 years of chronic transfusions in SCD patients when the serum ferritin exceeds 1000 ng/mL on at least two separate occasions, or after transfusion of approximately 120 mL/kg of red blood cells. Several agents are available for chelation therapy in patients with iron overload. Monotherapy is typically utilized but in some cases, combination therapy may be used [8]. The initial choice of chelation therapy depends on several factors such as the extent of iron overload, patient preference, and adverse effect profile. Generally, deferasirox therapy is associated with better adherence compared to deferoxamine therapy.

Deferoxamine is a clinically approved iron chelator in patients with iron overload states and can also remove iron directly from the myocardium [9]. Deferoxamine is administered by a continuous intravenous or subcutaneous infusion at a dose of 40–60 mg/kg infusion subcutaneously over 8–12 h at night for at least 4 days per week. This regimen usually leads to 600–1500 mg of iron loss per month.

Acute side effects of deferoxamine include abdominal discomfort, diarrhea, nausea, vomiting, hypotension, and anaphylaxis. Long-term side effects include visual and auditory neurotoxicity, which are reversible if identified early and if the drug is discontinued. Patients may also be at increased risk of developing infections due to the deferoxamine-iron chelate called feroxamine which acts like a siderophore for

microbes such as *Mucormycosis*, *Yersinia*, and *Vibrio* [9]. While patients are on deferoxamine, baseline and annual audiology examination, annual ophthalmology examination, assessment of renal and liver function every 3 months, and monitoring growth velocity should be performed.

Deferasirox is an oral iron chelator and is approved for the treatment of chronic iron overload with a liver iron concentration ≥5 mg iron/gram of liver dry weight and a serum ferritin >300 mcg/L [9]. Common adverse events include transient abdominal pain, nausea, vomiting, diarrhea, back pain, and skin rash. A newer formulation of deferasirox (Jadenu) has been associated is reported to have fewer GI side effects compared to the Exjade formulation. Serious adverse events include agranulocytosis, gastrointestinal hemorrhage, and renal toxicity including Fanconi syndrome and hepatic toxicity [9]. While patients are on deferasirox, renal function tests and complete blood counts monthly and liver function tests every 2 weeks for the first month followed by monthly and annual auditory and ophthalmologic evaluations should be performed. We have observed unexpectedly high busulfan clearance rates in patients on chronic chelation with deferasirox in beta-thalassemia patients. We recommend stopping oral iron chelation therapy with deferasirox at least a month before the HSCT conditioning regimen if the conditioning regimen contains busulfan, to reduce chances of altering busulfan pharmacokinetics.

Deferiprone is an oral chelation agent used in iron overload states, but is currently not FDA approved for SCD patients. Deferiprone is a useful alternative for those in whom current chelation therapy with deferoxamine has been inadequate or where deferoxamine and deferasirox are not available [9]. Deferiprone is administered at a dose of 75–100 mg/kg divided three times per day. Common adverse events include elevated liver enzymes, gastrointestinal discomfort, and arthralgia. Serious adverse effects include agranulocytosis and neutropenia with an incidence of 0.2 and 2.8 per 100 patients over 1 year that are reversible after stopping therapy [9]. While the neutropenia is reversible, frequent monitoring of CBCs should be performed while a patient is on deferiprone, especially when initiating therapy or changing doses. In addition to CBC monitoring, liver function testing, zinc levels, and clinical monitoring for development of arthropathy should be performed [9].

In some instances, patients with SCD and a high hemoglobin either at baseline or due to hydroxyurea treatment (which often raises hemoglobin by approximately1 g/dL) can be treated with therapeutic phlebotomy instead of iron chelation therapy. Alternatively, for patients on chronic transfusion therapy for primary/secondary stroke prevention, chronic RBC exchange (erythrocytapheresis) can be utilized as way to minimize iron overload (see Chap. 5 for further details). Monitoring of adequacy of iron chelation therapy should be performed routinely by serum ferritin levels at every blood transfusion and annual assessment of liver iron content, preferably by noninvasive R2-MRI methods.

Iron overload after successful HSCT can occur either due to preexisting iron overload or to transfusions after transplant. It is typical to initiate measures to reduce iron burden approximately 1–2 years after HSCT since most patients have normal

erythropoiesis and no longer require immune suppression. Phlebotomy is the preferred approach to remove excess iron as it is safe, inexpensive, and highly efficient. Phlebotomy is performed if the hemoglobin is >9.5 g/dL and the systolic blood pressure is >85–90 mmHg. One approach is to remove 5–6 mL/kg of blood every 14 days and, if indicated, replaced with normal saline though there are therapeutic phlebotomy regimens varying from center to center. Laboratory monitoring includes a CBC before each phlebotomy, liver and kidney function testing at baseline and then every 3 months, and serum ferritin every 2 months. The goal of phlebotomy is typically to reach a stable serum ferritin level of <100 mcg/L. Duration of treatment depends on the extent of iron overload and ranges from a few months to several years.

Neurovascular Complications After Allogeneic Hematopoietic Stem Cell Transplant

Children with SCD are at higher risk of neurovascular complications compared to children undergoing HSCT for different underlying indications. These complications include seizures and posterior reversible encephalopathy syndrome (PRES), which have historically been associated with a higher hemoglobin values, thrombocytopenia, or hypertension. As a result, all children with SCD undergoing HSCT should be started on anticonvulsant treatment throughout the period of calcineurin inhibitor use due to risk of seizures. Careful attention should be paid to hemoglobin and platelet levels and standard transfusion thresholds altered accordingly. It is recommended to maintain the platelet count >50,000/mm^3 until platelet engraftment, to prevent intracranial hemorrhage, especially in children with a history of strokes. Hemoglobin should be maintained between 9 and 11 g/dL to minimize severe anemia and hyperviscosity, respectively. Serum electrolytes should also be monitored closely with particular attention to magnesium levels. Lastly, aggressive monitoring and control of blood pressures in instances of hypertension are advocated for these children to prevent PRES and seizures from hypertension.

Allogeneic HSCT is considered to be effective as secondary prevention of strokes in children with either overt strokes or silent cerebral infarcts, but it is not universally protective [10]. The frequency of strokes after successful donor engraftment is very low but not completely absent [11]. In addition, there appears to be a risk during the peri-engraftment period when the evolution of brain injury by MRI can progress, before eventual stabilization. A previous report has also suggested that existing central nervous system parenchymal changes may continue after HSCT for up to 3 years after stem cell infusion [12]. These early progressive imaging changes noted after HSCT may be due to the natural history of neurovascular injury, including reactive gliosis related to the initial event. These children should be considered for annual neurovascular imaging and neuropsychiatric testing [13].

GVHD and Graft Failure

GVHD is the leading cause of morbidity and non-relapse mortality in patients with SCD who undergo an allogeneic HSCT. Several studies have reported varying rates of acute and chronic GVHD in their patient cohorts, as shown in Table 8.1. Myeloablative conditioning is associated with higher rates of acute GVHD compared to non-myeloablative or reduced intensity conditioning regimens [4, 28, 36]. Matched sibling donor HSCTs carry a lower risk of acute GVHD compared to unrelated bone marrow or umbilical cord blood transplants [28, 36]. On average, patients who received an unrelated cord blood transplant regardless of conditioning intensity experienced approximately a 20% incidence of grade II–IV acute GVHD. [4] Matched sibling donor transplant recipients who received a myeloablative conditioning had a 17% rate of grade II–IV acute GVHD [28, 36].

Severe chronic GVHD was observed in 38% of patients who underwent an unrelated donor reduced intensity HSCT, compared to 9% in haploidentical, 7% in myeloablative unrelated cord blood, and 3% in myeloablative matched sibling HSCT [4, 28, 32, 36]. The high rates of GVHD in the unrelated donor reduced intensity donor HSCT suggest that additional interventions to decrease the incidence of GVHD prophylaxis are needed in these patients. Current published results demonstrate that a non-myeloablative approach for matched sibling HSCT offers the best outcomes in terms of low incidences of acute (5%) and chronic (0%) graft-versus-host disease in young patients (Table 8.1). [28].

Graft failure/rejection is one of the biggest hurdles in HSCT in patients with SCD. The reported rate of secondary graft failure in the literature are as follows: 0–13% in patients who received an HLA-identical myeloablative HSCT, 13–14% of patients who received an HLA- identical non-myeloablative or reduced intensity HSCT, and 43% of patients who received a haploidentical donor HSCT [4, 28, 32, 36]. Graft failure was also high in the unrelated cord blood transplant setting at 37.5–62% [4]. Several reasons are postulated for this relatively higher incidence of graft failure in patients with SCD, including a “chemotherapy naïve” patient population, since higher rates of graft failure are seen in patients with hyperactive marrows who have no prior chemotherapy exposure prior to undergoing HSCT and high rates of alloimmunization due to multiple red blood cell transfusions [37]. Red blood cell alloimmunization is positively correlated with development of HLA antibodies in multiple reports of people with SCD. Heavily transfused patients with severe aplastic anemia have higher rejection rates, suggesting that exposure to foreign HLA antigens may predispose to high rejection rates.

Graft failure can be overcome by increasing the intensity of the conditioning regimen, but this benefit comes at the price of increased long-term toxicities including GVHD, end organ damage, and infertility. To ameliorate this complication, a conditioning regimen which reduces toxicity but achieves durable engraftment is needed. In the interim, strategies such as hydroxyurea pre-HSCT and early HSCT for patients before alloimmunization are to be tested or considered [38].

Table 8.1 Results pertaining to graft-versus-host disease and graft failure in case series or studies of allogeneic stem cell transplant in patients with sickle cell disease

Ref	Number of patients	Age (years)	Donor relation	GVHD prophylaxis	Preparative regimen	Acute GVHD at day + 100 (grades 1–4)	Chronic GVHD	Graft failure (primary or secondary)	Overall survival
[12]	14	5.4–17.4	Related	CSA MTX	MAC	42%	21%	0	93%
[14]	16	1.2–19.3	Related	CSA MTX	MAC	13%	0	0	100%
[15]	67	2–27	Related	CSA MTX	MAC	10%	22%	13%	97%
[16]	15	1.5–18	Related	Tacro MTX	MAC	26%	0	6.6%	100%
[17]	40	2–17	Related	CSA + MTX or MP	MAC	17.5%	5%	0	91%
[18]	10	2.8–16.3	Related	CSA MP	MAC	40%	10%	10%	90%
[19]	27	3.3–17.4	Related	CSA MTX	MAC	12%	0	0	96%
[20]	87	2–22	Related	CSA ± MTX	MAC	20%	12.6%	7%	93.1%
[21]	59	3.3–15.9	Related	CSA MTX	MAC	19% acute and chronic		9%	93%
[22]	11	2–13	Related	CSA MTX	MAC	55%	0	9%	90%
[23]	18	2.3–20.2	Related	Tacrolimus MMF	Reduced toxicity MAC	17%	11%	0	100%

(continued)

Table 8.1 (continued)

Ref	Number of patients	Age (years)	Donor relation	GVHD prophylaxis	Preparative regimen	Acute GVHD at day + 100 (grades 1–4)	Chronic GVHD	Graft failure (primary or secondary)	Overall survival
[24]	30	16–65	Related	Sirolimus	NMA	0	0	13%	96%
[25]	2	21–27	Related	MMF	NMA	0	0	0	100%
[26]	7	6–18	Related	CSA MMF	RIC	14%	14%	14%	100%
[27]	50	1.7–15.3	Related marrow and cord	CSA + MTX or MMF	MAC	20%	20%	8%	94.1%
[28]	43	3–20.3	Related marrow and cord	CSA or Tacrolimus	RIC	23%	13%	2%	93%
[29]	8	2.1–24.8	Related marrow and cord	CSA MMF	RIC	0	0	100% mixed chimerism	90%
[30]	11	2–20	Related cord	CSA	MAC	Incidences combined with thalassemia		9%	90%
[31]	30	1.7–18.8	Related and unrelated	CSA + MTX or MMF	MAC	7%	7%	6.6%	100%
[32]	30	4–19	Unrelated	CSA + MTX or MP	RIC	28%	62%	10%	86%
[33]	16	3–12	Unrelated cord	CSA + MMF or MP	MAC 9 RIC 7	Incidences combined with thalassemia		43%	94%
[34]	7	3.4–16.8	Unrelated cord	CSA + MP or MMF	MAC 4 RIC 3	71%	14%	57%	75%

[4]	8	7.4–16.2	Unrelated cord	CSA or Tacrolimus + MMF	RIC	25%	12.5%	62.5%	87.5%
[35]	8	1–10	Unrelated cord	Tacrolimus MMF	Reduced toxicity MAC	50%	12.5%	37.5%	62.5%
[12]	8	4.2–17.1	Haplo-identical	OKT3 MMF	MAC	50%	37.5%	38%	75%
[5]	14	15–42	Haplo-identical	Post HSCT Cy + MMF + Tacrolimus or Sirolimus	NMA	0	0	42%	100%

CSA cyclosporine, *MMF* mycophenolate mofetil, *MTX* methotrexate, *MP* methylprednisolone, *Cy* cyclophosphamide, *HSCT* hematopoietic stem cell transplant, *MAC* myeloablative, *NMA* non-myeloablative, *RIC* reduced intensity conditioning

Transplant-Associated Thrombotic Microangiopathy

Transplant-associated thrombotic microangiopathy (TA-TMA) is a serious complication after allogeneic HSCT occurring in 30–35% of HSCT recipients with 50% of these patients with TMA having life-threatening disease [39–41]. The incidence or severity of TA-TMA is unknown in children with SCD. Limited reports have reported the occurrence of TA-TMA in patients with SCD [42], but it is entirely plausible that the preexisting endothelial dysfunction in these children places them at higher risk of TA-TMA [43]. It has also been shown previously that TA-TMA incidence is greater in patients with gene variants related to complement activation [39]. TA-TMA incidence and number of complement gene variants have been shown to be higher in African-Americans, compared with white recipients, and the higher number of complement gene variants observed in African-American HSCT recipients is associated with a more severe TMA and higher mortality [39]. Patients with SCD should, therefore, be screened for TA-TMA by established criteria [40] and eculizumab therapy considered if they meet criteria for high-risk TA-TMA.

Vaso-occlusive Crisis After Allogeneic Hematopoietic Stem Cell Transplant

Vaso-occlusive crisis can occur in children who undergo an allogeneic HSCT for SCD despite having successful donor engraftment [24]. The etiology and incidence of a vaso-occlusive crisis after successful allogeneic HSCT are unknown. In a report of adult patients with SCD and with mixed donor–host chimerism [24], a prolonged period of pain treatment by opioids with a gradual taper in the opioid dosing over 6 months was reported. It is therefore suggested that pain threshold levels can be reset after HSCT which can result in tapering of opioid therapy. It is also hypothesized that remodeling of previously dysfunctional vascular or endothelial channels might occur after allogeneic HSCT to abrogate painful stimuli [13]. Families should be counseled that children may experience a vaso-occlusive crisis for approximately 6 months after HSCT, and treating physicians should be aware of this potential complication so that they may institute appropriate treatment with analgesics.

Challenges of Allogeneic Hematopoietic Stem Cell Transplant in Adult Patients

Hematopoietic stem cell transplant from HLA-matched siblings in children with SCD has shown promising results. Despite this, the applicability and use of the technique in children are not widespread due to the lack of HLA-identical sibling donors and parental concerns about complications. Older patients with SCD experience additional challenges due to the eventual cardiac dysfunction, renal sequelae,

Table 8.2 Studies reported in adult patients with sickle cell disease who underwent a myeloablative or reduced intensity conditioning regimen and their outcomes

Ref	Age (years)	Number of patients	Preparative regimen	Donor relation	GVHD prophylaxis	Acute GVHD	Chronic GVHD	Graft failure	Survival
[24]	16–65	10	RIC	Related	Sirolimus	0	0	10%	100%
[44]	17–40	13	RIC	Related	Sirolimus	0	0	7.6%	100%
[45]	16–26	12	MAC	Related	Cyclosporine methotrexate	53%	13%	0	91%

MAC myeloablative, *RIC* reduced intensity

iron overload, pulmonary fibrosis as sequelae of acute chest syndrome, or pulmonary hypertension that is part of the natural history of this disease [41]. In addition, a frequent manifestation in later years of patients with SCD is hemorrhagic stroke, and these patients are also at risk of neurovascular complications such as PRES or seizures after HSCT [10]. In addition, adult SCD patients are likely to have greater transfusions exposure, making them more allo-sensitized to foreign RBC and/or HLA antigens which likely contributes to the higher rate of graft failure that has been observed in adult patients, especially in those patients who have not received a myeloablative conditioning regimen. In general, all adult patients undergoing HSCT are at higher risk of developing acute and chronic GVHD, sinusoidal obstructive syndrome, and infertility from standard myeloablative conditioning regimens compared to pediatric patients. This increased risk has been observed in the adults with SCD who have undergone transplant to date. [36] Lastly, older patients are at risk of pre-HSCT lifestyle-induced complications such as diabetes, hypertension, and obesity which can also impair their ability to tolerate conditioning regimens and add to the morbidity after transplant. Table 8.2 shows results of studies from adult patients with SCD. Promising results are shown from the approach used by Hsieh et al. [36] which was replicated independently by Saraf et al. [44] using 1 mg/kg alemtuzumab intravenously and total body irradiation as a single dose of 300 cGy with sirolimus as GVHD prophylaxis in adults who received transplants from HLA-matched siblings. Stable engraftment was achieved in most patients, and importantly, the regimen was well tolerated with virtually no acute or chronic graft-versus-host disease or deaths. Future large-scale studies of this approach in adults are anticipated.

Summary

Allogeneic HSCT is a curative option for patients with SCD; however, SCD patients undergoing or wishing to undergo HSCT face unique challenges compared to other HSCT recipients. SCD patients are less likely to have MSD or unrelated donor options than patients undergoing HSCT for other conditions. SCD patients require careful attention in the pre- and post-transplant period to minimize complications and to maximize the likelihood of success of the transplant.

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Chapter 9
The Ethics of Hematopoietic Stem Cell Transplantation for Sickle Cell Disease

Robert Sheppard Nickel and Naynesh Kamani

Introduction

It has been over three decades since the first report of a successful hematopoietic stem cell transplant (HSCT) in a patient with sickle cell disease (SCD) resulted in a potential cure [1]. Despite the excellent outcomes observed after matched sibling donor HSCT for SCD during the past 20 years, the definitive indications for HSCT in this disease remain a topic of significant controversy. This lack of clarity regarding eligibility for HSCT fuels most of the ethical questions related to HSCT for SCD. Even if medical experts and patient advocates were to agree on indications for HSCT, other ethical issues related to this topic remain. This chapter highlights the ethical issues related to HSCT for SCD through five patient vignettes. While these scenarios are fictional, they illustrate real dilemmas faced by medical professionals, patients, and their families. We will analyze the issues raised by these vignettes through the principles of nonmaleficence, beneficence, respect for autonomy, and justice (Table 9.1) [2].

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Table 9.1 Four principles of biomedical ethics

Nonmaleficence	Obligation to not intentionally inflict harm (first, do no harm)
Beneficence	Duty to provide benefits (promote health)
Respect for autonomy	Right of a person to make his or her own choices (self-determination)
Justice	Responsibility to ensure persons are treated equally (fairness)

HLA-Identical Sibling HSCT for Children with Less Severe SCD

Micah is a 7-year-old with HbSS disease. He was briefly hospitalized for fever as an infant and had an emergency department visit for extremity pain requiring treatment with IV morphine at age 4 but has had no overt SCD complications since starting hydroxyurea at age 5. His growth is normal. He is doing well in school and annual transcranial Doppler (TCD) screenings have been normal. Micah's healthy 8-year-old sister is HLA-identical. Should transplant be offered to Micah?

Historically, eligibility for HSCT was restricted to children with SCD who had suffered severe complications from SCD. In general, HSCT for SCD was also previously only offered as part of a clinical research study. Table 9.2 lists the inclusion criteria for the first major international clinical trial of HLA-identical sibling HSCT for SCD [3]. At the time of this study in the 1990s, outcomes of HSCT for SCD were unknown; it was thus appropriate to only offer HSCT in the context of a research trial and restrict enrollment to patients who had experienced serious disease-related complications. These patients were at high risk of suffering further significant SCD-related complications and death at a young age, so it was ethical to consider what was then an experimental therapy (HSCT) that was potentially curative even though it was associated with a risk of death and major morbidity. Conversely, at that time it was also appropriate to exclude patients who had not suffered serious complications and were expected to do well at least in the short-term, because the risks of HSCT were unclear, and it was possible that many patients could have adverse outcomes.

Much has been learned since that first clinical trial and hundreds of individuals with SCD have subsequently undergone successful HSCT with HLA-identical sibling donors. A recent international registry study of 1000 patients with SCD who received HSCT using an HLA-identical sibling donor between 1986 and 2013 reported that greater than 90% of all patients were cured of SCD with even better outcomes for patients transplanted after 2006 [4]. Thus, pediatric HSCT for SCD using an HLA-identical sibling donor should no longer be considered experimental. In contrast, HSCT for SCD using alternative donors (unrelated bone marrow donors, unrelated cord blood, haploidentical related donors) currently remains experimental since fewer patients with SCD have undergone HSCT using these approaches and

Table 9.2 Study inclusion criteria to define severe SCD

	First collaborative group study of HSCT for SCD [3]	Haplo SCD Consortium HSCT trial (NCT01461837)	Children's National haploidentical HSCT trial (NCT02165007)
Neurologic	• Stroke	• Stroke • Abnormal TCD study requiring chronic transfusion therapy • Silent cerebral infarct	• Stroke • Elevated TCD velocity
Pulmonary	• Acute chest syndrome (ACS) with recurrent hospitalizations or previous exchange transfusion • Stage I or II sickle lung disease	• ≥2 ACS episodes	• >2 ACS episodes
Pain	• ≥2 pain events per year for several years • Recurrent priapism	• ≥3 pain events in the last 2 years	
Other	• Sickle nephropathy • Bilateral proliferative retinopathy and major visual impairment • Osteonecrosis of multiple joints • Red cell alloimmunization (≥2 antibodies) during chronic transfusion		• Chronic red cell transfusion for >6 months

these transplants have had worse outcomes compared to HLA-identical sibling HSCT [5–9]. The remainder of this discussion will thus pertain only to eligibility for HLA-identical sibling HSCT.

Disagreement exists in the medical community regarding eligibility criteria for HLA-identical sibling HSCT for SCD. Tasked with providing consensus-based recommendations on indications for HSCT, an expert international panel concluded that "young patients with symptomatic SCD who have an HLA-matched sibling donor should be transplanted as early as possible" [10]. Similarly, in a recent review, Walters et al. concluded that for children with SCD who have an HLA-matched sibling, "a broadened view about transplant eligibility is warranted" [11]. These recommendations contrast those of the National Institutes of Health Evidence-Based Management of Sickle Cell Disease expert panel report which did not offer any recommendations regarding HSCT, claiming that more research is needed before HSCT can become "a widely used therapy" [12]. On the other extreme, some have argued that HSCT "should be considered a first-line therapy for all children with a matched sibling," even those children with HbSS who have never had any

overt symptoms of SCD [13, 14]. These differences of opinion stem from two key points that complicate HSCT eligibility considerations for SCD:

1. *SCD disease severity is variable*. Two children with SCD can have very different clinical outcomes. Some young adults with SCD suffer daily chronic pain and have cognitive deficits due to SCD-induced cerebral infarcts, whereas others are thriving college students without significant disability. While many individuals with SCD die before age 40, some enjoy a normal life expectancy [15–21]. At the present time, despite research in this area, laboratory and clinical findings from early childhood have not been well validated to predict adult SCD severity [22–28]. Given advances in SCD care, including most recently the increased use of hydroxyurea starting in early childhood, historic predictive models also are likely not relevant to patients with SCD today [29]. In addition, patients with SCD who do well as children can still suffer major SCD complications as adults. Finally, it is important to acknowledge that patients with the HbSS and HbSβ^0 thalassemia genotypes have been shown to have a more severe clinical course than patients with the HbSC and HbSβ^+ thalassemia genotypes [30–32]. Yet, while as a group of patients with HbSC and HbSβ^+ thalassemia may fare better, many individuals with these genotypes still face serious problems from SCD similar to patients with HbSS and HbSβ^0 thalassemia [33–35].
2. *Long-term outcomes of HSCT compared to best supportive care remain unclear*. To date, no prospective study has directly compared outcomes of patients with SCD who have undergone HSCT to outcomes for similar patients who did not receive HSCT [36]. A recent retrospective study comparing survival of patients who underwent HSCT, those who were treated with hydroxyurea, and those who received no disease modifying therapy showed a survival advantage for the hydroxyurea treated group [37]. However, treatment was not assigned randomly, so confounding by indication likely influenced these groups' outcomes. In addition, with longer follow-up, patients cured through HSCT may demonstrate improved survival compared to patients treated with hydroxyurea who still have SCD. Finally, while survival is obviously critical, it is also extremely important to consider quality of life which both hydroxyurea and HSCT appear to improve [38–40].

Some have considered HSCT for children with less severe SCD as unethical because it violates the principle of nonmaleficence [41]. With current supportive care practices and hydroxyurea therapy, survival of children with SCD to adulthood approaches 100% in the developed world [30, 37, 42]. In contrast, HSCT does have a finite risk of causing death through infection or graft-versus-host disease (GVHD). While the risk of death from HLA-identical sibling HSCT is low (current estimate less than 5% risk of death), it is higher than the short-term risk of a child dying from SCD. Is it ethical to conduct a procedure such as HSCT that increases the short-term risk of death?

To answer the above question, it is important to consider the rule of double effect (Table 9.3) [2]. Applying this rule, if four conditions are satisfied, then an action is ethically permissible even if it causes harm. HSCT for children with SCD clearly

Table 9.3 Rule of double effect

Nature of act	Act must be good or at least neutral
Agent's intention	Agent intends only the good effect
Distinction between means and ends	Bad effect must not be the means to the good effect
Proportionality	The good effect must outweigh the bad effect

Table 9.4 Summary of key potential harms of HLA-identical sibling HSCT and SCD

	HSCT	SCD
Death	Low risk (<5%) of dying from transplant complication	Very low risk (<1%) of dying as a child with care currently available in high-income countries, high risk of dying prematurely as an adult
Infection	Severely immunocompromised for weeks posttransplant	Defective or absent splenic function
Acute complications	Intra-transplant risks of mucositis, hair loss, veno-occlusive disease, cerebral hemorrhage, posterior reversible encephalopathy syndrome	Lifelong risk of vaso-occlusive crisis, acute chest syndrome, cholecystitis, priapism, splenic sequestration, aplastic crisis, stroke
Chronic complications	Graft-versus-host disease (GVHD)	Chronic pain, avascular necrosis, progressive multi-organ damage (retinopathy, kidney dysfunction, pulmonary hypertension)
Reproductive problems	Possible secondary to gonadotoxic conditioning	Possible secondary to organ damage (men, erectile dysfunction; women, pregnancy complications) or hydroxyurea therapy
Social	Intense short-term burden due to transplant hospitalization and care posttransplant	Lifelong burden of chronic disease
Quality of life (QOL)	Poor QOL for the first 6–12 months post-HSCT, poor long-term QOL if chronic GVHD	Lifelong poor QOL for a significant number of patients

Table adapted from Nickel et al. The ethics of a proposed study of hematopoietic stem cell transplant for children with "less severe" sickle cell disease. Blood. 2014;124(6):861–6

satisfies the first three conditions: (1) the nature of HSCT is good, (2) the HSCT team intends only for good results, and (3) the bad effects of HSCT are not the means to achieve the good effect of cure. The final condition of proportionality is less clear—does the good effect of cure outweigh the bad effect of an increased risk of short-term mortality? While the potential harms of HSCT (most importantly the increased risk of short-term mortality) are significant, so are the potential harms of SCD that HSCT can ameliorate through cure (Table 9.4). It therefore can be asked, is it possible that we are harming children with SCD by not offering curative HSCT

so that they may face a future of SCD associated morbidity, decreased neurocognitive function, and poor quality of life?

For children with SCD who have already suffered major SCD complications (most specifically stroke), the good effects of HSCT are felt to outweigh its bad effects, but this balance is less clear for children with SCD who have not had significant complications. Given this uncertainty, further research in this area is clearly justified [43]. In the absence of such research, since it is possible that the benefits of HSCT outweigh the risks, HSCT for these children does not violate the principle of nonmaleficence.

It is important to emphasize the difference between recommending and offering HSCT to children who have not had significant complications. Given the above two key points (SCD disease severity is variable and long-term outcomes of HSCT compared to best supportive care remain unclear), at this time it seems inappropriate *to recommend* HSCT for children with SCD who have had less severe or no overt disease symptoms. In contrast, it is ethically permissible *to offer* matched sibling HSCT to a broader group of children (potentially all children with HbSS/Sβ^0 thalassemia and children with HbSC/Sβ^+ thalassemia who have symptomatic disease) so that families themselves can decide the best course of action based on their own individual values and thinking.

Offering HSCT to patients with SCD honors the principle of respect for autonomy. Our society has rejected medical paternalism, the practice in which a doctor alone decides and prescribes the treatment plan for a patient. Instead we now embrace shared decision-making, honoring the individual patient as the person who makes the final medical decisions regarding his or her treatment after being educated by his or her medical provider. In the case of children, parents are respected as the agents to make medical decisions for their children. This idea of shared decision-making between parents and providers has been advocated as an effective approach for offering hydroxyurea treatment to children with SCD [44].

Some may argue that parents' decision to have their child undergo HSCT violates that child's own autonomy. An informed adult with SCD may view HSCT as too dangerous and be unwilling to accept its risks—so why should a child who is doing well be subjected to an intervention that could cause death and have potential long-term side effects? This concern could be avoided if one simply waited for a child to become an adult (or at least an adolescent) who can make his or her own autonomous choice and provide informed consent (or true assent) regarding this very difficult decision.

If there was no potential downside in waiting until a child with SCD could consent and make his or her own decision regarding HSCT as an adult, then it could be considered unethical to transplant children with SCD. Studies have demonstrated, however, that there are medical benefits to performing HSCT at a younger age. Younger children have better HSCT outcomes, in particular less GVHD [45–48]. In the largest study of HLA-identical sibling HSCT for SCD to date, Gluckman et al. demonstrated that every year waited to perform HSCT was associated with higher mortality (HR 1.1, 95% CI 1.06–1.14, $p < 0.0001$) [4]. Post-HSCT splenic function is also better if HSCT for SCD is done at a younger age [49]. To decrease the risk of

permanent organ damage from SCD, HSCT should be performed early. Unfortunately SCD can cause harm to virtually every organ. Regarding the brain specifically, studies have shown that by the age of 6 years, greater than 25% of children with HbSS have evidence of cerebral infarction that has been associated with poor neurocognitive outcomes [50–52].

It is also prudent to consider the idea that restricting a child with SCD from undergoing HSCT violates that child's theoretical future autonomous choice to have wanted to pursue HSCT as a child instead of as an adult. As part of this consideration, it should be noted that adult SCD patients' desire to pursue HSCT and to accept the risk of transplant-related mortality does not appear to be associated with their disease severity or healthcare providers' assessment of risk [53]. Patients with SCD seem to have different perceptions about the acceptability of HSCT risk than physicians and are often willing to accept more risk to achieve cure. A recent study found that a majority of adolescents and parents of children with SCD were willing to accept the current risks associated with matched sibling HSCT irrespective of disease severity [54]. Rather than maintaining a paternalistic system in which physicians only consider patients with "severe" SCD eligible for HSCT, to promote the principle of autonomy, informed patients should be allowed to make their own decision regarding HSCT. Similarly to other difficult medical decisions, since children are not capable of making such a decision, parents should decide what is best for their child with the guidance of their healthcare providers. While some may worry that this decision may burden parents or that parents are not capable of properly weighing the risks and benefits for their child, parents are likely the best agents to represent their child. Of note, parents' willingness to accept hypothetical varying risks of transplant-related mortality for SCD is very similar to the risks that adolescent and adult patients with SCD would accept [53–55], supporting the concept that parents make reasonable decisions on behalf of their children.

Some have argued that parents of children with less severe SCD should not be offered HSCT because we do not offer HSCT to children with certain other conditions. For example, a child with low-risk acute lymphoblastic leukemia (ALL) has a greater than 95% chance of cure with conventional chemotherapy that involves 2–3 years of treatment [56]. HSCT could also achieve cure in ALL with a less prolonged treatment course. If respect for autonomy is paramount, why do we not offer the option of HSCT to parents of children with low-risk ALL? Some parents may prefer the more intense treatment option of HSCT since it is likely to decrease the length of time treatment is needed. The problem with this option is that the potential benefit of a less prolonged treatment course must be weighed against the increased short-term mortality and long-term risk of GVHD associated with HSCT. The survival of patients with ALL treated with HSCT is expected to be inferior to conventional chemotherapy, with both treatments offering cure. In this example, the risks of HSCT are much greater than the benefit of a shorter treatment course with no other significant benefit; it is thus ethical to not offer this treatment option even though it does restrict parental autonomy. Also, as noted above, in contrast to pediatric ALL, with the exception of TCD screening to predict stroke risk, a contemporary risk categorization for children with SCD has not been definitively established

Table 9.5 Potential reasons for early transplant versus reasons to wait for transplant

Transplant early to:
• Prevent early organ damage secondary to SCD
• Avoid SCD complications in childhood
• Achieve better HSCT outcomes secondary to less pre-HSCT organ damage, alloimmunization, and lower risk of GVHD
Wait to consider transplant because:
• Advances in HSCT technology may further improve HSCT outcomes in the future
• Other curative therapy (gene therapy) may be developed in the future with potentially less risks than HSCT
• Further improvement in SCD supportive care (new medications other than hydroxyurea) may make curative therapy less relevant

Table adapted from Nickel et al. The ethics of a proposed study of hematopoietic stem cell transplant for children with "less severe" sickle cell disease. Blood. 2014;124(6):861–6

to accurately predict later disease severity. Children with SCD who have not suffered overt complications may not be "low risk," in that they likely will suffer significant problems from SCD as adults due to the progressive nature of the disease.

