

The New England Journal of Medicine

© Copyright, 1996, by the Massachusetts Medical Society

VOLUME 335

AUGUST 8, 1996

NUMBER 6



BONE MARROW TRANSPLANTATION FOR SICKLE CELL DISEASE

MARK C. WALTERS, M.D., MELINDA PATIENCE, R.N., M.S.N., WENDY LEISENRING, PH.D., JAMES R. ECKMAN, M.D., J. PAUL SCOTT, M.D., WILLIAM C. MENTZER, M.D., SALLY C. DAVIES, M.D., KWAKU OHENE-FREMPONG, M.D., FRANÇOISE BERNAUDIN, M.D., DANA C. MATTHEWS, M.D., RAINER STORB, M.D., AND KEITH M. SULLIVAN, M.D.

ABSTRACT

Background We investigated the risks and benefits of allogeneic bone marrow transplantation in children with complications of sickle cell disease.

Methods Twenty-two children less than 16 years of age who had symptomatic sickle cell disease received marrow allografts from HLA-identical siblings between September 1991 and April 1995. The indications for transplantation included a history of stroke ($n = 12$), recurrent acute chest syndrome ($n = 5$), and recurrent painful crises ($n = 5$). Patients were prepared for transplantation with busulfan, cyclophosphamide, and antithymocyte globulin.

Results Twenty of the 22 patients survived, with a median follow-up of 23.9 months (range, 10.1 to 51.0), and 16 patients had stable engraftment of donor hematopoietic cells. In three patients the graft was rejected and sickle cell disease recurred; in a fourth patient graft rejection was accompanied by marrow aplasia. In 1 of the 16 patients with engraftment, there was stable mixed chimerism. Two patients died of central nervous system hemorrhage or graft-versus-host disease. Kaplan-Meier estimates of survival and event-free survival at four years were 91 percent and 73 percent, respectively. Among patients with a history of acute chest syndrome, lung function stabilized; among patients with prior central nervous system vasculopathy who had engraftment, stabilization of cerebrovascular disease was documented by magnetic resonance imaging.

Conclusions Allogeneic stem-cell transplantation can be curative in young patients with symptomatic sickle cell disease. (N Engl J Med 1996;335:369-76.)

©1996, Massachusetts Medical Society.

TRANSPLANTATION of hematopoietic stem cells from HLA-identical siblings can be curative in several nonmalignant hematologic disorders, including aplastic anemia, β -thalassemia major, congenital immunodeficiency disorders, and certain inborn errors of metabolism.¹⁻³ Pilot studies of bone marrow transplantation for the treatment of young patients with symptomatic sickle cell disease have demonstrated eradication of the underlying disease with low transplantation-related mortality.⁴⁻¹⁰ We undertook this study to determine whether these results could be reproduced in a multicenter trial.

Because the clinical course of sickle cell disease is highly variable and difficult to predict, we attempted to identify patients at risk for poor outcomes before extensive organ damage due to sickle cell disease occurred. This strategy was similar to that applied in patients with β -thalassemia major.¹¹ We selected patients with debilitating clinical events, such as stroke, recurrent acute chest syndrome, and recurrent pain-

From the Division of Clinical Research, Fred Hutchinson Cancer Research Center, Seattle (M.C.W., M.P., W.L., D.C.M., R.S., K.M.S.); the Departments of Pediatrics (M.C.W., D.C.M.) and Medicine (R.S., K.M.S.), University of Washington, Seattle; Emory University, Atlanta (J.R.E.); the Medical College of Wisconsin, Milwaukee (J.P.S.); the University of California, San Francisco (W.C.M.); Central Middlesex Hospital National Health Service Trust, London (S.C.D.); the University of Pennsylvania, Philadelphia (K.O.-F.); and Hôpital Henri Mondor, Creteil, France (F.B.). Address reprint requests to Dr. Sullivan at the Fred Hutchinson Cancer Research Center, Division of Clinical Research, FB600, 1124 Columbia St., Seattle, WA 98104.

Other contributing authors were George R. Buchanan, M.D., University of Texas Southwestern Medical Center, Dallas; Philip J. Darbyshire, M.B., Ch.B., Birmingham Children's Hospital National Health Service Trust, Birmingham, United Kingdom; Robertson Parkman, M.D., University of Southern California, Los Angeles; Roswitha Dickerhoff, M.D., University of Bonn, Augustin, Germany; Franklin O. Smith, M.D., Indiana University School of Medicine, Indianapolis; and Jean E. Sanders, M.D., Fred Hutchinson Cancer Research Center, Seattle.

Investigators and centers participating in this study are listed in the Appendix.

ful vaso-occlusive crises, which contribute to the high morbidity and early mortality among patients with sickle cell disease.¹² We report here the results of allogeneic stem-cell transplantation in 22 children with symptomatic sickle cell disease.

METHODS

Patients less than 16 years of age with symptomatic sickle cell disease (sickle cell anemia [hemoglobin genotype S/S], sickle cell-hemoglobin C disease [S/C], or sickle cell- β -thalassemia) and HLA-identical sibling donors (hemoglobin genotype A/A or A/S) were considered for marrow transplantation. Typing for HLA-A, HLA-B, and HLA-DR antigens was performed with a standard complement-dependent microcytotoxicity assay.¹³ All patients were required to meet the eligibility criteria outlined in Table 1^{10,14}; those with extensive end-organ damage (e.g., stage III or IV sickle lung disease or severe renal impairment) were excluded.^{15,16} Patients were enrolled at 15 centers in the United States and Europe (see the Appendix). The study was approved by the institutional review board of the Fred Hutchinson Cancer Research Center and by the institutional review boards or their equivalents at each of the

TABLE 1. 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 hours
 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 to 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*
 One or more of the following:
 Karnofsky or Lansky functional performance score < 70 †
 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

*Patients with HLA-matched related donors with the sickle-cell trait were not excluded.

