Allogeneic hematopoietic stem cell transplantation for sickle cell disease: the time is now

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Although sickle cell disease (SCD) has a variable clinical course, many patients develop end-organ complications that are associated with significant morbidity and early mortality. Myeloablative allogeneic HSCT (allo-HSCT) is curative but has been historically performed only in children younger than 16 years of age. Modest modifications in the conditioning regimen and supportive care have improved outcome such that the majority of children with a suitable HLA-matched sibling donor can expect a cure from this approach. However, adult patients have been excluded from myeloablative allo-HSCT because of anticipated excess toxicity resulting from accumulated disease burden. Efforts to use nonmyeloablative transplantation strategies in adults logically followed but were initially met with largely disappointing results. Recent results, however, indicate that nonmyeloablative allo-HSCT in adult patients with SCD allows for stable mixed hematopoietic chimerism with associated full-donor erythroid engraftment and normalization of blood counts, and persistence in some without continued immunosuppression suggests immunologic tolerance. The attainment of tolerance should allow expansion of these potentially curative approaches to alternative donor sources. Efforts to build on these experiences should increase the use of allo-HSCT in patients with SCD while minimizing morbidity and mortality. (Blood. 2011;118(5):1197-1207)

Introduction

Sickle cell disease (SCD) results from a single nucleotide mutation, which changes the glutamate for valine in the 6th position of the β-globin protein. This change results in a propensity for the hemoglobin protein to polymerize when deoxygenated and causes the characteristic sickle-shaped red cells. The disorder is characterized by anemia, ongoing hemolysis, along with acute and chronic complications affecting multiple organs. Although transfusions can prevent further neurologic events in patients at risk, iron overload is common, resulting in significant end-organ toxicity. The implementation of newborn screening, penicillin prophylaxis, and prompt evaluation and treatment for fever have improved the outlook for children with SCD. Specific treatment has remained limited and includes erythrocyte transfusions and more recently hydroxyurea.1-3 Hydroxyurea results in a significant reduction in the number of painful crises per year and a decreased frequency of acute chest syndrome; it has become the treatment of choice for many patients with SCD. Unfortunately, hydroxyurea is not curative and does not appear to reverse established end-organ damage. The medical costs of this disease are also enormous, with estimates of $40,000 per patient per year (year 2000 figures) for chronic transfusion therapy and chelation alone, but they do not include the impact on quality of life of those with the disease.4 It is astounding that a single nucleotide substitution can lead to a multigenorgan disease that dramatically reduces the quality of life and shortens the lifespan of those affected. Unfortunately, these existing treatments only ameliorate the manifestation of SCD, leaving an increasing number of adults with accumulating end-organ damage. Although longstanding efforts to deliver the correct β-globin gene through gene therapy approaches are progressing well, currently allo-HSCT remains the only immediate cure. Because patients with SCD are living longer and with chronic organ insufficiencies from sicklerelated organ damage or from transfusional iron overload, improving HSCT for children and adults with SCD has emerged as an area of research interest with immediate potential clinical benefit.

Indications for HSCT

Although the curative potential of HSCT has been well established in several nonmalignant disorders, there has always been difficulty in determining which patients with SCD warrant the potential risks of this technique. Early efforts to develop criteria to distinguish the more severe patients proved difficult in a disorder with chronic, yet mostly manageable complications, punctuated by rarer, more severe, even life-threatening complications. There remains a temptation to intervene early when patient status would improve the odds against complications from the conditioning regimen. Alternatively, intervening later when chronic, irreversible disease complications clearly establish the severity of the patient’s phenotype limits its application because the conditioning regimen is less likely to be well tolerated. Transcranial Doppler (TCD) examination has proven effective in predicting the risk of stroke and has been used to predict the severity of disease. Although abnormal TCD examinations predict subsequent development of stroke, and prompt red cell transfusion therapy dramatically reduces that risk,5 data are beginning to show that TCDs can remain abnormal in a small proportion of patients.6 Furthermore, among children with a first stroke on chronic transfusion therapy, a second event of overt stroke, silent stroke, or CNS vasculopathy can occur.7 These red cell transfusions, even when extensively matched, can lead to red...
hemorrhage, occurred in 7 of 21 patients with SCD who underwent myeloablative conditioning. The incidence of neurologic complications subsequently decreased by maintaining a platelet threshold of >50 000/μL and a hemoglobin level of 9-11 g/dL, adding phenytoin prophylaxis, and preventing significant hypertension and hypomagnesemia.

In one series, patients were divided into 2 groups by criteria reflective of their access to care. The first group consisted of patients who underwent transplantation according to traditional severity criteria and were permanent residents of a European country. The second group of patients underwent transplantation because they would subsequently be returning to their country of origin in Africa, where access to chronic SCD care may be limited. Although patients in the first group were older (median age 8.6 vs 2 years; P = .0016), the first group had a greater rate of graft rejection (25% vs 7%, P < .001), and all of the patients who developed severe GVHD were in group 1. These data suggest that transplantation for patients with SCD may be performed more safely in pediatric patients who have not experienced end-organ damage or other markers of disease severity.

The largest group to date was reported in 2007. Overall survival and event-free survival were 93% and 86%, respectively. A major finding of this study was that after the addition of antithymocyte globulin in 1992 to the conditioning regimen, the rejection rate decreased from 22.6% to 3%, with an event-free survival rate of 95% among patients who underwent transplantation after January 2000. These results establish that HLA-matched transplants that use myeloablative conditioning should be the standard of care for eligible pediatric patients with SCD. To date, ~300-400 affected subjects mostly younger than the age of 16 years have undergone fully myeloablative allo-HSCT.

