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Allogeneic Hematopoietic Stem-Cell Transplantation for Sickle Cell Disease

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Abstract

BACKGROUND—Myeloablative allogeneic hematopoietic stem-cell transplantation is curative in children with sickle cell disease, but in adults the procedure is unduly toxic. Graft rejection and graft-versus-host disease (GVHD) are additional barriers to its success. We performed nonmyeloablative stem-cell transplantation in adults with sickle cell disease.

METHODS—Ten adults (age range, 16 to 45 years) with severe sickle cell disease underwent nonmyeloablative transplantation with CD34+ peripheral-blood stem cells, mobilized by granulocyte colony-stimulating factor (G-CSF), which were obtained from HLA-matched siblings. The patients received 300 cGy of total-body irradiation plus alemtuzumab before transplantation, and sirolimus was administered afterward.

RESULTS—All 10 patients were alive at a median follow-up of 30 months after transplantation (range, 15 to 54). Nine patients had long-term, stable donor lymphohematopoietic engraftment at levels that sufficed to reverse the sickle cell disease phenotype. Mean (±SE) donor–recipient chimerism for T cells (CD3+) and myeloid cells (CD14+15+) was 53.3±8.6% and 83.3±10.3%, respectively, in the nine patients whose grafts were successful. Hemoglobin values before transplantation and at the last follow-up assessment were 9.0±0.3 and 12.6±0.5 g per deciliter, respectively. Serious adverse events included the narcotic-withdrawal syndrome and sirolimus-associated pneumonitis and arthralgia. Neither acute nor chronic GVHD developed in any patient.

CONCLUSIONS—A protocol for nonmyeloablative allogeneic hematopoietic stem-cell transplantation that includes total-body irradiation and treatment with alemtuzumab and sirolimus can achieve stable, mixed donor–recipient chimerism and reverse the sickle cell phenotype.

Sickle cell disease results from a single nucleotide substitution in which valine replaces glutamic acid at the sixth position of the β-globin chain of hemoglobin A.[1,2] This change causes a propensity toward polymerization of hemoglobin and, hence, sickle-shaped red cells. Anemia, increased hemolysis, and acute and chronic vaso-occlusive complications that affect multiple organs are the main features of sickle cell disease. At present, allogeneic

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hematopoietic stem-cell transplantation is the only curative option. [3–5] Approximately 200 children have undergone this procedure after myeloablative conditioning with busulfan and cyclophosphamide, with or without antithymocyte globulin, resulting in a rate of disease-free survival of 95% in the most recent series.[5] After transplantation, the donor's hematopoietic cells completely replace those of the recipient in most children who undergo this procedure, but some continue to have both recipient and donor cells in the blood (mixed chimerism).[6] This mixture is sufficient to reverse the sickle cell disease phenotype.

The development of safe, nonmyeloablative conditioning regimens that allow stable, mixed chimerism could facilitate allogeneic stem-cell transplantation in adults with severe sickle cell disease, in whom the toxicity of myeloablative conditioning can be prohibitive. Early attempts at such conditioning in sickle cell disease did not, however, reliably achieve long-term engraftment of donor cells.[7] Sustained engraftment of allogeneic stem cells in patients with other diseases after minimally toxic nonmyeloablative conditioning with fludarabine and cyclophosphamide has been reported,[8,9] although the mixed-chimeric state was temporary. In most cases, alloreactive donor T cells eradicated the recipient's stem cells, and the rates of graft-versus-host disease (GVHD), morbidity, and mortality were high.[8,9]

We sought to develop a means for performing hematopoietic stem-cell transplantation in adults with sickle cell disease that would allow engraftment and avoid GVHD in the presence of allogeneic donor T cells. On the basis of a novel mechanism for inducing immunologic tolerance, we chose low-dose radiation plus sirolimus (formerly known as rapamycin). Unlike calcineurin inhibitors such as cyclosporine, sirolimus does not block the process of T-cell activation through the T-cell receptor but rather inhibits T-cell proliferation by binding to the mammalian target of rapamycin. Activated T cells that cannot proliferate become anergic, and this property can promote T-cell tolerance. [10] We showed the feasibility of this approach in a murine model in which we administered a short course of either cyclosporine or sirolimus after a single dose of total-body irradiation (300 cGy). Long-term, high-level chimerism was attained only in the mice treated with sirolimus. This method can correct the sickle cell disease phenotype in transgenic mice with the sickle cell gene.[11] Here we describe our results with the application of this approach in 10 adults with severe sickle cell disease.