†The Lansky performance score is a measure of functional status in children.

collaborating sites. All patients or their parents or guardians gave written informed consent for their participation.

A Data Safety and Monitoring Board was appointed by the National Heart, Lung, and Blood Institute to monitor the safety of patients, adherence to ethical standards, and progress of this investigation. The board consisted of five hematologists, a biostatistician, and two patient advocates. Quarterly reports were submitted, and annual progress reports were presented to the board by the principal investigators.

Fourteen patients were prepared for transplantation with a combination of busulfan (total dose, 14 mg per kilogram of body weight, administered every six hours for 16 doses over four days), cyclophosphamide (total dose, 200 mg per kilogram, administered in 4 daily doses of 50 mg per kilogram), and horse antithymocyte globulin (ATGAM; total dose, 90 mg per kilogram, administered in 3 daily doses of 30 mg per kilogram). Four patients received CAMPATH antibody (10 mg per kilogram each day for five days) in lieu of antithymocyte globulin.¹⁷ Patients 9, 10, and 11 received busulfan (total dose, 500 mg per square meter of body-surface area, in 16 doses over four days), cyclophosphamide (200 mg per kilogram, as above), and rabbit antithymocyte globulin (total dose, 20 mg per kilogram, in 4 daily doses of 5 mg per kilogram), and Patient 6 received busulfan (16 mg per kilogram, in 16 doses over four days), cyclophosphamide (200 mg per kilogram, as above), and antithymocyte globulin (80 mg per kilogram, in 4 daily doses) at the discretion of the investigators at the center. Since November 1994, the pharmacokinetics of busulfan have been monitored in North American patients to maintain adjusted steady-state blood concentrations of 400 to 600 ng per milliliter in order to lower the risks of graft rejection and toxic effects. On the basis of their blood concentrations, we calculated that Patients 13, 19, and 20 (who were 5.2, 3.2, and 12.2 years of age, respectively) received a total dose of 14 mg of busulfan per kilogram, and Patient 17 (who was 4.2 years old) received 16 mg per kilogram. Patients received either a combination of methotrexate and cyclosporine (21 patients) or cyclosporine and prednisone (1 patient) for the prevention of acute graft-versus-host disease (GVHD).^{18,19} Prophylaxis with cyclosporine was given for six months after transplantation. The definitions and methods of grading acute and chronic GVHD have been described previously.^{18,19}

Before transplantation, six patients who were not receiving long-term transfusion therapy underwent a partial exchange transfusion to achieve a fraction of hemoglobin S in the blood of 30 percent or less. All patients who were seronegative for cytomegalovirus received seronegative blood products.²⁰ After transplantation, patients received prophylactic broad-spectrum antibiotics intravenously until the number of circulating neutrophils reached 500 per cubic millimeter and oral penicillin for at least two years after transplantation.

In response to an increased incidence of neurologic complications after transplantation, the following guidelines were adopted in June 1993: anticonvulsant prophylaxis with phenytoin was initiated during the administration of busulfan and continued for six months after transplantation, hypertension was strictly controlled, magnesium deficiency was promptly rectified, and hemoglobin concentrations were maintained at 9 to 11 g per deciliter (5.6 to 6.8 mmol per liter) and platelet counts above 50,000 per cubic millimeter.¹⁴ In addition, cerebral magnetic resonance imaging (MRI) and magnetic resonance angiography were performed in all patients before transplantation and 180 and 365 days after transplantation. Measurements of pulmonary function (total lung capacity, forced vital capacity, residual volume, and the ratio of forced expiratory volume to forced vital capacity) with pulse oximetry, arterial blood gas measurements, or both, were made annually.

Hematopoietic-cell chimerism was identified by in situ hybridization with a Y-chromosome-specific probe in cells from marrow and peripheral blood when the donor and recipient were mismatched for sex and by studies of restriction-fragment-length polymorphisms or tandem repeats in DNA when the donor and recipient were of the same sex. These analyses were performed 56 days, 84 days, and 1 year after transplantation.

Statistical Analysis

We used the Kaplan–Meier method to estimate survival and event-free survival (the events we studied were death, graft rejection, and recurrence of sickle cell disease).²¹ A cumulative incidence curve for graft rejection is also presented.²² Survival and graft rejection were evaluated in relation to each of the following factors with the log-rank test²²: the patient's age and sex, the donor's sex, the genotypes of both patient and donor, the number of transfusions received, the incidence of red-cell alloimmunization, previous chelation therapy, the presence of hepatomegaly, and the serum ferritin concentration before transplantation. We assessed the associations between various factors in contingency tables by Fisher's exact test.²³

RESULTS

Characteristics of the Patients

Twenty-one patients with sickle cell anemia and one patient with sickle β^+ -thalassemia received marrow allografts from HLA-identical siblings between September 1991 and April 1995 at 15 collaborating transplantation centers. The 8 girls and 14 boys ranged in age from 3.3 to 13.9 years (median, 10.4) (Table 2). The indications for transplantation included a history of stroke (12 patients), recurrent acute chest syndrome (5 patients), and recurrent painful episodes (5 patients). The pretransplantation hemoglobin values varied from 6.4 to 11.9 g per deciliter (4.0 to 7.4 mmol per liter), and the hemoglobin S fractions reflected the long-term transfusion therapy given to 16 patients.