*We feel that it is ethical for transplant to be offered to Micah. While Micah currently appears to be doing well on hydroxyurea therapy, it is quite possible that he will suffer SCD-related complications in the future and thus benefit from cure. Micah's medical team should acknowledge both reasons to consider HSCT now as well as to wait (*Table 9.5*). While his physician should offer his or her own advice to pursue or not pursue HSCT based on his or her interpretation of existing data, the decision to undergo or not undergo HSCT should be made by Micah's parents.*

Preimplantation Genetic Diagnosis to Conceive Stem Cell Donors

Aaliyah is a 5-year-old with HbSS currently receiving chronic transfusion therapy because of an abnormal TCD. Her parents are interested in having another child who could serve as a stem cell donor to cure Aaliyah's SCD. Should preimplantation genetic diagnosis (PGD) be offered to this family to help conceive a child who could serve as a stem cell donor for Aaliyah?

Assisted reproductive technology using in vitro fertilization (IVF) and PGD helps couples at risk of conceiving a child with an inherited disease like SCD, have a child without the disease. After IVF, embryos are tested for the indicated disease, and only unaffected embryos are then transferred to the uterus for implantation. The successful application of this technology was first reported in a couple with sickle cell trait in 1999 [57]. While some religious and cultural groups object to this technology, especially its creation and discarding of "affected" embryos, our society has largely accepted it as an ethically appropriate medical option. The use of PGD to conceive a child free of a certain disease is in accordance with the principle of

beneficence. The conceived child clearly benefits from not having the tested disease. The use of PGD is also ethically preferable to the alternative option of natural pregnancy and then prenatal diagnosis with the potential for pregnancy termination if the fetus is affected.

In addition to testing for diseases, PGD can also include HLA antigen testing. In 2001, the first PGD with HLA matching was reported in a child conceived to serve as a donor for a sibling with Fanconi anemia [58]. In this case, the umbilical cord blood of the conceived sibling was collected at delivery, a procedure that importantly involves no risks. The hematopoietic stem cells from this collected cord blood were then used for the actual transplant. Successful HSCTs from siblings conceived after PGD have been reported for a variety of conditions [59]. In regards specifically to SCD, HSCT using HLA-identical sibling umbilical cord blood has been shown to be as efficacious as bone marrow [60]. However, at times umbilical cord blood may not be sufficient for transplant depending on the quantity of the cord blood collection and the size of the patient. Thus, it is wrong to claim that PGD to conceive an HLA-identical sibling donor places this conceived child at no risk since additional stem cells may need to be collected via a bone marrow harvest at a later time. This risk of stem cell donation fortunately is small, and, while the nuances of protecting pediatric stem cell donors have been debated, it is generally accepted that children can participate as stem cell donors as long as certain conditions are satisfied [61–66]. Nonetheless, the use of PGD to create HLA-identical donors raises unique ethical concerns because, unlike PGD for disease testing, the conceived child does not directly benefit from HLA antigen testing. While some have argued that these children will later benefit psychologically from having served as a donor to cure a sibling, research has also suggested potential psychological harms of donation, especially if the transplant is unsuccessful [67, 68].

When PGD is used to conceive an HLA-identical sibling, some have argued that these children are potentially subject to objectification as the primary motivation for their conception was to create a hematopoietic stem cell donor. This thinking ignores the fact that individuals can decide to have children for many, sometimes selfish reasons. A couple may have a child in an attempt to save a failing marriage, or a single woman may become pregnant so she will have someone to take care of her when she is elderly. Kahn et al. aptly acknowledge: “Given the wide range of reasons and motivations for having children, it is difficult to argue convincingly that having a child to save the life of an existing sick child is such a bad parental motivation” [69]. Despite concerns associated with the use of PGD to conceive a HLA-identical sibling, the American Academy of Pediatrics supports its practice as long as the delivery is not modified to maximize the number of cells collected [61].

On a societal level PGD raises other issues, specifically with regards to the principle of justice. IVF with PGD is expensive and generally not covered by health insurance. This technology and the benefits it offers are therefore not available to all. Is it fair that a rich family can conceive an HLA-identical sibling to cure their child, but a poor family cannot? This question involves the difficult issue of the distribution of limited healthcare resources and does not have a simple answer. Yet, it should be acknowledged that the high up-front costs of IVF, PGD, and HSCT

using the conceived sibling to cure a patient with SCD may be less than the cost of lifelong medical care for a patient with SCD, especially a patient receiving chronic red blood cell (RBC) transfusion therapy [70]. Justice concerns regarding the use of PGD are especially relevant to SCD since the disease disproportionately afflicts individuals from minority racial groups that have a higher prevalence of poverty. While limited research has been conducted in this area, it appears that most families with SCD are unfortunately not aware of the option of PGD [71].

We feel that it is ethical to offer PGD to Aaliyah's parents to conceive a sibling who is both free of SCD and HLA-identical. Aaliyah's medical team should refer Aaliyah's family to an assisted reproductive technology center where they can receive further counseling regarding the details of IVF and PGD. Since many families will not be able to pay for the costs of this technology, we also feel that advocacy work is needed to ensure that this technology and its associated health benefits are fairly distributed to members of our society.

Transplant for Children with SCD and Social Concerns

Cedric is a 13-year-old with HbSβ^0thalassemia. He has had seven hospitalizations in the past year for pain. He also has a history of acute chest syndrome that required intubation. A recent brain MRI obtained due to his failing the sixth grade showed multiple, small infarcts consistent with "silent" strokes. He has been prescribed hydroxyurea but admits to missing multiple doses each month. He has five siblings, including a sister with SCD. One of his healthy brothers is HLA-identical. He lives with his mother and siblings in a two-bedroom apartment. His father is in prison. His mother has little support from family or friends and has missed hematology appointments because of her work schedule. Should transplant be offered to Cedric?

While it is the inherited biology of SCD that causes harm throughout a patient's body, it is important to also acknowledge that the social environment of a patient can significantly impact the disease. Like most diseases, if patients with SCD have a more difficult social situation (less financial and emotional support), they are more likely to have worse health outcomes. For example, a study in England found that patients with SCD living in the most socioeconomically deprived areas were at highest risk of readmission and inpatient mortality [72]. A vicious cycle can occur where complications of SCD cause social problems which in turn contribute to the occurrence of more disease complications (Fig. 9.1). Given this background, optimal care for individuals with SCD should include social support services.

Since HSCT is an intensive medical process that requires not only a lengthy hospitalization but also months of close outpatient follow-up with lifestyle restrictions and new medications, it is prudent to evaluate the social situation of potential transplant candidates and their ability to adapt to the requirements of HSCT [73]. If a patient undergoes HSCT and then does not take prescribed medications to prevent GVHD or follow recommendations to minimize the risk of infection, the patient could potentially die from HSCT complications. In one study, patients who

pre-HSCT had problematic adherence were significantly more likely to die 1-year post-HSCT, particularly secondary to infection or GVHD (complications which may have been prevented with improved adherence) [74]. One could argue that it is ethical to not offer HSCT to patients who have social concerns that will impact adherence since HSCT has a greater potential to harm these patients.

It is paternalistic for a provider to not offer HSCT to certain patients with social concerns that may impact adherence. In some instances this paternalism is definitely justified as the principle of nonmaleficence can trump respect for parental autonomy, but its application raises ethical issues. Who decides if a patient is likely to be able to follow the necessary HSCT care? How is such a decision made? Is it just to deny a child HSCT because of circumstances that he/she has no control over and his/her parent(s) may or may not be responsible for? Since almost all people with SCD are from minority groups that have suffered and unfortunately continue to face discrimination, safeguards need to be in place to ensure that such decisions are fair. Patients with SCD should also be evaluated fairly when compared to patients who are being considered for HSCT for other diseases. For example, if HSCT is medically indicated for a patient with relapsed leukemia, the evaluation of this patient's social situation in relation to eligibility for HSCT should be similar to that for a patient with SCD. There is a concern that resources will be mobilized to assist a patient with relapsed leukemia who has a challenging social situation to go to HSCT, but similar efforts may not be made available to a patient with SCD who has social concerns. One could argue that a patient with relapsed leukemia should be evaluated and treated differently since their disease is more imminently fatal without treatment. Yet, if patients with SCD for whom HSCT is medically indicated are denied HSCT because of social concerns and not provided similar support, then they are likely destined for a future of increased disease complications compounded by their social situation (Fig. 9.1). This future may not be as imminently fatal as the patient with relapsed leukemia but one could argue is just as bleak.

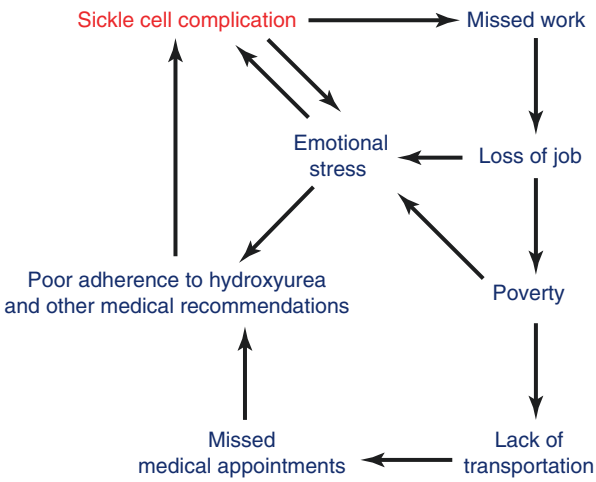

Fig. 9.1 Pathologic relationship of SCD complications and social struggles

We feel that Cedric and his mother should be educated about the option of HSCT especially given the severity of his disease. At the same time, they should be educated that improved adherence to hydroxyurea would also likely decrease Cedric's risk of certain complications. Given that Cedric has not been compliant with medical care (nonadherent to hydroxyurea, missed appointments), his medical team should be concerned about the family's ability to support Cedric through HSCT and explain the serious dangers of nonadherence during HSCT to the family. Social support services should be provided to the family to improve adherence. If the family is interested in HSCT, a fair evaluation of the family's ability to comply with the requirements of HSCT should be done per a set protocol before HSCT is offered. In addition, a lengthy hospitalization for a HSCT can be an opportune time to educate the patient and family about the benefits of compliance and the risks of nonadherence. If there are sufficient concerns regarding the family's social situation to not offer HSCT, then efforts should be made to help the family overcome these difficulties so that HSCT can be offered in the future.

Transplant for Children with SCD from Low-Income Countries

Kofi is a 4-year-old with HbSS disease from Ghana. He has had one hospitalization for a pain crisis. He has never had a TCD and has not been offered hydroxyurea treatment. His parents both had cousins with SCD who died in childhood. Kofi has a healthy, 6-year-old sister who has not had HLA typing. The family was able to raise funds to travel to the United States and pay for a potential HSCT. They want their son to be cured and then return to Ghana. Should transplant be offered to Kofi?

The majority of patients affected by SCD live in low-income countries. It is estimated that greater than 75% of babies with HbSS are born in sub-Saharan Africa. A baby with HbSS born in this part of the world has a very different prognosis than an American baby with HbSS. A large percentage of children with SCD die in Africa before age 5 years [75, 76]. Most African countries lack universal newborn screening to even identify children with SCD, so African children with SCD often do not receive critical preventive and acute medical care. With this background, currently HSCT is not a realistic option for most African children. Nonetheless, it is encouraging that the first child cured of SCD through HSCT performed in a low-resource country, Nigeria, was recently reported [77].

A few families from low-income countries will have personal or communal resources to travel to countries like the United States to undergo HSCT. Should these patients have different eligibility criteria for HSCT? Without readdressing the already discussed controversy inherently associated with HSCT eligibility for SCD, some have argued that patients from less developed countries who plan to move back to these countries should be evaluated differently. Since SCD has a much

higher risk of causing death in childhood in low-income countries, it may be appropriate to allow these children to take on more risk to achieve cure. This thinking was applied by one HSCT center in Belgium in the 1990s (when HLA-identical HSCT was still experimental for SCD). This center transplanted 14 young African patients who had minimal or no overt symptoms of SCD before their return to Africa [78]. These patients fortunately did well (100% survival, 93% cured), and today this approach should be accepted since HLA-identical HSCT is a proven therapy for SCD. Nonetheless, the ethical thinking that was used to support offering an experimental therapy at that time to children who were clinically doing well is problematic.

Treatment that is different from the standard of care in high-income countries is often ethically offered to patients with the same condition in low-income countries. For example, in high-income countries, chronic RBC transfusion therapy is the standard of care for children with SCD who have suffered a stroke because a randomized clinical trial has shown that hydroxyurea treatment with phlebotomy was inferior to transfusions with iron chelation for secondary stroke prevention [79]. In most low-income counties, blood transfusion support is limited, and it may not be possible to provide chronic RBC transfusion therapy, so in these countries it would be ethical to offer an alternative treatment (hydroxyurea) that is considered below a high-income country's standard of care for secondary stroke prevention. However, if a child from Africa who has suffered a stroke moves to the United States, it would be wrong for American physicians to treat this child with hydroxyurea rather than chronic transfusion (unless there was a medical contraindication to transfusion). The ethical standards of the location where the medical care is being provided should apply.

This thinking can be illustrated when considering HSCT for SCD using donors other than HLA-identical siblings. For example, HSCT for SCD using a haploidentical donor is a promising approach being studied in several clinical trials currently recruiting patients in the United States. These studies appropriately have strict eligibility criteria limiting enrollment to patients with significant complications from SCD (Table 9.2). Given that they have already had certain complications, it is likely that they will suffer ongoing, progressive complications of SCD, and thus the proportionality of potential benefit is felt justified to expose these patients to the risk of an investigational therapy. One could attempt to argue that an African child should also qualify for such a study even if he or she has not suffered certain disease complications because an African child with SCD is likely to have a poor outcome if not cured because of the lack of adequate supportive care in Africa. This rationale is problematic because it could lead to children from low-income countries who have not suffered significant complications being sent over to high-income countries to participate in these clinical trials. These trials will initially just benefit individuals in high-income countries since HSCT (especially haploidentical HSCT) is not reasonable to currently consider performing in most low-income counties. Such experimentation on members of a vulnerable group unlikely to benefit the larger vulnerable group is clearly ethically inappropriate. In addition, it should be noted that patients

may suffer GVHD or other late complications post-HSCT that cannot be safely managed in low-income countries [29].

We feel that since HLA-identical sibling HSCT for SCD is no longer experimental and it is reasonable to consider offering HSCT to all children with HbSS, Kofi's family should be offered HLA typing to determine if his sister is a match. His family should also be educated about TCD screening and hydroxyurea therapy.

Kofi had a TCD that did not show any concerning velocity measurements. His family is interested in hydroxyurea but declined starting treatment since they did not think it would be possible to actually obtain hydroxyurea and undergo the required laboratory monitoring in the part of Ghana where they plan to return. HLA typing is also performed, and Kofi's sister is not HLA-identical. His parents know about the possibility of HSCT using a parent as the donor and are very interested in this option of haploidentical HSCT even if it is considered very high risk. Should haploidentical HSCT be offered to Kofi?

We feel that since haploidentical HSCT for SCD is currently under investigation, it should only be offered as part of clinical research trials which have strict eligibility criteria to restrict enrollment to patients with severe disease complications. Since Kofi has not suffered severe complications, it is ethically problematic to subject him to an experimental therapy that could cause serious harm.

Transplant for Adults with SCD

Michelle is a 31-year-old with HbSC disease. She has had a total of five hospitalizations for pain crises in her life, only one of which was in the last 2 years. She has had a cholecystectomy for biliary colic and laser photocoagulation eye surgery for sickle retinopathy. She works as a computer programmer, but a few days each month, she misses work or must work from home due to pain. She has one brother who is healthy. She is very interested in a cure for her SCD. Should HSCT be offered to Michelle?

In the past, HSCT was not considered an option for adults with SCD. The first large study of HSCT for SCD restricted enrollment to children less than 16 years [3]. Adults were generally excluded due to legitimate concerns that older patients with SCD would not tolerate intensive, myeloablative HSCT conditioning because of the organ damage caused by SCD over time. Fortunately, recent clinical studies have demonstrated very good results of matched sibling HSCT with reduced intensity or nonmyeloablative conditioning for adults with SCD [39, 80, 81]. Thus, it is no longer appropriate to consider adults with SCD ineligible for HSCT.

In some respects, the ethical concerns associated with HSCT may be less troublesome for adults compared to children. While the medical decisions of parents should largely be respected as discussed above, it is simpler to accept the consent of a competent adult patient than a parent proxy for a pediatric patient when considering elective HSCT that has serious risks. An adult patient with SCD knows what it is like living with SCD since he or she has actually lived many years with the disease. One could argue that such an informed person is much better equipped than parents to decide based on his or her own experiences if the potential benefits of

curative HSCT outweigh its risks. Parents, in contrast, are making a decision for a child that has lived a limited number of years with SCD and may not have experienced significant SCD complications. Parents do not have the same wealth of personal knowledge about actually living with SCD to inform their decision.

The above argument that adult patients with SCD are better able to give informed consent for HSCT is complicated by the fact that many adults with SCD have suffered cerebral infarcts without overt neurologic deficits ("silent strokes"). Brain damage in patients with SCD progresses with time and increasing volume of cerebral infarction has been associated with lower IQ [82–84]. The resulting cognitive impairment can be significant and has been linked to the high prevalence of unemployment faced by adults with SCD [85]. Consequently, some adult patients with SCD who have suffered cerebral infarcts will have significant difficulty making complex medical decisions like the decision to undergo HSCT. Providers need to be aware of this potential issue and provide extra assistance to such individuals to ensure appropriate informed consent.

While Michelle has HbSC disease which has been associated with a milder phenotype (in particular a more normal life expectancy) than HbSS, she clearly has suffered both acute and chronic complications from her disease. Given the encouraging results of recent studies of HSCT specifically for adult patients with HLA-identical sibling donors, we feel it is appropriate to offer HLA typing and HSCT if her brother is a match after obtaining appropriate informed consent.

Michelle is educated about HSCT and her providers have no concerns regarding her understanding of the potential benefits and risks. Her brother had HLA typing, but he is not a match. Michelle remains very interested in HSCT and wants to pursue alternative donor HSCT. Should alternative donor HSCT be offered to Michelle?

Since alternative donor HSCT is investigational, it should only be offered as part of a clinical research trial. Michelle does not meet disease severity criteria for current alternative donor HSCT trials. While one could argue that disease severity criteria for eligibility defined by studies are somewhat arbitrary, we feel that some restrictions are appropriate given the lack of data on these transplants and the potential for serious harm.

Conclusion

The ethical issues explored through the presented vignettes help bring to light a number of ideas. First, although eligibility criteria for HLA-identical sibling HSCT remain controversial, until reliable markers to predict disease severity are validated and/or strong evidence directly comparing outcomes of HSCT versus supportive care is available, it is ethically permissible to offer HSCT to all children with HbSS or HbSβ^0 thalassemia who have matched siblings. Second, PGD is an ethically appropriate option for interested families to conceive a sibling who can serve as a matched donor for a HSCT. Third, while providers may be justified in not offering HSCT to patients because of social concerns, pre-HSCT evaluations must be fair, and efforts must be made to help families overcome or manage psychosocial

concerns so that HSCT can be offered and carried out. Fourth, different ethical standards should not be applied to children with SCD from a low-income country when they are treated in a high-income country. Finally, providers should make certain that even adult patients give true informed consent to pursue HSCT, but should not offer experimental HSCT to all interested patients.

The ethical issues involving HSCT for SCD will continue to evolve over time as supportive care for SCD continues to improve, better models to accurately predict disease severity are developed, and the promise of other curative therapy (gene therapy) is hopefully realized. While the ethical questions may change, the principles of biomedical ethics applied in this chapter should continue to serve as useful tools. Further scientific research is paramount to advance care, including HSCT, for people with SCD, but consideration of additional ethical issues raised by new research findings is also important to ensure that advances are appropriately and fairly realized.

Glossary

Beneficence Principle of biomedical ethics that refers to an obligation to act for the benefit of others

In vitro fertilization (IVF) Assisted reproductive technology procedure in which eggs are first harvested, and these eggs are then combined with sperm in the laboratory to create embryos

Nonmaleficence Principle of biomedical ethics that asserts an obligation not to inflict harm intentionally

Preimplantation genetic diagnosis (PGD) Process used to identify genetic defects or traits in embryos created through in vitro fertilization (IVF) prior to their transfer to the uterus. Embryos to transfer and implant are selected based on the results of the genetic testing

Respect for autonomy Principle of biomedical ethics that requires honoring a person's right to make his or her own choices

Justice Principle of biomedical ethics that ensures equals are treated equally and concerns the fair distribution of benefits and burdens

Rule of double effect Ethical doctrine used to justify claims that a single act having both good and harmful foreseen effects is not always morally prohibited if the harmful effect is not intended

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Chapter 10
Psychosocial Care and Education of Children with Sickle Cell Disease Undergoing Hematopoietic Stem Cell Transplant and Their Families

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Abbreviations

GVHD	Graft-versus-host disease
HSCT	Hematopoietic stem cell transplant
QoL	Quality of life
SCD	Sickle cell disease

Psychosocial support and education are critical components of care for young patients with sickle cell disease (SCD) undergoing hematopoietic stem cell transplant (HSCT) and their families. Although few studies have specifically examined the educational needs, psychosocial risk factors, and post-HSCT outcomes of children with SCD and their families, research is available in related patient groups (e.g., patients with SCD who have not undergone HSCT; patients with malignant diseases who have undergone HSCT) and can inform recommendations. Here, we review unique challenges and opportunities faced by medical teams in discussing the details of HSCT with patients with SCD and their families. Concrete recommendations for providing effective teaching about HSCT and for assessing comprehension and retention of this information are described. We also review the limited evidence on psychosocial outcomes in children with SCD undergoing HSCT and their families, highlight risk factors for difficulty coping and adjusting, recommend strategies for monitoring risk factors and psychosocial adjustment, and offer directions for future research.

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Communication and Education About HSCT for SCD

Deciding to pursue HSCT as a cure for SCD can be a lengthy and stressful process for patients and their families. In order to make an informed decision, patients and families must digest a great deal of complicated information on available treatment options, understand the short- and long-term impacts of SCD, and thoughtfully weigh the risks of HSCT against other treatments and the potential for lifelong cure. Patients and families must evaluate the recommendations of their hematology team, whom they have often known since the patient's birth, and their transplant team, with whom they may be far less familiar and comfortable, while taking into consideration patient preferences and familial, cultural, and religious values and beliefs. Unlike decisions to pursue HSCT for a malignant disease like cancer (where pursuing HSCT is often the only option to prevent death from disease), the decision to pursue HSCT for SCD is far less clear, and in many cases, HSCT could be considered an elective treatment option.

In general, the quality of the family-physician relationship impacts family decision-making. Studies of adults with SCD demonstrate that interactions with providers are not always positive and that poor clinical interactions can lead to patient-provider relationships lacking in trust [1]. Moreover, lack of trust in medical professionals and teams has been identified as a barrier to clinical trial participation among parents of youth with SCD [2] and appears to play a role in family decisions about HSCT [3]. Research also indicates that children with SCD and their parents often take a passive role during clinic visits, that physicians spend more time gathering information and less time building relationships, and that compared to visits of children with other chronic illness, physicians spend more time speaking to parents and caregivers than to the patients themselves [4]. Facilitating a more balanced exchange of information and active engagement of caregivers *and* patients may positively affect the family-provider relationship, which in turn may impact families' awareness of treatment options and the quality of decisions made about patient care.

To date, HSCT remains an underutilized treatment for SCD, which may be due, at least in part, to lack of information and limited understanding of both SCD and HSCT. Research indicates that patients and families lack accurate knowledge of the morbidity and mortality of SCD, of HSCT as a modality for cure, and of the risks associated with HSCT [2, 3, 5, 6]. Misinformation and significant gaps in SCD knowledge are common, as a majority of parents of children with SCD believe that their child's disease will get better, will not prevent their child from reaching their life goals, and will not shorten their child's life span [2, 6]. Further barriers to HSCT include reported lack of time, resources, and support for HSCT, belief that current therapy is adequate, fear of HSCT and related complications (including infertility, graft-versus-host disease [GVHD], and death), concerns about risk for the sibling donor and the emotional impact of HSCT on the patient, and costs associated with the procedure [3, 5].

While outcomes for HSCT for SCD are promising, HSCT is not without significant risks. As a result, patients and caregivers must understand and be willing to

accept a certain degree of risk in pursuit of a cure. Regardless of disease severity, the majority of adolescents with SCD and their parents are willing to accept mortality and GVHD risks that are consistent with current outcome data for matched sibling HSCT for SCD (5% and 10%, respectively; [7]). A significant subset of patients and caregivers, however, remain unwilling to accept any risk of HSCT-associated mortality or GVHD to pursue cure. It is unclear whether this represents an underappreciation for the severity of SCD or other unknown factors, as research is lacking on what distinguishes those who accept some risk and those who do not. Regardless, in order to appropriately tailor communication and education about HSCT, providers should consider patient and family perception of risks, assess willingness to accept known risks, and facilitate frank discussions weighing short- and long-term risks of both SCD and of HSCT.

Overall, education about HSCT for SCD should involve multiple conversations over time, rather than a singular discussion, with members of the hematology, transplant, and psychosocial teams. First and foremost, early and frequent communication with patients and families about morbidity and mortality associated with SCD will be critical to their ability to make informed decisions about future care. As such, comprehensive hematology clinic visits should include routine and honest education about long-term risks and shortened life expectancy of patients with SCD [7]. Family engagement in these pediatric visits is critical, so providers should devote time to relationship building and make a conscious effort to facilitate active participation of both patients and parents. In order to promote a reciprocal exchange of information, for example, providers may wish to encourage families to write down questions prior to their visit. Finally, communication and education should not just be directed at caregivers; child and adolescent patients should be provided with developmentally appropriate information about their disease and treatment and included in discussions about their care. As SCD can impact aspects of cognitive development, providers are encouraged to check frequently for patient understanding and provide re-education and clarification as necessary.

Currently, there is no standard guideline or recommendation for when or how HSCT as an option for cure should be discussed with patients and their families, as research on education about transplant for SCD is extremely limited. Clinical experience and principles of effective health communication, however, support initiating conversations with patients and families early, revisiting such conversation regularly, and encouraging patient and caregiver engagement in discussion [4, 8]. As risks of HSCT are not insignificant, families should be accurately informed of potential complications and toxicities so that they can make informed decisions about whether to pursue curative options for SCD. Opportunities for interaction with other families who have been through the transplant process may also be helpful.

Few opportunities typically exist for families of children with SCD to interact with HSCT experts or access accurate, up-to-date information about SCD and treatment options [2]. In order to address barriers of information, disseminate clinical research information, and provide a forum for direct interaction between families of pediatric SCD patients and the HSCT experts, group educational opportunities

should be considered. A larger-scale educational approach has been shown to be a successful and cost-effective strategy to increasing knowledge about transplant [3] and may be particularly helpful for patients and families who are just beginning to explore the option of HSCT for SCD cure.

More specifically, a symposium for patients and families summarizing research findings, evidence-based information, and the patient/family experience has been implemented annually at a large urban children's hospital and shown to increase knowledge of and interest in HSCT for SCD. The half-day symposium provides direct access to experts in HSCT and includes (1) topic lectures and breakout sessions on concepts and terminology of HSCT, current research trials in SCD and HSCT, chronic GVHD, ethical considerations in HSCT for SCD, psychosocial support of transplant patients and families, impact of HSCT on fertility, etc.; (2) a family panel where patients and families who have been through HSCT discuss their experiences, both positive and negative; and (3) a question and answer period during which family members interact with both HSCT experts and patient families. Pre- and post-symposium surveys have demonstrated effectiveness of this educational format, as scores reflecting knowledge of HSCT increased significantly following attendance at the symposium. Furthermore, attendees have reported a high level of satisfaction with the symposium, the belief that information received was helpful in making a decision for their children's future treatment, and an increased desire to discuss HSCT further with the transplant team directly [3]. Other modalities for disseminating information to wider audiences (e.g., webinars, social media) may prove to be similarly effective in educating patients and families and may help decrease the knowledge gap about HSCT as a curative option for SCD.

Considerations for Clinical Practice: Assessing Understanding of HSCT Teaching

There are several challenges to address when attempting to provide preparation that will help optimize psychosocial outcomes for a child with SCD and his or her family prior to HSCT. In order to ensure adequate transfer of knowledge about HSCT and determine the need for additional teaching, one must routinely conduct careful assessments of the patient's and family's understanding. In this section, we describe recommendations for the evaluation of patient and family knowledge of HSCT and offer guidance related to the effective communication of information about HSCT and its risks and benefits.

As previously described, numerous factors influence the transfer of knowledge from physician to patient and family regarding HSCT for pediatric SCD. The decision to pursue HSCT is significant and requires both the development of a trusting relationship and a grasp of complex medical terminology and procedures. While most hematology and transplant physicians are experienced at consenting patients and families to clinical trials and discussing the risks and benefits of various treatments, it is less common to have specific training in strategies for assessing compre-

hension and tactfully delivering developmentally, culturally, and linguistically appropriate teaching about complex topics such as HSCT. As such, it may be useful to recruit support staff (e.g., child life specialists, psychologists, social workers), if available, to provide direct teaching, reinforce physician teaching, or provide guidance about language to use and language to avoid.

Regardless of the method of instruction used to educate families about HSCT, it is imperative that providers carefully gauge patients' and families' understanding; doing so allows recognition of important gaps in understanding and opportunities for reteaching. Since discussions about HSCT for pediatric SCD often begin months or years in advance of the actual transplant, one strategy for ensuring adequate comprehension is to break down the larger discussion of HSCT into several small modules or chunks that are relatively narrow in scope. It is helpful to utilize concrete examples and analogies and stop frequently to redirect patients' and families' attention and inquire about comprehension. It is well documented that comprehension and retention of information is improved when larger topics are divided into smaller sections [9]. Allowing natural pauses to occur and inviting questions also emphasize a preference for a balanced, conversational approach rather than a one-sided lecture, which increases the likelihood that families will remain engaged [10, 11].

Engagement is critical, as it can be very difficult to assess a family's understanding of HSCT when they feel intimidated or overwhelmed and choose to ask few questions. Rather than posing close-ended questions to families ("Does this make sense?" or "Do you have any questions?"), which invite restricted and socially desirable responses [12], ask open-ended questions ("Tell me three things I said that did not make complete sense" or "I would be very happy to answer your questions now") that imply an expectation for the family to talk and that require marshaling of cognitive resources to both retrieve learned information and reorganize information using the family's own language preferences.

Another strategy for evaluating understanding is to request that patients and families paraphrase learned information. While certain details of HSCT and post-HSCT home care must be committed to memory, the ultimate goal in most aspects should not be to have families simply memorize terms and procedures. Instead, patients and families should be developing a deep understanding of and personal connection to the information provided, so that they can critically weigh costs and benefits for their family, generate questions, and form realistic appraisals of the transplant experience. Asking families to paraphrase chunks of information ("Using your own words, please tell me what you heard me say" or "How would you explain what I just told you to a friend of yours?") will not only reveal deficits in understanding but also further contribute to encoding of information in long-term memory [13, 14]. As inaccuracies or gaps in understanding are identified, providers should empathize with the challenge of digesting this complex information and praise the effort being exerted to acquire this new knowledge. Provide corrective information and again request that the patient or family member paraphrase the information presented until adequate comprehension is achieved. In cases where families are difficult to engage or indicate they have no questions, it may be useful to suggest they discuss the information as a family at home, write down at least five questions generated by

family members, and return at a subsequent visit to review their questions with the transplant team. It should be made clear that there is an expectation for families to have questions about HSCT and that ongoing bidirectional dialogue is typical and welcomed.

Building a trusting relationship with patients and families almost always involves a significant investment of time, patience, and empathy. However, such an investment must be made to move beyond possible mistrust of new medical providers and move forward with a possible cure for a patient with SCD [2, 15]. Adopting communication strategies that increase patient and family engagement is essential, but providers must also pay careful attention to the degree to which information conveyed to patients and families is comprehended and address gaps in understanding. Taking such steps is consistent with ethical commitments, may reduce feelings of deception in the future, and may increase compliance with posttransplant guidelines.

Psychosocial Adjustment and Outcomes in the Context of HSCT for SCD

Patient Adjustment. In addition to evaluating HSCT information uptake in the context of SCD, providers should monitor the psychosocial functioning of patients and caregivers throughout the transplant process, as these important patient-reported outcomes have implications for adherence to recommended behaviors and medications regimens [16]. Despite significantly limited research regarding psychosocial outcomes of patients with SCD who undergo HSCT, recent studies have helped clarify the course of psychosocial adjustment for pediatric patients with other life-threatening diseases who receive a HSCT. In the absence of empirical data on the psychological trajectory of patients with SCD who are treated with HSCT, relevant conclusions can be cautiously extrapolated from existing literature involving children with cancer who have undergone transplant and from literature describing adjustment issues commonly seen in SCD.

