

REVIEW

Hematopoietic cell transplantation for thalassemia and sickle cell disease: past, present and future

M Bhatia¹ and MC Walters²

¹Department of Pediatrics, Columbia University, New York, NY, USA and ²Department of Pediatric Hematology/Oncology, Children's Hospital and Research Center, Oakland, CA, USA

β -Thalassemia major and sickle cell disease (SCD) are among the most common hereditary disorders worldwide. The supportive treatment of β -thalassemia major requires chronic, life-long RBC transfusions, which cause progressive iron overload and the potential for impaired endocrine, cardiac and hepatic function. The phenotype of thalassemia major is reliably predicted by its genotype. In contrast, SCD is a variable genetic disease caused by a single amino acid substitution in the β chain of human hemoglobin. Manifestations of SCD are quite varied, but generally result from the tendency of Hb S to irreversibly polymerize under physiologic stressors such as hypoxemia and acidosis. The polymerization causes perturbations in the erythrocyte integrity that promote vaso-occlusion and which manifest as clinical events such as severe painful episodes, acute chest syndrome, splenic infarction, stroke and avascular necrosis of target joints. The only cure proved for these disorders is correction of the genetic defect by allogeneic hematopoietic cell transplantation (HCT). We illustrate the pediatric experience of HCT for hemoglobinopathies and discuss how these results affect future therapeutic decisions in children who inherit these disorders.

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Introduction

β -Thalassemia major and sickle cell disease (SCD) are hereditary anemias that decrease lifespan and reduce the quality of life. While supportive therapies are available that minimize long-term sequelae, the only cure for these

disorders is allogeneic hematopoietic cell transplantation (HCT). While the outcomes after HCT are quite similar in both disorders, the decision about when and if to pursue HCT follows very different time-lines. All patients with thalassemia are at risk for transfusion-related iron overload and its attendant negative impact on clinical well-being. As a result, many clinicians strongly consider HCT as a therapeutic option in children who are less than 17 years of age and who have an HLA-identical sibling donor, due to the excellent results of HCT in this setting. However, in patients with SCD, the clinical symptoms are diverse and most clinicians delay HCT until there is evidence of severe disease, such as in those who have had a stroke, even though the results after HCT for SCD are very similar to results after HCT for thalassemia. This review presents the current state-of-the-art of HCT for β -thalassemia and SCD and discusses future directions that might improve survival and decrease morbidity.

β -Thalassemia major

Background

Thalassemias result from mutations of the globin genes that cause reduced or absent hemoglobin production, reducing oxygen delivery.¹ The thalassemias are the most common single-gene disorders, with 4.83% of the world population carrying a globin gene variant.² To treat the anemia and restore oxygen delivery to tissues, chronic lifelong transfusions are required in those who have thalassemia major. However, this promotes progressive iron overload and organ damage. Regular iron chelation by deferoxamine B slows this process, but progressive iron overload contributes to portal fibrosis and cardiac failure, and the former may be enhanced by concomitant hepatitis C virus infection. The only definitive cure for thalassemia is to correct the genetic defect by HCT.

Indications for HCT

In most cases, a suitable HLA-matched identical family donor must be identified before HCT for thalassemia. In addition, the patient typically is assigned to 1 of 3 Pesaro risk classes. This classification is based upon clinical features of thalassemia that include: (1) adherence to a program of regular iron chelation therapy, (2) the presence

Correspondence: Dr M Bhatia, Pediatric Blood and Marrow Transplantation, Morgan Stanley Children's Hospital of New York-Presbyterian, Columbia University, 3959 Broadway, CHN 10-07, New York, NY 10032, USA.

E-mail: mb2476@columbia.edu

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or absence of hepatomegaly and (3) the presence or absence of portal fibrosis observed by liver biopsy (Table 1). The conditioning regimen is uniform for Classes 1 and 2 patients (those who have none or one risk factor, respectively), but is modified for those who have Class 3 features (those with more than one risk factor) due to an increased risk of transplant-related mortality (TRM).³

Hematopoietic cell transplantation for β -thalassemia

In December of 1981, a 1-year-old thalassaemic patient with no transfusion exposures was treated by HCT in Seattle using an HLA-identical sibling donor. The patient remains alive and well more than 20 years later with no sequelae of β -thalassemia. Shortly after the initial Seattle case, a 14-year-old heavily transfused thalassaemia patient underwent HCT in Pesaro, but in contrast, had recurrence of disease.⁴ Since these initial cases, more than 900 patients in Pesaro, Italy, and over 1600 patients worldwide have been treated by HCT for β -thalassemia (Table 2).¹⁰ However, these initial cases illustrate how the approach to HCT in terms of patient selection and transplantation preparation evolved in response to risk factors in transplant recipients.