Cerebral MRI was performed in all 22 patients before transplantation. Of the 12 patients with a history of overt stroke, all but 1 (Patient 13) had evidence of cerebral infarction, vasculopathy, or both on neuroimaging (Table 3). Among the remaining 10 patients, 2 (Patients 6 and 18) had evidence of a "silent" cerebral infarction on cerebral MRI. Pulmonary function, tested in 18 patients, was normal in 7; mildly-to-moderately or moderately restrictive pulmonary disease was present in 6 patients, mildly restrictive changes were present in 4 patients, and mild airway obstruction without restrictive changes was noted in 1 patient (Table 3). A history of recurrent acute chest syndrome was the chief indication for transplantation in five patients, and three others (Patients 2, 9, and 17) had previously had one or two episodes of the acute chest syndrome. In this subgroup, pulmonary function was impaired in four patients, normal in two, and not studied in two. Of the five for whom a history of recurrent painful episodes was an indication for transplantation (Patients 9, 10, 16, 21, and 22), the number of painful crises ranged from 3 to 13 per year; two of these patients also had osteonecrosis.

Sixteen patients were receiving monthly red-cell transfusions before transplantation (12 for stroke, 3 for recurrent painful episodes, and 1 for the acute chest syndrome). Six of these 16 patients had red-cell alloantibodies, and 10 were receiving chelation

TABLE 2. CHARACTERISTICS OF THE 22 PATIENTS BEFORE TRANSPLANTATION.

CHARACTERISTIC	MEAN \pm SD	MEDIAN	HISTORY OF TRANSFUSION		NO HISTORY OF TRANSFUSION	
			MEAN	MEDIAN	MEAN	MEDIAN
Age (yr)	9.9 \pm 3.3	10.4				
Mean corpuscular volume (fl)	85.0 \pm 7.1	87.5				
Hemoglobin F (%)	8.4 \pm 8.1	6.0				
White cells ($\times 10^{-3}/\text{mm}^3$)	13.0 \pm 3.8	12.0				
Platelets ($\times 10^{-3}/\text{mm}^3$)	355 \pm 32	371				
Serum ferritin (ng/ml)	1542 \pm 1730	1079				
Hemoglobin g/dl	9.7	9.5	8.3	8.5		
mmol/liter	6.0	5.9	5.2	5.3		
Hemoglobin S (%)	28	30	84	86		

treatment for iron overload (Table 3). The serum ferritin values in all 22 patients ranged from 58 to 6795 ng per milliliter (mean, 1542). Most of the 22 children had received frequent transfusions before transplantation, although 5 patients had received 10 or fewer units of red cells. Of the 16 patients who underwent serologic testing before transplantation, 3 had evidence of a previous viral hepatitis infection (hepatitis A in Patient 9 and hepatitis C in Patients 1 and 6). Liver biopsies were performed before transplantation in eight patients with a history of hepatitis or numerous transfusions. Four patients (Patients 13, 16, 21, and 22) had mild-to-moderate hemosiderosis without portal fibrosis, one (Patient 2) had minimal periportal inflammation, and two (Patients 5 and 6) had changes consistent with chronic hepatitis. Only one patient (Patient 20, who had a serum ferritin concentration of 6795 ng per milliliter) had evidence of portal fibrosis.

Clinical Course after Stem-Cell Transplantation

In all patients the absolute neutrophil count exceeded 500 per cubic millimeter by a median of 18 days after transplantation (range, 15 to 32). The platelet count rose above 20,000 per cubic millimeter, independently of transfusions, by a median of 26 days after transplantation (range, 11 to 40). Mild-to-moderate veno-occlusive disease of the liver was noted in two patients, who recovered without sequelae. Interstitial pneumonitis developed in Patient 15, who had no prior pulmonary complications. Bronchoscopy showed no infective cause, and he recovered uneventfully. Five patients had bacteremia, including one episode of *Haemophilus influenzae* bacteremia with pneumonia in Patient 9. Grade II or III acute GVHD developed in two patients. The pa-