Although the process of undergoing HSCT requires frequent medical tests, prolonged hospitalizations, and medications that can result in adverse side effects, very few children exhibit signs of depression, anxiety, or posttraumatic stress at the point of admission for HSCT [17]. Moreover, such symptoms typically remain low in the months following HSCT and, in some cases, decline [17, 18]. Rates of depressive symptoms, specifically, are comparable to or lower than those seen in healthy children and, while a notable subgroup of children report symptoms of posttraumatic stress at the time of admission for HSCT, these symptoms typically dissipate, returning to normal levels 6 months after transplant [17]. Although the majority of children who undergo HSCT seem to adjust reasonably well, there are some who continue to exhibit symptoms of anxiety, depression, or posttraumatic stress, and additional research is needed to clarify risk factors for psychological distress posttransplant, particularly in pediatric SCD.

In terms of quality of life (QoL), children undergoing HSCT tend to report significant increases in physical and emotional QoL between the time of HSCT and the third month after discharge [19]. In a sample of children with heterogeneous diagnoses undergoing HSCT, patients maintained normal levels of QoL in areas of mental health, self-esteem, behavior, and bodily pain and reported even better QoL 6 months post-HSCT [17]. However, patients endorsed poorer QoL in areas of physical functioning and general health relative to healthy comparisons [17]. In sum, patients describe their QoL as similar to that of healthy youth and indicate that QoL steadily improves after HSCT. Data also suggest that difficulties with physical functioning may be a salient concern for children receiving a HSCT, though such findings must be interpreted with caution given the dearth of literature specific to the experience of youth with SCD.

HSCT and associated treatments may also have direct and indirect impacts on patients' cognitive and social functioning. Factors such as treatment toxicities or complications, prolonged hospitalizations, and related school disruption likely contribute to these changes. Children's intelligence has been shown to remain stable after HSCT; however, patients are more likely to demonstrate increased difficulties in tasks of arithmetic [20], which may reflect a need for additional supportive services in the classroom. Post-HSCT, patients may struggle to integrate back into or be accepted by their peer groups, particularly if HSCT has caused changes in physical appearance or impacted their functional/athletic abilities [21]. Psychosocial oncology research suggests that while childhood cancer survivors treated with HSCT are not disliked by their peers, they are viewed by classmates as more isolated and withdrawn than children who have not undergone HSCT. Age seems to moderate these outcomes, as HSCT survivors who were older at the time of transplant may return to social activities more quickly [20], which may help to facilitate social adjustment post-HSCT.

Caregiver, Sibling, and Family Adjustment. Just as patients face multiple physical and psychological challenges throughout treatment, parents and caregivers also experience significant stressors at multiple time points during the HSCT process. They have to navigate a complex medical system, absorb large amounts of complicated medical information, repeatedly make significant decisions about treatment, take care of other family members, adjust to shifting roles and responsibilities within the family structure, negotiate for time off from work, and manage the financial impact of lost wages. As such, it is not surprising that some parents and caregivers experience increased emotional distress during their child's treatment. Barrera et al. [18], for example, reported that immediately prior to their child's HSCT, 50% of mothers had clinically significant levels of anxiety while 8% of mothers had clinically significant levels of depression. Multiple trajectories of adjustment over time have been identified; the most common course involves low rates of anxiety and depression at the time of HSCT that are stable or decrease during the first year after HSCT [22]. A less common but notable trajectory of adjustment involves caregivers with high levels of anxiety or depressive symptoms at the time of HSCT that decrease over time. Finally, a small percentage of caregivers experience high levels of anxiety at the time of HSCT that remain stable or increase over time. Additional

research is needed to further clarify factors that influence the course of psychosocial functioning in caregivers of children with SCD undergoing HSCT and, specifically, predict which caregivers will experience unremitting or worsening anxiety and depressive symptoms.

Siblings of children undergoing HSCT also experience significant changes in their environment, including disrupted routines, changes in family roles, temporary and sometimes unpredictable changes in caregivers, prolonged absences of one or more parents, and decreased contact with their sibling undergoing treatment [23]. Siblings who act as donors of their ill brother or sister are at particular risk for emotional difficulties, as they have been found to experience increased anxiety and decreased self-esteem compared to non-donor siblings [24, 25]. The potential for a negative emotional impact on the sibling donor is even greater in instances where the patient experiences significant prolonged complications or dies during or after transplant, as the sibling donor may feel a personal sense of responsibility for the outcome.

Finally, family functioning appears to impact a child's ability to cope with the stressors of HSCT. Specifically, family environments characterized by low conflict, high cohesiveness, and open and honest communication patterns are associated with lower levels of patient distress [25–27]. Higher family cohesion and family connectedness appear to be protective against the multiple stressors commonly associated with transplant [18]. High family cohesion, in particular, is correlated with improved QoL and behavioral adjustment of patients who have undergone HSCT, and high family connectedness and adaptability have been shown to moderate the effect of stress on parents of children with SCD [18, 28]. These findings underscore the need for family-level interventions aimed at increasing cohesion and connectedness and promoting positive relationships between patients undergoing HSCT and their caregivers.

Considering the dearth of literature concerning the psychosocial adjustment of patients with SCD undergoing HSCT and their families, further research is needed to provide clarity and depth to the knowledge about psychosocial risks and outcomes. Although resilience has been studied in children undergoing HSCT, there is little research regarding the impact of resilience among patients with SCD in this treatment context. Also, while the HSCT experience is somewhat similar regardless of diagnosis, questions remain regarding how HSCT may affect youth with SCD differently than other, more studied groups (e.g., cancer). For example, preparatory treatment for HSCT will likely constitute the first time patients with SCD lose their hair, which is often an event already experienced by children with cancer undergoing HSCT. Although children with SCD may have a history of frequent hospitalization, it is uncommon for them to have experienced as prolonged of a hospital stay as that which is typical of HSCT. Beyond these examples, researchers should consider the role of other unique experiences and characteristics of children with SCD and their families in affecting psychosocial outcomes during and after HSCT. There is also extensive room for research in the area of effective interventions for patients with SCD undergoing HSCT and validation of available interventions with this patient population. Considering the increasing number of patients with SCD being

treated with HSCT, it will be important for researchers to shed additional light on the psychosocial outcomes and associated risk factors specific to these patients so that early identification and intervention methods can be enhanced.

Considerations for Clinical Practice: Assessing Psychosocial Functioning

Although data are still accumulating on the factors that influence psychosocial outcomes in patients with SCD undergoing HSCT and their families, existing research provides guideposts to direct the monitoring of psychosocial risk factors and adjustment. An efficient approach to supporting the psychological, social, and behavioral functioning of children with SCD as they enter transplant is to screen for known risk factors that could predict subsequent distress. Assessment of risk factors prior to HSCT creates important opportunities, including (1) risk stratification and proper allocation of often limited support resources, (2) targeted monitoring and early intervention to prevent psychosocial problems from developing or worsening, (3) insight into baseline functioning that enables recognition of worsening symptoms upon reassessment, and (4) early introduction of psychologists or other mental health care providers as a standard of care, which may lessen stigma commonly associated with such services.

While most children undergoing HSCT adjust quite well, certain risk factors for poorer adjustment have been identified. These include patient, family, and disease factors. Patients with premorbid mental health disorders (e.g., anxiety, depression, substance abuse); lower pre-HSCT social, emotional, behavioral, and cognitive functioning; and low resilience appear to be at risk for lower QoL and poorer adjustment during and after transplant [19, 20, 29]. Poor medical outcomes post-HSCT (e.g., chronic GVHD, high levels of functional impairment) increase patients' risk for psychological distress [29]. Additional risk factors include high levels of caregiver stress, maternal anxiety and depressive symptoms at the time of transplant, and additional family stressors such as financial difficulties and lack of transportation [19]. Factors contributing to positive adjustment and improved QoL include family cohesion [18], family connectedness, and greater caregiver social support [19].

Aspects of psychosocial functioning such as anxiety, depressive symptoms, behavior problems, and posttraumatic stress are salient to assess in initial meetings about HSCT, as pre-HSCT mood and behavioral functioning are likely strong predictors of posttransplant functioning [20]. Numerous assessments are available for evaluating these symptoms and risks. Interested readers are referred to the *Journal of Pediatric Psychology's* special issue on evidence-based assessment in pediatric psychology [30], which provides a systematic, domain-specific review of measures of psychosocial adjustment and psychopathology, health-related QoL, and coping and stress [31–33]. Most of these domains can be assessed using well-established

self- or caregiver-report questionnaires that possess excellent psychometric properties; many have been previously used with patients with SCD. Moreover, some of these questionnaires have normative samples that allow for comparison to healthy populations or relevant disease groups to determine the degree to which symptom reports are abnormal and necessitate intervention.

Beyond measuring patients' mood and behavior concerns routinely, beginning prior to HSCT, research highlights the importance of considering familial and social risk factors for the worsening of psychological symptoms after HSCT [18–20]. Family functioning and relationships should be assessed (see [34] for a review of well-established measures), as these characteristics represent important risk and resilience factors associated with psychosocial outcomes in children undergoing HSCT. In addition, a wealth of literature documents the relationship between parent mental health and the psychological functioning of their children [35–37]. Therefore, assessing the psychological well-being of parents and caregivers (e.g., depressive symptoms, anxiety, stress, family history of mental illness and treatment) represents a prime opportunity to gather information about the possible trajectory of a patient's mental and behavioral health following HSCT. Mental and behavioral health screening surveys for adults are publicly available through various sources, including the Substance Abuse and Mental Health Services Administration (http://integration.samhsa.gov/clinical-practice/screening-tools). Rather than assessing each of these domains independently, some have advocated for the use of composite risk assessment measures. One such example with a strong evidence base is the Psychosocial Assessment Tool 2.0 [38], a caregiver-reported questionnaire that assesses a broad range of risk factors, takes approximately 10 min to complete, and provides specific recommendations regarding the degree of psychosocial intervention required to support the family. This measure has been used in families of children with SCD [39] and those undergoing solid organ transplant [40], among other pediatric populations.

Conclusion

Although we are still learning about the unique needs of patients with SCD and their families in the context of HSCT, available evidence suggests that we have to be thoughtful and deliberate in our approach to preparing patients and families for HSCT in order to optimize both medical and psychological outcomes. Specifically, providers must take care when educating patients and families about HSCT for SCD by: (1) fostering a trusting relationship, (2) using developmentally and culturally appropriate language, (3) encouraging patients and family members to actively engage in the discussion about HSCT, and (4) tactfully assessing for gaps in families' understanding of HSCT so that additional information can be provided. Further research is greatly needed to better understand psychological outcomes of patients with SCD who have undergone HSCT, but related literature indicates that patients tend to adjust well following HSCT. A subset of patients, however, will experience

social or academic problems, and some may exhibit symptoms of depression, anxiety, or posttraumatic stress that should be monitored and treated. Fortunately, evidence-based tools are available to assess known risks for adjustment problems before HSCT and can be used to monitor mood and behavior in a systematic way over the course of treatment. Furthermore, institutions offering HSCT for patients with SCD should establish multidisciplinary teams to support families before, during, and after transplant and continue to advance research describing risks for psychosocial maladjustment and interventions to promote positive outcomes.

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Chapter 11
Long-Term Effects of Hematopoietic Stem Cell Transplantation for Sickle Cell Disease

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Abbreviation

AML	Acute myelogenous leukemia
CHRIs	Child health ratings inventories
CNS	Central nervous system
EFS	Event-free survival
GVHD	Graft-versus-host disease
HRQoL	Health-related quality of life
HSCT	Hematopoietic stem cell transplant
IST	Immunosuppressive therapy
LIC	Liver iron content
OS	Overall survival
SCD	Sickle cell disease
SMN	Second malignant neoplasms
TBI	Total body irradiation
TRM	Therapy-related myelodysplasia
TRMo	Treatment-related mortality

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Introduction

The success of hematopoietic stem cell transplant (HSCT) in patients with sickle cell disease (SCD) with matched sibling donors has improved over the past two decades with event-free survival rates approaching 95%. However, pre-existing conditions and the preparative regimen itself can put patients at high risk for HSCT-related morbidity and mortality. Careful attention must be paid when considering these patients for HSCT. Iron overload, cardiac dysfunction, renal injury, pulmonary insufficiency, and baseline psychosocial functioning are all crucial elements that can determine the success of HSCT. In this chapter, we discuss the impact of the pretransplant health of the patient prior to undergoing HSCT, the influence of the conditioning on organ function, some of the long-term effects encountered post HSCT such as graft-versus-host disease and infertility, as well as the effect on the patient's health-related quality of life. Lastly, recommendations post HSCT are included in this review to help guide providers on the best way to care and monitor their patients post HSCT.

Impact of Pre-existing Conditions on Post-HSCT Care

After HSCT for SCD, long-term follow-up care must also include management of pre-existing conditions from prior to transplant, particularly those specific to SCD. These may include the sequelae of iron overload from repeated red blood cell transfusions, which will be reviewed later in this chapter. Chronic pain is also a common adverse effect in SCD that can persist through the peri- and posttransplant period [1–3]. Great advances have been made in understanding the etiology of chronic pain in SCD, and subsequently age-appropriate expertise in pain management has provided hematologists with a valuable partner in its evaluation and treatment [4, 5]. When available, the expertise of multidisciplinary pediatric-trained pain specialists can help minimize the impact of the many comorbidities of chronic pain, especially those that are related to mental health (depression, anxiety, and addiction or chemical dependency) [6–8]. Therefore, if a transplant candidate or eventual participant has been diagnosed with chronic pain, it is advisable to continue specialized multidisciplinary pain management prior to, during, and after the HSCT. Other sites of pre-existing organ dysfunction may include the central nervous system, eyes, lungs, and kidneys (Table 11.1). The corresponding specialists delivering care prior to HSCT should remain a part of the survivor's long-term follow-up care post HSCT.

Table 11.1 Pre-existing organ dysfunction seen in sickle cell patients prior to HSCT and the corresponding subspecialties that may be best suited to deliver care specific to the condition

Sickle cell-related pre-existing conditions	Corresponding subspecialties delivering care for this condition
Transfusion-related hemochromatosis	Hematology
Chronic pain	Pain/anesthesiology/psychology
Avascular necrosis	Orthopedics/physical medicine and rehabilitation
Pulmonary hypertension	Cardiology/pulmonology
Chronic kidney disease	Nephrology
Proliferative sickle retinopathy	Ophthalmology
Priapism	Urology
Stroke	Neurology/neurosurgery/hematology/transfusion medicine

Iron Overload

Red blood cell transfusion is an integral part of therapy for patients with SCD and inevitably leads to iron accumulation (see Chap. 5). With use of transfusions to prevent and treat SCD-related complications, there are an increasing proportion of patients at risk for iron overload. Several factors affect the rate and extent of iron accumulation in SCD including age at the start of transfusions, the type of transfusion (simple vs. manual exchange vs. automated exchange), and the target hemoglobin S level [9, 10]. After a successful HSCT, there is presumably no additional iron loading and fewer reactive iron species are generated, but the persistence of tissue iron overload can influence long-term HSCT risks [11].

Excess iron affects many different organ systems, including the liver, heart, and endocrine system. When not addressed, hepatic iron overload can lead to fibrosis and cirrhosis though the frequency of this complication is unknown in SCD patients. Compared to thalassemia major and Diamond-Blackfan anemia, extrahepatic iron deposition is relatively uncommon in SCD [9, 12].

Despite this, it is important to monitor for cardiomyopathy, hypothyroidism, gonadal dysfunction, growth delay, and bone health in SCD patients who receive transfusions. Importantly for SCD patients who undergo HSCT, toxicities related to the preparative regimen have the potential to exacerbate the effect of iron on all organ systems after HSCT.

Serum ferritin is a gross measure of iron burden but is not precise and needs to be used in conjunction with more objective measures of liver iron content (LIC) including MRI and liver biopsy. Indications for chelation in SCD have been based on those used for thalassemia major and include a serum ferritin >1000 μg/L on two separate measurements, LIC >7 mg/g dry weight or cumulative transfusions of

>120 mL of packed red blood cells/kg [9]. After successful HSCT, phlebotomy is preferred over chelators to treat iron overload because drug toxicity and interactions can be avoided with phlebotomy. Given the nature of SCD and the use of transfusions before HSCT, post-HSCT monitoring of iron burden and early therapeutic intervention when needed are a particularly important part of care for SCD patients who have undergone HSCT.

Effects of the Conditioning Regimen on Organ Function

Organ dysfunction specific to the HSCT itself is most directly related to the conditioning regimens used for transplant preparation and their respective intensity. A fully myeloablative preparative regimen typically includes a backbone of full-dose busulfan (12–16 mg/kg with a steady-state concentration of 600–700 ng/mL) and cyclophosphamide (200 mg/kg). Reduced-intensity and toxicity preparative regimens may include a combination of drugs such as alemtuzumab, anti-thymocyte globulin, busulfan, cyclophosphamide, fludarabine (140 mg/m^2), melphalan, and total body irradiation. Exact drug combinations and doses are specific to the ablative potential desired by the treating physician [13]. In these settings, cumulative cyclophosphamide and busulfan doses are typically lower than in myeloablative regimens and total body irradiation may be as low as 300 cGy as a single dose.

The greatest potential for long-term complications is seen after exposure to radiation and the alkylating agents used in fully myeloablative preparative regimens (cyclophosphamide and busulfan). The true long-term late effects of agents such as alemtuzumab, fludarabine, and anti-thymocyte globulin are still being described and not well characterized as of yet, but early evaluations of late effects offer promising hope for less long-term toxicity after reduced-intensity condition regimens and subsequently such regimens are becoming more common [14–18].

Cyclophosphamide (alkylating agent) may cause dysfunction across several organ systems [19–21]. Potential testicular dysfunction has been characterized as infertility from oligospermia or azoospermia, testosterone deficiency or insufficiency, and delayed or arrested puberty. Ovarian dysfunction may include delayed or arrested puberty, premature menopause, and infertility. Second malignant neoplasms (SMN) of the blood such as acute myelogenous leukemia (AML) and therapy-related myelodysplasia (TRM) are a well-described late effect of alkylators. Cyclophosphamide also may cause urinary tract toxicity such as hemorrhagic cystitis, bladder fibrosis, dysfunctional voiding, vesicoureteral reflux, and hydronephrosis. It has also been associated with late bladder malignancy. When combined with exposure to anthracyclines, there is also concern for heart dysfunction (cardiomyopathy).

Busulfan and melphalan are also both an alkylating agents and have the potential to affect many of the same organ systems as cyclophosphamide resulting in similar testicular and ovarian dysfunction [19–21]. There is also a similar risk for

SMN of the blood such as AML or TRM. Unique risks after busulfan exposure include the potential for lung and eye dysfunction with pulmonary fibrosis and cataracts, respectively. There may be a relationship between the pulmonary late effects and related cardiac sequelae as well as busulfan-related thyroid dysfunction (hypothyroidism) [22].

Total body irradiation (TBI) also has the potential to negatively affect several organ systems [19–21]. This association has historically been identified with higher cumulative doses of TBI than the 200–400 cGy dose that is typically used prior to HSCT for SCD. As more experience is gained using this lower dose of TBI, additional data will be generated to determine the true risks of TBI prior to HSCT for SCD patients. General risks associated with TBI at doses typically higher than 600 cGy are discussed here. Like alkylators, there is a risk for the previously described testicular and ovarian dysfunction and infertility. There is also additional risk for hormonal dysfunction such as growth hormone deficiency, thyroid dysfunction, and growth stunting. The SMN risk is different than alkylators as TBI exposure has been associated with a risk for breast, colorectal, thyroid, and central nervous system (CNS) malignancies. Additional risk for CNS late effects includes neurocognitive deficits and clinical leukoencephalopathy such as spasticity, ataxia, dysarthria, dysphagia, hemiparesis, and seizures. This is of particular importance to sickle cell patients who often have pre-existing conditions (such as overt stroke, silent cerebral infarction, or moyamoya disease) that impact CNS function. There may also be significant risk to oral health via dental abnormalities (tooth/root agenesis, microdontia, root thinning/shortening, enamel dysplasia, periodontal disease, dental caries, malocclusion, temporomandibular joint dysfunction) or xerostomia. Risk for lung dysfunction may exist including pulmonary fibrosis, restrictive/obstructive lung diseases, or interstitial pneumonitis. The potential cardiac dysfunction after TBI is broad: congestive heart failure, cardiomyopathy, pericarditis, fibrosis, valvular disease, myocardial infarction, arrhythmia, or atherosclerotic heart disease. These may be exacerbated by additional potential late effects such as dyslipidemia, renal dysfunction (hypertension and renal insufficiency), impaired glycemic control (diabetes mellitus), and veno-occlusive disease of the liver. TBI has also been associated with risk for the development of scoliosis and neuropathy as well as uterine vascular insufficiency that may result in adverse pregnancy outcomes.

Alemtuzumab and anti-thymocyte globulin have not been clearly associated with new-onset long-term treatment-related organ dysfunction [19]. The body of literature describing potential late effects will grow as reduced-intensity conditioning regimens are being used more frequently and survivors are living longer post HSCT. This will allow for additional follow-up that is both long-term and objective. Fludarabine is a purine analog antimetabolite but also does not have clearly associated risks for long-term complications [14, 17, 18]. Its acute and short-term side effects' profile is better understood and most notable for severe CNS toxicity (progressive multifocal leukoencephalopathy), eye dysfunction, and peripheral neuropathy. All of these, if severe enough, could result in chronic conditions but would be unlikely to occur as a new-onset late effect [23, 24].

Graft-Versus-Host Disease

While overall and event-free survivals after HSCT for SCD are improving, the incidence of both acute and chronic graft-versus-host disease (GVHD) remains a deterrent. In the myeloablative (MA) setting, various studies evaluating sibling donor HSCT have identified median rates of grades II–IV acute GVHD as 14.8% (range 0–20) and of chronic GHVD as 14.3% (range 0–22) [25–32]. More concerning is the TRM directly attributable to GVHD. Bernaudin et al. reported GVHD as the main cause of TRM in their cohort of 87 patients with four deaths due to GVHD [27]. In a retrospective study conducted by Gluckman, 1000 patients who had undergone HLA-identical sibling HSCT, the mortality rate was 7% (n = 70), with nine deaths directly attributable to GVHD [30]. Certain risk factors such as stem cell source (higher incidence in bone marrow recipients), donor or recipient age (those greater than 15 years of age), or male recipients with female donors have been seen in select studies; however, these are not consistent observations [27, 29]. Furthermore, the prolonged immunosuppressive therapy (IST) required to treat GHVD has been estimated at a median duration of 11 months (range 10–12) in those with acute GHVD and 12 months (6–16 months) in those with chronic GHVD [28]. Additional IST also has downstream effects such as increased susceptibility to infections and delayed immune reconstitution and return to activities such as work and school.

The use of lower-intensity conditioning regimens in HSCT is being studied with more interest in part to decrease the long-term effects often seen with myeloablative regimens. Krishnamurti et al. evaluated the efficacy of a reduced-intensity regimen in seven patients with matched sibling donor HSCT and found the overall (OS) and event-free (EFS) survivals to be 100% and 86%, respectively. The incidence of GVHD was quite encouraging as no patient developed greater than grade II acute GVHD or extensive chronic GVHD [33]. However, King et al. recently reported the results of a reduced-intensity conditioning regimen in 43 patients with SCD undergoing sibling HSCT. While the OS and EFS was quite encouraging and similar to that seen in myeloablative transplants, the incidence of acute GVHD (23%) and chronic GVHD (13.4%) was also comparable. Interestingly, all recipients who developed chronic GVHD were >14 years of age and three deaths were observed in this cohort—all related to GHVD [34].

To increase the pool of available donors, the use of alternate donors such as adult unrelated donors and haploidentical donors is being evaluated in clinical trials. In a pivotal trial by Shenoy et al., 29 children with SCD underwent a reduced-intensity conditioning regimen followed by HLA-matched unrelated donor HSCT. The rate of grades II–IV acute GVHD was 28% with a 1 year incidence of chronic GVHD of 62%, 38% of which was classified as extensive [35]. While the 1-year EFS did meet the prespecified target, the authors felt the morbidity associated with the regimen did not allow it to be sufficiently safe for widespread adoption.

In the haploidentical setting, GHVD rates have been variable. Dallas et al., using a CD34+ selected approach, studied eight patients with SCD, five of whom

engrafted. Of the five patients, four had acute GVHD and three of those four went on to develop chronic GVHD. Two of those three patients died from complications from GVHD [36]. Conversely, in 14 patients undergoing haploidentical HSCT using posttransplant cyclophosphamide, no patient developed any GVHD [37].

Given the morbidity and mortality seen with GVHD, more attention must be given to optimizing regimens and donor sources to promote widespread application of HSCT to those with SCD.

Gonadal Function and Fertility

Gonadal dysfunction occurs frequently in long-term survivors of HSCT [38–40]. Confounding matters, patients with SCD often have baseline issues with gonadal function related to their disease and chronic medical therapies they receive, including transfusions and hydroxyurea. Iron deposition and iron-induced oxidative stress can lead to hypothalamic-pituitary-gonadal dysfunction (most commonly hypogonadotropic hypogonadism) in those who are chronically transfused. Other potential contributing factors in men with SCD include priapism, testicular ischemia/infarction, and the use of hydroxyurea [41]. In patients with SCD, the adverse effects of iron overload and gonadotoxic therapy might be amplified by exposures in combination over a lifetime. Gonadal damage may manifest in several ways including delayed or arrested puberty, postpubertal gonadal insufficiency, and impaired fertility. The risk of gonadal dysfunction following HSCT depends on many factors including gender, the stage of puberty at the time of conditioning, the choice and dose of chemotherapy agents, and the use of TBI.

Females have a finite reserve of ovarian follicles, making them particularly sensitive to gonadotoxic therapy. Overall, ovarian failure is seen in 65–84% of HSCT recipients [38, 42]. Older age, myeloablative conditioning (high-dose Cytoxan, busulfan, or TBI), and pubertal status at the time of exposure increase the risk of ovarian dysfunction [38]. A significant percentage of those receiving myeloablative conditioning have primary amenorrhea and elevated gonadotropins [36, 43]. In males, spermatogonial germ cells are more sensitive to gonadotoxic therapy than testosterone producing Leydig cells so azoospermia or oligospermia are most common [38]. As in females, the risk of gonadal dysfunction is dependent on the type and intensity of the conditioning. TBI plays a central role in gonadal function in men, whereas chemotherapy-only regimens appear to have less of an impact (busulfan/Cytoxan carries a 50% and Cytoxan alone carries a 10% risk of dysfunction) [44]. Newer reduced-intensity conditioning regimens have the potential to have less of an impact on gonadal function [14, 45].

While infertility is not universal following HSCT for SCD, it remains a significant risk and an important issue to consider in the pretransplant phase. An individualized discussion about the risks of gonadal dysfunction/infertility and potential options for fertility preservation should take place in the pre-HSCT period so that

patients and their families can make informed decisions. Fertility preservation options vary based on gender and stage of puberty at the time of HSCT. Standard of care options include gonadal shielding in those receiving TBI, sperm cryopreservation, hormonal suppression, and embryo preservation. Experimental options for prepubertal children include oocyte cryopreservation and testicular tissue and ovarian tissue cryopreservation [46–50]. Successful oocyte preservation has recently been documented in a woman with SCD who underwent a matched sibling donor HSCT [51]. Additionally, live births have been reported with ovarian tissue cryopreservation and re-implantation after HSCT, including in a patient with SCD [52]. Importantly though when considering any of these options, financial implications need to be considered as many insurance companies will not cover fertility preservation procedures.

Health-Related Quality of Life

Health-related quality of life (HRQoL) is a patient's appraisal of how his or her well-being and level of functioning, compared to the perceived ideal, are affected by individual health. These assessments typically include physical, social, emotional, and school/work functioning. HRQoL can also be used to measure efficacy and effectiveness of treatment interventions, to predict outcomes and resource use, and to direct therapy. It can also help caregivers to understand the burden of disease that a patient experiences.

Previous studies in children with chronic diseases have shown that to get the most accurate assessments of HRQoL, both parent and child reports are necessary. Typically a parent's report of physical health equals that of a child's self-report, presumably, because physical health involves factors that can visibly be seen. Conversely, parent reports often differ from child reports of psychosocial health. The impact on HRQoL in patients with SCD has been well described. Using the CHQ-Child Form 87 and CHQ-Parent Form 50, 95 parents and 53 children were evaluated. Compared with the child report, parents reported worse HRQoL in the overall perception of health, physical functioning, behavior, and self-esteem ($p < 0.005$). Parent and child reports of HRQoL did strongly correlate in the assessment of the impact of bodily pain and moderately in physical functioning, behavior, general health, self-esteem, and changes in health domains [53]. In another study, 58 children with SCD were compared to 120 healthy children. Only parent reports were obtained using the CHQ-Parent Form 50. Caregivers reported children had more limited physical, psychological, and social well-being than healthy children. Older age of the child, female gender, and more disease-related complications predicted limitations in the physical health of children with SCD [54].

As more patients pursue curative options for hemoglobinopathies, the effects of these interventions will also need to be evaluated in the context of patient-reported outcomes. Kelly et al. serially evaluated the HRQoL of 13 children with hemoglobinopathies who received HSCT using the Child Health Ratings Inventories

(CHRIs). The CHRIs measure three general health status domains—physical, role, and emotional. These patients were compared to a group of children receiving HSCT for malignancies or severe aplastic anemia. There were similar rates of early infection, chronic GVHD, and all-cause mortality between the two groups. Children with hemoglobinopathies had higher HRQoL scores for physical (p = 0.01) and baseline emotional functioning (p = 0.03) than the comparison group. For all domains for both groups, parent reports demonstrated a nadir at 45 days with recovery to baseline by 3 months following transplant [55]. In a report by Bhatia et al., 17 patients with SCD undergoing HSCT and 23 caregivers were evaluated using the PedsQL™4.0 pre-HSCT, 6 months and 12 months post HSCT. At baseline, patients and primary caregivers reported a mean overall HRQoL of 66.05 (SD, 15.62) and 72.20 (SD, 15.50), respectively. In the patient-reported analysis, the estimated improvements in overall HRQoL were 4.45 (SE, 4.98; p = 0.380) and 16.58 (SE, 5.06; p = 0.003) at 180 and 365 days, respectively, after HSCT. For parent-reported overall HRQoL, the estimated improvements were 1.57 (SE, 4.82; p = 0.747) and 9.28 (SE, 4.62; p = 0.053) at 180 and 365 days, respectively, after HSCT. These results demonstrated a continued trajectory in HRQoL improvement for those undergoing HSCT [56]. Similar results were also seen in the recent study by Shenoy et al. evaluating matched unrelated donor HSCT in patients with symptomatic SCD [35]. Thirteen children and 21 caregivers were serially administered the CHQ-Child Form 87 and the CHQ-Parent Form 50, respectively, pre-HSCT and 100 days, 6 months and 12 months post HSCT. At 100 days, parents reported significantly worse self-esteem HRQoL scores but better general health perception. By 6 and 12 months post HSCT, children and parents reported significantly improved change in health scores. These improvements can also be seen in the adult patients undergoing HSCT for SCD. Thirteen patients undergoing a non-myeloablative HSCT for SCD had HRQoL assessments pre-HSCT and 1 year post HSCT using the SF36. At 1 year post HSCT, a significant improvement was seen in all HRQoL parameters including bodily pain, general health, and vitality [57].

Long-Term Follow-Up Screening Recommendations

Screening recommendations for the above potential late effects can be found in published reports created by various consortiums, professional societies, expert panels, and collaborative groups [19–21, 58]. In patients with SCD treated with a HSCT, it is critical to use not only these guidelines but also consider pre-existing comorbidities that must be followed longitudinally.

For example, using the consensus panel expert recommendations in selected areas from Pulsipher et al., a patient with SCD in a long-term follow-up clinic after HSCT with a myeloablative preparative regimen would receive the surveillance and screening outlined in Table 11.2 [19]. These recommendations would be, in addition to the lifelong follow-up care, necessary for the SCD-specific pre-existing comorbidities.