Table 1 Risk factors for BMT in hemoglobinopathies

Risk factors for BMT in thalassemia			
	Chelation	Hepatomegaly	Fibrosis
	Regular vs irregular	Absent vs present	Absent vs present
	Chelation	Hepatomegaly	Fibrosis
Risk classes for BMT in thalassemia			
Class 1	Regular	No	No
Class 2	Regular/irregular	No/Yes	No/Yes
Class 3	Irregular	Yes	Yes

Abbreviation: BMT = bone marrow transplantation.

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Table 2 Outcomes of hematopoietic cell transplantation in β -thalassemia (pediatric to young adults)

Author	N	Age median/range (yrs)	Stem cell source	TRM (%)	Survival (%)	aGVHD ≥ 2 (%)	cGVHD (%)
Lucarelli <i>et al.</i> ⁵	886	NA (1–35)	MSD/MRD	NA	TFS: 73	NA	NA
La Nasa <i>et al.</i> ⁶	68	15 (2–37)	MUD	20	OS: 79.3	40	18
Locatelli <i>et al.</i> ⁷	33	5 (1–20) ^a	Matched: 27 (61%) ^a	NA	2 years EFS: 79	11 ^a	6 ^a
	Thalassemia						
	11 SCD		Mismatched: 17 (39%) ^a		OS: 100 ^a		
Hongeng <i>et al.</i> ⁸	49	Related: 7.2 (0.5–18.7) Unrelated: 4 (0.7–12)	Related: 28 Unrelated: 21	10	OS (all patients): 89	Related: 32 Unrelated: 42	Related: 14 Unrelated: 14
Gaziev <i>et al.</i> ⁹	29	6 (1.1–33)	HLA-phenotypically identical relatives: 6 Mismatched relatives: 2 mMSD: 13 Mismatched parents: 8	34	OS: 65 EFS: 21	47.3	37.5

Abbreviations: aGVHD = acute graft-versus-host disease; cGVHD = chronic graft-versus-host disease; EFS = event-free survival; mMSD = mismatched sibling donor; MRD = matched related donor; MSD = matched sibling donor; MUD = matched unrelated donor; NA = not available; OS = overall survival; SCD = sickle cell disease; TFS = thalassaemia-free survival; TRM = transplant-related mortality.

^aIncludes total cohort (thalassaemia/SCD).

A study by Lucarelli *et al.*³ in 1990 reported the initial results after HCT involving 222 patients with β -thalassaemia. All patients received a uniform conditioning regimen of busulfan (14 mg/kg) (Bu14) and cyclophosphamide (200 mg/kg) (CY200) and GVHD prophylaxis consisted of CsA, MTX and in a few instances, antilymphocyte globulin (ALG). An analysis of a cohort of these patients ($n = 99$) showed that thalassaemia-free survival in Classes 1 and 2 patients was 94 and 77%, respectively (Figure 1), with Class 3 patients experiencing a decreased rate of thalassaemia-free survival (53%).³ Subsequently, Class 3 patients were conditioned with a lower dose of cyclophosphamide (160 mg/kg) in an effort to decrease TRM. This modification improved survival after HCT in Class 3 patients <17 years (79 vs 65%), but this was associated with a graft rejection (GR) rate of 30%, thus overall event-free survival (EFS) was not affected by the dose modification (Figure 2).¹¹ To decrease the rejection rate in this group of patients, a newer regimen was adopted in 1997. The aim of the novel approach was to accomplish bone marrow ablation and also enhance pre-HCT immunosuppression by administering hydroxyurea (30 mg/kg per day) and azathioprine (3 mg/kg per day) well in advance of HCT, from day –45 and also administer fludarabine (FLU) (20 mg/m² per day) from days –17 to –11. In addition, chelation and hypertransfusion were performed during the pre-HCT conditioning. The patients then received conventional conditioning with Bu14 and CY160 (patients <17 years) or CY 90 mg/kg (patients >17 years). A recent analysis of 29 Class 3 patients <17 years showed a thalassaemia-free survival of 90% and an overall survival (OS) of 96%.⁵ Furthermore, the probability of death and rejection were improved, with rates of 4 and 7%, respectively.⁵

The North American experience of HCT for β -thalassaemia, although limited, has been similar to the Italian series.^{12–14} At the University of California, San Francisco, 17¹² patients (ages 0.8–18 years) underwent HLA-matched sibling ($n = 16$) or phenotypic HLA-matched parental ($n = 1$) HCT. All were conditioned with Bu16, antithymocyte