TABLE 3. COMPLICATIONS OF SICKLE CELL DISEASE AND THERAPY RECEIVED BY THE 22 PATIENTS.*

PATIENT No.	AGE (YR)	INDICATION FOR TRANSPLANTATION	No. OF PULMONARY EVENTS†	No. OF PAINFUL CRISES OR OTHER EVENTS‡	>10 UNITS TRANSFUSED	RED-CELL ALLO-IMMUNIZATION§	SERUM FERRITIN (ng/ml)	REGULAR CHELATION	PULMONARY FUNCTION¶		CEREBRAL MRI OR MRA	
									BEFORE TRANSPLANTATION	AFTER TRANSPLANTATION	BEFORE TRANSPLANTATION	AFTER TRANSPLANTATION
1	10.5	Stroke	0	None	Yes	No	2600	Yes	Mild-to-moderate RPD	Mild RPD	Abnormal	No change
2	10.6	Stroke	1	<1/yr	Yes	No	3199	Yes	Normal	Normal	Abnormal	No change
3	10.2	Stroke	0	None	Yes	Yes (C,K)	1079	Yes	—	Moderate RPD	Abnormal	No change
4	10.1	Stroke	0	None	Yes	Yes (E,c)	1780	Yes	Mild obstructive changes with no RPD	—	Microvascular disease	ICH
5	11.3	ACS	5	3/yr with 3 episodes of priapism	Yes	Yes (E,K)	795	No	Mild-to-moderate RPD	Mild-to-moderate RPD	Normal	Normal
6	12.3	ACS	8	5/yr	No	No	484	No	Normal	Normal	Silent infarction	No change
7	5.2	Stroke	0	2/yr	Yes	No	—	No	—	Normal	Abnormal	No change
8	13.9	ACS	2	10–15/yr	No	No	133	No	Moderate RPD	Moderate RPD	Normal	Normal
9	13.2	VOC	2	>3/yr with osteonecrosis	No	No	167	No	Mild-to-moderate RPD	—	Normal	—
10	8.3	VOC	0	>3/yr	No	No	83	No	Mild-to-moderate RPD	Mild-to-moderate RPD	Normal	Normal
11	8.2	Stroke	0	None	Yes	No	150	No	Mild-to-moderate RPD	Mild-to-moderate RPD	Abnormal on TCD	No change
12	13.7	ACS	3	1/yr	Yes	No	982	Yes	—	Normal	Normal	Normal
13	5.2	Stroke	0	None	Yes	No	5066	No	Normal	—	Normal	—
14	12.3	Stroke	0	None	Yes	Yes (K,E,c,S)	1633	No	Normal	Normal	Abnormal	No change
15	13.3	Stroke	0	<1/yr	Yes	No	1236	Yes	Mild RPD	Mild RPD	Abnormal	No change
16	10.2	VOC	0	13/yr	Yes	No	1767	Yes	Normal	Normal	Normal	Normal
17	4.2	Stroke	2	3/yr	Yes	No	562	Yes	—	Normal	Abnormal	No change
18	8.2	ACS	2	4/yr	No	No	58	No	Mild RPD	Mild RPD	Silent infarction	No change
19	3.3	Stroke	0	None	No	No	656	No	Mild RPD	—	Abnormal	New CVA
20	12.3	Stroke	0	None	Yes	Yes (Jka,s,N, E,c,C,c)	6795	Yes	Mild RPD	Mild RPD	Abnormal	No change
21	13.9	VOC	0	5/yr with widespread osteonecrosis	Yes	Yes (I)	1140	Yes	Normal	Normal	Normal	—
22	6.9	VOC	0	7/yr	Yes	No	2723	No	Normal	Normal	Normal	Normal

*MRI denotes magnetic resonance imaging, MRA magnetic resonance angiography, RPD restrictive pulmonary disease, ICH intracranial hemorrhage, ACS acute chest syndrome, VOC vaso-occlusive crisis, TCD transcranial Doppler scanning, and CVA cerebrovascular accident. Dashes indicate that testing was not done.

†This column shows the total number of episodes of acute chest syndrome since birth.

‡Numbers of painful crises are reported as mean values over the past three years.

§The letters indicate the minor-blood-group surface antigen targets for alloantibodies.

¶For pulmonary function, restrictive pulmonary disease was categorized as mild (80 percent of the predicted normal values, or 1 SD below normal) or moderate (60 percent of the predicted normal values, or ≥2 SD below normal).

TABLE 4. RESULTS OF STUDIES OF CHIMERISM AND OUTCOMES AFTER TRANSPLANTATION.*

PATIENT NO.	TIME TO REJECTION (DAYS)	CURRENT CHIMERISM STATUS	CURRENT HEMOGLOBIN STATUS ON ELECTROPHORESIS			LANSKY PERFORMANCE SCORE†	CLINICAL OUTCOME	FOLLOW-UP (MO)‡
			HEMOGLOBIN F	HEMOGLOBIN S	DONOR GENOTYPE			
			percent					
1	—	96.5% donor§	0.7	0	A/A	100	Free of SCD	51.7
2	—	100% donor§	1.2	0	A/A	100	Free of SCD	35.7
3	164	VNTR not interpretable	9.0	88	A/S	90	GR; recurrent VOC; started hydroxyurea	37.5
4	—	ND	ND	ND	ND	ND	Died of intracranial hemorrhage	—
5	—	100% donor¶	2.4	41	A/S	100	Free of SCD	27.8
6	—	93% donor§	2.2	38	A/S	80	Free of SCD	24.7
7	—	100% donor§	0.3	34	A/S	100	Free of SCD	23.3
8	—	100% donor§	0	35	A/S	100	Free of SCD	25.6
9	—	ND	ND	ND	ND	ND	Died of GVHD	—
10	—	100% donor¶	ND	0	A/A	100	Free of SCD	39.2
11	—	95% donor¶	ND	31	A/S	100	Free of SCD	28.4
12	156	100% host	0	25**	A/A	100	GR; resumed long-term red-cell transfusion therapy	26.3
13	—	10–20% donor§	6.7	30	A/A	100	No symptoms with stable mixed chimerism	19.0
14	—	100% donor§	0	0	A/A	100	Free of SCD	15.9
15	—	100% donor§	0	0	A/A	100	Free of SCD	17.6
16	—	100% donor¶	0.9	0.5	A/A	100	Free of SCD	22.3
17	—	100% donor	ND	37	A/S	90	Free of SCD	13.3
18	—	100% donor¶	0	0.7	A/A	100	Free of SCD	15.3
19	91	Myeloid lineage: 6–8% donor§ Erythroid lineage: 18% donor§	0	20**	A/S	90	GR; recurrent CVA; resumed long-term red-cell transfusion therapy	13.5
20	270	10% donor (marrow)§ 66% donor (blood)§	ND	4**	A/A	100	Late graft failure requiring transfusion support	10.3
21	—	≥90% donor§	0	<0.5	A/A	100	Free of SCD	34.1
22	—	96% donor§	ND	1.5	A/A	100	Free of SCD	13.8

*Patient 1 has previously been described by Johnson et al.,⁸ Patients 1 and 4 by Walters et al.,¹⁴ and Patient 3 by Vermlyen and Cornu.⁹ SCD denotes sickle cell disease, VNTR variable number of tandem repeats, GR graft rejection, VOC vaso-occlusive crisis, ND not done, GVHD graft-versus-host disease, and CVA cerebrovascular accident.