The results of these studies are summarized in Table 2. However, the number of patients who have undergone transplantation is small compared with the estimated 70 000 patients with SCD in the United States alone, indicating that this curative therapy is underused. Furthermore, a 15% to 20% GVHD rate with cyclosporine-based postgraft immunosuppression in the myeloablative setting has been reported, with some deaths directly attributable to GVHD. What is remarkable is that despite myeloablative dosing in the conditioning regimens, a mixture of both donor and recipient hematopoietic cells, termed mixed donor chimerism, is consistently observed in approximately 10% to 20% of these children. Interestingly, this mixed chimeric state is sufficient to direct BM to produce donor-type hemoglobin and red cells, revert the SCD phenotype, and minimize the risk of GVHD. This

### HLA-matched sibling allo-HSCT

Allo-HSCT remains the only curative strategy for patients with SCD. The first successful HSCT in a patient with SCD was reported in 1984 in a pediatric patient with coexisting acute myeloid leukemia. Traditional myeloablative conditioning regimens that use BM as the HSC source and myeloablative doses of busulfan in combination with highly immunosuppressive doses of cyclophosphamide were used with transplants first reported in Europe, and then in the United States. Antithymocyte globulin was later added more consistently to decrease the risk of graft rejection in this population of patients who have been previously transfused and are frequently alloimmunized. In one early trial, neurologic complications, including seizures and fatal intracranial hemorrhage, occurred in 7 of 21 patients with SCD who underwent myeloablative conditioning. The incidence of neurologic complications subsequently decreased by maintaining a platelet threshold of >50 000/μL and a hemoglobin level of 9-11 g/dL, adding phenytoin prophylaxis, and preventing significant hypertension and hypomagnesemia.

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### Table 1. Indications for HSCT or hydroxyurea

<table>
<thead>
<tr>
<th>Indications to start hydroxyurea (patients ≤ 16 y)</th>
<th>Historical indications for HSCT (patients ≥ 16 y)</th>
<th>NIH indications for HSCT (patients &gt; 16 y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Three or more VOC requiring hospitalizations</td>
<td>Stroke or CNS event lasting longer than 24 hours</td>
<td>Irreversible end-organ damage</td>
</tr>
<tr>
<td>Two or more acute chest syndromes</td>
<td>Abnormal brain MRI</td>
<td>Stroke or clinically significant CNS event</td>
</tr>
<tr>
<td>Two or more joints with osteonecrosis</td>
<td>Elevated TCD</td>
<td>Elevated TRV ≥ 2.6 m/s</td>
</tr>
<tr>
<td>Symptomatic anemia</td>
<td>ACS with recurrent hospitalizations</td>
<td>Sickle-related renal insufficiency (Cr ≥ 1.5 times the upper limit of normal or biopsy proven)</td>
</tr>
<tr>
<td>Two or more VOC requiring hospitalizations for several years</td>
<td></td>
<td>Sickle hepatothropy (including iron overload)</td>
</tr>
<tr>
<td>Osteonecrosis of multiple joints</td>
<td>Reversible sickle complication not ameliorated by hydroxyurea</td>
<td></td>
</tr>
<tr>
<td>Red cell alloimmunization</td>
<td>Two or more VOC requiring hospitalizations for several years</td>
<td></td>
</tr>
<tr>
<td>Sickle cell lung disease</td>
<td>Any ACS while on hydroxyurea</td>
<td></td>
</tr>
</tbody>
</table>

ACS indicates acute chest syndrome; NIH, National Institutes of Health; MRI, magnetic resonance imaging; TCD, transcranial Doppler; TRV, tricuspid regurgitant velocity; and VOC, vaso-occlusive crises.

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Table 2. Myeloablative HSCT for children with SCD with matched related donors

<table>
<thead>
<tr>
<th>Reference</th>
<th>Transplantation regimen</th>
<th>Conditioning</th>
<th>Acute GVHD</th>
<th>Chronic GVHD</th>
<th>Death (cause)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bernaudin et al, 2007</td>
<td>Bu 16 mg/kg, Cy</td>
<td>ATG, CSA, MTX</td>
<td>10 (Gr 2-3: 7, Gr 4: 3)</td>
<td>6 (Gr 1-2: 2, Gr 3: 4)</td>
<td>1 (unknown)</td>
<td>At 5 y after HSCT; 6 had mixed chimerism (3: 50%-70%)</td>
</tr>
<tr>
<td>Panepinto et al, 2007</td>
<td>Mostly Bu 16 mg/kg, Cy</td>
<td>ATG, CSA, MTX</td>
<td>19 (79)</td>
<td>3 (Gr 2-3: 3, Gr 7)</td>
<td>1 (unknown)</td>
<td>At 8.7 y after HSCT; 1 had mixed chimerism (3: 50%-70%)</td>
</tr>
<tr>
<td>Brachet et al, 2004</td>
<td>Bu 14-16 mg/kg, Cy; 200 mg/kg, MTX, CSA</td>
<td>ATG, CSA, MTX</td>
<td>24 (2-5: 1, Gr 2: 20)</td>
<td>50 (2-5: 1, Gr 2: 46)</td>
<td>50 (2-5: 1, Gr 2: 46)</td>
<td>At 30 y after HSCT; 1 had mixed chimerism (3: 25%-40%)</td>
</tr>
<tr>
<td>Walters et al, 2001</td>
<td>Bu 16 mg/kg, Cy</td>
<td>ATG, CSA, MTX</td>
<td>19 (79)</td>
<td>1 (unknown)</td>
<td>1 (unknown)</td>
<td>At 5 y after HSCT; 6 had mixed chimerism (3: 50%-70%)</td>
</tr>
</tbody>
</table>

ATG indicates antithymocyte globulin; Bu, busulfan; CIBMTR, Center for International Blood and Marrow Transplant Research; CSA, cyclosporine A; Cy, cyclophosphamide; Gr, grade; MTX, methotrexate; and SCD, sickle cell disease.