METHODS

STUDY DESIGN AND PROCEDURES

We conducted a phase 1–2 study to determine the feasibility of nonmyeloablative allogeneic hematopoietic stem-cell transplantation for adults with severe sickle cell disease. It was approved by the institutional review board of the National Heart, Lung, and Blood Institute and was monitored by an independent data and safety monitoring board.

Patients 16 years of age or older were eligible for enrollment if they were homozygous for hemoglobin S or compound heterozygous for hemoglobins S and C, as confirmed by results on hemoglobin electrophoresis, identification of an HLA-identical family donor, and the presence of severe disease (Table 1). Written informed consent or assent was obtained for all patients and donors. Inclusion criteria were a severe end-organ complication (previous cerebrovascular event, sickle-cell nephropathy, or elevated tricuspid regurgitant jet velocity)[3,12,13] or a potentially reversible complication (frequent vaso-occlusive crises, the acute chest syndrome, osteonecrosis, or red-cell alloimmunization) [3–5] that was not ameliorated by treatment with hydroxyurea.

HLA typing at the molecular level was performed, and all donors were fully matched. Donors underwent 5 to 6 days of granulocyte colony-stimulating factor (G-CSF)

mobilization (10 to 16 μg per kilogram of body weight per day), followed by large-volume leukapheresis, with the goal of collecting at least 10×10^6 CD34+ cells per kilogram of the recipient's body weight; the donor's cells were cryopreserved.[14] The conditioning regimen for the recipient consisted of alemtuzumab, 1 mg per kilogram (total dose) given in gradually increasing doses on days 7 to 3 before transplantation, a single dose of 300 cGy of total-body irradiation on day 2 before transplantation, and oral sirolimus starting the day before transplantation (Fig. 1A). Alemtuzumab, a humanized monoclonal antibody directed against CD52 (expressed on lymphocytes), depletes T cells and B cells. It does not affect the development of hematopoietic stem cells[17] and has been used to prevent GVHD.[18,19] Tapering of sirolimus was to be initiated when donor chimerism reached 100%.

SUPPORTIVE CARE

We followed standardized, intramural guidelines for supportive care established at our facility for patients undergoing allogeneic hematopoietic stem-cell transplantation, with several modifications specific for sickle cell disease. Recipients were maintained on hydroxyurea until 24 hours before starting the preparative regimen. Red-cell exchange was performed to reduce hemoglobin S levels to 30% or less before the preparative regimen. Platelet and red-cell transfusions were used to maintain the platelet count at 50×10^9 per liter or more and the hemoglobin level at 9 to 10 g per deciliter. G-CSF was not administered because of its association with complications and death in sickle cell disease. Penicillin V potassium, 250 mg, was given twice daily from day 0 until pneumococcal vaccination was completed.

ANALYSIS FOR CHIMERISM

Engraftment of donor cells was assessed with the use of methods that detect informative polymorphisms in regions known to contain short tandem repeats.[20] Peripheral-blood CD3+ T cells and CD14+CD15+ myeloid cells were selected for analysis with the use of immunomagnetic beads (Dynal). On the basis of studies using mixtures of known proportions of allogeneic DNA samples, the lower limit of sensitivity for this method is 1 to 3% of donor-type polymorphic markers in the mixture.[20]

STATISTICAL ANALYSIS

Means (±SE) were calculated for chimerism, hemoglobin values, and hemolytic variables. A paired t-test was used to compare data obtained before and after transplantation.

RESULTS

CHARACTERISTICS OF PATIENTS AND TRANSPLANTS

During the 5 years of recruitment, we performed HLA typing on 169 siblings and 112 patients with sickle cell disease.[15,16] Of the 24 eligible recipients, 10 have undergone transplantation to date (Fig. 1B). Table 1 summarizes the clinical information and indications for these 10 patients, who ranged in age from 16 to 45 years (median, 26). One patient had hemoglobin SC; the other nine had hemoglobin SS. All donors, including those with sickle cell trait, tolerated G-CSF mobilization without adverse events, which yielded 5.51 million to 28.2 million CD34+ cells per kilogram of the recipient's body weight. There were no technical difficulties in thawing stem cells collected from donors with sickle cell trait.[14] Recipients received 5.51 million to 23.8 million CD34+ cells per kilogram of body weight.