†The Lansky performance score is a measure of functional status in children.

‡Follow-up is shown through May 1996.

§Determined by cytogenetic analysis.

¶Determined by VNTR analysis.

||Determined by analysis of restriction-fragment-length polymorphisms.

**The hemoglobin S fraction reflects the resumption of red-cell transfusions.

tient with grade III acute GVHD subsequently died of chronic GVHD.

Neurologic Complications

After four of the first seven patients enrolled had neurologic events, including two episodes of intracranial hemorrhage,¹⁴ we adopted the prophylactic measures described above. Three of the next 15 patients had seizures, but none had intracranial hemorrhage. Patient 19 had seizures during busulfan treatment and was noted to have a subtherapeutic plasma phenytoin concentration during oral loading. Three months later he had graft rejection and

subsequently had recurrent cerebral infarction when the hemoglobin S fraction rose to 60 percent. He later resumed long-term transfusion therapy. Seizures developed in Patient 22 during replacement of a central venous catheter, and Patient 20 had seizures after the reinstatement of cyclosporine therapy. Both patients recovered without sequelae.

Graft Rejection

Sustained engraftment with 85 percent or more donor-derived blood cells was demonstrated by tests for chimerism in 16 of the 20 surviving patients (Table 4). Sickle cell disease recurred in three patients, and

graft failure occurred in a fourth. One of the 16 patients with engraftment had stable mixed chimerism.

Patient 3 had evidence of rejection on day 164 and by eight months after transplantation had no detectable hemoglobin A. She remained asymptomatic for nearly two years until she had a recurrence of vaso-occlusive crises; treatment with hydroxyurea was initiated, and she is currently free of symptoms. In Patient 12, hemoglobin electrophoresis on day 156 showed 30 percent hemoglobin S. There was no evidence of donor cells in the peripheral blood; accordingly, long-term transfusion therapy was resumed, and the patient remains asymptomatic. In Patient 19, cytogenetic studies performed one month after transplantation showed 40 percent donor cells. In situ hybridization with a Y-chromosome-specific probe of marrow progenitor colonies revealed a disparity in the degree of myeloid and erythroid chimerism: 6 of 34 blast-forming unit–erythroid colonies were of donor origin (18 percent), whereas 6 to 8 percent of 600 nuclei of myeloid cells derived from a long-term marrow culture were scored as donor cells. Similar findings have been reported in patients with thalassemia who had mixed chimerism after marrow transplantation and in an animal model of thalassemia.^{24,25}

Patient 20 had 70 percent donor-derived hematopoietic cells in the bone marrow and 90 percent donor cells in the peripheral blood on day 180, but marrow aplasia developed 270 days after transplantation and she required red-cell and platelet transfusions. Chimerism studies demonstrated more than 90 percent host cells, a result that was consistent with graft rejection. After treatment with antithymocyte globulin and cyclosporine was instituted, the cellularity of the marrow improved and the need for transfusions diminished. Recent chimerism studies (315 days after transplantation) demonstrated 10 percent donor cells in the marrow and 66 percent donor cells in the peripheral blood.

Patient 13 had stable mixed chimerism. The hemoglobin S value was 12 percent three months after transplantation. Thereafter, serial studies revealed 10 to 20 percent donor cells and 30 percent hemoglobin S in the blood for nearly two years. As of this writing, the patient has no symptoms of sickle cell disease.

Outcome

After a median of 23.9 months (range, 10.1 to 51.0), 20 patients were alive, 16 of whom had stable engraftment with donor hematopoietic cells. Kaplan–Meier estimates of survival and event-free survival at four years were 91 percent and 73 percent, respectively; the cumulative incidence of graft rejection was 18 percent (Fig. 1). One patient died three months after transplantation from an intracerebral hemorrhage,¹⁴ and another died one year after trans-

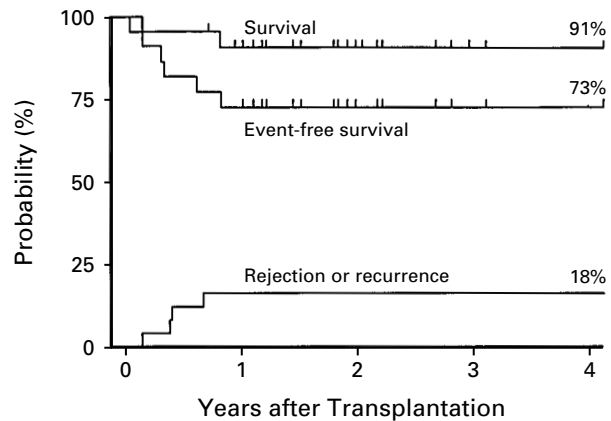


Figure 1. Kaplan–Meier Estimates of Survival and Event-free Survival after Bone Marrow Transplantation in 22 Patients with Sickle Cell Disease.