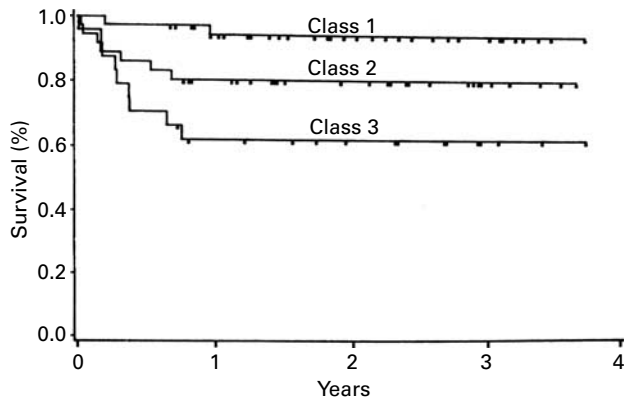


Figure 1 Probabilities of survival after transplantation in 99 patients with thalassemia. The patients in Class 1 ($n=39$) had neither hepatomegaly nor portal fibrosis, those in Class 2 ($n=36$) has only one of the risk factors, and those in Class 3 ($n=24$) had both. Lucarelli *et al.* Bone marrow transplantation in patients with thalassemia. *N Eng J Med* 1990; **322**: 417–421. © Copyright [1990] Massachusetts Medical Society. All rights reserved.³

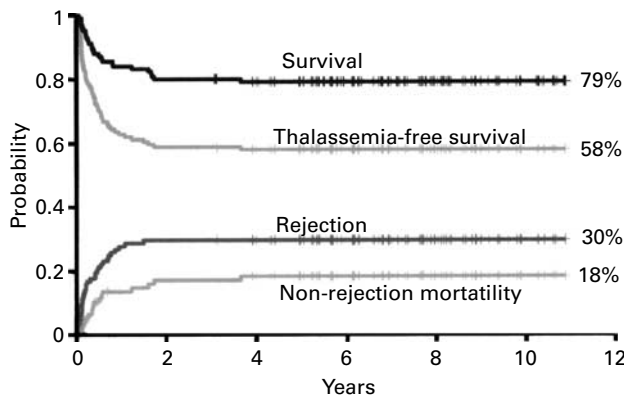


Figure 2 Survival and rejection rate in 122 Class 3 thalassemia age less than 17 years receiving busulfan 14 mg/kg and cyclophosphamide 120–160 mg/kg. Originally published in Lucarelli *et al.* The cure of the thalassemia with bone marrow transplantation. *Bone Marrow Transplant* 2001; **28** (Suppl 1): S11–S13, © Copyright (2001), with permission from Nature Publishing.¹¹

globulin (ATG) (80 mg/kg) and CY200. OS was 94%, with a thalassemia-free survival of 71%.¹⁴ Interestingly, 5 of these patients had Pesaro Classes 2 or 3 characteristics. Similarly, a report from Memorial Sloan Kettering Cancer Center included 13 children (age 1.2–15 years) who received an HLA-matched ($n=12$) or single HLA-antigen mismatched ($n=1$) family donor bone marrow transplantation following conditioning by Bu14 and CY200 ($n=9$) or TBI (720 cGy) and CY120 mg/kg ($n=4$). The OS was 92.3% and the thalassemia-free survival was 84.6%. A role for radiation in conditioning is suggested by this report, although earlier experience in Italy indicated that the mortality rate was higher in regimens with TBI.³

Alternate donor stem cell transplantation in thalassemia

Related donor umbilical cord blood transplantation (UCBT). While bone marrow as a source of hemato-

poietic cells from an HLA-identical sibling is the gold standard in patients with β -thalassemia, there is still a risk of TRM and GVHD after BMT. Cord blood is an alternative source of hematopoietic cells in pediatric patients with malignant and nonmalignant conditions. Several studies have suggested that UCBT recipients benefit from a lower risk of GVHD^{15,16} and a recent analysis comparing 113 children who received a UCBT from a compatible sibling with 2052 HLA-identical sibling marrow transplant recipients showed that children receiving UCB experienced a significantly reduced risk of developing acute GVHD (aGVHD) and chronic GVHD (cGVHD).¹⁷

Locatelli *et al.*⁷ retrospectively analyzed 44 patients who had thalassemia ($n=33$) or SCD ($n=11$). The median age was 5 years (1–20 years). All patients with thalassemia had Pesaro Classes 1 or 2 risk features. Conditioning regimens were variable with Bu14 or Bu16 as the backbone combined with CY, FLU or Thiotepa (TT). A total of 18 patients also received ALG or ATG. GVHD prophylaxis was also variable but the majority of patients ($n=30$, 68%) received CsA alone. The median nucleated cell dose was 4×10^7 total nucleated cells per kg recipient weight. The OS in both groups was 100%. The thalassemia disease-free survival was 79% and the SCD-free survival was 90%. Among the thalassemics, the use of BU, TT and CY, or BU, TT and FLU in the preparative regimen was associated with a significantly higher probability of EFS compared with the combination of BU and CY with or without ATG (94 vs 62%, respectively, $P=0.03$). The EFS in thalassemia patients who had Classes 1 and 2 features was 89 and 62%, respectively. Of the 38 patients who engrafted, only 4 developed grade 2 aGVHD. Among those patients who developed aGVHD, 2 received allografts from an HLA-disparate donor. Limited cGVHD was diagnosed in 2 of 36 evaluable patients, 1 of whom had aGVHD. The Kaplan–Meier estimate of probability of developing aGVHD and cGVHD was 6 and 11%, respectively.¹⁵