Table 11.2 Recommendations for follow-up in selected[a] areas from consensus panel experts for a patient with SCD after HSCT with a myeloablative preparative regimen [19]

Organ dysfunction	Screening recommendation
Iron overload	– Annual serum ferritin
Gastrointestinal	– Hepatitis virus infection screening – Annual hepatocellular carcinoma screening for high-risk patients (hepatitis C/B infection, obesity/diabetes, low platelet count)
Renal	– Annual urine albumin:creatinine ratio
Pulmonary	– Annual pulmonary function tests based on symptoms and past results/measurements
Cardiac	– Annual cardiovascular risk assessment – Annual blood pressure evaluation – Electrocardiogram/echocardiogram at least every 5 years, more frequently if exposure to anthracyclines, total body irradiation (TBI), or chest irradiation
Metabolic	– Lipid profile and fasting glucose at least every 5 years
Thyroid	– Annual physical exam (after TBI) – Annual thyroid-stimulating hormone and free thyroxine for 10 years after busulfan and at least 30 years after TBI
Growth	– Annual accurate measurement of growth through adolescence – Bone age as needed
Bone	– Dual-energy X-ray absorptiometry scan yearly if previous scans had z-score < -1 to assess bone mineral density – Early MRI screening for joint/groin pain or limping to assess for osteonecrosis
Reproductive system	– Women: monitor for ovarian failure with FSH screen and assessing menstrual cycle history – Men: semen analysis

[a]Pulsipher et al. [19] highlight that their recommended follow-up is not intended to be exhaustive, and additional areas should be included in comprehensive follow-up care such as screening for second malignancies, neurocognitive, functional, and quality of life effects

Another example of proposed follow-up care for a similar patient treated with busulfan and cyclophosphamide is summarized in Table 11.3 using the Long-Term Follow-Up Guidelines from the Children's Oncology Group [21]. These guidelines include specific questions to include when obtaining a routine history and specific findings to evaluate during a physical exam depending on the agent the survivor was exposed to during his preparatory regimen.

Ensuring longitudinal care across the lifespan is an emerging issue in long-term follow-up care for patients with SCD. Since almost all children with SCD now live beyond 18 years of age, a growing body of literature has developed examining the optimal approach to support the continuation of SCD-focused care for pediatric patients as they transition to young adult ages [59–70]. This transitional period is critical in SCD as patients have increased risk of health complications, hospitalizations, and death [71–75].

Introducing HSCT into the past medical history of children with SCD adds additional concerns when considering the transition of care to adult-centered providers.

Table 11.3 Recommendations for screening during periodic evaluations of a patient transplanted for sickle cell disease exposed to a myeloablative preparative regimen [21]

Organ dysfunction	Screening recommendation
Hepatic	– Baseline AST, ALT, bilirubin, and ferritin and then as clinically indicated
Bone	– Baseline dual-energy X-ray absorptiometry scan and then as clinically indicated – MRI screening as clinically indicated to assess for osteonecrosis
Renal	– Baseline BUN, creatinine, and electrolyte evaluation including magnesium and phosphorus and then as clinical indicated – Annual urinalysis
Pulmonary	– Baseline pulmonary function tests and then as clinically indicated
Reproductive system	– Women: monitor for ovarian failure with FSH screen and assessing menstrual cycle history – Men: semen analysis (FSH screen if sexually mature and unable to perform semen analysis)
Hormonal system	– Women: baseline FSH, LH, and estradiol at age 13 and then as clinically indicated – Men: baseline testosterone level at age 14 and then as clinically indicated
Dental	– Dental exam and cleaning every 6 months

Following the model of long-term follow-up care in childhood cancer, which also often includes survivors with a history of HSCT, there is clear evidence suggesting a need for improved models of care to ensure the continuation of survivor-focused care along the age continuum [76–78]. More than 60% of survivors will have a therapy-related late effect, and almost 30% will have one that is life threatening [76]. However, as young adults, only 19.2% report a visit at a cancer center where presumably long-term follow-up or HSCT programs are housed [77]. In addition, only 17.5% of survivors report receiving survivor-focused care that includes advice about risk reduction or discussion or ordering of screening tests [78]. As more children with SCD are cured with HSCT, long-term follow-up programs will begin to care for more of these patients, and in addition to providing risk-based post-HSCT care, providers will have to create models of care that both promote and ensure the continuation of survivor-focused care for children with SCD cured with HSCT.

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Chapter 12
Matched Sibling Donor Hematopoietic Stem Cell Transplantation for Sickle Cell Disease

Gregory M.T. Guilcher and John T. Horan

Abbreviations

AML	Acute myelogenous leukemia
ATG	Antithymocyte globulin
AUC	Area under the curve
EFS	Event-free survival
GVHD	Graft-versus-host disease
HLA	Human leukocyte antigen
HSCT	Hematopoietic stem cell transplant
HU	Hydroxyurea
MDC	Mixed donor chimerism
MRD	Matched-related donor
MRI	Magnetic resonance imaging
PK	Pharmacokinetics
rATG	Rabbit antithymocyte globulin
SCD	Sickle cell disease
SOS	Sinusoidal obstruction syndrome
STAR	Sickle Transplant Alliance for Research
TBI	Total body irradiation
UCB	Umbilical cord blood

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Early Experience with Matched Sibling Donor Hematopoietic Stem Cell Transplantation for Sickle Cell Disease

The first hematopoietic stem cell transplant (HSCT) for sickle cell disease (SCD) was reported in 1984 [1, 2]. An 8-year-old girl with acute myelogenous leukemia (AML) and known HbSS disease received a bone marrow allograft from her 4-year-old brother, who had sickle cell trait (HbAS). The indication for HSCT in this case was AML; she was transplanted in first complete remission as part of a St. Jude Children's Research Hospital clinical trial with myeloablative conditioning prescribed according to the AML study. This patient engrafted on day +12 and did not suffer any further SCD crises. She was successfully treated for acute and chronic graft-versus-host disease (GVHD) as well as *Streptococcal* pneumoniae sepsis. This case served as proof of principle that SCD could be cured with HSCT and that a donor with HbAS is acceptable, provided some clues about the unique supportive care needs of HSCT recipients with sickling syndromes [2].

The first cohort of children and young adults to undergo HSCT specifically for SCD was described by Vermylen et al. in Brussels, Belgium [3]. The 12 patients were returning to Africa, where mortality rates can exceed 50% for children under 5 years of age depending on access to care [4]. It should be noted that some of these children had mild phenotypes; the risks and uncertain efficacy of HSCT in SCD were justified by the authors due to the significant risks of morbidity and mortality of SCD in lower income countries [3]. Myeloablative conditioning consisting of oral busulfan, intravenous cyclophosphamide, and 750 cGy of thoracoabdominal radiation (for recipients over 12 years of age) was given; donors were mostly human leukocyte antigen (HLA)-matched siblings. Four donors had normal hemoglobin electrophoresis analyses, while the remaining eight had sickle cell trait. Eleven of these patients were cured of their SCD, while the remaining patient sustained secondary graft failure (i.e., initial donor hematopoietic engraftment with subsequent graft loss). This child underwent a second HSCT and was also cured. No sickling crises were noted post-HSCT, and hemoglobin electrophoresis levels reflected those of the donors. Only four patients developed grades I–II acute GVHD. The long-term follow-up of this cohort revealed no adverse outcomes secondary to HSCT.

A larger combined experience describing outcomes of 42 young patients in France and Belgium demonstrated sustained donor hematopoietic stem cell engraftment in 36; all those with sustained engraftment were free of ongoing crises due to SCD and achieved donor hemoglobin electrophoresis patterns [5]. All donors but one were matched siblings. One patient died; five had graft rejection, and of these, three had autologous marrow recovery and the other two had successful second HSCTs. The summarized Belgian experience of 50 patients demonstrated overall, event, and disease-free survival rates of 93%, 82%, and 85%, respectively, with the subgroup with milder phenotypes who were returning to Africa faring somewhat better [6].

These encouraging observations led to the era of multicenter clinical trials for matched sibling donor HSCT for SCD from the late 1980s to the present.

Clinical Trials Evaluating Myeloablative Conditioning Regimens for Matched Sibling Donor HSCT for Sickle Cell Disease

Multicenter investigation of hematopoietic stem cell transplantation for sickle cell disease. A landmark collaboration between HSCT centers in Europe, the United States, and Canada provided essential data published in several manuscripts which serves as the foundation of the practice of HSCT for SCD [7–10]. This was a prospective, nonrandomized clinical trial which included a myeloablative conditioning regimen with busulfan (14 mg/kg total over 4 days), cyclophosphamide (200 mg total over 4 days), and serotherapy with either horse antithymocyte globulin (majority) or alemtuzumab. GVHD prophylaxis consisted of methotrexate and cyclosporine. Donors were HLA-identical siblings with HbAA or HbAS.

Eligibility criteria. Patients less than 16 years of age with HbSS, SC, and Sβ thalassemia were considered. Strict inclusion and exclusion criteria were developed at an expert consensus meeting in Seattle in 1990; these criteria have since been used to determine HSCT eligibility for several subsequent clinical trials, as well as for both referring hematologists and consulting HSCT physicians in deciding which children and adolescents should be offered HSCT as part of routine care (see Sect. "Whom Should Be Offered Matched-Related Donor Transplantation?"). The data from this trial are presented here; it should be noted that these results have expanded the practice of HSCT for SCD worldwide.

Results. The outcomes for the first 22 subjects were reported in 1996 in the New England Journal of Medicine. With a median follow-up of 24 months, 20 subjects were alive with 16 demonstrating stable donor hematopoietic cell engraftment [7]. Three of the four with failed engraftment had autologous reconstitution; one subject had marrow aplasia. Stable mixed donor chimerisms (MDCs) were noted in one of the 16 survivors. Notably, stability of preexisting cerebrovascular and pulmonary disease was noted.

Updates on this cohort were published in 1997, 2000, and 2001 [8–10]. The most recent publication reports data for 59 subjects with a median follow-up of 42 months [10]. There are 55 survivors, with 50 cured of their SCD. Thirteen of the 50 children and adolescents cured of their sickle phenotype have stable MDCs (90–99% donor), while five had lower levels of MDCs ranging from 11% to 74%. Interestingly, four of these five recipients with low donor chimerisms had HbS levels reflective of their donor, while the subject with only 11% donor chimerisms had only 7% HbS with a donor with a normal hemoglobin phenotype. These important data support the investigation of reduced intensity approaches to HSCT, proving that full donor chimerisms are not required to cure SCD and that even 10% stable MDCs can be sufficient (see Sect. "Clinical Trials Evaluating Reduced-Intensity and Non-myeloablative Conditioning Regimens for Matched Sibling Donor HSCT for Sickle Cell Disease").

None of the 50 subjects who maintained donor engraftment have had SCD-related crises post-HSCT. In ten subjects with a history of stroke and stable donor engraftment with at least 2 years of follow-up, cerebral magnetic resonance imaging

(MRI) was improved or stable post-HSCT [10]. Of the 59 total subjects, 11 (19%) developed GVHD with three deaths due to complications of its therapy. No subject with stable MDCs experienced acute or chronic GVHD. Of the 26 subjects with follow-up of 2 years or longer, three developed grades I–III acute GVHD and two had chronic GVHD.

Supportive care lessons learned. While supportive care is discussed in detail in Chap. 8, it is important to emphasize that four of the first seven subjects had neurologic events, two of whom had intracranial hemorrhages (one fatal) [7]. The risk of intracranial hemorrhage, seizures, and posterior-reversible encephalopathy syndrome led to the development of updated supportive care guidelines in 1993. These included anticonvulsant prophylaxis during busulfan *and* for the duration of cyclosporine administration, maintenance of normal magnesium levels, maintenance of platelet counts greater than 50×10^9/L and Hb between 90 and 110 g/L, and control of hypertension [7, 10].

Other clinical trials of MRD HSCT for SCD. Data from single-institution experiences or registry-based reports complete the summary of existing literature with myeloablative HSCT for SCD. Many of these publications are listed in Table 12.1.

Table 12.1 Myeloablative hematopoietic stem cell transplantation for sickle cell disease

Authors	*N*	Median age (years)	Conditioning	OS	Graft rejection	DFS	cGVHD	TRM
Walters et al. (2000) [10]	50	9.4	Bu-Cy-ATG	94%	10%	84% at 3 years	12%	6%
Bernaudin et al. (2010) [46]	144	9	Bu-Cy-ATG	95%	<2%	93% at 3 years	10%	7%
Dedeken et al. (2014) [12]	50	8.3	Bu-Cy-ATG	94%	8%	86% at 8 years	20%	<5%
Lucarelli et al. (2014) [47]	40	12	Bu-Cy-ATG ± Flu	91%	–	91% at 5 years	5%	9%
McPherson et al. (2011) [15]	27	8.6	Bu-Cy-ATG	96%	0%	96% at 5 years	<5%	<5%
Vermylen et al. (1998) [6]	50	–	Bu-Cy ± TLI ± ATG	93%	10%	85% at 11 years	20%	2%
Bhatia et al. (2014) [17]	18	8.9	Bu-Flu-Alemtuzumab	100%	0%	100% at 2 years	11%	0%

OS overall survival, *DFS* disease-free survival, *cGVHD* chronic graft-versus-host disease, *TRM* transplant-related mortality, *Bu* busulfan, *Cy* cyclophosphamide, *ATG* antithymocyte globulin, *Flu* fludarabine, *TLI* total lymphoid irradiation

Importance of Serotherapy

French experience with antithymocyte globulin. The Société Française de Greffe de Moelle established a consensus approach to HSCT in major transplant centers in France in 1988 [18]. All young patients with severe SCD were recommended HSCT. Severe SCD was initially defined as having a history of stroke, three or more vaso-occlusive and/or acute chest crises per year, multiple sites of avascular necrosis, or red cell alloimmunization (two or more alloantibodies). After 1992, incomplete disease control with hydroxyrurea (HU) was required to meet the vaso-occlusive/acute chest crises criteria. Later, patients with elevated transcranial Doppler velocities or MRI angiographic evidence of cerebral stenoses despite chronic transfusion were also eligible. Results for HSCT for 87 consecutive patients ages 2–22 from 1988 to 2004 who received an HLA-matched sibling donor allograft were described. Conditioning consisted of busulfan and cyclophosphamide, with antithymocyte globulin (ATG) given to patients after 1992 (69/87 received ATG). Rabbit ATG (Thymoglobuline; Genzyme, Saint-Germain en Laye, France) was given at a total dose of 20 mg/kg divided equally and given on days −6 to −3, inclusive. GVHD prophylaxis consisted of cyclosporine and methotrexate in 62 and methotrexate alone in 25. The 5-year cumulative incidence of rejection was 2.9% in those who received ATG serotherapy compared to 22.6% who did not ($p = 0.002$). An increased incidence of mixed but stable donor chimerisms was noted in the ATG group. Improved outcomes since the year 2000 were also reported; only 1/43 patients allografted with ATG after 2000 experienced graft rejection. The event-free survival was 95.3% after January 2000, and no deaths after the 40th HSCT were reported. Rates of acute and chronic GVHD were only reported for the entire cohort; 17/86 (20%) developed grades II–IV acute GVHD, with 5.8% and 2.3% having grades III and IV, respectively. The cumulative incidence of chronic GVHD was 12.6%. Of 83 evaluable patients, 11% had limited, and 2.4% had extensive chronic GVHD.

This experience has since been updated with a total of 215 patients who received either (1) no ATG, (2) 10–15 mg/kg, or (3) 20 mg/kg of rabbit ATG [19]. Similar to the earlier report, disease-free survival was 95% with ATG, with the higher dose reducing the risk of chronic GVHD without increasing the risk of viral infection.

These impressive results have led to consideration of HSCT as standard of care for those with a matched sibling donor. Such low rates of morbidity and mortality compare favorably with risks of death and life-threatening and life-limiting complications of SCD with best supportive care practices.

Alemtuzumab. A reduced-toxicity, busulfan-based myeloablative conditioning regimen using alemtuzumab serotherapy resulted in similar rates of acute GVHD (17%, 3/18) compared to ATG-based myeloablative approaches [17]. Alemtuzumab was given days −6 to −2 (total dose 54 mg/m^2). All subjects survived with long-term donor hematopoietic engraftment, with four cases of cytomegalovirus (CMV) reactivation—including one case of CMV disease—and

two cases of respiratory syncytial virus upper respiratory tract infections. The timing and dose of alemtuzumab can greatly impact engraftment, immune reconstitution, and risk of opportunistic viral and fungal infections. While alemtuzumab serotherapy is the focus of many research efforts as a component of reduced-intensity conditioning, the data describing this drug as a component of myeloablative regimens is limited. The impressive French data warrant consideration of ATG serotherapy as the standard when myeloablative conditioning is given.

Role of Pre-HSCT Immune Suppression

Hydroxyurea. HU is used to prevent complications of SCD and is currently routinely recommended for all children and adolescents with HbSS or HbSβ^0 thalassemia 9 months of age and older, regardless of clinical severity [20]. Despite its widespread use as an immunosuppressive agent pre-HSCT (in addition to SCD control), there is scant data to support the use of HU to promote engraftment. While there is theoretical benefit to reduce marrow hypercellularity and in the provision of some degree of immunosuppression pre-HSCT for SCD to promote donor hematopoietic cell engraftment, the data justifying this widespread practice is relatively weak.

The potential benefit of pre-HSCT HU exposure was first described by a Belgian group in 2004 [21]. Routine administration of HU to all children with SCD was not standard practice at the time pioneering HSCTs were performed in children in Belgium between 1988 and 2002. While numbers are small in this report, of five patients who received busulfan (16 mg/kg), cyclophosphamide (200 mg/kg), and ATG, two failed to engraft and a third required donor lymphocyte infusion and steroids for secondary graft rejection. In contrast, none of the 13 patients who received the same conditioning with pre-HSCT HU had failed engraftment or late graft rejection. HU dosing was 20–35 mg/kg/day for a median of 2.16 years pre-HSCT (minimum 0.6 years). It should be noted, however, that the group of five patients who did not receive HU had relatively higher rates of red blood cell alloimmunization, strokes, and acute chest syndrome pre-HSCT. The same group updated their experience in 2014 [12]. They report 97.4% event-free survival (EFS) and no episodes of graft failure since HU was introduced pre-HSCT for all patients; all patients received busulfan, cyclophosphamide, and ATG conditioning since 1991. It should be emphasized that this cohort includes patients transplanted between 1988 and 1991 without ATG, with the SFGM in France having published improved outcomes with ATG (introduced in 1992) and in those patients undergoing HSCT after 2000, thus emphasizing the influence of HSCT-era on outcomes [18].

The optimal duration of HU exposure pre-HSCT is also not clearly defined, and in many studies, its prescription is at the discretion of treating physicians as a disease-modifying therapy for SCD, as opposed to mandated immune suppression prior to transplant [18, 22–24].

Busulfan Dosing and Pharmacokinetics

The practice of busulfan dosing adjustments to achieve targeted area under the curve (AUC) values has become standard practice in most HSCT centers. Levels are targeted to maximize the donor hematopoietic stem cell engraftment while minimizing toxicities, notably sinusoidal obstructive syndrome (SOS), also known as veno-occlusive disease [25]. Busulfan is the myeloablative agent used most commonly in MSD allogeneic HSCT for SCD; targeted dosing has become standard practice whether the drug is administered every 6 h or once a day [26].

McPherson et al. described the Children's Healthcare of Atlanta experience with busulfan pharmacokinetics (PK) between 1993 and 2007 [15]. Of 27 patients to undergo myeloablative MSD HSCT for SCD, 25 had PK measurements. Dosing of busulfan was 0.875 mg/kg/dose for 16 doses, combined with cyclophosphamide and ATG. It should be noted that oral busulfan was administered during the initial study period to 17 subjects. The busulfan area under the curve (AUC) was then calculated after the first dose administered, with adjustments made to target AUCs which varied based on different time periods within the period of study: 585–877 μmol min/L (1996–1999), <1500 μmol min/L with no lower limit (1999–2004), and 900–1100 μmol min/L from 2004 onward. Any subsequent AUC measurements were at the discretion of the treating physician.

All patients had donor hematopoietic stem cell engraftment, with full donor chimerisms in 21/25 (84%). AUC was associated with donor chimerisms: AUC of 862 ± 73 μmol min/L for those patients with MDCs compared to 1018 ± 122 μmol min/L for recipients with full donor chimerisms. Eight patients developed SOS (32%); SOS was not associated with busulfan AUC levels. All but one patient is a long-term survivor.

A multicenter experience of myeloablative busulfan-based conditioning by Maheshwari et al. describes a cohort of 16 children and adolescents with SCD who underwent matched-sibling donor HSCT [25]. Conditioning consisted of 0.8–1 mg/kg/dose busulfan (age-based initial dosing) given every 6 h for 16 doses, combined with cyclophosphamide and equine ATG. The first-dose PK analysis was performed to target an AUC of 877–1023 μmol min (steady-state concentration of 600–700 ng/mL). Subsequent dose adjustments were made as required.

Dose adjustments were required for 14/16 (88%) of patients, emphasizing the importance of PK analysis. Nine patients required dose increases, five decreased dosing, and two had no change in dose. The median total dose of busulfan given was 14.7 mg/kg. No patient developed SOS; all had successful long-term engraftment (median 100%, range 80–100) with a 3-year SCD-free survival of 100%.

Myeloablative Reduced-Toxicity Approaches

Fludarabine. Fludarabine, an antimetabolite drug with immunosuppressive properties, can potentiate the effects of alkylating agents such as busulfan and cyclophosphamide when dosed and timed appropriately, yet is associated with lower rates of

toxicity when compared with cyclophosphamide [26]. When combined with busulfan, rates of SOS are lower when fludarabine replaces cyclophosphamide, perhaps due to glutathione-independent metabolism of fludarabine.

The immunosuppressive properties of fludarabine—with comparatively lower rates of toxicity—can be applied synergistically with other immunosuppressing medications and have prompted its study with an aim to reduce doses of alkylating agents [23]. A study of the addition of fludarabine to a myeloablative busulfan-based conditioning regimen with cyclophosphamide and horse ATG in MRD HSCT for SCD aimed to reduce dosing of both busulfan and cyclophosphamide, without compromising day +28 donor hematopoietic cell engraftment. Four dose levels were developed, with initial reduction in cyclophosphamide dosing followed by busulfan dose reductions. Cyclophosphamide dosing was reduced from 200 to 90 mg/kg without adverse impact on donor chimerisms. The trial was stopped when the first two patients to undergo busulfan dose reduction from 12.8 to 9.6 mg/kg had less than 50% donor-derived T-cell engraftment on day +28 post-HSCT (as per study design). All 14 subjects on the trial are alive without SCD, with no reported regimen-related toxicity. These data warrant consideration for future study with the aim of further reducing alkylating agent exposure and their inherent risks of infertility.

With the widespread adoption of myeloablative busulfan, fludarabine, and ATG reduced-toxicity conditioning for hematologic malignancies, this regimen forms the backbone of a current large multicenter trial of MSD HSCT in adolescents and young adults with SCD (clinicaltrials.gov, #NCT02766465).

Treosulfan. This myeloablative alkylating agent with immunosuppressive properties has been of increasing interest due to linear pharmacokinetics, less variability in metabolism between patients, and a favorable side effect profile when compared with busulfan [28]. Notably, the risk of hepatotoxicity- with risks of SOS- has raised concerns regarding busulfan-based conditioning for patients with thalassemia, particularly those with high pre-HSCT iron burden. However, while encouraging results from Italy with a treosulfan-based regimen have been reported, the low rates of hepatotoxicity reported for recipients of busulfan-based regimens for SCD combined with increasing patient and provider interest in reduced-intensity conditioning approaches might limit the interest in a large-scale study of treosulfan for SCD [28].

Clinical Trials Evaluating Reduced-Intensity and Non-myeloablative Conditioning Regimens for Matched Sibling Donor HSCT for Sickle Cell Disease

While rates of successful cure have been high with myeloablative matched-sibling donor HSCT for SCD, there are still short- and long-term toxicities that remain barriers to widespread application of this curative therapy for those with sibling HLA matches. The risk of infertility with myeloablative busulfan, particularly for

adolescent females, is of major concern [29, 30]. In general, outcomes of HSCT are better in younger recipients with notably lower rates of GVHD compared to recipients who are adolescents or young adults [27]. However, younger children cannot participate meaningfully in discussions about risks of infertility weighed against benefits of cure. Decision-making for families and care providers is influenced greatly by risks of end-organ toxicities, GVHD, as well as that of infertility.

With the knowledge that even 10–20% MDCs can result in a cure of the SCD phenotype, the applicability of less intensive conditioning regimens is even more appealing [8, 31]. This principle was applied to the first described non-myeloablative conditioning approaches to cure SCD [31]. However, with this relatively new approach to HSCT, less is known about long-term outcomes, including graft survival and late toxicities [24, 31]. Some of the approaches studied to date are summarized here. Table 12.2 includes the published studies with larger numbers of subjects.

Low-dose busulfan. Doses of busulfan ≥600 mg/m^2 (~20 mg/kg) have been associated with male infertility, and combined with data suggesting adolescent females are vulnerable to busulfan gonadotoxicity and that males with sickle cell disease may already have compromised spermatogenesis, the appeal of dose reduction of busulfan is apparent [33]. Cyclophosphamide, historically commonly administered with busulfan for HSCT for SCD, is also gonadotoxic. Horan et al. described a busulfan dose de-escalation approach to myeloablative, cyclophosphamide-containing conditioning with fertility preservation in mind [23]. Further such efforts include those of Krishnamurti et al., who published a series of seven children and adolescents with severe SCD who received conditioning with lower doses of busulfan (total dose 8 mg/kg) [34]. Minimal regimen-related toxicity was reported, and all patients are alive. Six of seven have long-term donor hematopoietic cell engraftment; two have full donor chimerisms, the remaining with MDCs. One patient had secondary graft failure with autologous hematopoietic reconstitution, with a history of cyclosporine nonadherence.

Fludarabine/low-dose total body irradiation. A cohort of six children and young adults with SCD who underwent MSD bone marrow allografting who received

Table 12.2 Reduced intensity conditioning/non-myeloablative conditioning for children, adolescents, and young adults with sickle cell disease

Authors	*N*	Median age (years)	Conditioning	OS	Graft rejection	DFS	cGVHD	TRM
King et al. (2015) [22]	43	13	Flu-Mel-Alemtuzumab	93%	<2%	91%	13%	7%
Hsieh et al. (2014) [24]	29	28.5	TBI-Alemtuzumab	97%	14%	86%	0%	0%
Saraf et al. (2016) [38]	13	30	TBI-Alemtuzumab	100%	8%	92%	0%	0%

OS overall survival, *DFS* disease-free survival, *cGVHD* chronic graft-versus-host disease, *TRM* transplant-related mortality, *Flu* fludarabine, *Mel* melphalan, *TBI* total body irradiation

fludarabine (30 mg/m^2/day), 200 cGy total body irradiation (TBI) with or without equine ATG [35]. Horan et al. also described three patients with SCD who received very low intensity consisting of fludarabine (25 mg/m^2/day × 5 days), rabbit ATG (daily × 4 days), and 200 cGy (TBI) [36]. While toxicities were minimal, all but one patient in these papers had autologous recovery and recurrence of SCD, demonstrating the need for either additional myelosuppression, immunosuppression, or both.

Alemtuzumab/melphalan/fludarabine. A multicenter study included 43 children with symptomatic SCD and nine with thalassemia major who received this combination of conditioning agents and MRD allografts (bone marrow in the majority, with some sibling umbilical cord blood (UCB) products given) [22]. Alemtuzumab was given "early," i.e., days −21 to −19. GVHD prophylaxis varied during the life of the study. The OS and EFS for those children and adolescents with SCD were 93% and 90.7%, respectively, at a median of 3.4 years. Graft rejection occurred in the one recipient who received only sibling UCB. Three deaths due to GVHD occurred in recipients 17–18 years of age. Rates of acute and chronic GVHD for all subjects were 23% and 13%, respectively; all cases of chronic GVHD were extensive and seen in recipients greater than 14 years of age. Of those with SCD and thalassemia who had follow-up of at least 1 year, 36/50 had full donor chimerisms (four with thalassemia). Two patients with SCD required donor lymphocyte infusion, neither of whom developed GVHD nor have evidence of SCD. Of those recipients with MDCs, immune suppression was withdrawn successfully without evidence of GVHD or graft rejection. Delayed lymphocyte recovery was noted, and *Staphylococcus* bacteremia and cytomegalovirus preemptive therapy were noteworthy. While these results are encouraging for successful donor hematopoietic cell engraftment with reduced-intensity conditioning, GVHD rates remain a concern.

National Institutes of Health Protocol. Hsieh et al. presented provocative data in the New England Journal of Medicine describing reduced intensity conditioning in adolescents and adults with symptomatic SCD [37]. Conditioning consisted of "late" alemtuzumab (1 mg/kg total dose) and 300 cGy TBI, followed by prolonged sirolimus exposure for GVHD prophylaxis and to prevent secondary graft failure due to delayed lymphocyte recovery (related to the timing of alemtuzumab). Donor-recipients with major ABO incompatibilities were excluded due to the risk of pure red cell aplasia. The NIH group expanded on their experience in 2014 with an updated description of 29 young adults and adults who received the same conditioning regimen [24]. Of these subjects, 25 (86%) had stable long-term donor engraftment. No acute or chronic GVHD was noted. There was only one death in a subject with recurrence of SCD who died of intracranial hemorrhage. Fifteen of the twenty-six subjects with stable donor engraftment had sirolimus withdrawn successfully with ongoing stable donor chimerisms and no GVHD. This protocol originally mandated 100% donor CD3$^+$ chimerisms prior to sirolimus weaning; since no patients achieved this degree of donor T-cell chimerisms, the study was amended to allow for weaning after 50% donor CD3$^+$ cells were achieved. Adverse events included pain, infections, thyroiditis, and sirolimus toxicities. The toxicities noted were quite acceptable given the SCD comorbidities noted in many subjects at baseline. No malignancies secondary to the radiation therapy have been noted, but longer follow-up is required. Pregnancies have been documented in recipient females.

An additional 13 high-risk young adult and adult patients with SCD have undergone this approach in Chicago, including two cases in which a major ABO incompatibility existed between the donor and recipient [38]. All patients are alive, with 12/13 having stable MDCs. One patient sustained secondary graft failure with a history of sirolimus nonadherence. No acute or chronic GVHD was noted. Four patients have since discontinued sirolimus without graft loss or GVHD. This approach has also been used with success in children and adolescents, with most recipients eligible for weaning of immune suppression at 1-year post-HSCT [39]. Given the absence of reported acute or chronic GVHD with this regimen, the possibility of fertility preservation in addition to high rates of cure of SCD, this regimen warrants further multicenter evaluation. Long-term graft survival data following sirolimus withdrawal will be essential.

Summary of Key Matched Sibling Donor HSCT Clinical Trials for SCD

Myeloablative conditioning regimens remain the most studied and most commonly prescribed for MSD allogeneic HSCT for SCD [27]. With the high rates of EFS and OS with busulfan, cyclophosphamide, and ATG conditioning, this regimen is widely considered the standard of practice [18, 19]. Fludarabine has been increasingly used to replace cyclophosphamide [17]. Efforts to reduce long-term toxicities—particularly infertility—are ongoing, with reduced-intensity conditioning regimens the focus of ongoing investigation. However, sustained donor engraftment and graft-versus-host disease remain barriers to their widespread implementation [22].

Impact of Hematopoietic Stem Cell Source

While bone marrow is the most common hematopoietic stem cell source used in MRD transplantation of children, UCB has shown to be a viable alternative for many children [40]. Because of their association with chronic GVHD and mortality, the use of peripheral blood stem cells have been avoided in transplantation for pediatric SCD [41].

Most of what we know regarding the suitability of UCB in MRD transplantation for pediatric SCD comes from registry studies of patients with thalassemia or SCD performed by Eurocord. In 2003, this group reported on outcomes in 44 patients (33 with thalassemia, 11 with SCD) [32]. In 2013, Eurocord published the results of larger study in patients with hemoglobinopathies comparing bone marrow and cord blood [40]. This study included 485 patients, 130 with SCD. Cord blood grafts were given to 96 of the 485 recipients. The distribution of patients with SCD between the two groups was similar. The cord blood recipients were significantly younger (5.9 vs 8.1 years, $p = 0.02$). Various forms of myeloablative, busulfan-based conditioning were used in all cases. In cord blood recipients, the infused cell dose was ample (median = 3.9×10^7 nucleated cells/kg recipient weight).

There were no differences in the incidence of graft failure between the two groups. Recovery of neutrophil and platelet counts was slower in UCB transplant recipients. Acute and chronic GVHD were more common in the marrow transplant group. Remarkably, no extensive chronic GVHD was observed in the cord blood transplant recipient group. Disease-free survival, event-free survival (defined as the absence of graft failure, extensive chronic GVHD, and death), and overall survival did not differ (Fig. 12.1).