Conditioning regimens

As depicted in Tables 2 and 3, myeloablative doses of busulfan and high-dose cyclophosphamide have produced the most consistent results in children with SCD. However, the persistent finding of graft rejection and GVHD indicates that further improvements must be made. Reduced doses of busulfan (from 14 mg/kg to 6.4 mg/kg) appeared to allow engraftment, whereas a similar reduction in melphalan dosing did not. Since then, a bewildering number of conditioning regimens have been developed, mostly in the setting of malignant disease, in an attempt to bring transplantation procedures to a greater number of patients and potentially to those with SCD. To allow comparisons across studies, a recent categorization of conditioning regimens was proposed that used the terms myeloablative (MA), reduced-intensity conditioning, and nonmyeloablative (NMA).

In the proposed classification, MA regimens were defined as those that produce minimal cytopenia with no need for stem cell support. NMA regimens were defined as regimens that produce minimal cytopenia with no need for stem cell support. Those regimens remaining, which fit neither classification, were termed reduced-intensity conditioning. Although this classification should prove useful in comparative analyses, additional conceptual factors could be considered in comparing and even constructing regimens that are disease-dependent. The emphasis currently is placed on lowering toxicity, yet further contemplation on mechanism of engraftment and desired use of the allograft should direct further research in the area. Indeed, not all conditioning regimens are equivalent, and the era of one-size-fits-all is certainly behind us. We propose a further division of the classification of conditioning based on the mechanism of engraftment: immune ablation or myelosuppression with tolerance induction.

Immune ablation

In patients with malignancies, myeloablative conditioning regimens designed to both eradicate host hematopoiesis (including...
Table 3. Nonmyeloablative HSCT in children and adults from matched related donors

<table>
<thead>
<tr>
<th>Reference</th>
<th>Transplant regimen</th>
<th>Number of patients (age range, y)</th>
<th>Alive without SCD</th>
<th>Acute GVHD</th>
<th>Chronic GVHD</th>
<th>Death</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hsieh et al, 2009&lt;sup&gt;48&lt;/sup&gt;</td>
<td>TBI 300 cGy, alemtuzumab 1 mg/kg, sirolimus</td>
<td>10 (16-45 y)</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>At 3 y after HSCT, mixed chimerism in all</td>
</tr>
<tr>
<td>Krishnamurti et al, 2008&lt;sup&gt;50&lt;/sup&gt;</td>
<td>BU 6.4 mg/kg IV or 8 mg/kg PO, FLU 175 mg/kg, ATG, 500 cGy of TLI, CSA, MMF</td>
<td>7 (6-16)</td>
<td>6</td>
<td>1 (Gr 2 skin)</td>
<td>1 (limited skin)</td>
<td>0</td>
<td>Mixed chimerism in 4</td>
</tr>
<tr>
<td>Horwitz et al, 2007&lt;sup&gt;51&lt;/sup&gt;</td>
<td>TBI 200 cGy, FLU 24 mg/m², Cy 500 mg/m², alemtuzumab 100 mg, MMF</td>
<td>2 (21 and 27)</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>N/A</td>
<td>Mixed chimerism in both</td>
</tr>
<tr>
<td>Horan et al, 2005&lt;sup&gt;55&lt;/sup&gt;</td>
<td>TBI 200 cGy, FLU 125 mg/m², ATG, CSA, MMF</td>
<td>3 (9-30)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Shenoy, 2005&lt;sup&gt;57&lt;/sup&gt;</td>
<td>Melphalan 140 mg/m², FLU 150 mg/m², alemtuzumab 48 mg, CSA + MTX</td>
<td>1 (2)</td>
<td>N/A</td>
<td>0</td>
<td>0</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Jacobsohn et al, 2004&lt;sup&gt;63&lt;/sup&gt;</td>
<td>BU 6.4 mg/kg IV, FLU 180 mg/m², ATG, CSA, MMF</td>
<td>1 (22)</td>
<td>0</td>
<td>1 (Gr 1)</td>
<td>1 (extensive)</td>
<td>1 (GVHD)</td>
<td></td>
</tr>
<tr>
<td>Iannone et al, 2003&lt;sup&gt;34&lt;/sup&gt;</td>
<td>TBI 200 cGy, FLU 150 mg/m², CSA or tacrolimus, MMF</td>
<td>6 (3-20)</td>
<td>0</td>
<td>1 (Gr 2)</td>
<td>0</td>
<td>0</td>
<td>Transient engraftment in 5 patients for 97-441 days</td>
</tr>
<tr>
<td>van Besien et al, 2000&lt;sup&gt;38&lt;/sup&gt;</td>
<td>Melphalan 140 mg/m², FLU 120 mg/m², ATG, tacrolimus, MTX</td>
<td>2 (40 and 56)</td>
<td>0</td>
<td>1 (Gr 2 skin)</td>
<td>1 (severe lung)</td>
<td>2 (lung GVHD, liver GVHD and CNS infection)</td>
<td></td>
</tr>
</tbody>
</table>