All recipients tolerated the conditioning regimen. The median duration of neutropenia (defined as a neutrophil count of less than 0.5×10^9 cells per liter) was 15.5 days (range, 10 to 21) (Fig. 2 and Table 2). Only three patients had a neutrophil count below 0.1×10^9 cells

per liter, which was the nadir value lasting for 1, 4, and 7 days. The median duration of lymphopenia ($<0.75\times10^9$ cells per liter) was 4 months (range, 1.5 to 8), and thrombocytopenia ($<50\times10^9$ cells per liter) lasted a mean of 7 days (range, 1 to 19). The median numbers of packed red-cell units and single-donor apheresis platelet units were 6.5 (range, 4 to 11) and 5.5 (range, 1 to 9), respectively. Intravenous antibiotics were administered for a median of 7 days (range, 0 to 15). No patient received antimicrobial agents for presumed fungal disease, and there was no need for parenteral nutrition.

OUTCOMES

All 10 patients were alive at a median follow-up of 30 months (range, 15 to 54) (Table 2). The graft was retained in nine of the patients. At a median of 30 months after transplantation among these nine recipients, the mean percentage of circulating donor T cells was 53.3±8.6% (range, 7 to 72), and the mean percentage of circulating donor myeloid cells was 83.3±10.3% (range, 19 to 100). The increase in circulating donor myeloid cells was more rapid than the increase in donor T cells (Fig. 3A, and Fig. 1 in the Supplementary Appendix, available with the full text of this article at NEJM. org). Since no patient reached 100% donor chimerism in T cells, sirolimus therapy was continued throughout the study. Patients 5 and 8 had undetectable levels of sirolimus on multiple occasions during the most recent 6 months and acknowledged noncompliance with sirolimus therapy.

Hemoglobin levels gradually improved after transplantation (Fig. 3B, and Fig. 1 in the Supplementary Appendix). Respective mean hemoglobin levels for women and men were 8.8±0.3 and 9.3±0.5 g per deciliter before transplantation and 12.6±0.6 and 12.7±1.1 g per deciliter at the most recent follow-up. To date, four patients have completed therapeutic phlebotomy to correct transfusion- related iron overload, and four more are undergoing this treatment. Hemoglobin electrophoreses performed on blood samples collected 6 months or more after transplantation showed the same percentage of hemoglobin S as in the donors for all these patients except Patient 2 (Table 3).

Markers of increased hemolysis declined (Fig. 3C, and Fig. 1 in the Supplementary Appendix). The respective mean values before and after transplantation were as follows: reticulocyte counts, $200\pm35\times10^9$ per liter and $60\pm12\times10^9$ per liter; total bilirubin levels, 3.03±0.72 and 0.69±0.28 mg per deciliter; and lactate dehydrogenase levels, 302±48 and 186±17 units per liter.

Patients with a history of cerebrovascular injury before transplantation did not have cerebrovascular events after transplantation. The findings on brain magnetic resonance imaging and magnetic resonance angiography remained stable at 1 and 2 years after transplantation in patients with abnormalities on brain imaging. Patients with elevated tricuspid regurgitant jet velocity or a history of the acute chest syndrome had no episodes of dyspnea or documented declines in oxygenation during conditioning. The mean tricuspid regurgitant jet velocity before transplantation (2.8±0.1 m per second) did not differ significantly from the mean values 1, 12, and 24 months after the transplantation (Table 1 in the Supplementary Appendix). Although a 6-minute walk test was not performed routinely before transplantation, values after transplantation compared favorably with those in historical controls at our institution. [21] In three patients with sickle cell nephropathy and proteinuria, the slope of decline in renal function did not exceed the slope before the transplantation. Patient 3 became pregnant 30 months after the transplantation; she delivered a healthy daughter at 38 weeks of gestation (Table 2 in the Supplementary Appendix).

ADVERSE EVENTS

Table 3 summarizes the adverse events. Acute or chronic GVHD did not develop in any patient.