Alternate related donor HCT

Generally, the results after HCT using HLA-mismatched donors are inferior compared to HLA-identical sibling HCT due to an increased incidence of and severity of GVHD, infections and GR.^{18,19} Gaziev *et al.*⁹ analyzed 29 patients with β -thalassemia who received HCT from phenotypically HLA-matched relatives ($n=6$), HLA-mismatched relatives ($n=2$), mismatched siblings ($n=13$) or mismatched parents ($n=8$). The median age was 6 years (1.1–33 years; only one patient was greater than 16 years). Pesaro classification was as follows Class 1 ($n=6$), Class 2 ($n=17$) and Class 3 ($n=6$). The majority of patients ($n=15$, 52%) had a single HLA-antigen mismatch. Conditioning regimens were variable but all received Bu8, 14 or 16 mg/kg in combination with CY, TBI or TLI. Nine patients received ALG. GVHD prophylaxis was variable with the majority receiving CsA, methylprednisolone and MTX ($n=22$, 77%). After HCT, there was sustained engraftment of donor cells in only 13 patients (44.8%). A total of 62% of recipients experienced an infectious complication. A total of 10 patients (34%) experienced TRM, and 7 died during the first 100 days post-HCT. Of 19 patients evaluable for aGVHD, 9 developed grade II–IV

aGVHD (47.3%) and of the 8 patients evaluable for cGVHD, 3 developed severe cGVHD (37.5%) which was fatal in two cases. Overall survival and EFS were 65 and 21%, respectively, which is significantly lower compared to results after HLA-identical sibling HCT for thalassemia. This study demonstrated higher than acceptable rates of mortality and GVHD and at present, HLA-mismatched related donor HCT is not a suitable option for patients who lack an HLA-identical sibling donor.

Unrelated donor HCT for thalassemia

In an effort to expand HCT to those who lack a suitable sibling donor, unrelated donor HCT has been pursued.^{6,8} In one recent report, 68 thalassemic patients were treated by unrelated volunteer donors using donor selection criteria that relied on high-resolution molecular testing at HLA Class 1 and 2 loci. Patients had a median age of 15 years (range 2–37 years). A total of 14 patients were classified in risk Class 1, 16 in Class 2 and 38 in risk Class 3 using the Pesaro classification. Overall and thalassemia-free survival was 79.3 and 65.8%, respectively, and those with Classes 1 and 2 risk features had an overall and thalassemia-free survival of 96.7 and 80%, respectively. A total of 45 patients had full donor chimerism after HCT. A total of 24 of 59 evaluable patients (40%) developed grade II–IV aGVHD and 10 patients (17%) developed grade III–IV aGVHD. Among the 56 evaluable cases, 10 developed cGVHD (18%), limited in five cases and extensive in five cases.⁶

Similar results were reported in another analysis that included 49 children with thalassemia. In this series, 28 patients received a related donor allograft and 21 had an unrelated donor. With respect to engraftment, transplant-related complications, and thalassemia-free survival, there was no superior donor source identified. The 2-year thalassemia-free survival for recipients of related donor SCT was 82% as compared with 71% in the unrelated donor stem cell group. Three patients in the unrelated group who had GR experienced autologous recovery and are alive. Two patients in the related group died and three in the unrelated group died ($P=0.63$) of sepsis, GVHD or bleeding.⁸ Together, these data strongly suggest that improvements in donor selection and transplantation preparation have improved the safety of unrelated donor HCT for thalassemia, and in selected patients, this is an approach to pursue when there is no suitable sibling donor.

Sickle cell disease

Background

SCD contrasts with thalassemia major by its variable course of clinical severity. Its typical clinical manifestations include anemia, severe painful crisis, acute chest syndrome, splenic sequestration, stroke (clinically overt and silent), chronic pulmonary and renal dysfunction, growth retardation, neuropsychological deficits and premature death. Historically, the mainstays of treatment are both preventive and supportive. A number of specific milestones have been responsible for improving outcomes and these include

penicillin prophylaxis, improved management of febrile episodes and the recognition of acute chest syndrome as an important cause of morbidity and mortality. For those children affected by SCD, three major therapeutic options are available: chronic blood transfusion, hydroxyurea and HCT. Of these options, only HCT affords patients the possibility of cure. The first allogeneic BMT for SCD was reported in 1984 in a patient with AML who also had SCD.²⁰ Since this initial report more than 250 patients with SCD have been treated by HLA-identical sibling HCT.