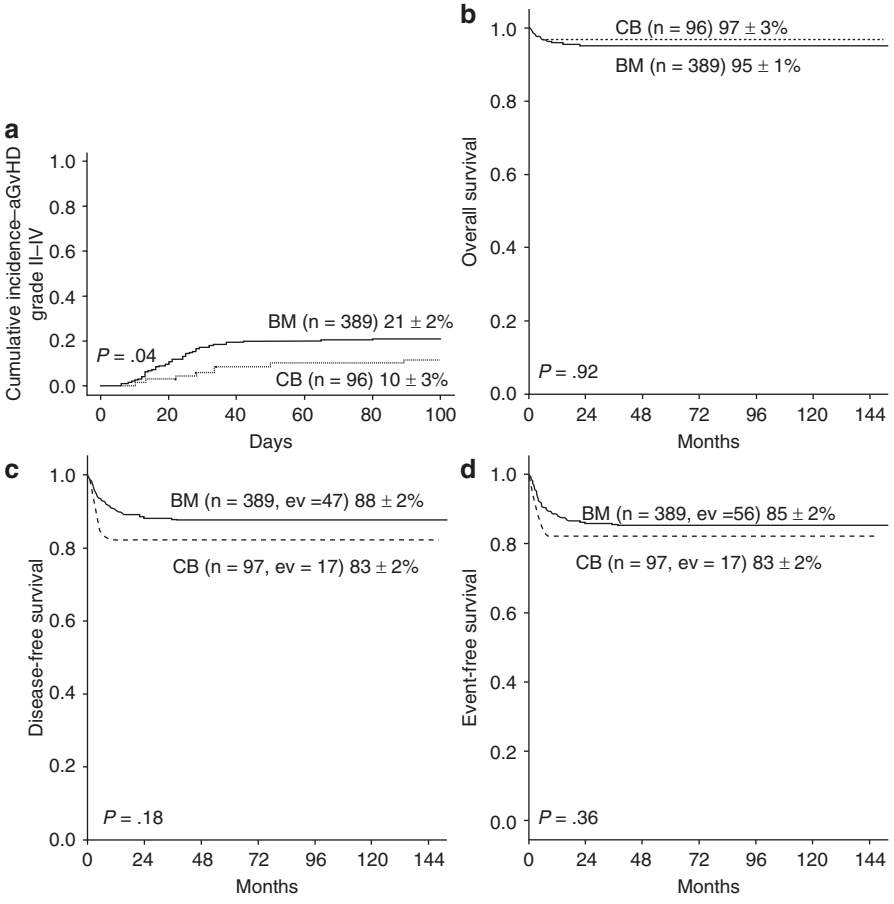

Fig. 12.1 Cumulative incidence of grade II–IV acute GVHD and Kaplan–Meier estimates of OS, DFS, and EFS. (**a**) Cumulative incidence of grade II–IV acute GVHD (aGVHD) for patients given BM and CB transplantation. (**b**) Kaplan–Meier estimate of OS for patients given BM and CB transplantation. (**c**) Kaplan–Meier estimate of DFS for patients given BM and CB transplantation. In the calculation of DFS, both death and graft failure were considered events. (**d**) Kaplan–Meier estimate of EFS for patients given BM and CB transplantation. In the calculation of EFS, death, graft failure, and extensive chronic GVHD were considered events. *BM* bone marrow, *CB* cord blood, *DFS* disease-free survival, *EFS* event-free survival, *GVHD* graft-versus-host disease, *OS* overall survival. © 2013 by The American Society of Hematology. Used with permission

One limitation of this study from the perspective of transplantation for SCD is that the patient sample was comprised predominantly of patients with thalassemia. The investigators did, however, perform a multivariate analysis of disease-free survival, examining the influence of graft type, recipient age, diagnosis, and year of transplant. Those with SCD were less likely to experience treatment failure (graft failure or death, HR = 0.52, 95% CI 0.28–0.97, $p = 0.04$).

This experience indicates that matched-related cord blood grafts are an excellent option for children with SCD. Transplant providers, however, should be careful in applying this experience to their own patients, being mindful of the ample cell dose received by most and myeloablative conditioning received by all recipients. We believe that matched-related cord blood transplantation is a good option when the cryopreserved cell dose is at least 3.9×10^7 nucleated cells/kg recipient weight and myeloablative conditioning is appropriate.

Whom Should Be Offered Matched-Related Donor Transplantation?

The eligibility criteria utilized in the international trial of MRD transplantation for SCD conducted in the 1990s [7, 10] have long guided the determination of whom should be offered transplantation. The consensus eligibility criteria (disease and patient) have been widely adopted for subsequent clinical trials as well as for patients offered transplantation as routine care.

This trial restricted enrollment to children who were severely affected by their SCD, utilizing nine disease-based criteria. Of the nine, three—clinical stroke, recurrent vaso-occlusive pain, and recurrent acute chest syndrome—accounted for nearly all the enrollment to the trial; clinical stroke accounted for over half. Patient-related criteria were designed to exclude patients more likely to suffer transplant-related complications, such as GVHD and SOS of the liver. Eligibility was limited to patients under the age of 16 years with a Lansky performance status of at least 70. Patients with bridging portal fibrosis/cirrhosis, significant renal insufficiency, and other organ dysfunction were excluded [7].

Since this study opened in 1991, there have been a myriad of changes in the care of patients with SCD as well as in the field of transplantation. While the consensus criteria developed for this trial remain relevant, they have been modified and supplemented in an effort to keep pace with advances in care. The most prominent changes in the care of children with SCD relate to the early detection of cerebrovascular disease and the widespread adoption of hydroxyurea. Cerebrovascular disease is now routinely identified before children suffer clinical stroke through transcranial Doppler ultrasound screening and the detection of silent cerebral infarcts by MRI scanning. Chronic transfusion therapy, in turn, is used to prevent the occurrence of clinical stroke and additional silent ischemic injury [11, 13]. Hydroxyurea is widely used to lessen the frequency of vaso-occlusive pain and

acute chest syndrome [14]. It is also now increasingly being initiated early in childhood to prevent disease-related complications [16, 20]. Elevated transcranial Doppler velocities and severe or progressive silent cerebral infarcts are now being recognized by some groups as indications for transplantation [22, 23]. Similarly, some have broadened criteria relating to vaso-occlusive pain and acute chest syndrome to offer transplantation to severely affected children who experience incomplete relief with hydroxyurea [23].

The Sickle Transplant Alliance for Research consortium (STAR, curesicklenow.org) employs a set of criteria to define severe disease that have been updated to account for advances in the field of SCD. These are shown in Table 12.3.

One of the chief advances in the field of transplantation since the Multicenter Investigation of Bone Marrow Transplantation for Sickle Cell Disease has been the development less intensive and safer forms of conditioning. With reduced intensity conditioning now having been studied successfully for MRD transplantation for SCD, it has made it possible to offer transplantation to some patients who would have been ineligible for the international trial, which used myeloablative conditioning. For example, in a multicenter trial of alemtuzumab, fludarabine, and melphalan, the lower limit for performance status was dropped from 70 to 40 [22].

Table 12.3 Indications for matched-related transplantation: updated criteria for defining severe disease

Previous clinical stroke, as evidenced by a neurological deficit lasting longer than 24 h, which is accompanied by radiographic evidence of ischemic brain injury and cerebral vasculopathy
Asymptomatic cerebrovascular disease, as evidenced by one the following:
Progressive silent cerebral infarction, as evidenced by serial MRI scans that demonstrate the development of a succession of lesions (at least two temporally discreet lesions, each measuring at least 3 mm in greatest dimension on the most recent scan) or the enlargement of a single lesion, initially measuring at least 3 mm. Lesions must be visible on T2-weighted MRI sequences
Cerebral arteriopathy, as evidenced by abnormal TCD testing (confirmed elevated velocities in any single vessel of TAMMV ≥200 cm/s for non-imaging TCD) *or* by significant vasculopathy on MRA (greater than 50% stenosis of ≥2 arterial segments or complete occlusion of any single arterial segment)
Frequent (≥3 per year for preceding 2 years) painful vaso-occlusive episodes (defined as episode lasting ≥4 h and requiring hospitalization or outpatient treatment with parenteral opioids). If patient is on hydroxyurea and its use has been associated with a decrease in the frequency of episodes, the frequency should be gauged from the 2 years prior to the start of this drug
Recurrent (≥3 in lifetime) acute chest syndrome events which have necessitated erythrocyte transfusion therapy
Any combination of ≥3 acute chest syndrome episodes and vaso-occlusive pain episodes (defined as above) yearly for 3 years. If patient is on hydroxyurea and its use has been associated with a decrease in the frequency of episodes, the frequency should be gauged from the 3 years prior to the start of this drug

TCD transcranial Doppler ultrasound, *TAMMV* time average mean of the maximal velocity

Future Directions

As more experience with MRD HSCT for SCD is garnered, it has become increasingly clear that outcomes are better in children compared to adolescents [27]. This recognition, we believe, presents both an opportunity and a challenge—an opportunity to expand the use of transplantation by extending it to children who are less severely affected by their disease and a challenge to improve outcomes in adolescent patients.

Extending Transplantation to Less Severely Affected Children

One of the chief advantages of performing transplantation prior to adolescence is a markedly lower risk of graft-versus-host disease. While it is unclear in MRD transplantation—where recipient and donor ages are closely correlated—whether it is the age of the recipient, the donor, or both that matter, studies in transplantation for pediatric leukemia clearly demonstrate that children are at lower risk for acute and chronic GVHD [42, 43].

While this issue has not been rigorously examined in HSCT for pediatric SCD, the published experience in SCD is consistent with that in leukemia. In the aforementioned multicenter trial of alemtuzumab, fludarabine, and melphalan conditioning, for example, there were three transplant related deaths, all in adolescent patients due to chronic GVHD. Furthermore, no chronic GVHD was observed in patients less than 14 years [22].

The safety afforded by earlier transplantation during childhood, coupled with the recognition that SCD produces significant suffering in most adults and greatly reduces life expectancy, provides a basis for extending MRD to less severely affected children. Experience from three STAR centers suggests that MSD HSCT could be a safe and effective treatment for these patients. This series included 25 patients with a median age of 5.3 years, most transplanted after 2010. Twenty-two had HbSS, and three patients had HbSC. While none of the patients met the criteria for severe disease listed in Table 12.3, most had a history of recurrent vaso-occlusive pain or acute chest syndrome. Most of these patients received myeloablative conditioning and a marrow allograft. One patient developed acute GVHD (grade III), one chronic GVHD, and one patient developed posterior-reversible encephalopathic syndrome. With a median follow-up of 28 (range 12–142) months, all 25 patients are off immune suppression and free of SCD. These findings must be confirmed by larger, prospective clinical trials. To this end, STAR recently developed a multicenter trial of HLA-matched MSD HSCT for children with less severe disease. This trial will be open to children who are moderately affected by SCD. To minimize the risk of GVHD, only patients less than 10 years with donors less than 10 years are eligible.

Providers need to be mindful that the decision to offer transplantation to less severely affected children is ethically more ambiguous than the decision to offer transplantation to those with severe complications. The benefits are less certain and less immediate, and while the risks for graft-versus-host disease and transplant related mortality are low, they still exist. Moreover, myeloablative conditioning carries a high risk of gonadal failure [29, 30]; and while the risk of gonadal failure with reduced intensity conditioning is likely to be lower, it remains poorly defined [22]. Finally, by limiting transplantation for less severe disease to children (excluding adolescents)—which we believe is a reasonable approach at this time—patient participation in the decision-making process is largely or completely precluded, depending on the age and capacity of the recipient. Given this ambiguity, it is imperative that painstaking care is taken by transplant providers to thoroughly inform families of the risks of transplantation and by hematologists to thoroughly inform them of alternatives to transplantation.

Improving Outcomes in Adolescents by More Effectively Preventing Graft-Versus-Host Disease

While the need for more effective approaches to preventing graft-versus-host disease in unrelated donor transplantation for SCD is widely recognized, the need for more effective strategies in adolescents with related donors also deserves greater attention. Future trials of GVHD prophylaxis in this age group are needed.

One strategy that merits consideration is pretransplant administration of higher-dose rabbit antithymocyte globulin (rATG, Thymoglobulin®). In a retrospective review of 236 MRD transplants (median age = 9.7 years) performed in France, using high dose busulfan and cyclophosphamide and varying doses of rATG for conditioning, the investigators demonstrated that the incidence of chronic GVHD was inversely related to rATG dose. Patients receiving the highest total dose 20 mg/kg (n = 160) had a very low incidence of chronic GVHD (6.4%). Higher rATG dose was associated with mixed donor chimerisms, but not rejection or serious infections [19]. Given the strong association of high-dose rATG (15 mg/kg) with infectious mortality in a randomized controlled trial in adults receiving unrelated donor transplants for hematologic malignancies, however, underscores the need for this to be examined in the context of a carefully conducted clinical trial [44].

STAR is currently investigating another strategy, the use of the co-stimulation blocking agent abatacept (CTLA4-Ig), an agent that has shown promise in early phase trials in mismatched unrelated donor transplantation for hematologic malignancies [45]. The STAR trial (NCT02867800) is open to patients receiving MRD transplants who are at least 10 years or whose donor is at least 10 years. It is also open to patients of all ages undergoing matched unrelated donor transplants. Patients receive IV abatacept on days −1, +5, +14, and +28 in addition to standard GVHD prophylaxis with a calcineurin inhibitor and methotrexate.

Conclusion

Matched sibling HSCT for SCD has evolved in the last three decades from being considered an experimental treatment for the sickest patients to now being viewed as a therapeutic option which should be considered early in the disease course to prevent the development of SCD-related organ injury. The field now focuses on reducing the intensity of the conditioning regimen with the hope of reducing HSCT toxicites, thereby making transplantation more acceptable to patients and families. Low toxicity HSCT approaches also allow adults with SCD who had been previously ineligible for HSCT due to concerns of exacerbating preexisting organ injury to now consider this curative treatment. With the impressive outcomes of non-myeloablative matched-sibling donor HSCT, notably the absence of any GVHD or significant toxicities, patients in the future with available donors may routinely undergo this curative treatment before the development of any complications of SCD.

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Chapter 13
Unrelated Donor Hematopoietic Stem Cell Transplantation for Sickle Cell Disease

Alexander Ngwube and Shalini Shenoy

Introduction

Sickle cell disease (SCD) is a chronic disorder of variable severity that has seen major strides in successful conservative management such as penicillin prophylaxis, hydroxyurea, and chronic red cell transfusion therapy [1, 2]. Patients with hemoglobin SS and $S\beta^0$thalassemia have severe phenotypes; other sickle hemoglobinopathies are rarer, but may also manifest with severe symptoms. SCD severity is determined by organ toxicity caused by endothelial damage and vasculopathy from misshapen red cells especially in deoxygenated state. The resultant ischemia causes severe pain episodes and damages the central nervous system (overt and silent strokes), lung (acute chest syndrome, pulmonary hypertension), cardiovascular system, kidney (renal failure, nephropathy), retina, bones, and joints (osteonecrosis, avascular necrosis) as outlined in Chap. 1. Though conservative therapy has come a long way in managing SCD complications thus extending lifespan in the modern age, a fraction of patients will continue to experience serious symptoms either due to inadequate access to care or progression despite adequate intervention. Progression can manifest as multiple overt or silent strokes despite transfusions, progressive pulmonary hypertension and irreversible pulmonary disease, and end stage renal failure [3–6]. This could result in poor quality of life, early morbidity, or premature mortality often in early adulthood [7, 8].

The only curative therapy currently available for SCD is hematopoietic stem cell transplantation (HSCT) from a normal or donor with sickle cell trait, though other

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experimental interventions such as gene modification and gene therapy efforts are under investigation. Several independent groups have vetted human leukocyte antigen (HLA)-matched sibling donor (MSD) HSCT using varied transplant approaches that range from fully ablative HSCT to reduced intensity and very low intensity non-ablative HSCT. All have produced disease-free survival (DFS) rates of over 90% and graft rejection and treatment-related mortality rates of approximately 5% [9–14]. A major drawback to offering MSD HSCT especially to symptomatic patients is donor availability. The likelihood of patients with SCD having a HLA-identical MSD is approximately 18% [15]. HSCT toxicities such as treatment-related mortality (TRM), graft versus host disease (GVHD), and late effects such as sterility also deter patients from transplant [16, 17]. However, in the last decade, promising outcomes after MSD HSCT have prompted investigation of alternative donor transplantation in smaller numbers. These efforts are especially directed at patients with severe symptoms despite conservative medical care who lack a MSD. Such patients are predicted to have poor long-term prognoses due to progressive SCD complications.

Donor Availability

With more than 21 million volunteer unrelated donors listed and with continuing increase in voluntary donors from minority communities, the probability of finding a HLA-matched unrelated donor (MUD) should increase with time. Unfortunately, the number of donors of African origin is currently a small fraction of the total; however, the National Marrow Donor Program (NMDP) continues to make efforts and inroads into increasing these numbers. Significant genetic variability within African populations as well as an increase in interracial births further complicates the ability to find suitable volunteer donors for HSCT. In 2008, Dew et al. noted that only 3 of 50 (6%) African American patients requiring HSCT for malignant and nonmalignant disease found an HLA-identical donor by high-resolution molecular typing [18]. A more recent examination of the donor pool at the NMDP revealed that based on ethnicity, 19% of African Americans and 18% of Africans would have an 8/8 HLA-match available. The probability of identifying a suitable donor increased to 76% for African Americans if 7/8 HLA matches were considered acceptable [19]. The likelihood of identifying a suitable unrelated donor stem cell source increased, especially in children, if umbilical cord blood (UCB) was included as a potential stem cell source. The likelihood of identifying a 6/6 or 5/6 HLA-matched cord-blood unit in children, inherently accepting higher levels of mismatch from low-resolution typing at the class I locus and taking cell dose into account, revealed a 64% chance of identifying such a product in African American children. These numbers corroborated with another independent analysis that projected 20% availability of 8/8 HLA-matched donors. There was an 84% chance of identifying a donor matched at 7/8 HLA loci. Similarly, 90% of children <43 months old would find an “adequate” single cord defined as having an ideal total nucleated cell count

of >5 × 10^7/kg and matched at five or six of the six major loci (HLA-A, -B, -DRB1). This probability increased to 97% if the cell dose could be smaller (intending the use of double cords or a product that was expanded with in vitro culture) or if four of six HLA-locus matching was acceptable [20]. Since fully HLA-matched donor sources continue to remain relatively scarce for patients of African origin presumably due to the low number of donors of like ethnicity available in registries utilized by the National Marrow Donor Program, there continues to remain a need to expand the registry by recruiting minority donors. We found that education regarding the process of donation is the single major factor that promotes donation [21].

Each stem cell source has nuanced differences in engraftment capacity, GVHD risks, and immune reconstitution. Once engrafted successfully, all have the capacity to support normal hematopoiesis in the SCD patient. We will review the outcomes of URD HSCT from each stem cell source in this chapter. Haploidentical stem cell transplantation from marrow or peripheral blood cells can serve as an additional alternate donor source and will be reviewed in Chap. 14.

Indications for Transplant

Modern supportive care measures available in a country like the USA have reduced the mortality rate from SCD complications in childhood to 0.5 per 100,000 persons but remains high at >2.5 per 100,000 persons in early adulthood resulting in median survival of 42 and 38 years for women and men, respectively, in 2005 [8, 22]. Factors that predict early morbidity and mortality such as neurologic, cardiopulmonary, hematologic, or renal complications largely justify HSCT. Apart from early mortality, these patients have poor outcomes for quality of life, employment, and societal function and benefit from considering the pros and cons of alternate donor HSCT. Unfortunately, a major obstacle to providing HSCT options prior to the development of serious disease sequelae, when the procedure may be safer, is our inability to predict severity in advance.

As the safety profile of MSD HSCT continues to improve, one might consider this curative option in the majority of patients with hemoglobin SS or Sβ^0thalassemia and related genotypes that are associated with severe SCD phenotype during later decades of life if not during childhood [23]. The higher risks associated with alternative donor HSCT demands stricter criteria for consideration of this intervention, and the declaration of disease severity is imperative. Improving outcomes will alter the risk-benefit ratio of HSCT, and a reconsideration of indications is prudent periodically. Age, SCD complications, and donor source and availability are important variables to consider in alternate donor HSCT. Currently, unrelated donor HSCT is reserved for patients who do not have a matched sibling donor, do not respond well to supportive care measures, or develop toxicities from the same. Disease scenarios that warrant consideration of unrelated donor HSCT are listed in Table 13.1. They include severe pain symptoms that significantly alter quality of life, progressive neurologic involvement or organ function impairment such as

Table 13.1 Indications for unrelated hematopoietic stem cell transplant in sickle cell disease

Matched unrelated donor marrow (8/8 loci match with high-resolution tying)
Umbilical cord blood (5–6/6 loci match; total nucleated cells $\geq 5 \times 10^7$/kg)
Mismatched unrelated donor marrow (7/8 loci match with high-resolution typing)
Central nervous system: Overt stroke; recurrent silent strokes with deteriorating performance
Lung: Severe acute chest syndrome >2 with respiratory compromise; pulmonary hypertension
Renal: Sickle nephropathy
Immune: Red cell alloimmunization with established indication for chronic transfusion therapy
Pain: Severe episodes with prolonged (>72 h) hospitalizations for parenteral narcotics >2/year

retinopathy or nephrotoxicity, and red cell alloimmunization in the setting of an absolute requirement for red cell transfusion therapy. HSCT should be considered in a study setting wherein toxicities are tracked closely and stopping rules regarding organ toxicities, mortality, and GVHD (particularly severe chronic GVHD) risks are set to apply particularly if these risks are very high and perceived to outweigh benefits [10, 23–25].

HLA-Matched Unrelated Donor Transplantation

Transplantation for SCD was considered worthy of clinical trials after 1984 when it was first reported that the procedure cured both SCD and acute myeloid leukemia in a patient affected by both disorders [26]. A recent report of 1000 MSD transplants performed subsequently between the years 1986 and 2013 documented outcomes from 106 centers in 23 countries reported to the European and United States transplant registries to confirm benefit from the procedure and event-free survival (EFS) of >90% [9]. Overall survival (OS), EFS, and GVHD-free survival at 5 years post-HSCT were lower with increasing age and were 81%, 81%, and 77%, respectively, in patients over 16 years of age. More recently, alternative donor HSCTs have been performed in much smaller cohorts. The indication to pursue alternate donor HSCT for SCD is more stringent as mentioned.

HLA matching in the conventional sense is restricted to high-resolution typing at the eight major HLA loci (A, B, C, and DRB1). Extending typing in the unrelated donor setting to include DPB1 and additional loci could improve outcomes further as reported in thalassemia transplants and more recently in malignant disorders, but would further jeopardize our ability to find unrelated donors in SCD patients. The first US report of URD HSCTs performed within the Bone Marrow Transplant Clinical Trials Network (BMT CTN) was reported in 2016; used reduced intensity conditioning (RIC) consisting of hydroxyurea, fludarabine, melphalan, and alemtuzumab; and had 1- and 2-year EFS rates of 76% and 69%, respectively; the corresponding OS was 86% and 79%, respectively [27]. Outcomes were compromised by high rates of chronic GVHD (38% developed extensive chronic GVHD) and was responsible for mortality. Importantly, except one, all deaths occurred in recipients

Table 13.2 Summary of patients with sickle cell disease undergoing matched unrelated donor hematopoietic stem cell transplant

Reference	*N*	Type of conditioning	Age range (years)	Graft rejection (%)	OS (%)	EFS (%)	aGVHD grade II–IV (%)	cGVHD (%)
Mynarek et al. (2013) [29]	**2**	Reduced intensity	6–8	0	100	50	0	50
Strocchio et al. (2015) [28]	**6**	Myeloablative	27–48	17	100	83	0	0
Shenoy et al. (2016) [30]	**29**	Reduced intensity	4–19	10	79	69	28	38

≥16 years of age, mirroring the poorer results in older patients after MSD transplants. Thus, though this RIC regimen, which was used to offset late effects after myeloablation, proved capable of achieving engraftment unlike previous RIC transplant efforts, GVHD remained a problem. The focus of subsequent studies that are in progress are directed at reducing GVHD rates especially in older children. For example, a new pilot study within the Sickle Transplant Alliance for Research Consortium (STAR; www.curesicklenow.org) is exploring the application of modern GVHD prophylaxis to offset GVHD in the same RIC setting as used in the BMT CTN trial. In Italy, treosulfan in combination with antithymocyte globulin, fludarabine, and thiotepa was used prior to MUD HSCT in six patients, all under 16 years of age [28]. At a median of 3 years, EFS was 83% in this younger cohort; notably the incidence of GVHD was 0% in this small initial cohort transplanted from unrelated donors. A third report from Germany included two children who received MUD bone marrow and had stabilization of CNS manifestations after MUD HSCT. Published experience with unrelated donor marrow HSCT for SCD is summarized in Table 13.2. A new trial called the Sickle Cell Transplantation to Prevent Disease Exacerbation (STRIDE, NCT01565616) has commenced within the BMT CTN and will test the ability of myeloablative busulfan, fludarabine, and antithymocyte globulin in achieving desired transplant outcomes following matched related and unrelated donor marrow transplants in adolescents and young adults 15–40 years of age. This study will compare HSCT outcomes to SCD patients without HSCT to determine differences between the two groups.

Mismatched Unrelated Donor Transplantation

Acceptable alternate-unrelated donor stem cell sources in other disorders have routinely included marrow or peripheral blood products that are matched at 7/8 HLA (A, B, C, and DRB1) loci by high-resolution typing, especially in malignant disorders. Given the higher risk of chronic GVHD, we do not support the use of mismatched peripheral blood cell HSCT without T cell depletion in the nonmalignant disorder

setting. However, in the modern era, in vivo or in vitro immunologic T cell manipulation strategies may be utilized to employ one-antigen mismatched (7/8 HLA matched) products in HSCT. These include T cell depletion, CD34 selection, or αβCD3+ T cell depletion mechanisms. These manipulations can increase the availability of donors in patients that with severe SCD could benefit from HSCT and lack a MUD. As mentioned previously, 76% of patients of African American origin are likely to find a one-antigen mismatched donor. An increased risk of GVHD, TRM, and graft loss were previously associated with mismatched donor transplants [31]. However, there are currently clinical trials in progress evaluating mismatched unrelated donor HSCT due to the availability of effective GVHD prophylaxis with newer agents or stem cell manipulation. Both strategies are in clinical trials with reduced toxicity or reduced intensity conditioning regimens (NCT01966367 and NCT00920972).

Unrelated Umbilical Cord Blood Transplantation

Table 13.3 summarizes the unrelated donor UCBT experience to date. The initial report of three children undergoing UCBT from unrelated donor sources matched at 4/6 loci in 2004 was updated in 2007 [33, 40]. Busulfan-based reduced intensity

Table 13.3 Summary of patients with sickle cell disease undergoing unrelated cord blood donor hematopoietic stem cell transplant

Reference	*N*	Type of conditioning	HLA match	Graft rejection (%)	OS (%)	EFS (%)	aGVHD grade II–IV (%)	cGVHD (*n*)
Mazur et al. (2006) [32]	1	Reduced intensity	4/6	0	100	0	0	0
Adamkiewicz et al. (2007) [33]	7	Myeloablative (4), reduced intensity (3)	5/6(2) 4/6 (5)	14	86	38	57	1
Sauter et al. (2010) [34]	1	Reduced intensity	5/6	0	100	0	0	0
Ruggeri et al. (2011) [35]	16	Myeloablative (9), reduced intensity (7)	6/6 (2) 5/6 (4) 4/6(10)	44	94	50	23[a]	16[a]
Kamani et al. (2012) [36]	8	Reduced intensity	6/6 (1) 5/6 (7)	63	88	25	25	1 (extensive)
Radhakrishnan et al. (2013) [37]	8	Reduced intensity	NR	50	63	50	50	1 (limited)
Kharbanda et al. (2014) [38]	2	Reduced intensity	4/6	100	100	0	0	0
Abraham et al. (2017) [39]	9	Reduced intensity	5/6	22	100	78	42	2 mild 1 moderate

[a]Reported for a combined cohort of sickle cell disease and thalassemia transplant recipients

regimens failed to support engraftment. Of four myeloablative busulfan-based HSCTs, three engrafted and two remained disease-free long term; another successfully underwent a second HSCT using a RIC regimen consisting of hydroxyurea, rituximab, alemtuzumab, low-dose irradiation, and thiotepa. All patients who engrafted developed acute GVHD and one developed extensive chronic GVHD. These HSCTs taught us the proof of principle that, if successful, unrelated cord transplants eradicated SCD. An outcome analysis from US and European cord registries showed that while OS was 94% following unrelated UCBT, DFS was 53% predominantly due to high graft rejection rates [35]. A BMT CTN trial was undertaken in 2008–2009 to evaluate the efficacy of RIC in URD HSCT-restricted UCB products to 5–6/6 HLA-matched donors and a higher cell dose requirement of 4×10^7/kg TNC. Graft rejection again necessitated closure of the cord blood arm of this trial early when a DFS of 37.5% at one-year was noted [36]. A reduced toxicity conditioning approach (alemtuzumab, busulfan, and fludarabine) was evaluated in a clinical trial at the Columbia University, New York. Again, predominantly due to graft rejection, DFS was 50%; OS was compromised by viral infections, predominantly cytomegalovirus and adenovirus [37]. An attempt to combine mesenchymal stem cells with cord products to enhance engraftment failed in two patients undergoing unrelated UCBT and resulted in autologous reconstitution [38]. These outcomes provided a framework for improvement directives in the arena of URD UCBT, and several efforts are underway to make outcomes more comparable with UCBT from HLA-matched sibling donors where DFS was 90% [41].

UCBT efforts to improve upon existing outcomes are multidirectional. The reduced toxicity regimen that failed to provide engraftment in the BMT CTN trial has been modified to include thiotepa and has a DFS of 78% in early analysis (Abraham et al.; ASBMT/CIBMTR Tandem Meetings 2017). Infectious complications can be better tackled in this era of cellular therapy with low toxicity interventions such as antivirus-specific cytotoxic T lymphocytes. Cord blood product expansion techniques are under investigation to reduce the period of neutropenia and enhance engraftment, to offset both infection and graft rejection risks [42]. Late immune reconstitution following UCBT necessitates good supportive care plans when investigating URD UCBT. While chronic GVHD risks are lower than with marrow or peripheral blood stem cell sources, acute GVHD risks persist with UCBT as with other stem cell sources. Better methods of anticipatory prediction and newer agents that combat GVHD are other interventions that can help improve outcomes. If these complications are successfully tackled, cord blood products that have lower cell doses and those that are more HLA-mismatched than in use currently could become standard of care in the future even for SCD HSCT.

The use of UCB in transplantation for SCD offers additional advantages over other stem cell sources: the donor search time is expeditious making the time to transplant shorter compared to MUD HSCT, there is no risk to the donor, and a greater degree of mismatch may be acceptable given the low incidence of chronic GVHD. Cord products have been utilized with increased efficacy compared to marrow stem cells in HSCTs for neurologic disorders such as Hurler syndrome and Krabbe disease; this feature may prove to be an advantage in SCD patients with

neurologic sequelae. Disadvantages of UCB include delayed engraftment, higher risk of graft rejection, and immaturity of the transplanted immune system resulting in delayed immune reconstitution. Cell dose is critical for a successful UCBT, and engraftment rates and outcomes are better with higher cell doses (TNC $\geq 5 \times 10^7$/kg), especially with UCB products mismatched at more than one locus [43]. In vitro cord blood expansion techniques prior to HSCT are now in clinical trials for SCD and other disorders. Successful engraftment of UCB cells in seven of eight SCD patients was reported by Parikh et al. at the American Society of Hematology meeting in 2016 (abstract #3651) on behalf of Gamida-cell Ltd. The expanded UCB product Nicord® was co-transplanted with an unmanipulated product to facilitate engraftment.

A discrepancy between the patient's body weight and the number of hematopoietic cells in the cord blood unit can make a cord product ineligible for use. A very high-cell dose can trade off with HLA matching to some extent, but it is important to remember that graft rejection rates are higher in patients with SCD than described in malignant disorders due to recipient immune competence and potentially previous exposure and alloimmunization against both HLA and hematopoietic antigens.

Conclusions

Matched sibling donor HSCT is a reasonable consideration especially in the young, in this modern era of transplantation, based on efficient ways of achieving engraftment, reducing toxicity, and avoiding complications, thus achieving curative outcomes in >90% of patients. Current data extrapolated from the fewer URD HSCTs performed suggest that similar outcomes can be expected following successful URD transplantation. Limitations of URD transplantation include limited donor availability and the higher risk of HSCT-related toxicities such as GVHD and infections. The immunologic milieu of SCD is one of inflammation where innate stimulation of graft-versus-host and host-versus-graft reactions is likely to occur with high frequency. Ongoing clinical studies are already involved in efforts to decrease complications and increase safety and success. Such trials are directed at making SCD HSCTs safer and more accessible to a larger population of patients. Alternate donor HSCT is experimental at the current time and should be reserved for those patients with severe SCD unresponsive to conservative management. Further, alternate donor HSCT for SCD should be performed in the context of formal studies that target achieving outcomes closer to MSD HSCT outcomes using state-of-the-art HSCT knowledge, have built in safety parameters, and targeted supportive care. These efforts also demand allocation of adequate funding to continue this work and develop centers with expertise in caring for these complex patients aiming for cure as an end result.

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Chapter 14
Haploidentical Hematopoietic Cell Transplantation for Sickle Cell Disease

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Introduction

Hematopoietic stem cell transplantation (HSCT) is the only curative therapy for sickle cell disease (SCD), but its use has been limited due to donor availability. Outcomes following matched sibling donor HSCT are excellent in adult and pediatric patients, with 5-year event-free survival of 91.4% and overall survival of 92.9% recently reported in an international retrospective study including 1000 patients, the majority receiving myeloablative conditioning [1]. While one-quarter of patients should have a human leukocyte antigen (HLA)-identical sibling, studies of children with SCD found that only 14% of those meeting study entry criteria had an HLA-identical sibling [2]. Other donor options include HLA-matched unrelated donors or umbilical cord blood, but low numbers of black donors in the National Marrow Donor Program registry and a high degree of genetic polymorphisms limit these donor options [3–5]. As a result, recent studies found that only approximately 20% of African-Americans have an 8/8 HLA-matched unrelated donor, with only about half (9%) being a true 8/8 match (upon confirmatory testing) that proceed to donation [3, 6]. Based upon these data (and excluding unrelated umbilical cord blood, given suboptimal outcomes with high rates of rejection; see Chap. 13), approximately three-quarters of patients with SCD considered for HSCT will consequently be unable to undergo HSCT because they lack an available HLA-matched donor.