Only one of the nine patients for whom the donor, recipient, or both were positive for cytomegalovirus (CMV) had reactivated CMV infection. One seropositive recipient with a seropositive donor had transient reactivation of CMV infection at approximately day 14 after transplantation, while receiving valacyclovir; the infection resolved after 1 week of intravenous foscarnet therapy and maintenance treatment with valacyclovir.

Six patients had minor ankle arthralgia and nonpitting edema, starting approximately 1 month after transplantation and continuing for approximately 5 months. These events were attributed to the administration of sirolimus. Two patients had arthralgia severe enough to alter activities of daily living: one was admitted to the hospital to rule out causes other than sirolimus and the other was treated as an outpatient. In both patients, the symptoms resolved with supportive care and a reduced dose of sirolimus to obtain trough levels of 5 to 10 ng per milliliter. Two patients presented with exertional dyspnea 4 and 16 months after transplantation; pulmonary-function tests showed obstructive and restrictive abnormalities. After infection had been ruled out, sirolimus-associated pneumonitis was diagnosed in both patients. Cyclosporine was substituted for sirolimus in one patient,[22] and the sirolimus dose was lowered in the other. There was complete resolution 3 months later in both patients with no further intervention.

Before transplantation, four patients had a clinically significant need for narcotics to alleviate recurrent pain crises. Two required repeated hospital admissions for the narcotic-withdrawal syndrome, with symptoms resembling the pain of vascular occlusion after stem-cell transplantation. These episodes necessitated a gradual tapering of narcotics, which was successfully completed in all four patients approximately 6 months after transplantation (Fig. 2 in the Supplementary Appendix).

DEVIATIONS IN PROTOCOL

Patients 2 and 3 inadvertently received 200 cGy of total-body irradiation instead of the planned 300 cGy. Patient 2 had donor engraftment for about 4 months, followed by loss of the graft, autologous hematopoiesis, and recurrent sickle cell disease. One year later, after undergoing conditioning with a modified regimen consisting of 400 cGy and the dose of alemtuzumab administered previously, he received a second transplant from the same donor; this transplantation was successful. Patient 3 has persistent stable, mixed chimerism.

DISCUSSION

Sickle cell disease affects multiple organs and shortens the life span of those affected.[23–26] In our study of allogeneic hematopoietic stem-cell transplantation in 10 adults with severe sickle cell disease, the procedure itself was not associated with serious adverse events, and there were no treatment-related deaths. The duration of cytopenias was 10 to 21 days for neutropenia, 1.5 to 8 months for lymphopenia, and 1 to 19 days for thrombocytopenia. A neutrophil count nadir of less than 0.1×10^9 per liter, which lasted for 1 to 7 days, occurred in 3 of the 10 patients. The relatively brief periods of neutropenia probably accounted for the absence of overwhelming bacterial or invasive fungal infections in our patients. Our rate of successful engraftment (9 of 10 patients), is similar to the reported rates after myeloablative conditioning in children with sickle cell disease who underwent transplantation in France (95.5%),[5] the United States (91.5%),[6] and Belgium (94%).[4] Remarkably, acute or chronic GVHD, the major contributors to the morbidity and mortality associated with stem-cell transplantation, did not occur in any of our transplant recipients during a median follow-up

of 30 months. The absence of GVHD in our patients compares favorably with the results of myeloablative stem-cell transplantation in children with sickle cell disease, in whom the low frequency of GVHD has been attributed to age and the use of bone marrow or cord blood as stem-cell sources.

We believe that our results are due to the conditioning regimen. Total-body irradiation at a dose of 300 cGy must have created enough bone marrow space to allow a degree of hematopoiesis from donor stem cells that was sufficient to reverse the sickle cell disease phenotype. In addition, this level of radiation supplements the immunosuppression required to prevent rejection of the allograft. Alemtuzumab, with detectable lymphocytotoxic plasma drug levels for several weeks after administration,[27,28] reduced the numbers of circulating lymphocytes in the recipient before stem-cell infusion, thereby helping to prevent graft rejection, and also depleted alloreactive T cells after transplantation, which probably prevented the development of GVHD. Sirolimus for prophylaxis against GVHD, a relatively new use of the drug, promotes the differentiation of regulatory T cells and helper T cells, both of which play key roles in minimizing the risk of GVHD.[29–31] These benefits of sirolimus in conjunction with gradual reconstitution of donor T cells support a graft-tolerant milieu in which stable, mixed donor–recipient chimerism was sufficient to achieve erythropoiesis entirely from donor cells. In contrast, calcineurin inhibitors inhibit the induction of immune tolerance.[10,32] Most of our patients have continued to receive sirolimus because of their mixed chimeric state. Frequently reported side effects of sirolimus include myelosuppression, increased serum triglyceride levels, hypercholesterolemia, and hyperglycemia.[33–35] Prolonged administration of sirolimus increases the risk of viral reactivation, opportunistic infections, pneumonitis, and post-transplantation lymphoproliferative disorder. We have not observed myelosuppression in any of the patients; we are treating hyperlipidemia in three patients. We are gradually decreasing sirolimus doses to minimize side effects and are monitoring the patients for infection, changes in organ function, and tumors. The stability of donor chimerism in the two patients who did not comply with sirolimus treatment has prompted us to amend the protocol so that the dose of sirolimus is tapered in patients in whom over 50% of T cells are of donor origin.