ATG indicates antithymocyte globulin; cGy, centi-Gray; CSA, cyclosporine A; Cy, cyclophosphamide; FLU, fludarabine; Gr, grade; IV, intravenous; MMF, mycophenolate mofetil; MTX, methotrexate; N/A, not applicable; PO, per orally; SCD, sickle cell disease; TBI, total body irradiation; and TLI, total lymphoid irradiation.
malignant cells) and to ensure complete donor immune reconstitution have historically been used with busulfan and cyclophosphamide or cyclophosphamide and total body irradiation (TBI). A graft-versus-malignancy effect has long been recognized and adds to the curative potential of such approaches, securing complete donor immune reconstitution as a necessary goal. Similarly, nonmyeloablative transplantation strategies in malignant disease share the goal of complete eradication of host hematopoiesis as well as replacement of the immune system with that of donor. A graft-versus-malignancy effect can then be harnessed to achieve long-term disease-free survival. With these goals in mind, we and others have developed NMA conditioning regimens that target the host immune system, allowing engraftment to proceed through a donor allo-immune response. The use of strategies such as high-dose cyclophosphamide and fludarabine proved highly successful in attaining engraftment without myeloablation in virtually all patients. Early engraftment profiles after these highly immunosuppressive agents showed high degrees of donor T-cell chimerism and were contrasted by lower levels of donor myeloid chimerism. However, this mixed chimeric state was temporary, and in most cases, alloreactive donor T cells appeared to eradicate recipient stem cells, converting patients from mixed- to full-donor chimerism. In situations in which less than complete donor engraftment was obtained, especially in the presence of residual malignant disease, donor lymphocytes were often used to tip the balance in favor of donor.

Regimens that rely on donor lymphocyte infusions to establish full donor engraftment are thus of the immune ablative classification. Of note, this immune effect proved successful in eradicating malignant disease in a sizable fraction of recipients, yet was frequently associated with the occurrence of GVHD. Furthermore, although GVHD correlates with graft-versus-tumor effects, it provides no benefit to patients with nonmalignant hematologic disorders and is associated with considerable morbidity and mortality. In patients with nonmalignant hematologic disorders, complete eradication of host hematopoiesis is usually not necessary, and we submit that the conditioning regimen should be tailored specifically for the targeted patient population, especially when there is evidence that mixed donor chimerism (MDC) could revert the disorder. Indeed, reflexive application of regimens developed for malignant disorders would likely lead to greater than necessary and, we submit that the conditioning regimen should be tailored specifically for the targeted patient population, especially when there is evidence that mixed donor chimerism (MDC) could revert the disorder. Indeed, reflexive application of regimens developed for malignant disorders would likely lead to greater than expected transplantation-related morbidity in patients with SCD. We thus favor developing specific regimens, reflecting different patient ages, disease indications, disease burden, cell sources, and degree of HLA matching. In disorders such as SCD and the thalassemias, where low levels of donor chimerism are sufficient to reverse the disease, regimens intentionally aimed at a mixed chimeric state warrant investigation.

Myelosuppression with tolerance induction

Given the limitations of immune ablative regimens for nonmalignant disease, we sought to develop a transplantation regimen for adults with SCD for whom engraftment is not dependent on areoreactivity. Early attempts by others using regimens previously attempted in malignant disorders proved unsuccessful at achieving reliable long-term donor engraftment after nonmyeloablative conditioning in patients with SCD. Dampering enthusiasm for this approach. We therefore went back to the murine model to tailor a regimen for SCD. We specifically sought to develop a regimen that would allow sufficient myelosuppression to achieve the modest levels of donor myeloid chimerism required for disease reversion while also achieving immunologic tolerance through pharmacologic manipulation. This approach is similar to that sought in solid organ transplantation, whereby myelosuppression represents the equivalent of the organ transplant and the pharmacologic immunomodulatory approach the equivalent of tolerance induction. Several regimens tested in patients with SCD could fit this classification. Indeed, the work of Iaconne et al, in which they used 200 cGy of TBI, fludarabine, and immunosuppression with cyclosporine, showed that patients had temporary donor engraftment. However, when cyclosporine was tapered as per protocol, donor grafts decreased to undetectable levels, suggesting that immune tolerance had not been achieved.

Realizing the important finding of this study, we too chose a low-dose radiation approach, but used sirolimus (rapamycin) instead of cyclosporine, on the basis of a novel mechanism of tolerance induction. Unlike calcineurin inhibitors (eg, cyclosporine and tacrolimus), sirolimus does not block T-cell activation (signal 1) but binds to the mammalian target of rapamycin blocking proliferation (signal 2). Signal 1 in the absence of signal 2 renders T cells anergic, promoting T-cell tolerance. In a murine peripheral blood stem cell low-dose TBI transplantation model comparing a 30-day course of CSA to sirolimus, long-term, high level chimerism was attained only in sirolimus-treated mice, and mixed lymphocyte reactions demonstrated tolerance to donor cells. Correction of disease was also demonstrated in sickle cell transgenic knockout mice by use of the sirolimus-based regimen.

Given the almost exclusive use of G-CSF–mobilized peripheral blood as the source of HSCs for adult transplantation applications along with the 50% probability for heterozygosity for the sickle mutation among sibling donors with sickle cell trait, and the known adverse effects of G-CSF mobilization in SCD along with the 50% probability for heterozygosity for the sickle mutation among sibling donors with sickle cell trait, and the known adverse effects of G-CSF mobilization in SCD along with potential problems encountered with cryopreservation of SCT products, we initiated a prospective, controlled clinical trial of peripheral blood hematopoietic stem cell mobilization and processing in patients with sickle cell trait with age- and race-matched controls. Symptoms were similar between the 2 arms, and there was no evidence for G-CSF–induced sickling in subjects with sickle cell trait. HSC mobilization, collection, and cryopreservation also were similar, and a superior cryopreservation method was developed that constitutes our new standard.