The use of haploidentical donors has the potential to dramatically improve the HSCT donor pool for patients with SCD. Using a haploidentical or half-matched

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relative is an attractive donor option, as most patients have an available parent or half-matched sibling donor. This has led to its increasing use initially in the malignant disease setting, which has been subsequently translated for nonmalignant diseases including SCD. The occurrence of graft-versus-host disease (GVHD) was previously a major obstacle to the use of haploidentical donors for HSCT. The safety and efficacy of haploidentical HSCT have been greatly enhanced by the development of novel strategies for the prevention of GVHD, which have focused on ex vivo or in vivo T-cell depletion. Two of these approaches, infusion of a CD34 positively selected donor graft (ex vivo depletion) and of post-HSCT cyclophosphamide (in vivo depletion), are subsequently reviewed in detail. Consideration is given to the particular conditioning (e.g., myeloablative versus reduced intensity), and GVHD prophylaxis regimens are utilized in both completed and ongoing clinical trials for both these approaches.

Posttransplant Cyclophosphamide Approach

Rationale

In vivo T-cell depletion has been one of the major strategies used to overcome the significant HLA disparity in haploidentical HSCT both to reduce GVHD and to improve engraftment. Historically, this has been accomplished through the use of anti-thymocyte globulin (ATG), whose disadvantages include its long half-life (particularly in the case of rabbit ATG) [7] and nonselective elimination of T cells which may lead to delayed immune reconstitution and opportunistic infections. Based upon a large body of preclinical studies [8–12], the administration of high-dose cyclophosphamide post-HSCT offers the potential for more targeted depletion of T cells, by more selectively eradicating alloreactive T cells (in both graft-versus-host and host-versus-graft directions) while preserving immune reconstitution and protective immunity (Fig. 14.1a). Compared to ex vivo T-cell depletion strategies, the simplicity and low cost of posttransplant cyclophosphamide (PTCy) infusion have accounted for the rapid expansion of its use in a wide range of both malignant and, more recently, nonmalignant diseases.

Preclinical Background

The administration of PTCy takes advantage of the differential expression of the cellular enzyme aldehyde dehydrogenase (ALDH), which converts the active form of cyclophosphamide to its inactive form, carboxyphosphamide. Thus, cells with a high expression of ALDH (including hematopoietic stem cells) are less sensitive to cyclophosphamide's effects, whereas cells with a low expression of ALDH (including activated T and natural killer (NK) cells) are more sensitive to its effects [13, 14].

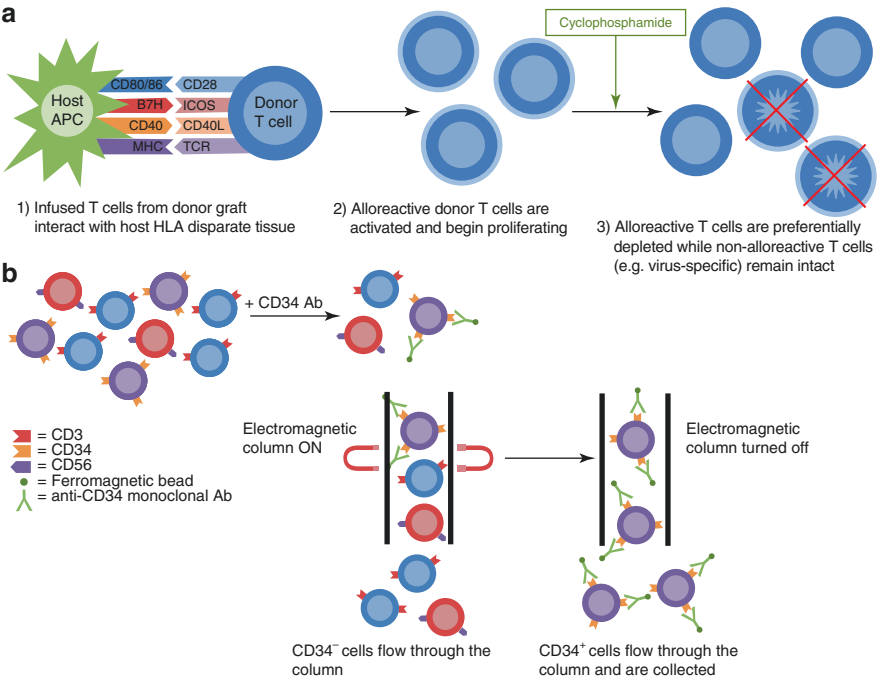

Fig. 14.1 Mechanistic schematic of posttransplant cyclophosphamide (PTCy) versus CD34-positive selection as strategies to deplete T cells in haploidentical hematopoietic stem cell transplantation. (**a**) PTCy takes advantage of the fact that T cell infused from the donor become alloreactive following interaction with recipient-disparate HLA tissues. The peak of this donor T-cell activation and proliferation occurs approximately 48–72 h post donor HSC infusion, accounting for the timing of PTCy administration (e.g., days +3 and +4 post-HSCT). Due to their differential expression of aldehyde dehydrogenase (compared to resting cells including HSCs), these alloreactive donor T cells are particularly sensitive to the effects of Cy, therefore undergoing a more selective apoptosis. (**b**) In CD34-positive selection, first, HSCs are labeled with a ferromagnetic bead that targets CD34. Second, the cells are passed through an electromagnetic column, on which $CD34^+$ cells (e.g., HSCs) stick when the magnet is turned on. Finally, the magnet is turned off and the column is washed, allowing $CD34^+$ cells to flow through the column and to be selectively collected

While the tolerance-promoting potential of cyclophosphamide has been known since the 1970s, preclinical studies over the last two decades have significantly enhanced our mechanistic understanding of this effect. Early studies were performed in a murine skin transplant model [8], wherein a single dose of cyclophosphamide given after allogeneic spleen cell administration led to the destruction of alloreactive T cells derived from both host and recipient. This effect was lost when cyclosporine was administered prior and concurrent to cyclophosphamide, which is thought to be due to interference of proliferation of donor-derived, antigen-stimulated alloreactive T cells [9]. Based upon these latter results, the start of traditional post-HSCT immunosuppression in clinical trials has been delayed until after PTCy administration.

Subsequent studies in murine models of HSCT demonstrated that the addition of PTCy allowed dose reductions of pre-HSCT radiation which could be enhanced with the administration of pre-HSCT fludarabine or cyclophosphamide [10–12]. Further, this effect was lost when the single dose of PTCy was given outside the range of 48–72 h post stem cell infusion [12], accounting for the timing of its administration in subsequent clinical studies.

Conditioning Regimens

The initial clinical studies utilizing PTCy were performed in adult patients with high-risk malignancies [15, 16], where a reduced-intensity conditioning regimen was used to decrease the risk of conditioning-related morbidity and mortality. In the initial phase I trial [15], the addition of cyclophosphamide on days −6 and −5 (14.5 mg/kg/dose) to PTCy on day +3 (50 mg/kg) improved engraftment compared to fludarabine and low-dose total-body irradiation (TBI) alone. This fludarabine, pretransplant cyclophosphamide, and low-dose TBI conditioning regimen was utilized for the subsequent multicenter trials, including the Blood and Marrow Transplant Clinical Trials Network (BMT CTN) 0603 trial in the same adult malignant disease population [16, 17]. In patients with graft failure, the majority had autologous reconstitution, consistent with this being a very-reduced-intensity, nonmyeloablative regimen [15–17].

The same conditioning regimen (fludarabine, low-dose TBI, and pretransplant cyclophosphamide) was thus extended from the malignant disease population [15–17] to the first SCD patient to receive haploidentical HSCT using PTCy [18]. This approach was then extended to an additional 13 patients with SCD [19], with rabbit ATG added on days −9 through −7 based upon data from Bernaudin et al. [20]. Ultimately, 6 of the 14 patients had graft rejection (42.9%), albeit all with autologous reconstitution [19]. These results have led to further adjustments to the conditioning regimen (backbone of fludarabine, pretransplant cyclophosphamide, ATG, and low-dose TBI) in both ongoing and planned haploidentical HSCT trials (NCT01850108 [21] and NCT00489281). The upcoming multicenter clinical trial of haploidentical HSCT for SCD using PTCy (BMT CTN 1507) will utilize this backbone (fludarabine, pretransplant cyclophosphamide, ATG, and low-dose TBI) with the addition of (1) hydroxyurea pretreatment (days –70 to 10 at 30 mg/kg) and (2) thiotepa (day −7 at total dose of 8 mg/kg divided over two doses).

Donor Selection

As in the PTCy trials for malignant diseases [15–17], the source of haploidentical hematopoietic stem cells in the SCD-specific trials to date has been the bone marrow, with standard cell processing protocols used, as discussed in Chap. 5 [19].

As is typical for HSCT in a nonmalignant disease, a target cell dose of 4×10^8 nucleated cells per kilogram recipient weight has been specified [19]. In the last three patients reported by Johns Hopkins [19], donor granulocyte-colony stimulating factor (G-CSF) stimulation was added to improve engraftment by increasing cell dose, a technique that was successful in murine models [11, 12]. Because G-CSF stimulation and higher cell dose did not impact engraftment in subsequent patients (per personal communication, Dr. Robert Brodsky), neither are being used in ongoing trials [21] or in the upcoming BMT CTN 1507 trial. Other donor selection considerations in this setting include excluding donors to whom the recipient has HLA antibodies and prioritizing donors based upon red blood cell compatibility (with a crossmatch compatible donor selected first, followed by minor and then major compatible donors) and/or non-inherited maternal antigens.

GVHD Prophylaxis

Due to a high combined incidence of graft rejection and severe GVHD (approximately 60% at 6 months) seen in the initial phase I trial with a single dose of PTCy [15], subsequent cohorts of patients treated for malignant and nonmalignant diseases at Johns Hopkins have received two doses (days +3 and +4) of PTCy (50 mg/kg per dose) [16, 18, 19]. A comparison was made between the two dosing strategies (on two separate trials) which demonstrated no difference in acute GVHD but a trend ($p = 0.05$) toward less-extensive chronic GVHD in the group receiving two doses of PTCy [16]. Thus, subsequent completed and ongoing trials including the BMT CTN (0603, 1507) have utilized the two dosing PTCy strategies [17].

In the initial malignant disease trials [15, 16], PTCy was combined with tacrolimus and mycophenolate mofetil (MMF), both starting the day following the completion of PTCy due to concerns that earlier administration could impact the effect of PTCy [9]. Several modifications have been made to this approach in subsequent trials. First, the duration of tacrolimus was extended to day +60 in the initial phase I trial in malignant disease patients due to a high incidence of late acute GVHD in the setting of high donor chimerism [15]. In subsequent trials, including the initial SCD patient, tacrolimus administration was extended through day +180 [16–18]. This approach was then further modified in the larger SCD patient cohort, where tacrolimus was continued through 1-year post-HSCT [19]. Tacrolimus was subsequently switched to sirolimus mid-protocol due to the development of posterior reversible encephalopathy syndrome (PRES) in three of the first nine SCD patients [19]; this approach had been successful in the previous reduced-intensity conditioning matched related donor HSCT trial at the NIH [22]. Second, the dose of MMF was increased in the midst of the malignant disease trials [15, 16], with the higher dosing (15 mg/kg TID) carried forward into SCD-specific trials [19].

Clinical Trials

While this approach of in vivo T-cell depletion with PTCy has been studied more extensively in patients with malignant diseases, including an ongoing randomized trial comparing haploidentical HSCT to double cord blood (BMT CTN 1101; NCT01597778), its evaluation in SCD patients is ongoing. The largest experience is from the ongoing Johns Hopkins trial [19], with their initial report including 14 adult SCD patients undergoing haploidentical HSCT (with an additional three patients reported using a matched related donor). As per Table 14.1, patients were conditioned with fludarabine/cyclophosphamide/200 cGy TBI with (majority) or without ATG, and GVHD prophylaxis consisting of MMF, sirolimus, and PTCy (days +3 and +4). All patients had count recovery with neutrophil engraftment (including the three related donors) at a median of 24 days post-HSCT; however graft rejection occurred in 42.8% (n = 6) of the haploidentical cohort [19]. In the remaining eight patients with engraftment reported, it is notable that one patient remained on immune suppression with very low donor chimerism (5–6%) and hemoglobin (6.2 g/dL) at 1-year post-HSCT [19]. Importantly, no patients developed either acute or chronic GVHD, and all patients were reported alive with a median follow-up of 711 days [19]. Consistent with a very-reduced-intensity conditioning regimen, all patients with graft rejection had autologous reconstitution [19]. The regimen was also well tolerated, with Epstein-Barr virus (EBV) or cytomegalovirus (CMV) reactivation (without disease) occurring in only three patients [19]. The latest iteration of this trial has increased the dose of TBI from 200 to 400 cGy (NCT00489281), with updated results not yet officially reported.

With adaptations in the conditioning regimen as detailed above (and per Table 14.1), encouraging results from an ongoing multicenter and international collaboration were reported at the 2016 American Society of Hematology meeting [21]. In the first cohort (including 12 pediatric and adult patients from two centers), the addition of thiotepa to the cyclophosphamide/fludarabine/TBI/ATG backbone conditioning regimen decreased the rate of graft rejection from 60% (3 of 5) to 0% (0 of 7), with >grade II acute GVHD seen in two patients and "no significant chronic GVHD" [21]. In seven patients who received thiotepa, the regimen was well tolerated with no deaths and viral reactivation at a comparable rate to the Johns Hopkins report [19, 21]. The second cohort included 22 pediatric patients from a single center, who received thiotepa, azathioprine, and hydroxyurea in addition to the cyclophosphamide/fludarabine/TBI/ATG backbone [21]. Graft rejection occurred at a rate of 9.1%, with 18.2% of patients developing treatment-responsive acute and chronic GVHD (no description of severity) [21]. Unfortunately, there have been three deaths in this cohort, secondary to infection and/or macrophage activation syndrome (without additional details of such), leading to an overall survival of 86.4% [21].

Given these encouraging results from a small number of centers and patients, this approach will be assessed in an upcoming multicenter clinical trial through the BMT CTN (1507). The trial will use a unified regimen adding a single day of thiotepa to the published Johns Hopkins conditioning regimen (cyclophosphamide/fludarabine/200 cGy TBI/ATG and 2-days of PTCy) [19] plus preconditioning with

Table 14.1 Clinical trials utilizing posttransplant cyclophosphamide approach for haploidentical HSCT for SCD

Location	Genotype	Age	Conditioning[a]	HSC source	TNC dose (10e6/kg)	CD34 dose (10e6/kg)	CD3 dose (10e8/kg)	Neutrophil engraftment	1° Graft rejection	2° Graft rejection	aGVHD	cGVHD	EBV/CMV reactivation	OS[b]	EFS[b]	Median follow-up
Hopkins; *n* = 14 (Ref. 19)	SS (12), SC (2)	23.5 y (15–42)	w/o rATG (2), w/ rATG (12)	BM (11)	4.6 (3.2–5.2)	5.22 (2.84–8.68)	3.83 (1.69–6.59)	24 d (0–35)[c]	7.1% (1)	35.7% (5)	0%	0%	21.4% (3)	100%	57.1% (8)	711 d (224–1981)[c]
				G-CSF stim BM (3)	11.4 (10.4–21.6)	6.02 (4.1–8.85)	10.9 (4.06–16.3)									
Multicenter; *n* = 12 (Ref. 21)	NR	26.4 y (12–50)	w/o TT (5)	G-CSF stim BM	8.4	3.63	NR	NR	40% (2)	20% (1)	NR	NR	NR	NR	NR	20.3 m (1.4–37.5)
		18.1 y (7–26)	w/ TT (7)					25 d	0%	0%	28.6% (2)[d]	0%[e]	28.6% (2)	100%	100%	
Imperial College; *n* = 22	NR	10 y (3–18)	w/ HU/Aza + TT					17 d	0%	9.1% (2)	18.2% (4)	18.2% (4)	NR	86.4% (19)[f]	81.8% (18)	

HSCT hematopoietic stem cell transplantation, *SCD* sickle cell disease, *HSC* hematopoietic stem cell, *TNC* total nucleated cell dose, *1°* primary, *2°* secondary, *aGVHD* acute graft-versus-host disease, *cGVHD* chronic graft-versus-host disease, *EBV* Epstein-Barr virus, *CMV* cytomegalovirus, *OS* overall survival, *EFS* event-free survival, *SS* hemoglobin SS, *SC* hemoglobin SC, *y* years, *rATG* rabbit anti-thymocyte globulin, *w/o* without, *w/* with, *BM* bone marrow, *G-CSF* granulocyte-colony stimulating factor, *d* days, *NR* not reported, *TT* thiotepa, *HU* hydroxyurea, *Aza* azathioprine

[a]Conditioning backbone of fludarabine, cyclophosphamide, 200 cGy total-body irradiation, and ATG, unless otherwise indicated

[b]Both EFS and OS calculated, at time of last follow-up

[c]Includes *n* = 3 patients using MRD

[d]Both >grade 2 involving gut

[e]Reported as "no significant chronic GVHD"

[f]Deaths "mainly from infectious complications and MAS"

hydroxyurea. Both pediatric and adult patients will be eligible in separate cohorts, with adults (defined as 15–45.99 years of age) eligible based upon standard disease severity criteria and children (5–14.99 years of age) eligible with either silent or overt CNS infarct or hemorrhage, based upon a differential age-dependent assessment of the risk-benefit ratio of HSCT.

Disadvantages

The primary limitation to the PTCy approach in SCD patients has been the high rate of graft rejection with data from the most recent alterations in conditioning too premature to make any conclusions. In adult SCD patients with higher rates of multiorgan dysfunction and/or lower performance scores, this historically very-reduced-intensity conditioning regimen has been preferable, as patients with graft rejection have survived with autologous constitution in most instances [19]. Additional limitations to this approach include exposure to cyclophosphamide, with associated risks including infertility and secondary neoplasms, albeit at a lower total dose (129 mg/kg) than the myeloablative dosing used in initial matched related donor trials in SCD [20]. Finally, although PTCy has been promoted as a more selective in vivo T-cell depletion strategy, it does not fully eliminate the risk for posttransplant infections or prevent GVHD.

Ex Vivo T-Cell Depletion Approach

Rationale

In vivo T-cell depletion strategies such as with anti-T-cell antibodies (e.g., alemtuzumab, ATG) or chemotherapy (e.g., cyclophosphamide) have been shown to decrease GVHD risk in allogeneic HSCT [15, 19, 23–27]. However, these in vivo approaches put the patient at risk for off-target toxicities such as anaphylaxis-like reactions or serum sickness in the case of antibodies and infertility and other organ toxicity in the case of chemotherapeutic agents. Performing T-cell depletion of the transplant ex vivo has the potential to avoid the toxicities of in vivo depletion agents (Fig. 14.1b). In addition, more sophisticated methods of targeted depletion could allow for reducing specific T-cell subsets that cause GVHD while maintaining the populations and precursors that speed recovery of antimicrobial immunity after HSCT. One could also envision enriching or expanding populations (such as virus-specific T cells), to be added back to the hematopoietic stem cell graft to further enhance recovery. Ex vivo approaches also allow for larger doses of hematopoietic stem cells while maintaining a fixed T-cell dose, such that hematopoietic engraftment and count recovery can be achieved more quickly than seen with in vivo T-cell depletion, but without increasing GVHD risk.

Preclinical and Early Clinical Background

The discovery of the cell surface protein CD34 allowed for the identification (and eventual selection) of a cell population enriched for hematopoietic stem cells [28]. Using this marker, a minimum dose of cells allowing successful engraftment could be determined. In the autologous setting, where hematopoietic stem cells are collected and then reinfused after high-dose chemotherapy, a dose of 2×10^6/kg $CD34^+$ cells is adequate for recovery of hematopoiesis [29]. In the HLA-matched and closely matched (7/8 or 8/8) allogeneic setting, this threshold is also considered adequate for engraftment. However, in a highly HLA-mismatched setting (e.g., haploidentical transplant) where rejection is a significant concern, higher doses are needed. As reviewed by Reisner et al. [30], preclinical murine studies in the 1980s demonstrated that transplantation of a sufficiently high number of hematopoietic stem cells (after ex vivo T-cell depletion) could overcome the HLA barrier and allow engraftment without GVHD.

With this premise, ex vivo T-cell depletion that enriched the $CD34^+$ population was used in the HLA-mismatched familial donor HSCT setting for patients with malignant and nonmalignant diseases [31]. "Megadoses" of $CD34^+$ cells, in the order of 20×10^6/kg and collected by donor peripheral blood apheresis following G-CSF mobilization, were associated with quicker time to lymphocyte recovery without severe acute GVHD (e.g., grade III–IV). This was achieved in the absence of T cell add back and despite no long-term post-HSCT immunosuppression [32]. CD34-positive depletion has FDA approval for use as GVHD prevention in patients undergoing matched related HSCT for AML, but no FDA approval has yet been obtained in the setting of haploidentical HSCT. This method of ex vivo depletion with CD34-positive selection was utilized in the initial haploidentical HSCT trials for SCD [33], likely due to this strategy being most familiar.

One of the primary drawbacks of the CD34-positive selection approach is the removal of desirable immune cells from the graft, such as natural killer (NK) cells and γδ T cells that are not known to cause GVHD but are important for immune reconstitution. To address this issue, subsequent T-cell depletion strategies continue to be explored in HSCT for malignant disease and are undergoing investigation in hemoglobinopathies. These newer approaches (in increasing order of selectivity) include CD3-positive depletion that decreases all $CD3^+$ T-cell subtypes, αβ-T-cell depletion that decreases conventional T cells, and more recently CD45RA depletion approaches that deplete primarily naïve T cells [34–37].

Conditioning Regimens

In the initial trial using ex vivo depletion for haploidentical HSCT, the indication for HSCT in the majority of patients was a malignant diagnosis. It is likely for this reason that most of the conditioning regimens used involved myeloablation and/or radiation, with the primary goal being prevention of relapse. Reduced-intensity

conditioning, or the use of less intense agents to limit toxicity to the HSCT recipient, is a major focus of modern HSCT for nonmalignant disorders including SCD. However, reducing conditioning intensity increases the risk of rejection due to inadequate eradication of recipient lymphocytes. In SCD, there is less need to completely eradicate recipient hematopoiesis, as *stable* mixed donor chimerism as low as 10–20% has been associated with SCD symptom resolution [38, 39]. As residual alloreactive T cells were associated with rejection in the early trials of $CD34^+$ megadose transplantation [31], ongoing trials of haploidentical HSCT for SCD have elected to utilize either (1) a more intense myeloablative regimen including total lymphoid radiation (NCT01461837) or (2) a less intense regimen with ATG or alemtuzumab to deplete recipient T cells (NCT02165007). The optimal conditioning regimen, with low toxicity and low rate of rejection, is yet to be determined for SCD.

Donor Selection

Individual patients will often have more than one haploidentical family donor available, with no standard approach for selecting the best donor. As cell dose is a critical determinant of overall success with an ex vivo T-cell depletion approach, donor size is often the most important selection criteria. Other factors for consideration include the absence of SCD or thalassemia (sickle trait is not a contraindication); the presence of donor-directed anti-HLA antibodies in the recipient, donor, and recipient CMV serostatus; gender; ABO blood type; and the presence of anti-red cell antibodies in the donor and recipient.

NK cell alloreactivity in the donor has been shown to reduce relapse in HSCT for malignant disease and may reduce rejection in nonmalignant diseases; however, research is ongoing regarding its importance [40–42]. Additionally, there are differing theories on how to predict NK cell alloreactivity [43]. In one approach, the "best" donor is selected based on the killer immunoglobulin-like receptor (KIR) repertoire of the donor and the HLA-B and HLA-C alleles of the recipient. In this model, recipient HLAs serve as ligands for donor NK cell inhibitory KIRs, with the best donor one where the ligands of the recipient are not cognate to the donor inhibitory KIRs, allowing donor NK cells to remain reactive [44]. In a second predictive model, the haplotype of the donor KIR is determined (A or B), with donors with higher B content considered better [45]. NK alloreactivity has been extensively reviewed elsewhere [43] and is beyond the scope of this chapter.

GVHD Prophylaxis

Given that T-cell depletion is so effective with these ex vivo approaches, one of the potential advantages is eliminating the need for standard post-HSCT GVHD prophylaxis (e.g., cyclosporine). This advantage further includes less concern about

medication adherence, lower risk of PRES (a common issue in SCD from calcineurin inhibitors), decreased risk of hypertension and kidney dysfunction, and possibly faster immune recovery. Thus, in the absence of need for post-HSCT donor lymphocyte infusion(s), the risk of acute and chronic GVHD with ex vivo T-cell depletion is extremely low [31, 32, 36, 46–48]. However, this GVHD data comes mainly from patients with malignant disorders and is yet to be shown convincingly for SCD. Additionally, there is concern for higher rejection in haploidentical HSCT for SCD than for malignant disorders, and post-HSCT immunosuppression may be used to prevent rejection in addition to prevent GVHD. Nevertheless, ex vivo T-cell depletion has the potential to allow for a calcineurin-free regimen, thereby reducing the toxicity of the HSCT process particularly for SCD.

Clinical Trials

For hemoglobinopathies specifically, these ex vivo T-cell depletion approaches were investigated initially in thalassemia patients. In an Italian study, 31 thalassemia patients were transplanted with ex vivo T-cell depleted grafts, either G-CSF-stimulated apheresis or bone marrow products [49]. Graft processing included CD34-positive selection in addition to some grafts also undergoing CD3/CD19 depletion. Conditioning consisted of hydroxyurea, azathioprine, fludarabine, busulfan, cyclophosphamide, thiotepa, and ATG-Fresenius S, and GVHD prophylaxis consisted of cyclosporine for 2 months. Complications included graft rejection in seven patients (22.6%) and two deaths (6.5%) from viral complications of CMV and EBV; however, no patients developed GVHD and 22 patients (71.0%) were cured [49].

In SCD, only one single-center experience using this approach has been published to date (Table 14.2) [33]. In this report, eight patients received conditioning with either (1) fludarabine, thiotepa, busulfan, rabbit ATG, and OKT-3 ($n = 3$) or (2) hydroxyurea, azathioprine, busulfan, cyclophosphamide, thiotepa, and OKT-3; all patients received MMF for GVHD prophylaxis. Patients received two different ex vivo T-cell depleted grafts, with the first undergoing CD34-positive selection and the second undergoing CD3 depletion, with a goal of infusing a target 5×10^6 $CD34^+$ cells/kg recipient weight and a maximum of 1×10^5 $CD3^+$ T cells/kg recipient weight. Three of the eight patients (37.5%) had sustained engraftment with resolution of SCD, while three patients (37.5%) rejected the graft. Two patients (25%) died of cGVHD – one had received a large $CD3^+$ T-cell dose ($>1 \times 10^5$/kg) and the second developed GVHD after receiving a donor lymphocyte infusion to prevent rejection. In this latter patient, the development of severe GVHD and death following nonspecific T-cell infusion suggests that this treatment is not justified to prevent rejection, a considerably less significant complication.

A second SCD-specific study has adapted the pre-HSCT preparative regimen used in the Italian thalassemia experience [49] (NCT01461837), and preliminary

Table 14.2 Clinical trials utilizing CDER$^+$ selection approach for haploidentical HSCT for SCD

Location	Genotype	Age	Conditioning	HSC source	TNC dose (10e6/kg)	CD34 dose (10e6/kg)	CD3 dose (10e8/kg)	Neutrophil engraftment	1° Graft rejection	2° Graft rejection	aGVHD	cGVHD	EBV/CMV reactivation	OS[a]	EFS[a]	Median follow-up
St Jude; $n = 8$ (Ref. 33)	SS (6), Sβ^0 (2)	9 y (4.2–17.1)	rATG/Flu/TT/Bu/OKT3 (3) HU/Aza + Bu/TT/Cy/OKT3 (5)	PBSC	0.93 (0.14–18.9)	n/a	0.06 (0.01–0.17)	12.5 d (11–16)	0%	50% (4)	50% (4)[b]	37.5% (3)[c]	NR	75% (6)[d]	50% (4)	5.3 y (0.9–9)
Multicenter; $n = 7$ (Ref. 50)	NR	13 y (8–20)	HU/Aza + Flu/Bu/TT/Cy/rATG/TLI	PBSC	NR	NR	NR	9 d (9–13)	0%	0%	0%	0%	NR	86% (6)[e]	86% (6)	93 d (24–710)

HSCT hematopoietic stem cell transplantation, *SCD* sickle cell disease, *HSC* hematopoietic stem cell, *TNC* total nucleated cell, *1°* primary, *2°* secondary, *aGVHD* acute graft-versus-host disease, *cGVHD* chronic graft-versus-host disease, *EBV* Epstein-Barr virus, *CMV* cytomegalovirus, *OS* overall survival, *EFS* event-free survival, *SS* hemoglobin SS, *Sβ^0* hemoglobin Sβ^0 thalassemia, *y* years, *rATG* rabbit anti-thymocyte globulin, *Flu* fludarabine, *TT* thiotepa, *Bu* busulfan, *PBSC* peripheral blood stem cells, *d* days, *HU* hydroxyurea, *Aza* azathioprine, *Cy* cyclophosphamide, *NR* not reported, *TLI* total lymphocyte irradiation

[a]EFS defined as alive without graft rejection, both EFS and OS calculated at time of last follow-up

[b]Grade 1 ($n = 2$), grade 2 ($n = 2$)

[c]Limited ($n = 1$), extensive ($n = 2$)

[d]Deaths from complications of GVHD

[e]Death from sinusoidal obstruction syndrome

results are encouraging [50, 51]. As shown in Table 14.2 [50], more detailed results have been reported in the first seven patients treated, where engraftment occurred in all patients and no patients developed GVHD. Infections were uncommon, with only one viral infection and no serious bacterial infections. One patient died of sinusoidal obstruction syndrome, leading to overall and event-free survival of 85.7%. In an updated report that included 13 patients [51], there were no additional deaths, leading to a 92% event-free survival at 1 year; [51] additional details related to GVHD have not yet been reported in this larger cohort.

Additional clinical trials using ex vivo T-cell depletion approaches have the goal of finding a reduced-intensity conditioning regimen that avoids radiation but maintains a high rate of sustained engraftment; this includes both ongoing (NCT02165007) and completed but not yet published (NCT00968864) clinical trials.

Disadvantages

The ex vivo T-cell depletion approach does have drawbacks that have limited its more widespread adoption. First, the graft processing requires specialized and costly laboratory equipment and consumable reagents (antibodies, buffers, tubing, immunomagnetic selection columns) as well as specialized technician training and expertise. The processing itself is labor intensive and can take an entire day of technician's time per graft processed, thus requiring 2 days of processing with patients typically requiring two infusions. This is expected to change, however, as fully automated machines that require significantly less technician hands-on time are entering the market; these machines have the additional benefit of generating other cell therapies, such as virus-specific and tumor-specific T cells. As these latter cell therapies appear to be the future of HSCT, it is expected that centers will increasingly acquire such automated machinery, thus increasing the availability for ex vivo T-cell depletion of HSCT grafts. For centers without such machinery, grafts can alternatively be shipped to a central processing center, with the graft shipped post-processing back to the referring center for infusion.

A second significant disadvantage of ex vivo T-cell depletion is delayed immune recovery due to the extensive T-cell depletion of the graft. This is more of a concern with the CD34-positive selection approach, as immune recovery must be derived from hematopoiesis from the graft containing only hematopoietic stem cells (e.g., not containing more mature immune cells). Newer approaches are being investigated to more selectively deplete the graft (αβ T-cell depletion, CD45RA depletion, alloreactive T-cell depletion) to at least partially overcome this negative effect [52].

Some of these disadvantages are highlighted in Table 14.3, in which a comparison (of major factors to consider) is made between the PTCy and ex vivo T-cell depletion strategies.

Table 14.3 Comparison of haploidentical HSCT approaches

	Posttransplant Cy (PTCy)	Ex vivo T-cell depletion
Cost	Low cost (cyclophosphamide only)	High cost (equipment and supplies)
Feasibility	No additional facilities needed	Requires expertise for graft manipulation, ownership of device for T-cell depletion
Toxicity	High-dose cyclophosphamide toxicity to the gonads and other organs, possibly increased risk of malignancy	Very rare anaphylactic reaction to reagents used in depletion process
Graft source	Mostly bone marrow requiring bone marrow harvest of donor in operating room under general anesthesia	G-CSF mobilized peripheral blood stem cells collected by apheresis in outpatient setting
Neutrophil engraftment	~3 weeks	~1.5 weeks

HSCT hematopoietic stem cell transplantation, *Cy* cyclophosphamide, *G-CSF* granulocyte-colony stimulating factor

Potential Newer Approaches for Haploidentical HSCT

For a haploidentical HSCT approach to become a widely accepted and utilized treatment for patients with SCD, key outcomes such as sustained engraftment and survival must be maximized. Additionally, short- and long-term side effects must be minimized, so that the cure is not viewed as worse than the disease. First, GVHD, if it should occur, should be low grade (as can be achieved with both in vivo and ex vivo T-cell approaches); second, immune recovery should be rapid to avoid infection risk; and third, HSCT-related organ toxicity must either be temporary or not worsen organ function from baseline. To achieve these goals, it is likely that attempts to either lower doses of conditioning agents and/or replace more toxic medications (such as alkylating agents) with less toxic agents (such as antimetabolites and monoclonal antibody preparations) will continue. As graft rejection risk in general increases with reduction in conditioning intensity, modifications to regimens are likely to be stepwise, with incremental improvement over time.