In summary, we have developed a simplified regimen of hematopoietic stem-cell transplantation that allows for stable, mixed donor–recipient chimerism and reverses the sickle cell disease phenotype. The simplicity, low toxicity, and high efficacy of this approach make it feasible for use at most transplantation centers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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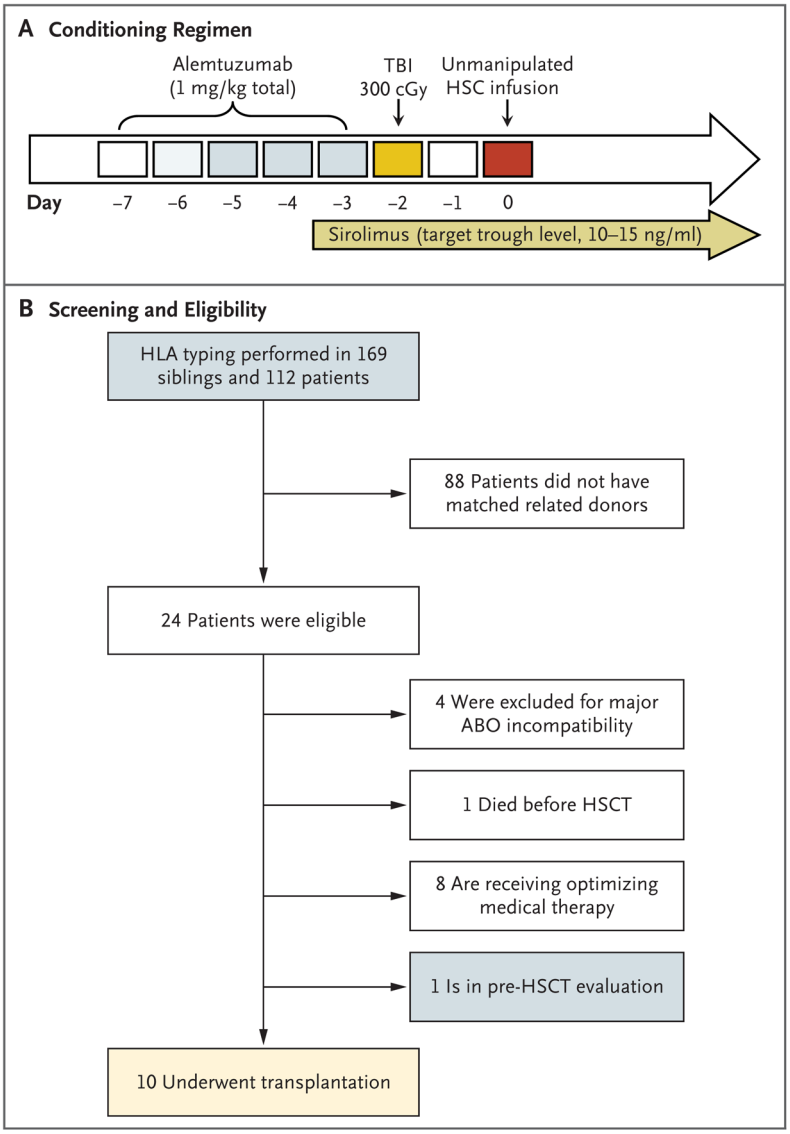