We then considered how adult patients with accumulated organ damage might tolerate any given conditioning regimen. Those with renal insufficiency may experience worsening renal function with calcineurin inhibitors; those with a history of cerebrovascular or Moy-Myo disease may have severe neurologic complications from posterior reversible encephalopathy syndrome attributable to calcineurin inhibitor use; and those with severe sickle hepatopathy may be at significant risk for veno-occlusive disease from traditional alkylating agents. Thus, we built on the results of our previous work and on the previous nonmyeloablative experiences for adults and devised a regimen on the basis of 300 cGy of TBI. As additional GVHD prophylaxis, we chose alemtuzumab over antithymocyte globulin for better infusional toxicity profile in patients with SCD and superior prophylaxis against GVHD. Within days of administration, alemtuzumab depletes majority of circulating host and infused donor lymphocytes. Alemtuzumab remains detectable for several weeks and further depletes alloreactive T cells during subsequent donor engraftment and initial immune reconstitution. Both components are preferable over more technically challenging ex vivo T-cell depletion procedures to allow engraftment and minimize early GVHD. We hypothesized...
that long-term administration of sirolimus (for 6-12 months) could then further induce sufficient number of regulatory T cells to promote tolerance in a mixed chimeric setting during the remainder of donor immune reconstitution to reduce delayed GVHD. Thus low-dose TBI, alemtuzumab, and sirolimus theoretically provide the necessary environment for stable mixed chimerism and minimal GVHD.

Ten adult patients with severe disease initially underwent transplantation, and their outcomes were reported recently. All patients tolerated the conditioning regimen, with infrequent serious adverse events found. All 10 patients are alive at a median follow-up period of 30 months and all but one have long-term donor engraftment. No patients have experienced acute or chronic GVHD. The kinetics of donor myeloid chimerism were rapid and reached nearly 100% early in the majority of patients, whereas the kinetics of donor T-cell engraftment was less rapid and reached a plateau after 12 months. No patient reached 100% donor chimerism in both compartments, and immunosuppression was continued on the basis of our initial withdrawal plan requiring full donor lymphoid chimerism in the absence of GVHD, but a modification of this plan to allow tapering with lymphoid chimerism levels of 50% or greater has allowed the withdrawal of immunosuppression in 4 patients to date. These 4 patients continue to demonstrate stable mixed chimerism in the absence of immunosuppression. The mixed chimeric state allowed complete replacement by donor-type hemoglobin to levels that gradually improved to the normal range post-HSCT. Importantly, the replacement by donor type red cells allowed complete withdrawal of chronic narcotic therapy. The use of nonmyeloablative regimens in this and other studies will also have the benefit of preserving fertility. Because the duration of immunosuppression may be longer in our study than in children who underwent myeloablative conditioning, we are following our patients closely for long-term effects of sirolimus, the effect of our conditioning regimen on pulmonary function and endocrine organs, and the development of secondary malignancies.

## MDC is sufficient and can be stable

MDC has frequently been described not only in patients with SCD but also in those with thalassemia after HSCT, and most patients with MDC are free of their underlying hemoglobin disorders, making MDC a minimal necessary goal. Certainly, the notion that less-than-complete donor engraftment may revert several disorders such as the severely combined immunodeficiencies has fostered work in genetic modification of autologous HSCs that has recently proven effective and continues to support this type of strategy for eventual application in SCD. However, the minimum percentage of donor cells that defined MDC differed in SCD and thalassemia patients undergoing allo-HSCT, and the cell populations (total leukocytes, mononuclear cells, or lineage specific cells) that were assayed for chimerism also varied; thus, the threshold percentage of donor cells sufficient to ameliorate the hemoglobin disorders has not yet been firmly established. This minimum, however, has been suggested to be as low as 10% in patients with SCD. This minimum has been corroborated in thalassemic mice, in whom 10% to 20% is sufficient, and 10% to 20% in thalassemic patients after myeloablative HSCT.

Others have questioned whether MDC can in fact be stable. Andreani et al recently reported their experience in myeloablative HSCT in patients with thalassemia major. They demonstrated that the risk of graft rejection is the greatest in the first 2 months after HSCT when MDC levels decrease to < 75%. However, this risk dramatically decreases after 1-2 years, even when the MDC levels decrease < 75%, indicating the first several months after transplantation are the most critical, when either rejection may occur or recipient hematopoiesis may directly compete with the donor hematopoietic contribution. After this period, patients with MDC appeared stable, with long-term reversal of their disease state.

There are several potential factors that seem to be associated with MDC. Less-intensive conditioning regimens in general are associated with a greater proportion of MDC. Interestingly, the addition of ATG to busulfan and cyclophosphamide increased the percent of French children with MDC (50% to 95% of donor cells) from 5% to 35% undergoing myeloablative HSCT but reduced the graft rejection rate. Similarly, the addition of antilymphocyte globulin also increased the proportion of Italian children with MDC undergoing myeloablative HSCT for thalassemia. The low-dose TBI, graded doses of alemtuzumab, large doses of G-CSF–mobilized peripheral blood HSCs, and sirolimus were proposed to induce stable MDC in the National Institutes of Health cohort of adults undergoing nonmyeloablative HSCT. These results together suggest that in vivo T-cell depletion may be a central component in inducing MDC. No doubt the mechanism(s) of MDC and the characteristics of tolerance between donor and recipient cells need to be elucidated. HSCTs in hemoglobin disorders are then poised to lead the field of tolerance induction because there are many eligible patients, there are shared interests for HSCT between the community and transplant physicians, and most importantly, tolerance without GVHD is the ultimate goal. The stable MDC observed, even in patients weaned from immunosuppression, provides indirect evidence for graft-specific tolerance, opening the possibility of expanding to alternative donor sources.

## Barriers to HSCT for patients with SCD

Although the results of HSCT for SCD in the HLA-matched sibling setting have been encouraging, many barriers to transplantation exist. To explore how much risk they would be willing to accept to cure their children of SCD, parents were provided a questionnaire specifically designed to quantitate this risk. Fifty-four percent of parents were willing to accept some short-term mortality risk, but only 13% could accept the estimated risks at the time of a 15% mortality risk with an additional 15% risk of developing GVHD. Another large barrier exists with regard to pursuing HLA typing. In another series, among 4848 patients with SCD who were younger than 16 years of age, 315 (6.5%) met entry criteria for transplantation. However, only 128 (41% of eligible or 2.6% of total) underwent HLA typing. Reasons for not performing HLA typing included lack of a sibling donor (24%), lack of financial or psychosocial support (10.5%), parental refusal (9.5%), and physician refusal (4%). Further studies in the new era of HSCT for patients with SCD with the improved overall survival and decreased risk of GVHD seen in more recent years to further explore parental and physician opinions and provide education are thus indicated.