Indications

While more than 1600 patients worldwide with β -thalassemia major have undergone allogeneic HCT, many fewer patients with SCD have done so.^{4,21,22} This is due in part to the more variable clinical course of SCD and limitation of patient eligibility to individuals with advanced stage disease often involving neurological and pulmonary vasculopathy.^{4,23,24}

The preliminary experience of HCT for β -thalassemia major has in part provided the rationale for extending this treatment to sickle cell anemia. Walters *et al.*²⁴ used selection criteria similar to that applied to patients with β -thalassemia major and chose patients with debilitating clinical events, including stroke, recurrent acute chest syndrome and recurrent painful vaso-occlusive crises, but selected children rather than adults and before the development of permanent end organ damage (Table 3). These complications are associated with significant morbidity and early mortality among patients with SCD²⁶ and are the criteria upon which most early studies are based.

Hematopoietic cell transplantation for SCD

Three major clinical series account for most of the experience of HCT for SCD. The multicenter collaborative study enrolled 59 patients with symptomatic SCD^{25,27} and the Belgian group reported results in 36 patients with previous sickle-related morbidity and 14 asymptomatic patients.²⁸ The largest series to date was published recently by Bernaudin *et al.*²⁹ in which 87 symptomatic patients with SCD were treated. The majority of patients received HLA-identical sibling donor allografts. The results of these three studies were very similar. OS was 92–94% and EFS was 82–86% with a median follow-up range of 0.9–17.9 years. Figure 3 depicts the outcomes for the first 50 patients with SCD transplanted in the multicenter collaborative study.²⁷ All patients with stable donor engraftment survive free of the clinical manifestations of SCD. Interestingly, the results in patients with asymptomatic disease were superior to those with symptomatic disease, with an OS of 100 vs 88% and EFS of 93 vs 76%, respectively, in the Belgian cohort (Table 4).^{28,30}

TRM from all three series was also similar and was approximately 7% with infections as the chief cause. Similarly, the incidence of aGVHD > grade II was approximately 15–20%. The rate of cGVHD was 20% in Vermeylen *et al.* study compared to 12 and 13.5% in the Walters *et al.* and Bernaudin *et al.* reports, respectively.

In these three series, all patients were conditioned with Bu 14–16 mg/kg or 485 mg/m² with CY200; ATG was also

Table 3 Criteria for eligibility for transplantation in children with sickle cell disease

Criteria for inclusion

- Sickle cell disease (sickle cell anemia, sickle cell–hemoglobin C disease or sickle cell– β -thalassemia)
- Age less than 16 years
- HLA-identical related donor
- One or more of the following
 - Stroke or central nervous system event lasting longer than 24 h
 - Acute chest syndrome with recurrent hospitalizations or previous exchange transfusions
 - Recurrent vaso-occlusive pain (≥ 2 episodes per year for several years) or recurrent priapism
 - Impaired neuropsychological function and abnormal cerebral MRI scan
 - Stage I or II sickle lung disease
 - Sickle nephropathy (moderate or severe proteinuria or a glomerular filtration rate 30–50% of the predicted normal value)
 - Bilateral proliferative retinopathy and major visual impairment in at least one eye
 - Osteonecrosis of multiple joints
 - Red-cell alloimmunization (≥ 2 antibodies) during long-term transfusion therapy

Criteria for exclusion

- Age greater than 15 years
- Lack of availability of HLA-identical donor^a
- One or more of the following
 - Karnofsky or Lansky functional performance score $< 70^b$
 - Acute hepatitis or evidence of moderate or severe portal fibrosis or cirrhosis on biopsy
 - Severe renal impairment (glomerular filtration rate, $< 30\%$ of the predicted normal value)
 - Severe residual functional neurologic impairment (other than hemiplegia alone)
 - Stage III or IV sickle lung disease
 - Demonstrated lack of compliance with medical care
 - Seropositivity for the human immunodeficiency virus

Walters MC, Patience M, Leisenring W, *et al.* Bone marrow transplantation for sickle cell disease. *N Engl J Med* 1996; **335**: 369–376. Copyright © 1996 Massachusetts Medical Society. All rights reserved.²⁵

^aPatients with HLA-matched related donors with the sickle-cell trait were not excluded.