Figure 1. Conditioning Regimen and Screening of Patients

Panel A shows the conditioning regimen used before hematopoietic stem-cell transplantation (HSCT). The regimen consisted of alemtuzumab, at a total dose of 1 mg per kilogram of body weight, given over a period of 5 days in gradually increasing doses: 0.03 mg per kilogram (test dose) on day −7, 0.1 mg per kilogram on day −6, and then 0.3 mg per kilogram per day on days −5 through −3. A single dose of 300 cGy of total-body irradiation (TBI) was administered on day −2 (with gonadal shielding applied for men). Treatment with oral sirolimus was initiated on day −1 at a dose of 5 mg every 4 hours for three doses, then 5 mg daily starting on day 0, modified to achieve target trough levels of 10 to 15 ng per milliliter of whole blood. Panel B shows the numbers of patients and their siblings who were screened, the number of eligible patients, and the number of patients who underwent transplantation. Among a total of 24 eligible patients, 6 of 6 HLA-matched siblings were identified. Four patients were excluded owing to major ABO incompatibility and a previously reported high incidence of pure red-cell aplasia from residual recipient lymphoid cells in nonmyeloablative HSCT,[15,16] and one patient died of suspected sudden arrhythmia from severe iron overload 1 month before the planned HSCT. Eight patients are currently

undergoing optimization of their medical therapy, including hydroxyurea therapy, iron chelation, and pain management, and one patient is currently undergoing evaluation for HSCT.

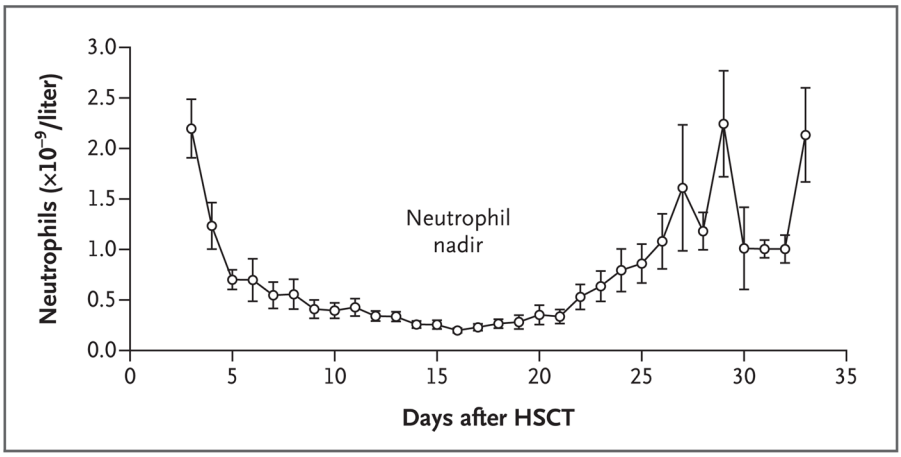

Figure 2. Neutrophil Counts after Hematopoietic Stem-Cell Transplantation (HSCT)
Each circle represents the mean neutrophil count for all patients after the transplantation procedure. I bars indicate standard errors.