However, the main limitation to HSCT for patients with SCD has been a scarcity of HLA-matched sibling donors. From our own experience, where 112 patients had SCD severe enough to be eligible for our transplantation protocol, only 24 patients (21%) had a histocompatible sibling donor. Furthermore, this percentage reflected a numerator that included patients referred solely because...
<table>
<thead>
<tr>
<th>Reference (Year/Author)</th>
<th>Transplant regimen</th>
<th>Median TNC dose, $/10^7$/kg (range)</th>
<th>HLA match</th>
<th>Number of patients (age range)</th>
<th>Alive without SCD</th>
<th>Acute GVHD</th>
<th>Chronic GVHD</th>
<th>Death (cause)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Related cord blood donors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brichard et al, 1996</td>
<td>Bu 16 mg/kg, Cy 200 mg/kg, ATG, CSA</td>
<td>4.6</td>
<td>6/6</td>
<td>1 (5)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>N/A</td>
</tr>
<tr>
<td>Miniero et al, 1998</td>
<td>Bu 16 mg/kg, Cy 200 mg/kg, CSA + MTX</td>
<td>(3.5-6.0)</td>
<td>6/6</td>
<td>3 (3-11)</td>
<td>2</td>
<td>2 (Gr 1)</td>
<td>0</td>
<td>N/A</td>
</tr>
<tr>
<td>Gore et al, 2000</td>
<td>Bu 726 mg/m², Cy 200 mg/kg, ATG, CSA</td>
<td>2.3</td>
<td>6/6</td>
<td>1 (9)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>N/A</td>
</tr>
<tr>
<td>Locatelli et al, 2003</td>
<td>Mostly Bu, Cy, ATG/ALG, CSA + MTX</td>
<td>4.0 (1.2-10)*</td>
<td>6/6 41 pts, 5/6 3 pts*</td>
<td>11 (1-20)*</td>
<td>10</td>
<td>11% (Gr 2)*</td>
<td>6% (limited)*</td>
<td>N/A</td>
</tr>
<tr>
<td>Walters et al, 2005</td>
<td>NR NR</td>
<td>6/6 42 pts, 4/6 4 pts†</td>
<td>8 (NR)</td>
<td>6</td>
<td>NR NR</td>
<td>1 (intractable seizures)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Unrelated cord blood donors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adamkiewicz et al, 2007</td>
<td>Mixed, 4 pts myeloablative, 3 pts reduced intensity</td>
<td>(1.5-9.3)</td>
<td>5/6 2 pts, 4/6 5 pts</td>
<td>7 (3.4-16.8)</td>
<td>3</td>
<td>1 (Gr 1); 2 (Gr 2); 1 (Gr 3); 1 (Gr 4)</td>
<td>1 (extensive)</td>
<td>1 (multiorgan failure)</td>
</tr>
<tr>
<td>Mazur et al, 2006</td>
<td>Rituximab, alemtuzumab, thiopeta (600 mg/m²), 600 cGy TBI, tac + MMF‡</td>
<td>4.2</td>
<td>4/6</td>
<td>1 (9)</td>
<td>1</td>
<td>Gr 1</td>
<td>0</td>
<td>N/A</td>
</tr>
<tr>
<td>Sauter et al, 2010</td>
<td>Reduced intensity</td>
<td>3.8 and 2.0§</td>
<td>5/6</td>
<td>1 (22)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>N/A</td>
</tr>
</tbody>
</table>

ALG indicates antilymphocyte globulin; ATG, antithymocyte globulin; Bu, busulfan; cGy, centi-Gray; CSA, cyclosporine A; Cy, cyclophosphamide; Gr, grade; MMF, mycophenolate mofetil; MTX, methotrexate; N/A, not applicable; pts, patients; NR, not reported; SCD, sickle cell disease; tac, tacrolimus; TBI, total body irradiation; and TNC, total nucleated cell.

*Includes 44 patients in the entire cohort: 33 patients with thalassemia and 11 patients with SCD.
†Includes 47 patients in the entire cohort: 14 patients with thalassemia, 8 patients with SCD, and 25 other.
‡This represents a second unrelated umbilical cord blood transplantation.
§Patient received a double umbilical cord unit transplantation.
of an already-established matched sibling donor. In the aforementioned study, where 315 patients with SCD were found to be eligible for transplantation, only 44 (14% of eligible patients) had an HLA-matched sibling. Therefore, there is an urgent need to increase the donor pool so that HSCT can be made available to the majority of patients with severe SCD.

Alternative donor HSCT

Cord blood (CB) transplantation (CBT), which was first successfully reported in a patient with SCD in 1996 (Table 4), offers the opportunity to obtain the donor source at a much faster rate compared with matched unrelated donor grafts and theoretically allows for a greater degree of mismatching. The experience of CBT has, however, been limited in patients with SCD, with a total of 33 cases reported, two-thirds of whom were in the related donor setting. The majority of related CBT have been 6/6 HLA-matched. Conditioning has predominantly consisted of busulfan and cyclophosphamide with or without ATG and calcineurin inhibitor–based GVHD prophylaxis. There has only been one reported death, and event-free survival in these small studies has ranged from 66% to 100%, with only a total of 3 patients developing grade 1-2 acute GVHD, and one patient developing limited chronic GVHD. Although these results on the whole are remarkable, the chances of finding a related HLA-identical CB unit are no better than that of finding an HLA-matched sibling. In addition, the relatively low cell dose attainable with CB restricts broad application.