^bThe Lansky performance is a measure of functional status in children.

administered in the French and multicenter studies to mitigate the risk of rejection and unstable mixed chimerism. GVHD prophylaxis consisted of CsA or CsA with MTX.

Alternate donor stem cell transplantation in SCD

While HCT is curative in patients with SCD, only 14–18% of patients with SCD are estimated to have a suitable family donor. Cord blood and marrow donations from family donors have been used with equal success in SCD.³¹ In the analysis noted earlier that included 11 patients with SCD, the OS was 100% and the EFS was 90% after UCBT for SCD.⁷ A single patient with SCD had GR after UCBT and there were low rates of acute and cGVHD.

The use of unrelated donors in HCT for SCD is under development. There are several limitations which restrict the uniform utilization of allogeneic adult donors that include donor availability, and the high risk of severe aGVHD. Recently, unrelated donor UCB safely reconstituted recipients with malignant and nonmalignant diseases after 2–3 HLA-antigen disparate HCT with a median time to myeloid engraftment of 24–28 days.^{32,33} Limited results

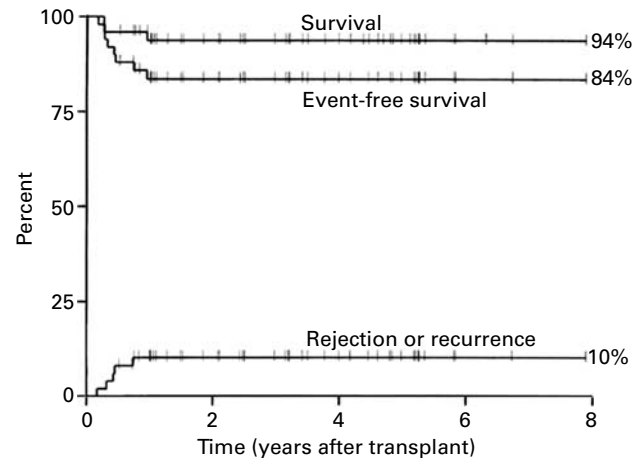


Figure 3 Outcome after transplantation for 50 children with advanced, symptomatic sickle cell disease. Kaplan-Meier estimates for survival and event-free survival following marrow transplantation are shown. An event is defined as death, graft rejection or recurrence of sickle cell disease. A cumulative incidence curve for graft rejection and return of sickle cell disease is also depicted. This research was originally published in *Blood*. Walters *et al.* Impact of bone marrow transplantation for symptomatic sickle cell disease: an interim report. *Blood* 2000; **95**: 1918–1924. © The American Society of Hematology.²⁷

of unrelated donor UCBT for SCD show promise for the future, but infection, GR and GVHD remain obstacles. Adamkiewicz recently published results on seven children with SCD and stroke receiving chronic transfusions who underwent unrelated UCBT. Five patients received an HLA 4/6 antigen matched UCBT and two patients received a 5/6 HLA-antigen matched UCBT. The regimens were variable with four receiving a myeloablative regimen and three patients receiving a reduced intensity regimen. Of these seven patients, three had sustained engraftment, all receiving a myeloablative preparative regimen. Among the patients who engrafted, two of three developed grade III–IV aGVHD, one patient developed cGVHD and the other patient died. Four patients developed (57%) viral infections. This study was limited in sample size and indicates that a prospective, multicenter trial with similar treatment guidelines might be more effective and informative.³⁴

Reduced intensity conditioning regimens

Efforts to expand the application of HCT for SCD and thalassemia have been restricted not only by lacking suitable donors, but also by the risk of significant toxicity that accompanies this intensive procedure.³⁵ A recent review of 175 SCD patients treated by conventional HCT observed complications of GR and disease recurrence in 16 patients (9%) with 17 patients (9%) dying of transplant-related causes. To date, this therapy is generally reserved for those who have experienced significant sequelae of SCD. To consider HCT for SCD before irreversible vital organ damage has occurred and thereby expand its availability, it is necessary to improve first the risk–benefit ratio.^{35,36}

Recently, reduced toxicity regimens, often employing FLU, have been tested in allogeneic HCT to reduce the

Table 4 Pediatric hematopoietic cell transplantation in sickle cell disease

Author	Center/ Group	N	Age median/range (years)	Follow-up median/range (years)	Graft rejection (%)	TRM (%)	EFS (%)	aGVHD ≥ 2 (%)	cGVHD (%)
Vermeylen <i>et al.</i> ²⁸	Belgium	50	7.5 (0.9–23)	5 (0.9–15)	10	7	82	20	20
Walters <i>et al.</i> ²⁷	USA	59	9.4 (3.3–14)	3.2 (0.5–7.9)	10	6	84	15	12
Bernaudin <i>et al.</i> ²⁹	SFGMTC	87	8.8 (2.2–22)	6 (2–17.9)	7	6.9	86.1	20	13.5