An event was defined as death, graft rejection, or recurrence of sickle cell disease. A cumulative incidence curve for graft rejection and recurrent sickle cell disease is also shown. Tick marks represent surviving patients and show the duration of follow-up after transplantation. Percentages above each curve indicate the estimates of survival and event-free survival and the cumulative incidence of graft rejection or recurrence of disease for the entire cohort.

plantation from complications related to chronic GVHD and obliterative bronchiolitis. No risk factors for rejection were identified in the four patients who had graft rejection. Busulfan concentrations in four patients were judged adequate for donor engraftment; two of these four (Patients 19 and 20) had graft rejection despite having steady-state busulfan concentrations of 429 and 565 ng per milliliter, respectively.²⁶

The effects of marrow transplantation on end-organ function were examined in patients with engraftment who were followed for at least 12 months (Table 3). All eight survivors with a history of stroke and stable donor-cell engraftment were studied with serial cerebral MRI and magnetic resonance angiography. As of this writing, they have had no progression of cerebral disease. The two patients with “silent” cerebral infarction before transplantation have had no changes on cerebral MRI. Pulmonary function could be evaluated in four of five patients with recurrent episodes of the acute chest syndrome before transplantation. All these patients had stable pulmonary function. No patient with engraftment had vaso-occlusive crises after transplantation.

DISCUSSION

In this collaborative multicenter trial we investigated the risks and benefits of allogeneic bone marrow transplantation in children with symptomatic sickle cell disease. Our findings indicate that transplantation of hematopoietic stem cells can cure sick-

le cell anemia. End-organ damage was stabilized in patients with sustained engraftment of donor cells, but evaluation of the ultimate effect of transplantation on such damage requires longer follow-up. Transfusions have been discontinued in 16 patients who had engraftment; all have remained free of disabling symptoms. These results are especially encouraging in view of the severity of sickle cell disease in these patients.

There was allograft rejection followed by a recurrence of sickle cell disease or marrow aplasia in 4 of the 22 patients, a result similar to the 12 percent rejection rate reported among children with β -thalassemia major who received HLA-matched marrow grafts from siblings.² Graft rejection after transplantation of hematopoietic stem cells occurs in patients with hemoglobinopathy more frequently than in patients with other hematologic disorders, such as aplastic anemia, severe combined immune deficiency, and malignant conditions, in whom the rate of rejection after the transplantation of marrow from HLA-identical siblings is less than 3 percent.^{27,28} The cause of this difference is not understood. Transfusion-induced sensitization could be a contributing factor, because among patients with aplastic anemia, the incidence of graft rejection is lower when transplantation is performed before transfusions are given.²⁹

The frequency of graft rejection appears to be lower among patients with β -thalassemia who undergo transplantation early in the course of the disease, before red-cell transfusions lead to iron overload or liver disease.^{11,30} Lucarelli et al. reported a graft-rejection rate of 4 percent and event-free survival of 94 percent among such patients,¹¹ and a similar outcome has been reported in patients with sickle cell disease.^{7,9,31} Among 28 Belgian patients with sickle cell anemia in an earlier report, 3 had graft rejection (1 of whom underwent a successful second transplantation), 1 died from transplantation-related complications, and 25 survived with stable donor engraftment (event-free survival, 89 percent).⁹ More recently, a Belgian bone marrow-transplantation center has required more severe clinical disturbances as a criterion for transplantation than were previously required.³² Of 12 patients who met these strict criteria, 4 had graft rejection, 1 died suddenly four years after transplantation, and 7 have survived free of sickle cell disease (Ferster A: personal communication). Giardini et al. reported that of four patients with advanced symptomatic sickle cell anemia, one is alive after marrow grafting, whereas four of four patients with less advanced sickle cell- β^0 -thalassemia have survived with donor-derived hematopoiesis.³³ These data suggest that patients who have not undergone long-term transfusion therapy and who do not have severe complications related to sickle cell disease are optimal candidates for stem-cell transplantation.

We observed one case of stable mixed chimerism (in Patient 13) in which the reduction in hemoglobin S was sufficient to render the patient asymptomatic. Stable donor-host chimerism was also reported in patients with thalassemia.^{24,34} In such patients the risk of graft rejection appeared highest when more than 30 percent residual host cells were present two months after transplantation, although in three patients stable mixed chimerism persisted for four to seven years.²⁴ Our data confirm that erythropoiesis by a minority of engrafted donor cells can lead to a majority of circulating normal erythrocytes.³⁵ This observation has implications for the design of less cytotoxic pretransplantation conditioning regimens, which may result in stable mixed chimerism.

The optimal timing of marrow transplantation in the course of sickle cell anemia remains uncertain, in part because of the unpredictable nature of the disease. We selected patients who appeared to have a high risk of severe morbidity and early death. Only 6 percent of patients with sickle cell anemia who were less than 16 years old and were followed in the collaborating centers met the criteria for transplantation specified in the study protocol.³⁶ But by the time these patients were selected, many had such severe vasculopathy and organ damage that they were predisposed to adverse events after transplantation. Marrow transplantation might therefore be better if performed early in the course of sickle cell disease, especially in patients with neurovascular disease (silent stroke) or pulmonary disease.³⁷

The long-term administration of hydroxyurea to stimulate the production of fetal hemoglobin and the transfusion of red cells to lower sickle hemoglobin levels are other therapies now available for the treatment of patients with symptomatic sickle cell disease.^{38,39} The evolving role of the transplantation of hematopoietic stem cells and the availability of other therapeutic options underscore the need for controlled clinical trials to investigate the risks and benefits of and the indications for therapeutic intervention in sickle cell disease.