There has been one report of double umbilical cord transplantation, which has been used in an attempt to augment the total cell dose, in a young adult patient with SCD and Hodgkin lymphoma. However, double umbilical cord transplants may not provide sufficient cell dose to overcome the graft rejection barrier in nonablative studies, compared with the large doses of CD34 cells that can be collected from G-CSF–mobilized peripheral blood. Certainly, in the nonmyeloablative setting, cell dose may be even more critical, and our success in the HLA-matched sibling setting may relate in part to the high doses of CD34+ cells (>2 × 10^9 CD34+ cells per kilogram of recipient body weight) obtained from our donors. As more related CBT are performed in patients with SCD, we may find that the CD34 dose collected may not be sufficient to overcome the host versus graft barrier, even in the HLA-identical setting. Given the nonacucate need for transplantation in this disorder, a greater dose of cells could easily be collected from the cord donor at a later date, potentially improving outcome in the nonmyeloablative setting.

In unrelated CBT, conditioning regimens have varied immensely, and the results have been much less successful (Table 4). The largest study consisting of 7 patients reported one death, an event-free survival of 43%, and 5 patients developed acute GVHD (2 with grade 3-4 acute GVHD) and one patient chronic extensive GVHD. These discouraging results compared with related CBT are likely because none of the transplantations were 6/6 HLA-matched. In addition, because of the African ancestry of most Americans with SCD, the chance of finding a 6/6 HLA-matched umbilical cord unit is dismal. Further, to our knowledge, there are no reports of matched unrelated donor HSCT for patients with SCD, again likely because of the difficulty finding a suitable matched unrelated donor. Although the National Marrow Donor Program (NMDP) has registered >4 million volunteer donors, only a small minority have been underrepresented minority donors. The number of African-American volunteer donors has, however, increased from 59,104 to 345,236 between December 1993 and April 2001, respectively. Between 1989 and 2001, the NMDP performed donor searches for 77 patients with SCD. Presumably, the majority of patients were children or young adults; 34% had 1-2 possible HLA-matched donors, and 26% of patients had at least 3 potential HLA-matched donors. A total of 46% of patients had 1-2 possible CB units available, and 71% of patients had at least 3 potential CB units available. Others have theorized that 54% of patients weighing 40 kg would match at least 4 HLA CB unit loci at a cell dose of at least 5 × 10^7 nucleated cells/kg. These results reflect HLA-typing at the antigen level only and are thus an overestimate of the probability of finding a donor.

In 2007, we initiated a donor search through the NMDP and Bone Marrow Donors Worldwide for 10 adult patients with severe SCD who lacked HLA-matched sibling donors to evaluate the feasibility of matched unrelated donor or CB transplantation for adult patients with SCD. Although potential donors were identified by serotyping in the majority, we found that only one patient had a greater than 1% chance of having a 6/6 HLA-matched donor according to Haplogic algorithm. Further, Dew and colleagues performed unrelated donor searches for 51 African-Americans with malignant or nonmalignant hematologic diseases and who were a median age of 38 years. Only 3 patients (6%) were found to have an HLA-matched donor by molecular typing at all loci. However, only 50 of the patients (98%) had at least one and frequently >1 (median 14, range 1-158) 4/6 HLA-matched CB units available with a median cell dose of 2.85 × 10^7 total nucleated cells per kilogram body weight. However, in our donor search, only a median of one suitable CB unit was available per patient (≥2.5 × 10^7 total nucleated cells per kilogram body weight, at least 4/6 HLA-matched, and ABO-matched). Greater degrees of matching reduced this median to zero. The discrepancy between the 2 searches likely exists because of our exclusion of major ABO mismatched cords because of the possible risk for the development of pure red cell aplasia posttransplantation in the nonmyeloablative setting because of persistent recipient anti-donor red cell antibodies should stable mixed chimerism ensue.

Therefore, although some patients have been successfully treated with CBT, alternative donor sources are limited principally by availability, GVHD, and graft rejection. HLA-identical related CBT, which may have a lower risk of graft rejection and GVHD, will be severely limited by the majority of patients not having a matched related donor and the limited cell count collected. Matched unrelated donor and matched unrelated CBT will likely not be applicable to sufficient number of patients with SCD because of a lack of suitable donors in the national and worldwide registries. Mismatched unrelated CBT, although more feasible for pediatric patients on the basis of the availability of 4/6 HLA-matched cord blood units, appears to be associated with a greater risk of graft rejection and GVHD on the basis of the limited data. Further, CBT for adults will further be limited by the necessary total nucleated cell count per kilogram of body weight for engraftment to occur and the difficulty in achieving this goal in adult patients.

Haploidentical transplantation, a more suitable option

In contrast, haploidentical donors would greatly increase the donor pool because the majority of patients will have a suitable parent, child, or haploidentical sibling donor. Haploidentical HSCT is invariably associated with a greater risk of graft rejection and GVHD compared with the matched related or matched unrelated donor setting. The authors of one study reported that in patients with hematologic malignancies, HLA-mismatched compared with HLA-matched family members led to an incidence of acute grade 3-4 GVHD of 31% of patients compared with 9%,
respectively (P < .0001), and engraftment failure occurred in 6.3% versus 2.4%, respectively (P = .01).76 There was no significant difference between the incidence of chronic GVHD between groups (60% vs 47%, respectively, P = .11). In another study also in patients with hematologic malignancies, the authors reported that graft failure occurred in 12.3% of haploidentical HSCT.79-83