Abbreviations: aGVHD = acute graft-versus-host disease; cGVHD = chronic graft-versus-host disease; EFS = event-free survival; SFGMTC = Société Française de Greffe de Moelle et de Thérapie Cellulaire; TRM = transplant-related mortality.

cytotoxic/morbid effects of traditional myeloablative conditioning regimens. These range in the degree of intensity from being minimally toxic and nonmyeloablative (NMA), and conducted in the outpatient setting to being moderately ablative and requiring hospitalization. However both are associated with fewer side effects compared to standard myeloablative regimens. The rationale for lower intensity conditioning regimens in hemoglobinopathies stems from the historical experience indicating that stable mixed chimerism is sufficient for cure.^{37,38} Thus, myeloablation may not be necessary for a successful outcome. Iannone *et al.*,³⁵ in a retrospective multicenter series, described a NMA HCT approach in seven pediatric patients with SCD ($n=6$) and thalassemia ($n=1$). This very low intensity conditioning regimen consisted of minimal intensity FLU/2GY TBI regimen. Two patients also received ATG. Regimen-related toxicity was minimal but resulted in only transient donor engraftment in six of seven patients. These results suggested that more intensive conditioning is required in individuals with pre-HCT transfusion exposures. This is also supported by the observation of frequent GR after myeloablative allogeneic HCT (5–10%). Thus, it is not likely that a minimal intensity conditioning regimen will sustain donor chimerism that is sufficient for a therapeutic effect, especially in transfused recipients. Another factor that affects outcome is the timing and tempo of immunosuppression withdrawal after NMA HCT. Studies in patients with malignant diseases typically follow a rapid taper of post-grafting immunosuppression to elicit a graft-versus-leukemia effect. However, a rapid taper of immunosuppressive therapy appears to increase the likelihood of GR in patients with SCD. An emphasis on immunosuppression rather than myeloablation may be worthy of consideration in future studies to reduce the toxicity of HCT for SCD. If these studies are able to show sustained mixed donor chimerism with low regimen-related toxicity, the paradigm that only severely affected patients with SCD be offered HCT may shift thus allowing more low-risk patients the alternative of cure without long-term morbidity.

Autologous gene therapy for hemoglobinopathies

In the absence of suitable donors, alternate curative therapies are under investigation. The genetic correction of autologous hematopoietic stem cells (HSCs) represents an attractive treatment option.³⁹ Issues common to all stem cell-based gene therapies include isolation and transduction

of HSCs, the design of vectors that provide therapeutic expression with a minimal risk of insertional oncogenesis, and the implementation of nontoxic transplant conditions that permit host repopulation.⁴⁰ Furthermore, globin therapy ideally should be erythroid specific, differentiation and stage specific, elevated, position independent and sustained.⁴⁰ Achievement of therapeutic β -globin expression has been an obstacle in the success of gene therapy, but recent studies have indicated that therapeutic levels of hemoglobin synthesis can be attained in the progeny of virally transduced HSCs. Using lentiviral vectors to obtain high-level expression of β -globin genes, therapeutic correction of several murine models of both β -thalassemia and SCD has been accomplished.⁴¹ Research has recently focused on safety issues with the initiation of the first phase I/II clinical trials in Europe in 2006.⁴²

Preimplantation genetic diagnosis for hemoglobinopathies

Hemoglobin disorders are among the most frequent indications for preimplantation genetic diagnosis (PGD). Polymerase chain reaction (PCR) technology has been used to detect point mutations or deletions in chorionic-villous samples, enabling first trimester, DNA-based testing for β -thalassemia and SCD.⁴³ Because pregnancy termination is unacceptable to some persons, methods have been developed to perform diagnostic testing before implantation. Briefly, PGD requires *in vitro* fertilization followed by removal of one or two cells from the blastomere on day 3.⁴⁴ PCR is then used to detect mutations within the cells that have been removed so that unaffected blastomeres may be selected for implantation. Recently, PGD has been extended to include HLA typing of embryonic biopsies, which facilitates the selection of an embryo that is not affected by either thalassemia or SCD and may also serve as an HLA-identical donor for an affected sibling. Reports have confirmed the feasibility of this approach.^{45–48} The financial cost is considerable and serious ethical concerns have been raised by the practice of selecting an embryo solely for the purpose of HSC donation.

Complications after HCT

β -Thalassemia

The long-term consequences of HCT are influenced by the transplant treatment and by the complications secondary to thalassemia and its treatment.