Exciting advances in the field of cellular therapy show the potential to significantly improve outcomes following haploidentical HSCT for SCD, with the likely advantage of low additional toxicity. Coupled with the T-cell depletion techniques, alloreactive T cells responsible for GVHD could potentially be selectively removed from a donor graft, or donor or recipient cells could be generated and infused to prevent rejection, to prevent and/or treat GVHD, and/or to rapidly restore antimicrobial immunity. For example, this includes mesenchymal stromal cells (MSCs) which are pluripotent progenitors present in small numbers in the bone marrow niche where they support hematopoietic progenitors [53]. They have also been shown to downregulate the immune system and function in ways to modulate an overactive immune response [54–57], as occurs in allograft rejection and GVHD after HSCT. Preclinical studies have demonstrated that MSCs are able to improve marrow engraftment [58, 59] as well as block T-cell proliferation and activation,

inhibit monocyte proliferation and cytokine production, and induce regulatory T cells leading to the release of anti-inflammatory T-cell cytokines [54–57]. MSCs could be administered after donor graft infusion to suppress alloreactive T cells driving rejection as well as GVHD, which is the focus of an upcoming trial of haploidentical HSCT for SCD (IND 16872).

Although T-cell depletion can be quite effective, it can markedly delay virus-specific immunity (up to 1 year), leaving patients susceptible to life-threatening viral infections and disease, particularly from CMV, EBV, and/or adenovirus [60–62]. These viruses are almost universally acquired in childhood, remain dormant in host and donor tissues, and reactivate during the period of immunodeficiency after HSCT. For example, CMV and adenovirus can be detected in the blood or stool in up to 83.3% of pediatric patients undergoing haploidentical HSCT, with mortality (in HSCT in general) as high as 45% in patients who progress to overt disease [60, 63]. Virus-specific T-cell recovery has been shown to protect from life-threatening viral disease (e.g., adenoviral disease), making approaches to speed this recovery highly desirable [64]. Thus, ensuring immune recovery after haploidentical HSCT that is specific to multiple viruses will be vital, especially if it is to become more broadly acceptable for SCD patients.

Virus-specific T cells (VSTs) can be expanded ex vivo from healthy donors for clinical use to prevent or treat viral infections in post-HSCT patients [65–70]. By exposing donor-derived peripheral blood mononuclear cells to viral antigens in specific cell culture conditions, VSTs can be generated. In this technique, viral antigens provide a selective advantage that favors VST expansion and attrition of nonspecific and alloreactive T cells. VSTs generated from both the HSCT donor and third-party donors have been shown to effectively treat viral infections without causing significant GVHD [67, 71]. For patients undergoing HSCT for malignancy, VST infusions have rapidly restored immunity to the multiple viruses (e.g., CMV, EBV, and adenovirus) that cause appreciable morbidity in HSCT recipients [67, 72]. Given these promising results, donor-derived VSTs to prevent viral infections after haploidentical HSCT for patients with SCD could further improve the efficacy of the approach.

Conclusion

Haploidentical HSCT has the potential to expand a curative option to virtually all patients with SCD, given that almost every patient will have a suitable donor. Unfortunately, published approaches to date have not shown the tremendous success seen with matched related donor HSCT; thus optimization of this approach is required. Such improvements include reducing the rate of rejection while still maintaining a low rate of GVHD, enhancing immune recovery, and ensuring a high survival rate with minimal long-term side effects. The most recent approaches, largely using reduced-intensity conditioning, are encouraging with improved engraftment rates, but larger multicenter clinical trial results are needed to confirm these improvements. Newer low-toxicity methods of HSCT that include cellular therapies and

modern graft manipulation techniques are likely to be employed to achieve the desired excellent outcomes. Ultimately, it will be crucial to compare long-term outcomes of haploidentical HSCT to other standard non-HSCT medical approaches in a multicenter clinical trial (such as is being done for matched related and unrelated donor HSCT in BMT CTN 1503 (NCT02766465) in order to determine if this curative approach is worthy of widespread use.

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Chapter 15
Gene Therapy: The Path Toward Becoming a Realistic Cure for Sickle Cell Disease

Alexis Leonard and Allistair Abraham

Abbreviations

ACS	Acute chest syndrome
ADA	Adenosine deaminase
BM	Bone marrow
Cas9	Bacterial CRISPR-associated protein 9
CGD	Chronic granulomatous disease
cPPT	Polypurine tract
CRISPR	Clustered, regularly interspaced palindromic repeats
crRNA	CRISPR-targeting RNA
DSB	Double-stranded break
gRNA	Guide RNA
GVHD	Graft-versus-host disease
HbF	Fetal hemoglobin
HDR	Homology-directed repair
HEK293T	Human embryonic kidney cells 293
HPFH	Hereditary persistence of fetal hemoglobin
HS	Hypersensitivity site

This chapter is dedicated to Derek Persons, M.D., Ph.D. (1962–2015). A pioneer in the field of gene therapy for sickle cell disease, he made key contributions to the field, most important of which was to inspire the next generation to persevere in the goal of developing a widely available cure for this devastating disease.

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HSCs	Hematopoietic stem cells
HSCT	Hematopoietic stem cell transplantation
IDLV	Integrase defective lentiviral vector
LCR	Locus control region
LTR	Long terminal repeat
NHEJ	Nonhomologous end joining
PAM	Protospacer adjacent motif
PB	Peripheral blood
PBS	Primer binding site
PBSCs	Peripheral blood stem cells
PID	Primary immunodeficiencies
RRE	Rev response element
SCD	Sickle cell disease
SCID	Severe combined immune deficiency
SCID-X1	X-linked SCID
SIN	Self-inactivating
ssRNA	Single-stranded RNA
TALENs	Transcription activator-like effector nucleases
tat	Trans-activator of transcription
VCN	Vector copy number
VOC	Vaso-occlusive crisis
WAS	Wiskott-Aldrich syndrome
WPRE	Woodchuck hepatitis virus posttranscriptional regulatory element
ZFNs	Zinc-finger nucleases
Ψ	Packaging element

Historical Perspective

The concept that gene therapy may ameliorate human genetic diseases emerged in the 1970s. It has taken three decades for gene therapy to become a reality given a limited understanding of gene regulation and genetic recombination in the 1970s. The early days of gene therapy research were limited in the knowledge of how the molecular defect directly related to the disease state and inability to predict the short- and long-term effects of gene therapy in human patients [1]. Much of the literature in the early 1980s focused on the impact of genetic manipulation on society and medicine [2], and the moral and ethical issues surrounding human gene therapy [3], some of which continues today. While there are less ethical concerns for gene therapy used to treat somatic cells of patients with hemoglobinopathies compared to genetic modification of germ line cells, issues surrounding safety, efficacy, and feasibility have limited clinical applicability [4]. Many of the obstacles in gene therapy over the last three decades have broadened our knowledge, advanced the science of gene therapy, and made the concept of gene therapy to alleviate human disease a reality.

The idea that viruses could serve as a gene delivery system was first reported in 1968 [5]. Proof of principle was later demonstrated after the introduction of a bacterial gene into tumor-infiltrating lymphocytes tracked the persistence and localization of cells after reinfusion into patients with advanced melanoma [6]. Due to ethical concerns and the basic scientific knowledge to manipulate viral systems being in its infancy, it wasn't until 1990 that the first clinical trial in gene therapy opened [7]. Children with severe combined immune deficiency (SCID) due to mutations in the adenosine deaminase (ADA) gene were treated using ex vivo gene transfer into umbilical cord blood cells or autologous T lymphocytes. The earliest clinical trials in gene therapy focused primarily on gene therapy to correct primary immunodeficiencies (PID) such as X-linked SCID (SCID-X1), ADA SCID (SCID-ADA), chronic granulomatous disease (CGD), and Wiskott-Aldrich syndrome (WAS). PIDs were an attractive first step for gene therapy as they require ex vivo delivery of a single gene into stem cells, and for some, there is a natural in vivo selection for gene-corrected cells. This contrasts with hemoglobinopathies where selection for the corrected cell emerges post-stem cell differentiation in the erythroid lineage and does not provide selective advantage for the corrected stem cell.

The early PID gene therapy trials brought rare successes and major yet critically informative setbacks in the field of gene therapy. Murine gammaretroviruses (specifically the murine leukemia virus) were utilized as the ex vivo delivery system into autologous stem cells. In addition to low efficiency of viral transduction, lack of sustained engraftment, and incomplete reversal of phenotype, the biggest setback in the use of these gammaretroviral vectors was the transformation toward genotoxicity. Despite cure in some boys with SCID-X1 [8, 9], 5 of 20 patients developed leukemia with retroviral gene insertion near the LMO2 proto-oncogene requiring the trial to close early [10]. One of these five patients died, while the other four were able to be treated with chemotherapy. Seven of ten patients with WAS treated with a gammaretrovirus developed leukemia, two of whom died [11, 12]. The first three trials for CGD were unsuccessful due to poor engraftment [13–15], with 4 of 14 patients in later trials developing myelodysplastic syndrome [16–18]. For reasons that are not completely understood, more than 40 patients with SCID-ADA have been treated with gammaretroviral vector without the development of malignancy from vector-related genotoxicity [19–21].

Whereas the death of a patient on an in vivo gene therapy trial for ornithine transcarbamylase deficiency in 1999 lead to the NIH, FDA, and US attorney general launching investigations into the practice of gene therapy [22], investigators continued to learn from these setbacks and improve design, safety, and efficacy of viral vector-mediated gene transfer. For example, due to the development of insertional leukemia in the SCID-X1 trial [10], methods were developed to detect viral integration sites via highly sensitive polymerase chain reaction techniques. Integration site analysis in addition to basic changes to improve vector design substantially increased the safety of gene therapy for monogenic disorders. After learning from these setbacks came major successes throughout the 1990s and early 2000s. In 2009, *Science* magazine listed the "return of gene therapy" as a runner-up in the major scientific breakthroughs of the year [23], which included the successes of the SCID-ADA patients.

Effective gene therapy for hemoglobinopathies, including β-thalassemia and SCD, has always been an area of significant interest given the fact that, as monogenic disorders, they are amenable to gene modification and are diseases that carry significant morbidity, early mortality, and have no ideal long-term treatment for patients. While more than 300 gene therapy trials were registered with the NIH by 2000, the first trial in β-thalassemia was not opened until 2007, with first results published in 2010 [24]. SCD presents an even bigger challenge for effective gene therapy, with the first case report published in 2017 [25], though more clinical trials are currently underway. There is exciting preliminary data; however, unique challenges associated with gene therapy in hemoglobinopathies remain in the present day.

Premise of Gene Therapy

Gene therapy involves the delivery of a functional copy of the defective gene or manipulation of regulatory genes that are known to influence disease phenotype into a patient's own HSCs. This correction is achieved either via gene editing, gene silencing, or by gene insertion/addition. The delivery of the corrective gene relies primarily on a viral vector delivery system, though recent advances in human genome manipulation suggest nonviral forms of genome editing could have therapeutic success (electroporation, nucleofection, and lipofection). Current gene therapy is aimed at many genetic and acquired diseases where site-specific modification of the genome is possible and has since expanded to more widespread applicability as the science has flourished. Gene therapy provides the possibility for cure for patients with immunodeficiencies, CGD, hemophilia, and hemoglobin disorders. In addition, gene therapy can be a treatment for malignant hematologic disorders and viral infections and provide a suicide gene "kill switch" to treat GVHD.

The most widely used approach for gene therapy, and the method that is currently in clinical trials for patients with SCD, utilizes a viral vector system for the addition/insertion of targeted gene transfer. There are multiple gene delivery systems available that can be utilized ex vivo in the case of SCD or in vivo for the targeted correction of monogenic disorders in postmitotic tissues such as hemophilia. Ex vivo manipulation has the advantage of stable gene transfer with a lowered risk for off-target effects compared to in vivo gene transfer. In early gene therapy trials, T lymphocytes were transduced with the gene of interest [7], but now curative therapy for PID and hemoglobinopathies is focused on transduction of bone marrow CD34+ HSCs.

In brief, ex vivo gene transfer occurs after a patient's HSCs are harvested from bone marrow, peripheral blood, or umbilical cord blood (Fig. 15.1). HSCs are then genetically modified either by the addition, manipulation, or correction of the gene of interest via a viral vector system that integrates into the host genome. The modified cells are then transplanted back into the patient after myeloablative conditioning in order to facilitate as much marrow repopulation with genetically modified cells as possible. Genetic modification of induced pluripotent stem cells (iPSCs) is

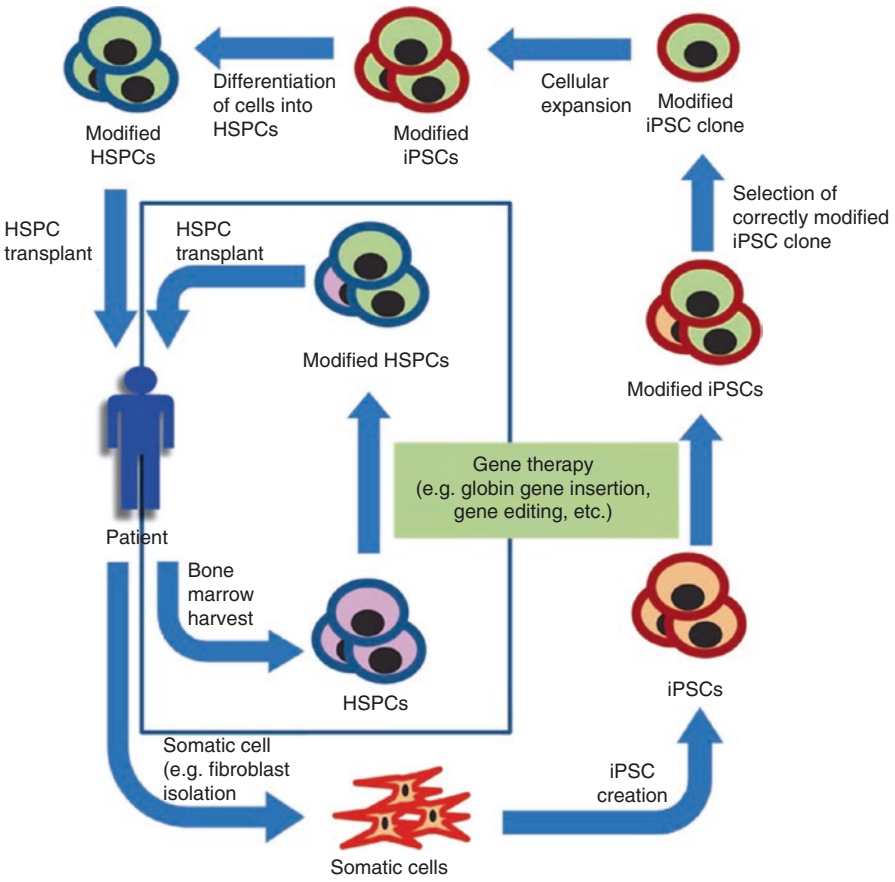

Fig. 15.1 Typical process of gene therapy for hematopoietic disorders. Hematopoietic stem and progenitor cells (HSPCs), induced pluripotent stem cells (iPSCs). HSPCs can be modified directly, as is the case for currently used therapies. Alternatively, somatic cells, e.g., fibroblasts, can be first reprogrammed into iPSCs before being modified. A correctly modified iPSC clone is then selected and expanded before being differentiated into HSPCs. In either case, the genetically modified HSPCs are then transplanted back into the patient. When HSPCs are modified directly, modification may not occur in every cell (Published in: Michael A. Goodman; Punam Malik; Therapeutic Advances in Hematology 7, 302–315. Copyright © 2016 SAGE Publications)

a future method for gene correction that is still in its infancy (Fig. 15.1). This method involves isolating a patient's somatic cells, reprogramming them into a pluripotent state, genetically modifying cells with the gene of interest, and expanding in vitro [26]. The expanded iPSCs are then differentiated into HSCs and transplanted back into the patient. While this method would reduce the need for a high-efficiency model that is currently required, this approach is currently limited by a poor repopulation potential.

Virally mediated gene transfer is an effective strategy for genetic modification as it takes advantage of the viral vector system that inserts its genetic material into the

target cell. The basic strategy of viral vector-mediated gene transfer is to package the transgene of interest into producer cells coupled with the individual viral proteins required for viral replication on independent expression cassettes (i.e., separate vector systems) thereby rendering the produced viral vector replication incompetent. The viral vectors produced from these packaging cells are then complete with the transgene of interest and all the packaging signals required for transduction and integration. They can then be transduced into the cell of interest for stable gene expression without an ability to form new virus.

The setbacks of the early clinical trials in gene therapy revealed the most significant risk of virally mediated gene transfer: genotoxicity. The integration of retroviruses is inherently mutagenic. In general, without a guide RNA (gRNA) directing the integration of the viral genome into a site-specific location, retroviral vectors integrate randomly but show a preference for transcriptionally active host genes. Furthermore, retroviruses contain sequences that are prone to activating nearby genes encoded by the host chromosome, creating the setup for transcriptional dysregulation. Alternative splicing, gene inactivation, truncation of cellular messenger RNA or protein, and microRNA activation via the generation of a truncated messenger RNA with missing regulatory elements are some of the risks of viral integration in the host genome. Most concerning in the SCID-X1 and CGD trials, however, was the tendency for integration in close proximity to gene regulatory regions such as promoters, enhancers, locus control regions (LCRs), or oncogenes that led to dysregulation, clonal dominance, leukemogenesis, and myelodysplasia.

Despite these risks, the advantages for gene correction in SCD are multiple, and the importance of this science has kept researchers actively involved in making gene therapy safer, more efficient, and available for patients. The burden of hemoglobinopathies worldwide as estimated by the World Health Organization in 2011 suggests that 5% of the world's population carries trait genes for hemoglobin disorders, mainly, SCD and thalassemia, and that approximately 300,000 babies with severe hemoglobin disorders are born each year [27]. In the United States, SCD affects approximately 100,000 Americans who have less access to comprehensive care teams than people with other genetic disorders like hemophilia and cystic fibrosis [28]. SCD is associated with early mortality [29] and is a public health concern with high health-care costs [30]. Curative therapy, either by HSCT or gene therapy, is an attractive option to reduce disease burden and improve outcomes for patients in addition to significantly reducing health-care costs. The lack of suitable marrow donors for the majority of patients with SCD, in addition to the morbidity and mortality associated with allogeneic transplantation, makes genetic modification of autologous stem cells attractive. Long-term engrafting HSCs, whether from a non-sickle donor in allogenic transplantation or from genetically modified autologous HSCs, allow for the expression of non-sickle β-globin in erythroid daughter cells. Stable mixed chimerism is enough to reverse the sickle phenotype in allogeneic transplantation [31]; therefore, if expressed at a high enough level, gene therapy is a potential curative option with single-use transplantation. In order for gene therapy to be a realistic curative therapy for SCD, there must be safe and efficient gene transfer into long-term HSCs, and there must be a high enough level of appropriately regulated and stable gene expression.

Successful gene therapy for SCD would therefore include four basic principles: (1) high-efficiency gene transfer into HSCs that is safe with minimal genotoxicity, (2) lineage-specific and developmental stage-specific expression of the inserted gene (i.e., erythroid lineage in hemoglobinopathies), and (3) consistent integration with high levels of expression of the inserted gene such that there is (4) phenotypic correction and disease amelioration.

Concept of Viral Vectors

In contrast to the gammaretroviral vectors that were utilized in many of the early clinical trials that led to genotoxicity, another vector derived from the family member of the *Retroviridae* family of retroviruses, the lentiviral vector, is known to have improved safety and efficacy of gene transfer. Unlike gammaretroviral vectors that led to the oncogenesis and myelodysplasia in the PID trials, lentiviral vectors tend to integrate into the body of genes rather than into regulatory regions, lowering the risk of genotoxicity associated with virally mediated gene transfer. Other advantages of lentiviral vectors are numerous: low immunogenicity and lack of prior immunity, ability to efficiently translocate the intact nuclear membrane and therefore transduce nondividing/quiescent cells, ability to persist in transduced cells for long prior to integration allowing for a longer window for integration, ability to carry a larger genetic material, and ability to be designed with alternative envelopes such as with vesicular stomatitis virus glycoproteins, providing broad tropism and enabling effective transduction of target cells like CD34+ HSCs. Still, the greatest risk of gene therapy is oncogenic transformation, which is not entirely eliminated by lentiviruses. To understand the risks of gene therapy despite the viral vector of choice, the concepts of gene therapy must be understood at a molecular level.

Retroviruses

Retroviruses are the preferred vector for stable gene transfer into proliferating cells primarily for their ability to integrate into the host cell genome. This contrasts with genetic correction of monogenic disorders in postmitotic tissues such as hemophilia where adeno-associated virus-derived vectors modify hepatocytes without integrating into the genome. A non-integrating approach is not applicable to gene therapy for SCD as integration into HSC genome is needed for gene modification to persist in red cell progenitors that differentiate from the HSCs. Retroviruses are named for their unique reverse transcriptase which allows permanent incorporation of its genetic material into the DNA genome of an infected cell. In addition to two identical copies of a positive sense single-stranded RNA (ssRNA), ranging between 7 and 12 kb, all retroviruses contain three major coding domains with all the information for virion production: gag, pol, and env (Fig. 15.2a) [32]. The gag coding domain contains the nucleoprotein structures and virion proteins that form the capsid; pol

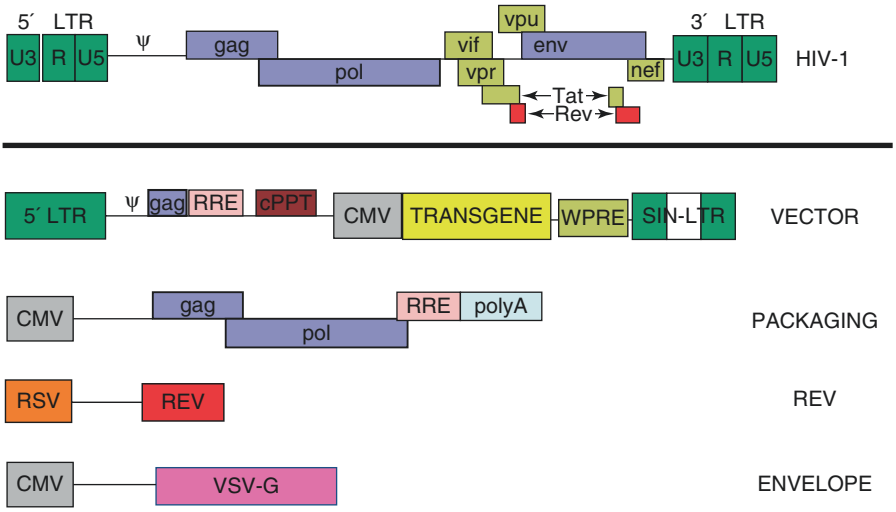

Fig. 15.2 Schematic of HIV-1 and third-generation lentiviral packaging system. (**a**) The HIV-1 contains three gene regions gag, pol, and env along with accessory proteins and the flanking long terminal repeats (LTR); (**b**) The lentiviral components found in the four plasmids used in generating third-generation lentiviral vectors. The vector plasmid contains a self-inactivating 3′ LTR (SIN-LTR), a Rev. responsive element (RRE), a central polypurine tract (cPPT), and the Woodchuck hepatitis virus posttranscriptional response element (WPRE). The psi sequence (Ψ) allows for efficient incorporation of the vector RNA genome into particles. In this schematic, the CMV early promoter is used for transgene expression, but other promoters are commonly substituted. The packaging plasmid expresses the gag and pol gene regions of HIV-1 which encode proteins required for virion formation and vector processing. This plasmid also contains an RRE. A plasmid-expressing rev is provided to facilitate nuclear transport of RRE-containing transcripts. The fourth plasmid is the envelope plasmid. Lentiviral vectors are commonly pseudotyped with the vesicular stomatitis virus G glycoprotein (VSV-G) as an alternative to the native HIV-1 envelope to increase the range of cell types and animal species susceptible to vector transduction (Shaw A, Cornetta K. Design and potential of non-integrating lentiviral vectors. Biomedicines 2014, 2(1), 14–35)

contains the reverse transcriptase and viral integrase; and env derives the surface and transmembrane components of the viral envelope that confers retroviral specificity. Additional, smaller, coding domains that flank the gag coding region are pro and psi (Ψ), encoding the virion protease and packaging signal that targets the RNA genome to the capsid, respectively (Fig. 15.2a).

The HIV lentiviral vector system is uniquely suited for gene editing. It contains the additional protein regulators of expression of virion proteins (rev), which regulate viral protein expression, and trans-activator of transcription (tat), which drastically enhances the efficiency of viral transcription (Fig. 15.2a). The rev regulatory domain encodes a nuclear localization signal, allowing the rev protein to be localized to the nucleus, in addition to harboring a nuclear export signal [33]. Once in the nucleus, rev interacts with an HIV rev response element (RRE) allowing HIV messenger RNA to be exported from the nucleus to the cytoplasm for downstream

events such as translation and virion packaging [34]. In the absence of rev, HIV messenger RNA required for elements such as structure, packaging, and release is retained in the nucleus, preventing translation. Such ability to translate in the nucleus for integration is essential during the creation of the viral vector; however, given the desire to create a replication incompetent vector once in the target cell of interest (i.e., HSCs), it is important that the rev element is separate and not included in the vector with the transgene of interest.

Retroviral Life Cycle

After entry into a target cell (via env), viral RNA is reverse transcribed into a double-stranded cDNA (via pol). This reverse-transcribed cDNA then translocates to the nucleus (via rev) and integrates into the host cell genome through the action of retroviral integrase (via pol), resulting in stable and permanent integration of the viral genome into the host cell. Viral transcription is then accomplished by host cellular RNA polymerase, generating full-length and spliced messenger RNAs as well as full-length progeny virion RNA. Rev + RRE allows viral messages to be translated in the cytoplasm where virion proteins and progeny RNA assemble at the cell periphery and the plasma membrane (via gag, pro, Ψ), and progeny virus is released by a process of budding and subsequent maturation into infectious virus [32]. Tat enhances efficiency of viral transcription, yet none of this would be accomplished without a strong signal for gene expression, located in the viral LTRs.

Long Terminal Repeats

The entire retroviral genome encoding its structural and enzymatic proteins is flanked by two LTRs that are essential for the initiation of viral DNA synthesis, integration of proviral DNA, and the regulation of viral gene expression. All of the required signals for gene expression are found in the long-term repeats (LTRs): enhancers, promoters, regulatory elements, areas of transcription initiation and termination, and capping with a polyadenylation signal [35]. Each LTR is segmented broadly into a U3, R, and U5 region (Fig. 15.2a). The 5′ U3 region encodes the viral enhancer/promoter elements which are required to generate a full-length viral transcript. The R region is a repeat region where genomic transcription begins. Once the provirus has undergone reverse transcription and integration, the LTR on the 5′ end serves as the promoter for the entire retroviral genome. The entire LTR is flanked by a primer binding site (PBS), which binds to the tRNA-Lys primer required for initiation of RT, and the packing element, Ψ, which is required for incorporation of vector RNA into particles.

Modification of Retroviruses for the Purpose of Gene Therapy

The major safety concerns with utilizing retroviral vectors in human therapy are twofold: (1) the risk generating a replication-competent virus and (2) creating insertional mutagenesis. To prevent the creation of a replication-competent virus, removal of the genetic elements responsible for viral pathogenicity and virulence is necessary. To avoid insertional mutagenesis, removal or silencing of the LTR enhancer/promoter that upregulates gene expression to very high levels is necessary to avoid simultaneously activating proto-oncogenes that may flank the insertion site.

Replication-Incompetent Viral Vector

The concept of gene therapy to correct monogenic disorders is made possible by taking advantage of a retrovirus's ability to infect and permanently integrate a gene of interest into the host cell. Once integration occurs, however, there is no need for the creation of viral progeny, and the use of retroviral machinery is no longer needed. To accomplish this, the viral vector must contain the gene of interest with the ability to integrate, but all the necessary elements for packaging and processing during vector production must remain separate in order to remain replication incompetent.

To make a replication defective vector, gene therapy systems utilize separate vector plasmids that are introduced into packaging cells [most commonly human embryonic kidney cells 293 (HEK293T) which have reliable growth and propensity for transfection] by transient transfection (Fig. 15.2b). The primary plasmid removes the major coding regions gag, pol, and env and replaces them with the therapeutic transgene of interest. The LTR and Ψ elements are retained to allow transgene expression and packaging into viral capsids during vector production; however, by removing the gag, pol, and env proteins, daughter viral progeny can no longer be produced. Often a polypurine tract (cPPT) and enhancer located in the 3′ untranslated region of coding sequences [i.e., Woodchuck hepatitis virus posttranscriptional regulatory element (WPRE)] is added to increase vector production and expression in a transgene-, promoter-, and vector-independent manner [36].

Because gag, pol, and env are essential coding regions to produce a transgene vector, a second separate packaging plasmid with gag, pol, and RRE provides the structural proteins for the initial virion formation and genome integration and is critical for generating a high-titer vector (via gag) (Fig. 15.2b). A third plasmid contains rev to activate the RRE engineered into the transgene, acting with the gag/pol plasmids, facilitates nuclear transport, and acts as a critical safety feature as it is not incorporated into the final viral genome. The fourth plasmid contains the env coding region, allowing engagement of the receptors on the target cell. By separating the machinery necessary to create a replication-competent virus, multiple separate recombination events would be required to generate a replication-competent vector which is highly unlikely.

Modifications of the LTR

For a replication-competent retrovirus, the LTR serves as an essential enhancer that initiates viral production and upregulates viral expression to a very high level. In the application of gene therapy, an intact LTR is unethical for human application given its propensity to integrate in gene bodies of expressed genes with an ability to simultaneously activate cellular proto-oncogenes [37]. Furthermore, methylation of the LTR can lead to the inactivation of the promotor and silencing of long-term transgene expression [38]. A critically important safety feature is therefore to silence the LTR and instead rely on an internal cellular promoter to drive transgene expression, such as ankyrin or a promoter of hereditary persistence of fetal hemoglobin (HPFH) in the case of β-hemoglobinopathies. To create this safety feature, the generation of a self-inactivating LTR (SIN-LTR) incorporates a large deletion in the U3 region of the 3′ LTR (U3 region of the 3′ LTR is copied into the 5′ LTR after reverse transcription) such that any progeny will contain two inactivated LTR after reverse transcription. Transgene expression is therefore no longer dependent on viral LTR and instead under the control of the added internal promoter [39, 40].

Production of Drug Product

Viral vectors are made by transfecting packaging cells, most commonly the cell line HEK293T cells, with plasmid DNA encoding the therapeutic transgene of interest and separate retroviral plasmids containing the necessary packaging elements (Fig. 15.3). Maximal vector production occurs 48–72 h after transfection, whereupon vector supernatant is collected. Vector particles are purified and concentrated to approximately 10^5–10^7 infectious units/mL and are ready to be used for transduction into the target cell, CD34+ HSCs.

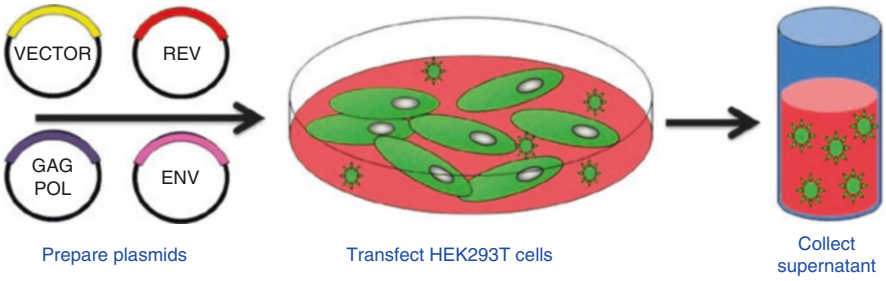

Fig. 15.3 Generation of lentiviral vector by transient transfection. The four packaging plasmids are transfected into cells that have a high capacity for vector production. The most commonly used cell line is HEK293T. Maximal vector production occurs 48–72 h after transfection. The vector particles are released into the media which is collected and clarified of cell debris. Vector particles can be further purified and/or concentrated (Shaw A, Cornetta K. Design and potential of non-integrating lentiviral vectors. Biomedicines 2014, 2(1), 14–35)

It is generally agreed upon that CD34+ HSCs need to leave G0 and be in G1 phase for optimal retroviral transduction. Cytokine stimulation has historically been used to recruit quiescent HSCs into the active cell cycle to increase transduction efficiency; however, this must be balanced with overstimulation as this will induce cell differentiation and ultimately reduce engraftment by reduction of long-term populating HSCs (CD34+/CD38−/lin−). One of the advantages of using a lentiviral vector over another retroviral vector such as the gammaretroviral system is that lentiviral vectors can transduce both proliferating and non-proliferating cells [41]. Previous data suggests improved human CD34+ cell transduction with lentiviral vector systems after cytokine stimulation compared to no stimulation as evaluated by colony-forming unit assay and long-term culture-initiating cell assay [42, 43].