Supported in part by grants from the National Heart, Lung, and Blood Institute (HL36444) and the National Cancer Institute (CA18221 and CA15704).

We are indebted to Beverly Torok-Storb, Ph.D., for performing studies of marrow-progenitor chimerism and to the physicians and nurses at the sickle cell and transplantation centers for their support of this study.

APPENDIX

The following investigators and centers made up the collaborative study group: *Atlanta* — J.R. Eckman and J. Wingard, Emory University; *Augustin, Germany* — R. Dickerhoff and T. Klingebiel, University of Bonn; *Birmingham, United Kingdom* — P.J. Darbyshire, Birmingham Children's Hospital National Health

Service Trust; *Boston* — J. Antin, E. Guinan, B. Gee, and O. Platt, Dana-Farber Institute, Children's Hospital, and Harvard University, and L. McMahon, Boston Comprehensive Sickle Cell Center; *Chapel Hill, N.C.* — E. Orringer and J. Wiley, University of North Carolina; *Creteil, France* — F. Bernaudin, M. Kuentz, and J.P. Vernant, Hôpital Henri Mondor; *Dallas* — G.R. Buchanan, Z.R. Rogers, and E. Sandler, University of Texas Southwestern Medical Center; *Durham, N.C.* — M.L. Graham, T. Kinney, and J. Kurtzberg, Duke University; *Houston* — K.W. Chan, University of Texas; *Indianapolis* — F.O. Smith and W. Rackoff, Indiana University School of Medicine; *London* — S.C. Davies and I.A.G. Roberts, Royal Postgraduate Medical School; *Los Angeles* — R. Parkman, D. Powars, and C. Lenarsky, University of Southern California; *Milwaukee* — B. Camitta and J.P. Scott, Medical College of Wisconsin; *New Haven, Conn.* — J. Rapoport, Yale University; *Oakland, Calif.* — R. Vichinsky and L. Styles, Children's Hospital of Oakland; *Philadelphia* — K. Ohene-Frempong and N. Bunin, University of Pennsylvania; *St. Louis* — D. Wall, St. Louis University; *St. Petersburg, Fla.* — A. Wayne and M. Klemperer, University of Southern Florida; *Sao Paulo, Brazil* — S. Brandalise and R. Pasquini, University of Campinas; *San Francisco* — W.C. Mentzer and M. Cowan, University of California, San Francisco; *Seattle* — K.M. Sullivan, M.C. Walters, D.C. Matthews, J.E. Sanders, and F.R. Appelbaum, Fred Hutchinson Cancer Research Center and University of Washington; *Stanford, Calif.* — M. Amylon, B. Glader, and K. Blume, Stanford University; *Toronto* — N.F. Olivieri, Hospital for Sick Children; *Washington, D.C.* — P. Dindorf and O. Castro, Children's Hospital National Medical Center, George Washington University, and Howard University.