Iron overload. Almost all patients with thalassemia have significant iron overload after HCT. The iron burden slowly decreases after HCT, but the rate of decline is accelerated by regular phlebotomy or iron chelation therapy after HCT.^{49–51} In Pesaro, a program utilizing regular phlebotomy has been successful in Classes 2 and 3 thalassemics after HCT. Reduction in the body iron burden was accompanied by lowering of the serum alanine aminotransaminase level and improvement in the histologic changes of chronic hepatitis C, observed in 85% of these patients.⁴⁹ Furthermore, investigators found that reducing iron overload by phlebotomy restored left ventricular diastolic function and indices of contractility when the liver iron level was decreased in the range of 3.2–7 mg Fe per gram liver dry weight.⁵²

Growth and development. The adverse effects on growth, development and fertility after transplantation conditioning are often difficult to distinguish from effects of pre-existing iron overload. Failure of growth and sexual development occurs in as many as two-thirds of individuals with thalassemia major.⁵³ For children treated by HCT before 8 years of age, growth is usually normal in contrast to older children or those with Class 3 features, who tend to experience impaired growth.^{54,55} Endocrine dysfunction in patients with β -thalassemia major usually is a consequence not only of iron overload after regular RBC transfusions, but also of the preparative conditioning before HCT. While the data are limited, studies of endocrine function after HCT have been performed. Li *et al.*⁵⁶ reported results in 15 patients (10 boys and 5 girls) who were pubertal at HCT and who were followed for longer than 12 years after HCT. During the follow-up, 80% (8/10) boys had spontaneous onset of puberty and 20% (2/10) developed gonadal failure requiring hormonal replacement. The two males who required androgen replacement were 15 years old at HCT and had no signs of pubertal development. Among the eight males with spontaneous puberty, three showed some degree of gonadal impairment. Interestingly, these three males were 12 years of age at HCT. Two had elevated follicle stimulating hormone levels, but normal testosterone levels, and one had decreased testosterone, but increased luteinizing hormone levels. Of the 5 females, 100% had ovarian failure and required hormonal replacement to induce puberty. In addition, five girls who had entered puberty before HCT all developed ovarian failure after BMT and required estrogen replacement.

Sickle cell disease

Growth and development. Eggleston *et al.*⁵⁷ recently studied linear growth in patients with SCD after HCT. Results showed improved growth after HCT in patients with SCD. Myeloablative conditioning did not appear to inhibit growth provided that it was not carried out near or during the adolescent growth spurt.

Gonadal failure and delayed sexual development were delayed after HCT, although reports are limited in size. Of six females who received Bu16/CY200 from the Belgian series, five had primary amenorrhea and one underwent

spontaneous menarche.²⁸ Similarly in the multicenter collaborative study, six of the seven evaluable females had primary amenorrhea.²⁷ The majority of evaluable males who received Bu16/CY200 had normal sexual development and this was a consistent finding in all three major series.

CNS Disease. Walters *et al.*²⁷ have recently evaluated CNS disease in 26 patients with at least 2 years post-HCT follow-up. A total of 19 of the patients had evidence of CNS abnormalities before HCT. Of the 22 engrafted patients, none had SCD-related neurological events post-HCT and the majority had stabilization or improvement of cerebral vasculopathy on MRI and MRA, these results are similar to observations in the French and Belgian series.^{28,29}

Splenic dysfunction. The Belgian group reported splenic reticuloendothelial recovery post-HCT for SCD: an increase in the splenic red cell pool in 7/10 patients studied 3–36 months post-HCT suggests that splenic dysfunction may be reversible after successful HCT.²⁸

Conclusion

Allogeneic HCT is curative in selected patients with clinically significant hemoglobinopathies. For those with β -thalassemia, results have indicated a thalassemia-free survival and EFS over 70% in patients reported worldwide. When stratifying patients, initially those with Pesaro Class 1 characteristics <17 years had a superior thalassemia-free survival; however, recent updates show that outcomes are very similar across all three risk categories after employing risk-based conditioning regimens. Alternatively, the use of HCT in SCD is applied infrequently compared with β -thalassemia, although outcomes are similar. The application of HCT for SCD is currently reserved for those with severe disease. Even so, the OS and SCD-free survival is 93 and 85%, respectively. However, not all patients who might benefit are able to pursue this option due to a lack of suitable donors. With the advent of alternate donor HCT and improving HLA-typing methodology, more appropriate donors may be identified, thus affording more patients the option of cure. In addition, patient selection is still controversial although there is a trend to treat younger patients in order to decrease the risk of GR and transplant-related morbidity and mortality. With the advent of lower intensity conditioning regimens which rely on less myeloablation and more immunosuppression, many of the long-term effects, such as growth and endocrine dysfunction observed after myeloablative conditioning regimens, may be ameliorated. Furthermore, older patients who would have not been considered for HCT may benefit, although this approach is still under development.

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