While the viral vector is being produced, a patient's CD34+ HSCs are collected via bone marrow harvest or peripherally via apheresis following mobilization. The HSCs are isolated via immunoselection and cultured in flasks using serum-free media containing stem cell factor, FMS-related tyrosine kinase 3 ligand, and thrombopoietin for approximately 24 h [44]. Prolonged cytokine stimulation increases transduction efficiency (% expression of transgene) but at the expense of reduced HSC repopulating ability. The ideal transduction methods that preserve engraftment while improving HSC transduction remain under investigation.

Following stimulation, CD34+ cells are transduced with the viral vector particles at a multiplicity of infection (MOI) of 5–50. High-vector MOI yields much lower transduced cells because many CD34+ cells with high viral vector concentrations die after transduction with high MOI [44]. After 24 h of transduction, transduced cells are collected and ready for infusion into the patient who has already undergone myeloablative conditioning in preparation for transplantation.

Beta-Globin Expressing Vectors

Genetic correction of the sickle cell allele in a patient's autologous HSCs is a promising potential cure for patients with SCD. Potential methods for gene therapy in SCD include addition of β-globin to make adult hemoglobin (HbA), addition of γ-globin to increase fetal hemoglobin (HbF) expression, direct gene editing of the sickle mutation, editing of globin regulatory elements, or knockdown of HbF repressors to increase HbF expression (Fig. 15.4). Several groups have been working on gene therapy for SCD for the last three decades [45] and have made many critical advances in our understanding of the β-globin gene cluster and the modifications required for effective gene therapy for hemoglobinopathies. Critical regulatory elements required for high level of globin expression are better understood, and the improvement in both viral vector systems and methodologies for gene therapy has enabled gene therapy for SCD to now enter clinical trials and reach patients.

There have been several challenges associated with β-globin expressing vectors. As mixed chimerism studies have taught us from allogenic HSCT studies [46, 47], gene therapy for SCD must attain efficient, high-level erythroid-specific expression

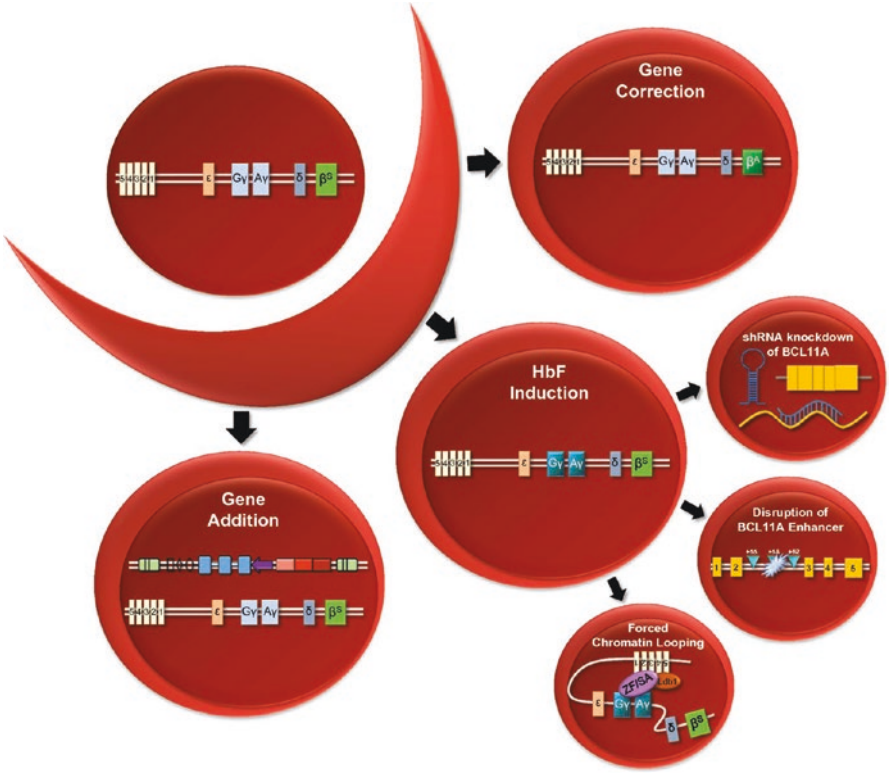

Fig. 15.4 Strategies for gene therapy for SCD: schematic overview of various approaches for correcting the sickle phenotype via gene therapy. Gene correction: targeted genome engineering leads to correction of the sickle mutation such that β^S is repaired as β^A. HbF induction: multiple strategies for induction of γ-globin expression include shRNA-mediated knockdown of BCL11A, targeted disruption of the +58 DNase I HS site in the BCL11A erythroid-specific enhancer, and forced chromatin looping to promote association of the β-globin LCR with the γ-globin genes. Gene addition: integrating lentiviral vector carrying a β-globin, γ-globin, or anti-sickling β-globin cassette. Ldb1, Transcription factor; ZF/SA, Zinc-finger self-association domain. Hoban M, Orkin S, Bauer D. Genetic treatment of a molecular disorder: gene therapy approaches to sickle cell disease (Blood. 2016 Feb 18; 127(7): 839–848)

of the corrected hemoglobin to overcome the pathologic, shortened lifespan of sickle cell RBCs. At least 15–20% of all engrafted HSCs must express the therapeutic globin gene in order to overcome the pathologic phenotype [48]. The identification of critical regulatory elements needed for this high expression of β-globin has made gene therapy for SCD possible. The β-globin locus control region (LCR) is a powerful erythroid-specific enhancer required for high-level globin gene expression. It is 40–60 kB upstream of the β-globin gene and is composed of five DNAase-1 hypersensitivity sites (HS) linked to downstream globin genes. An intact LCR (5′HS1–5) maintains an open chromatin conformation for globin expression. HS2, HS3, and HS4 carry the enhancer activity of the LCR and are the binding sites for

ubiquitous and erythroid-specific transcription factors. Early retroviral studies without the LCR elements showed <1% β-globin expression [49].

In 2000, the first group to cure β-thalassemia in a mouse model demonstrated transgene expression of nearly 20% of the total hemoglobin using a human β-globin lentiviral vector with LCR fragments (TNS9 vector) (Fig. 15.5) [50, 51]. A similar β-globin vector with a shortened LCR sequence (β^A) was also able to cure

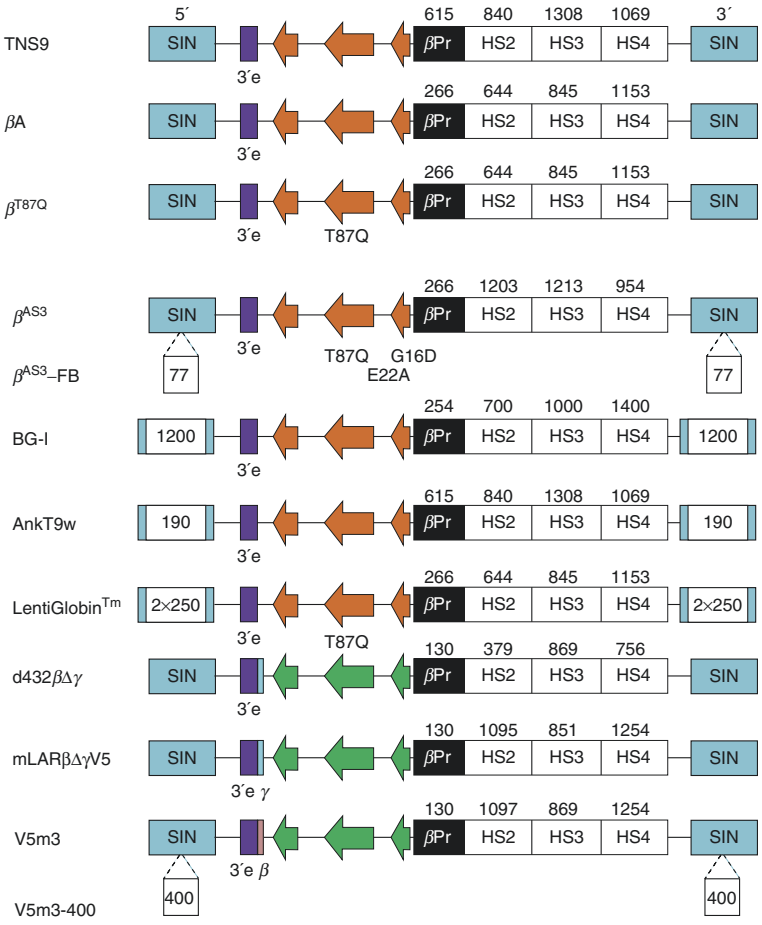

Fig. 15.5 Globin lentiviral vectors used to correct murine and human models of β-thalassemia and sickle cell disease. Schematics of the integrated provirus genome for lentiviral vectors used by different groups. All vectors are SIN. Highlighted are the constellation of the DNase I HS2, HS3, and HS4 for each LCR and β-globin promoter (βPr, *black box*) sequences that are critical for high-level, erythroid-specific expression; the genomic globin sequences (*orange* or *green*); 3′ UTR sequences (γ: *turquoise*, β: *pink*); 3′ enhancer (3′e: *purple box*) and insulator elements (*white boxes*). Therapeutic globin sequences are in reverse orientation and include β-globin (*orange arrows*) or γ-globin (*green arrows*) with amino acid mutations indicated. The length (in base pairs) of each HS, β-globin promoter, and insulator element is indicated (Villamizar, O., Chambers, C. B. and Wilber, A. 2014. Gene therapy for severe haemoglobin disorders. eLS. 15 OCT 2014)

β-thalassemia in a mouse model but also demonstrated that the hematologic and pathologic improvement depended on a vector copy number (VCN) of at least three in each HSC [52]. Based on the success of the TNS9 and β^A lentiviral vectors which utilized a wild-type β-globin gene, a similar lentiviral vector using a mutant β-globin gene where glutamine is substituted for threonine at amino acid 87 (β^{T87Q}) was attempted in an SCD mouse model [53]. This substitution confers two advantages: (1) glutamine is present in γ-globin and is thought to promote anti-sickling activity and (2) the modifications confer ability to distinguish β^{T87Q} from wild-type adult β-globin (i.e., from potentially recently transfused blood) by HPLC, allowing for direct quantification of hemoglobin production from the transduced drug product. Studies showed that the β^{T87Q} vector, which contains a smaller 2.7k kb LCR, had enough β^{T87Q} expression to resolve anemia and reduce organ damage in mice with three vector copies per HSC. A different vector (β^{AS3}) added additional changes to β^{T87Q} by adding two additional γ-globin-based substitutions that may further limit sickling of RBCs and used a 3.4 kb LCR. This construct was able to express 20–25% β^{AS3} at 2.2 vector copies per HSC. Organ damage was reduced, but anemia was only partially improved [54]. Other vectors that introduce the γ-globin gene to increase fetal hemoglobin have been successful and are illustrated in Fig. 15.5 [48, 55].

Other challenges associated with β-globin expressing vectors that have been addressed include size of the transgene and reduction of gene silencing. For hemoglobinopathies in general, viral vectors were initially insufficient to carry the large globin gene and the regulatory elements required for high-level expression. Vector design has since improved with the use of human immunodeficiency virus (HIV)-based lentiviral vectors and modified LCRs. As for retroviral potential to undergo expression variation and silencing, the addition of insulators such as the chicken hypersensitive site-4 (cHS4), ankyrin (AnkT9W), and FB (β^{AS3}-FB) insulators may reduce gene silencing and reduce position-dependent variability in gene expression, therefore promoting safety and efficiency of gene expression [56–59].

The ideal β-globin expressing vector would therefore include, in reverse orientation, the genomic globin sequence of interest, the human β-globin LCR and promoter that contains HS2, HS3, and HS4 for high-level, long-term, erythroid-specific expression from the β-globin promoter, a 3′ untranslated SIN region, and potentially an insulator element in the 3′ LTR to prevent gene silencing or variable activation of the transgene itself, or of nearby genes.

Newer Genomic Engineering Approaches

Much that has been discussed for gene therapy in SCD above relates primarily to the concept of gene addition, the current approach for gene therapy in SCD that has moved into clinical trials. There are, however, advances in genomic engineering that have expanded the possible genetic therapeutic options for many hematopoietic disorders. These methods include gene knockdown of HbF regulators to improve the β-hemoglobinopathy phenotype, gene editing of globin regulatory elements, and direct globin gene editing with targeted nucleases such as zinc-finger nucleases

(ZFNs), transcription activator-like effector nucleases (TALENs), meganucleases, and the clustered, regularly interspaced palindromic repeats (CRISPR)-associated nuclease Cas9 (Figs. 15.4 and 15.6).

Targeted Gene Editing

Targeted gene editing has revolutionized the field of gene editing and is ideal given the ability to perform precise genome manipulation. ZFNs, TALENS, meganucleases, and the CRISPR-Cas9 system each have designable DNA-binding motifs that allow for double-strand breaks (DSB) in the human genome with site specificity (Fig. 15.6). The advantages over gene addition include precise gene correction and the ability to significantly reduce or entirely avoid nonspecific integration that may lead to insertional oncogenesis.

ZFNs and TALENs are artificial fusion proteins that are coupled with a nonspecific nuclease (FokI restriction enzyme). ZFNs have inherent difficulties in design and validation and are limited by their coupling with the FokI nuclease. The FokI nuclease cleaves without sequence specificity and instead requires dimerization,

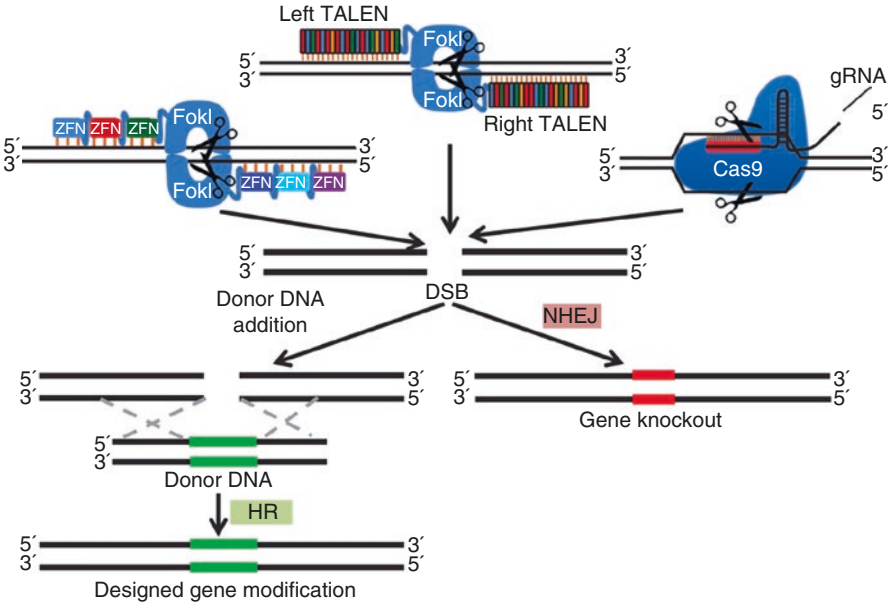

Fig. 15.6 *Cas* CRISPR-associated protein, *CRISPR* clustered regularly interspaced short palindromic repeats, *DSB* double-strand break, *gRNA* guide RNA, *HR* homologous recombination, *NHEJ* nonhomologous end joining, *TALEN* transcription activator-like effector nuclease, *ZFN* zinc-finger nuclease (Published in: Michael A. Goodman; Punam Malik; Therapeutic Advances in Hematology 7, 302–315. Copyright © 2016 SAGE Publications)

creating the potential for undesired breaks. Similarly, TALENs are chimeric nucleases that require engineering a DNA-binding domain coupled with a FokI nuclease. The highly repetitive nature of TALEN-coding sequences creates barriers to delivery using certain viral vectors, such as lentiviruses. Meganucleases, which recognize relatively long (14–40 bp) target sequences, are limited in their application by low frequency of target site presence at most genes, and thus many genes of interest cannot be edited.

Unlike the aforementioned protein-based editing mechanisms, the major advantage of CRISPR-Cas9 is its RNA-guided system. Instead of using a protein-guided dimer for sequence recognition, CRISPR/Cas9 technology uses a short gRNA with a defined 20 bp sequence complementary to the DNA sequence to be targeted. The system consists of a gRNA and a Cas protein, which is also known as a bacterial CRISPR-associated protein 9 nuclease from *Streptococcus* pyogenes. Bacteria have adapted to express genomically encoded RNAs to guide nuclease cleavage to matching sequences of invading phage and plasmid DNA. These gRNAs are essential for protecting against invasion by viruses by introducing DSBs in invading plasmids. The gRNAs are also well designed for genomic manipulation in human cells given the unique and flexible RNA moiety that targets the nuclease to a desired DNA sequence as opposed to the creation of a novel protein to be engineered for each new target site that is required for ZFNs, TALENs, and meganucleases. The critical components of the *Streptococcus* pyogenes CRISPR-Cas9 system include three necessary components: Cas9, the large multifunctional protein nuclease which produces blunt double-stranded breaks; CRISPR RNA/gRNA, the DNA-binding 20 nucleotide gRNA sequence that has precise complementarity to its DNA target and forms a complex with Cas protein to direct the protein to the correct genomic location; and the trans-activating CRISPR-targeting RNA (crRNA) bridging the crRNA to Cas9. Recognition of a target site by Cas9 depends on the presence of a protospacer adjacent motif (PAM) sequence NAG or NGG immediately downstream of the gRNA target sequence.

The crucial first step in gene editing is the creation of a DNA DSB at the genomic locus to be modified. From there, nuclease-induced DSBs can be repaired by one of two different pathways via endogenous cellular repair machinery: (a) nonhomologous end joining (NHEJ), which is the direct fusion of the nuclease cleaved ends, or (b) homology-directed repair (HDR), which relies on an exogenous DNA template flanked by homologous arms that align with the DNA sequences surrounding the region to be modified for targeted insertion (Fig. 15.6). Genetic correction is dependent on HDR; however, NHEJ is the default repair mechanism and is favored by the cell over correction by HDR which is dependent on the timing in the cell cycle. The genetic material for HDR must be present in the nucleus at the time of repair, and if no donor DNA is provided, DSBs will be corrected by NHEJ. NHEJ is the dominant pathway in G1, S, and G2 phases, whereas HDR preferentially occurs during the late S and G2 phases which are less favorable for maintaining quiescent long-term repopulating CD34+ HSCs. Furthermore, NHEJ is error prone; a significant proportion of repair events result in insertions or deletion of base pairs (indels) at the site of the break and can easily disrupt the translational reading frame. Such disruption

can lead to the inadvertent generation of β-thalassemia alleles in the process of β-globin repair.

The advantages to site-specific gene correction include the reduction in off-target insertion, transient delivery of the engineered nuclease and repair template to achieve correction, and lack of permanent insertion of foreign DNA into the genome. A side-by-side comparison of ZFNs, TALENs, and CRISPR/Cas9 in their ability to modify the β-globin locus in iPSCs demonstrated superiority in the CRISPR/Cas system [60]. ZFNs have demonstrated ability to correct human CD34+ HSC both from umbilical cord blood and mobilized peripheral blood; however, the rates of correction in long-term HSCs were suboptimal and well below levels necessary for therapeutic benefit [61]. A unique TALEN system that inserted the entire β-globin cDNA immediately in front of the native β-globin gene prevents read through of the native β-globin given a polyadenylation signal at the end of the engineered cDNA and allows for applicability for treatment of any mutation found within the β-globin gene [62].

Reactivation of Fetal Hemoglobin

Given decreased clinical severity in patients with SCD and high fetal hemoglobin levels, including HPFH, researchers have attempted to exploit many of these genetic engineering methods to increase the production of fetal hemoglobin. Such methods include a short hairpin RNA-mediated knockdown of BCL11A (a known repressor of fetal hemoglobin production) [63], targeted disruption of the +58 DNase I HS site in the BCL11A erythroid-specific enhancer [64], and forced chromatin looping to promote association of the β-globin LCR with the γ-globin genes [65]. Other attempts have focused on modifying globin regulatory elements. Kruppel-like factor 1 (KLF1) is an erythroid-restricted transcription factor that is critical for β-globin expression. γ-globin levels were increased 18-fold in a mouse model that inserted a binding site for the KLF1 transcription factor into the promoter region for γ-globin and corrected the pathologic anemia [66]. A combined approach of gene addition with HbF induction may prove superior to a single modality, and proof of principle has been shown [67].

Current Clinical Approaches for Gene Therapy in Hemoglobinopathies

Many years of preclinical work and advances in gene therapy for hemoglobinopathies have enabled this critical research to now reach patients. Several clinical trials for SCD gene therapy are ongoing, with preliminary results showing promise in a cure for SCD.

The first clinical trial evaluating patients with transfusion-dependent β-thalassemia was a phase I/II trial that opened in 2007 (HGB-204, NCT01745120) [24]. To date, 18 patients aged 12–35 years have been treated per HGB-204 [24, 68–71]. In this trial, patient CD34+ HSCs were collected via bone marrow harvest, stimulated and transduced with the β^{T87Q} vector carrying two copies of a 250 bp cHS4 insulator in the 3′ LTR (LentiGlobin™) and were reinfused after myeloablative busulfan conditioning. The first report of HGB-204 noted appearance of a partially dominant population of myeloid cells secondary to alternative splicing, though this particular population lost dominance, and the patient remains blood transfusion independent [24, 68]. Of the remaining patients, all individuals with non-Hb $\beta^0\beta^0$ thalassemia (HbEβ^0, Hb$\beta^0\beta^+$, $\beta^+\beta^+$ thalassemia) became transfusion independent within a year of transplant, with a median vector-derived increase in hemoglobin of 4.7 g/dL and a median total hemoglobin of 11.6 g/dL [69–71]. Though the patients with Hb$\beta^0\beta^0$-thalassemia remain transfusion dependent, they had a similar vector-derived hemoglobin increase and experienced a 60% reduction in their transfusion requirements. The follow-up study, HGB-205 (NCT02151526), which includes transfusion-dependent β-thalassemia or severe SCD, has enrolled four transfusion-dependent thalassemia patients in addition to the first sickle cell patient, a 13-year-old male with HbSS whose individual results were recently published [25]. He had a history of recurrent vaso-occlusive crises (VOCs), two episodes of acute chest syndrome (ACS), cerebral vasculopathy, bilateral hip osteonecrosis, and had undergone cholecystectomy and splenectomy. Given lack of reduction of symptoms despite being on hydroxyurea from ages 2–9 (average of 1.6 SCD-related events annually), he was started on prophylactic, monthly red cell transfusions in 2010. He enrolled on HGB-205 in May 2014 and received the drug product, LentiGlobin BB305, in October 2014 after myeloablative busulfan conditioning. β^{T87Q} levels steadily increased and contributed 5.7 g/dL (48%) of the patient's hemoglobin at 15 months posttransplant with a reciprocal decrease in HbS levels to 5.8 g/dL (49%) (Fig. 15.7). Red cell transfusions were discontinued on day 88, and total hemoglobin levels were stable between 10.6 and 12.0 g/dL 6 months post-gene therapy. Most importantly, the patient remained free of any SCD-related clinical events or hospitalizations 15 months post-gene therapy, and no adverse events related to the LentiGlobin-BB305 transduced stem cells have been reported to date. As for the remaining thalassemia patients on HGB-205, three patients with Hb$\beta^E\beta^0$-thalassemia are transfusion independent with total hemoglobin of 10.9, 11.3, and 13.5 g/dL, respectively, with HbA-T87Q expression levels of 7.7–10.1 g/dL. The fourth patient is homozygous for a severe β-globin mutation and has not needed a transfusion in the 3 months since drug product transfusion (total Hb of 8.3 g/dL) [72].

Following the success of the European HGB-205 trial, HGB-206 was opened as an open-label Phase 1 study in the United States designed to evaluate the safety and efficacy of LentiGlobin BB305 product in up to 29 adults with severe SCD (NCT02140554). As of December 2016, seven patients with severe SCD have been enrolled [73]. Subjects range between 18 and 42 years of age and have histories of recurrent VOCs (6), stroke (1), ACS (6), and chronic transfusion (1). Follow-up is

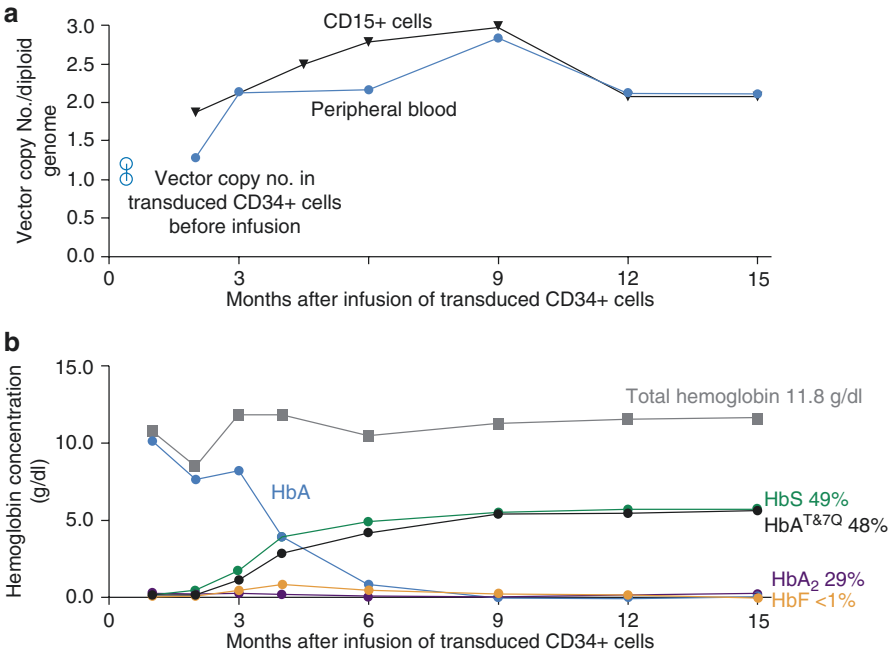

Fig. 15.7 Engraftment of transduced cells and therapeutic gene expression in a patient with SCD successfully treated with gene therapy. Panel **a** shows vector copy number values in blood nucleated cells and the short-lived CD15+ (neutrophils) fraction thereof over 15 months after infusion of transduced CD34+ cells. Initial values in transduced cells before the infusion are shown. Panel **b** shows total hemoglobin levels and calculated levels of each hemoglobin fraction based on high-performance liquid chromatography measurements of globin chains. The percent contribution of hemoglobin fractions at month 15 is also indicated. The hemoglobin A (HbA) levels are derived from the regular red-cell transfusions received by the patient before gene therapy and briefly thereafter (the last red-cell transfusion occurred on day 88). HbA2 is an alternative adult hemoglobin that is not derived from transfused blood. HbF denotes fetal hemoglobin, and HbS sickle hemoglobin. Ribeil JA, Hacein-Bey-Abina S, Payen E, Magnani A, Semeraro M, Magrin E, et al. Gene therapy in a patient with sickle cell disease (N Engl J Med. 2017 Mar 2;376(9):848–855)

more limited for this study, but initial reports show no safety concerns but lower gene transfer efficiency. All subjects express β^{T87Q}, with a median of 0.4 g/dL (0.1–1.0 g/dL) at 3 months, with two subjects with the longest follow-up expressing 0.31 and 1.2 g/dL β^{T87Q} at 9 months.

Notable differences in the SCD patient described in HGB-205 and the patients in HGB-206 include median cell dose (5.6×10^6 CD34+ cells per kg vs. 2.1×10^6 CD34+ per kg), drug product VCN (1.2 vs. 0.6) or average number of vector integrations per cell in the gene-modified product prior to infusion, and peripheral blood VCN (0.2–3.4 vs. 0.05–0.13) or average number of vector integrations per cell in the peripheral blood after infusion. Obvious differences in patient demographics include age, and the use of chronic transfusions for 4 years prior to autologous transplantation, invoking interesting questions and hypotheses on the

appropriate patient that may have maximal potential for success in therapeutic genetic correction.

For both HGB-205 and HGB-206 thus far, patient CD34+ HSCs were collected via bone marrow harvest. Mobilized peripheral blood stem cells (PBSCs) have several advantages over traditional bone marrow-derived HSCs for most gene therapy applications. However, mobilization of PBSCs with G-CSF is contraindicated for use in patients with SCD given the risk for severe adverse events, including VOC, ACS, multi-organ system failure, and death [74]. Studies are ongoing for alternative methods of PBSC mobilization for people with SCD since PBSC collection has several advantages: (1) larger harvest of CD34+ HSCs resulting in higher yield of CFU-GM and progenitor cells [75]; (2) faster rates of granulocyte, platelet, and T lymphocyte recovery after G-CSF mobilization [76]; and (3) PBSC collection is safer than bone marrow harvest in other patient populations. A combination of G-CSF and plerixafor in the rhesus macaque model demonstrated a proof of principle for plerixafor use, as G-CSF and plerixafor-mobilized CD34+ cells accelerate lymphocyte engraftment and contain HSCs capable of reconstituting multilineage blood cells [77]. An open trial is investigating the escalation of plerixafor for mobilization of CD34+ HSCs and evaluating globin gene transfer in patients with SCD (NCT02193191), which has shown successful HSC mobilization without evidence of platelet, endothelial, or neutrophil activation or brain VOC in a mouse model (as measured by radiographic brain tissue perfusion before and after treatment) [78]. A recent study examined steady state bone marrow (BM) in a rhesus macaque model and demonstrate similar gene marking in vitro and in vivo, as compared with mobilized peripheral blood (PB) CD34+ cells [79]. In SCD patients, PB and steady-state BM had a higher frequency of CD34+ cells compared to controls, likely due to stress erythropoiesis; however, CD34+ cell counts were reduced in both the PB and BM in SCD patients treated with hydroxyurea. This lower CD34+ percentage observed with hydroxyurea treatment may warrant withholding hydroxyurea temporarily prior to harvesting HSCs. Whether patients should be on chronic transfusions prior to gene therapy, as was done for the patient described in HGB-205 [25], is yet to be determined. Given the potential advantages of mobilized peripheral blood HSCs, HGB-206 has included a plerixafor-mobilized arm into its trial design.

Other ongoing gene addition trials investigating variations of the corrective hemoglobin vectors include a β^{AS3} vector with an insulator following myeloablative conditioning (NCT02247843) and a lentiviral vector with γ-globin following reduced intensity conditioning (NCT02186418). Gene editing strategies with CRISPR/Cas9 is promising but has not yet been performed in vivo in humans. Preclinical work has shown that CRISPR/Cas9-mediated correction of the sickle mutation in human CD34+ cells results in over 18% gene modification in vitro [80]. This is in contrast to ZFN modification which has shown rates of correction of long-term HSCs well below levels necessary for therapeutic benefit (estimated minimum of 15–20% of all engrafted HSCs). In preclinical models, multiple pairs of TALENs and several gRNAs were designed to cleave in exon 1 of human β-globin, the location of the glutamic acid to valine mutation underlying SCD. TALENs produced an

average gene modification rate between 8.2 and 26.6%, whereas CRISPR/Cas9 gRNAs demonstrated a higher rate of up to 64.3% gene modification of the β-globin locus. Electroporation only resulted in <5% allelic disruption, therefore an integrase defective lentiviral vector (IDLV) containing the gRNA was used, followed by homologous donor template. Human CD34+ HSCs harvested from patients with SCD were tested with electroporation with a Cas9 messenger RNA and transduced with an IDLV carrying the gRNA and wild-type human β-globin. Gene correction rates averaged 20.6%, while the rates of indels averaged 16.3%. At the end of erythroid differentiation in vitro, SCD bone marrow CD34+ cells treated with Cas9 messenger RNA and IDLV demonstrated 7.3–12.6% wild-type hemoglobin A. There were no off-target cleavage sites detectable using CRISPR gRNAs. Unlike data with ZFNs, gene correction rates in BM CD34+ HSCs from SCD patients were higher than indel percentages and may be sufficient to overcome the pathologic phenotype [61]. This work is ongoing and is promising for an additional genetic therapeutic option for cure in patients with SCD.

Conclusions

Based on the results of a single case study [25], cure of SCD from gene correction is now a reality. Genetic correction of patient CD34+ HSCs is an exciting and promising field of clinical research that is aimed at providing patients with SCD another option for cure apart from allogenic HSCT which carries morbidity and mortality and is not universally available. Many obstacles in the field of gene therapy have been overcome, but many hurdles remain for people with SCD. Challenges for gene therapy specific to patients with SCD include a hypoxic and inflamed marrow, low percentage of long-term progenitor CD34+ HSCs, low transduction efficiency, and suboptimal engraftment of transduced cells. Attempts to improve each of these challenges are underway and include modification of pre-gene therapy patient management (i.e., holding hydroxyurea and initiating chronic transfusions), investigation of plerixafor mobilization or other techniques to maximize cell dose, creating safe viral vectors, adding transduction enhancers to increase viral VCN and total transduced cell dose, and creating successful engraftment of transduced cells. All of these revisions, based on lessons of previous trials and work of today, come with the fundamental goal of providing relief of the devastation of SCD. Ultimately if gene therapy proves to be effective in larger clinical trials, the next challenge facing researchers will be how to export and adapt this therapy to developed and underdeveloped nations alike. Patients with SCD may one day no longer have to face a small chance of cure due to an inability to find a suitable allogenic match. The dream of autologous gene therapy for the cure of SCD is now an actuality, and it is our responsibility to continue striving for better, safer, and more efficient methods that may one day be available to all patients regardless of circumstance.

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