GVHD ranging from 13% to 48% for patients with hematologic malignancies undergoing haploidentical HSCT.79-83 an incidence of grade 2-4 acute GVHD ranging from 6% to 13%, and chronic extensive GVHD ranging from 13% to 48% for patients with hematologic malignancies undergoing haploidentical HSCT.79-83

Unlike hematologic malignancies, where survival is expected to be poor and the graft-versus-leukemia (GVL) effect is beneficial, SCD is in general a chronic illness, and substituting one chronic illness with another in the form of GVHD is highly undesirable. Perhaps the risk of GVHD will be lower for patients with SCD versus patients with hematologic malignancies because of the absence of irradiation and chemotherapy before transplantation. Instead, patients with SCD may be at an increased risk for graft failure because of the strong hematopoietic drive and high incidence of alloimmunization. Indeed, one case report demonstrated failure to engraft in a 14-year-old boy with severe SCD after receiving a BM transplant from his mother (Table 5).84 Because the patient was only conditioned with fludarabine and 200 cGy of TBI, a more intensive regimen is likely necessary to overcome the engraftment barrier. However, the authors of another report cited 3 patients with paroxysmal nocturnal hemoglobinuria, one of whom also had SCD, who underwent haploidentical HSCT and were conditioned with fludarabine, 200 Gy of TBI, and pre- and posttransplant cyclophosphamide (Table 5).85 Two of the patients, including the patient with SCD, engrafted with no evidence of GVHD, and both patients were free of disease at 1 and 4 years after transplantation. The development of a safe and effective low-intensity regimen that can be applied to the majority of patients with severe SCD with the use of haploidentical donors is necessary, and further research in this area is currently ongoing.

### Gene therapy for SCD

For those lacking a suitable allogeneic HSC donor, autologous HSC gene transfer with the use of viral vectors designed to permanently integrate a therapeutic gene into the target cell chromosomal DNA remains a rational alternative. Proof of concept through clinical successes in the immunodeficiencies and storage disorders has recently renewed enthusiasm for this approach,86-90 although vector-mediated insertional mutagenesis has also proven to be more than a theoretical risk in several patients. The globin disorders have long been held as a therapeutic target but have proven much more difficult because of the requirement for regulated, lineage-specific, high-level globin expression. The discovery of the human β-globin locus control region (LCR), which functions as a tissue-specific enhancer, led to the evolution of a new generation of globin retroviral vectors incorporating 4 or 5 hypersensitive LCR elements able to direct erythroid specific globin expression; however, these vectors were highly unstable and proved unreliable. The stable incorporation of these large LCR fragments became feasible with the development of vector systems based on HIV1, allowing for the first-time regulated human β-globin expression sufficient to revert the phenotype in a β-thalassemia mouse model,91 with confirmation soon after for both thalassemia and SCD.92,93 Recently, promising results were reported in a single patient with severe β^A/β^A thalassemia after autologous HSC gene transfer with the use of an HIV1-based vector encoding a mutant human β-globin.94 At 1 year after transplantation, hemoglobin levels increased to ~10 g/dL, allowing therapeutic phlebotomy, with an estimated 3 g/dL contribution by the therapeutic vector. This increase was, however, accompanied by an increase in the contribution by a clone bearing an integration in the HMGA2 gene, a gene that when overexpressed results in myeloproliferation.95 The significance of this clonal expansion will require long-term follow-up, and efforts to improve on the safety of integrating vectors remain a priority.

### Summary and future directions

HSCT for patients with SCD, although highly effective, is underused. The cumulative data on myeloablative HSCT in children and nonmyeloablative HSCT in adults indicate that there are shared interests in the sickle cell community to use HSCT as an immediately available curative therapy for patients who are at high risk for SCD-related morbidity and mortality. In these high-risk children and adults, enrollment in well-designed clinical studies can be justified, and SCD-specific transplantation regimens can emerge. We need successive regimens to be tested that use G-CSF–mobilized versus steady-state BM-derived HSCs and promote MDC as a minimum without exchanging for GVHD or graft failure. Future well-designed studies are poised to lead the field in immune tolerance and provide insight to differentiate graft-versus-host and graft-versus-malignancy effects. Indeed, achieving tolerance should allow extension of these potentially curative approaches to alternative donor sources. These efforts no doubt will continue to benefit children and now adults with chronic organ damage and in the future may allow application earlier in the disease. HSCT by the use of regimens developed in hematologic malignancies have brought cures to patients

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### Table 5. Mismatched related HSCT for SCD

<table>
<thead>
<tr>
<th>Reference</th>
<th>Transplant regimen</th>
<th>Number of patients (age, y)</th>
<th>Alive without SCN</th>
<th>Acute GVHD</th>
<th>Chronic GVHD</th>
<th>Death (cause)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raj et al, 2004</td>
<td>Flu 90 mg/m², 200 cGy TBI, CSA + MMF</td>
<td>1 (14 y)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Not available</td>
<td>Graft failure</td>
</tr>
<tr>
<td>Brodsky et al, 2008</td>
<td>Cy 29 mg/kg, Flu 150 mg/m², 200 cGy TBI, Cy 100 mg/kg, tac + MMF</td>
<td>1 (33)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>N/A</td>
<td>Pt with concomitant PNH</td>
</tr>
</tbody>
</table>

cGy, centi-Gray; CSA, cyclosporine A; Cy, cyclophosphamide; Flu, fludarabine; MMF, mycophenolate mofetil; N/A, not applicable; PNH, paroxysmal nocturnal hemoglobinuria; SCD, sickle cell disease; tac, tacrolimus; and TBI, total body irradiation.
with SCD; it is time to build on these experiences with SCD-specific issues in mind to increase the use of HSCT while minimizing morbidity and mortality.

Acknowledgments

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Allogeneic hematopoietic stem cell transplantation for sickle cell disease: the time is now

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