REFERENCES

- Parkman R. The application of bone marrow transplantation to the treatment of genetic diseases. *Science* 1986;232:1373-8.
- Lucarelli G, Galimberti M, Polchi P, et al. Bone marrow transplantation in patients with thalassemia. *N Engl J Med* 1990;322:417-21.
- Camitta BM, Storb R. Aplastic anemia: pathogenesis, diagnosis, treatment, and prognosis. *N Engl J Med* 1982;306:645-52, 712-8.
- Johnson FL, Look AT, Gockerman J, Ruggiero MR, Dalla-Pozza L, Billings FT III. Bone-marrow transplantation in a patient with sickle-cell anemia. *N Engl J Med* 1984;311:780-3.
- Vermilyen C, Robles EF, Ninane J, Cornu G. Bone marrow transplantation in five children with sickle cell anemia. *Lancet* 1988;1:1427-8.
- Sullivan KM, Reid CD. Introduction to a symposium on sickle cell anemia: current results of comprehensive care and the evolving role of bone marrow transplantation. *Semin Hematol* 1991;28:177-9.
- Ferster A, De Valck C, Azzi N, Fondu P, Toppet M, Sariban E. Bone marrow transplantation for severe sickle cell anaemia. *Br J Haematol* 1992;80:102-5.
- Johnson FL, Mentzer WC, Kalinyak KA, Sullivan KM, Abboud MR. Bone marrow transplantation for sickle cell disease: the United States experience. *Am J Pediatr Hematol Oncol* 1994;16:22-6.
- Vermilyen C, Cornu G. Bone marrow transplantation for sickle cell disease: the European experience. *Am J Pediatr Hematol Oncol* 1994;16:18-21.
- Beutler E, Sullivan KM. Bone marrow transplantation for sickle cell disease. In: Forman SJ, Blume KG, Thomas ED, eds. *Bone marrow transplantation*. Boston: Blackwell Scientific, 1994:840-8.
- Lucarelli G, Galimberti M, Polchi P, et al. Marrow transplantation in patients with thalassemia responsive to iron chelation therapy. *N Engl J Med* 1993;329:840-4.
- Platt O. The sickle syndromes. In: Handin RL, Lux SE, Stosel TP, eds. *Blood: principles & practice of hematology*. Philadelphia: J.B. Lippincott, 1995:1645-700.
- Hopkins KA. Basic microlymphocytotoxicity test. In: Zachary AA, Teresi GA, eds. *Laboratory manual*. 2nd ed. Lenexa, Kans.: American Society for Histocompatibility and Immunogenetics, 1990:195-201.
- Walters MC, Sullivan KM, Bernaudin F, et al. Neurologic complications after allogeneic marrow transplantation for sickle cell anemia. *Blood* 1995;85:879-84.
- Powars D, Weidman JA, Odom-Maryon T, Niland JC, Johnson C. Sickle cell chronic lung disease: prior morbidity and the risk of pulmonary failure. *Medicine (Baltimore)* 1988;67:66-76.
- Powars DR, Elliott-Mills DD, Chan L, et al. Chronic renal failure in sickle cell disease: risk factors, clinical course, and mortality. *Ann Intern Med* 1991;115:614-20.
- Dyer MJS, Hale G, Hayhoe FGJ, Waldman H. Effects of CAMPATH-1 antibodies in vivo in patients with lymphoid malignancies: influence of antibody isotype. *Blood* 1989;73:1431-9.
- Storb R, Deeg HJ, Whitehead J, et al. Methotrexate and cyclosporine compared with cyclosporine alone for prophylaxis of acute graft versus host disease after marrow transplantation for leukemia. *N Engl J Med* 1986;314:729-35.
- Chao NJ, Schmidt GM, Niland JC, et al. Cyclosporine, methotrexate, and prednisone compared with cyclosporine and prednisone for prophylaxis of acute graft-versus-host disease. *N Engl J Med* 1993;329:1225-30.
- Bowden RA, Sayers M, Flournoy N, et al. Cytomegalovirus immune globulin and seronegative blood products to prevent primary cytomegalovirus infection after marrow transplantation. *N Engl J Med* 1986;314:1006-10.
- Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 1958;53:457-81.
- Kalbfleisch JD, Prentice NL. *The statistical analysis of failure time data*. New York: John Wiley, 1980:79-81, 168-71.
- Fisher LD, van Belle G. *Biostatistics: a methodology for health sciences*. New York: John Wiley, 1993:185-6.
- Andreani M, Manna M, Lucarelli G, et al. Persistence of mixed chimerism in patients transplanted for the treatment of thalassemia. *Blood* 1996;87:3494-9.
- van den Bos C, Kieboom D, Wagemaker G. Correction of murine β -thalassemia by partial bone marrow chimerism: selective advantage of normal erythropoiesis. *Bone Marrow Transplant* 1993;12:9-13.
- Slattery JT, Sanders JE, Buckner CD, et al. Graft-rejection and toxicity following bone marrow transplantation in relation to busulfan pharmacokinetics. *Bone Marrow Transplant* 1995;16:31-42.
- Schuening F, Deeg HJ, Bean MA, Storb R. Prevention of graft failure in patients with aplastic anemia. *Bone Marrow Transplant* 1993;12:Suppl 3:S48-S49.
- Anasetti C, Amos D, Beatty PG, et al. Effect of HLA compatibility on engraftment of bone marrow transplants in patients with leukemia or lymphoma. *N Engl J Med* 1989;320:197-204.
- Anasetti C, Doney KC, Storb R, et al. Marrow transplantation for severe aplastic anemia: long-term outcome in fifty "untransfused" patients. *Ann Intern Med* 1986;104:461-6.
- Andreani M, Manna M, Nesci S, Fattorini P, Graziosi G, Lucarelli G. Persistence of mixed chimerism in class 3 thalassemic patients following BMT. *Bone Marrow Transplant* 1991;7:Suppl 2:75.
- Bernaudin F, Souillet G, Vannier JP, et al. Bone marrow transplantation (BMT) in 14 children with severe sickle cell disease (SCD): the French experience. *Bone Marrow Transplant* 1993;12:Suppl 1:118-21.
- Ferster A, Corazza F, Bujan W, et al. Patients with sickle cell anemia who present graft failure after bone marrow transplantation may develop increased levels of fetal hemoglobin associated with improved clinical presentation. In: Beuzard Y, Lubin B, Rosa J, eds. *Sickle cell disease and thalassemias: new trends in therapy*. London: John Libbey, 1995:579-80.
- Giardini C, Galimberti M, Lucarelli G, et al. Bone marrow transplantation in sickle-cell anemia in Peasaro. *Bone Marrow Transplant* 1991;7:Suppl 2:122-3.
- Nesci S, Manna M, Andreani M, Fattorini P, Graziosi G, Lucarelli G. Mixed chimerism in thalassemic patients after bone marrow transplantation. *Bone Marrow Transplant* 1992;10:143-6.
- Kapelushnik J, Or R, Filon D, et al. Analysis of β -globin mutations shows stable mixed chimerism in patients with thalassemia after bone marrow transplantation. *Blood* 1995;86:3241-6.
- Walters MC, Patience M, Leisenring W, et al. Barriers to bone marrow transplantation for sickle cell anemia. *Biol Blood Marrow Transplant (in press)*.
- Piomelli S. Sickle cell diseases in the 1990s: the need for active and preventive intervention. *Semin Hematol* 1991;28:227-32.
- Charache S, Terrin ML, Moore RD, et al. Effect of hydroxyurea on the frequency of painful crises in sickle cell anemia. *N Engl J Med* 1995;332:1317-22.
- Styles LA, Vichinsky E. Effects of a long-term transfusion regimen on sickle cell-related illnesses. *J Pediatr* 1994;125:909-11.