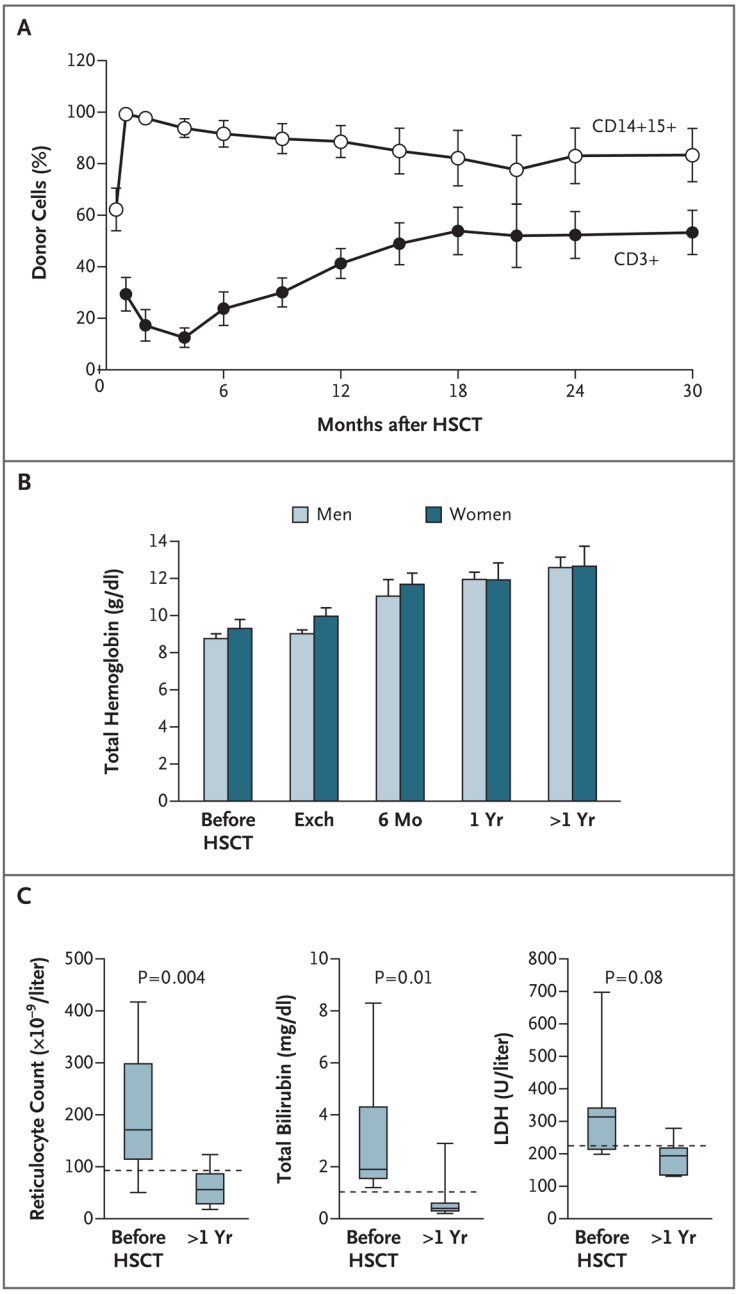

Figure 3. Donor-Cell Chimerism and Laboratory Measurements after Hematopoietic Stem-Cell Transplantation

Panel A shows the mean (±SE) percentage of donor cells after hematopoietic stem-cell transplantation (HSCT). Donor chimerism among CD3+ T cells and CD14+15+ myeloid cells was assessed by means of a polymerase-chain-reaction assay to determine minisatellite polymorphisms between patient and donor. Panel B shows the laboratory measurements obtained one month before HSCT and those obtained at the most recent follow-up (median, 30 months; range, 15 to 54). The bar graphs in Panel B show mean values for total hemoglobin before, during (Exch), and after HSCT for male and female recipients; T bars indicate standard errors. For the reticulocyte count, total bilirubin level, and lactate dehydrogenase (LDH) level shown in Panel C, the horizontal lines within the box plots

indicate the mean values, the lower and upper ends of the boxes represent the 25th and 75th percentiles, and the I bars represent the 10th and 90th percentiles; the dashed lines represent the upper limits of the reference ranges.

Table 1

Characteristics of 10 Patients Undergoing Nonmyeloablative Hematopoietic Stem-Cell Transplantation (HSCT).*

Patient No.	Age at HSCT *yr*	Sex	Type of Sickle Hemoglobin	Coexisting Conditions and Indications for HSCT	Medical Management before HSCT
1	24	F	SS	Recurrent TIA and stroke, elevated TRV	Simple and exchange red-cell transfusions
2	27	M	SS	Frequent VOC, priapism, proteinuria (1.7 g/24 hr)	Hydroxyurea, simple and exchange red-cell transfusions
3	21	F	SS	TIA, frequent VOC, acute chest syndrome	Hydroxyurea, exchange red-cell transfusions
4	16	M	SS	Frequent VOC, acute chest syndrome, narrow CNS arteries on MRA	Hydroxyurea, exchange red-cell transfusions
5	21	M	SS	Frequent VOC, acute chest syndrome	Hydroxyurea
6	40	M	SC	Frequent VOC, priapism, narrow CNS arteries on MRA, lacunar infarcts	Hydroxyurea
7	26	F	SS	Frequent VOC, elevated TRV	Hydroxyurea
8	26	F	SS	Frequent VOC, elevated TRV	Hydroxyurea and simple red-cell transfusions
9	45	F	SS	Sickle-cell–related FSGS (baseline creatinine, 2.5–2.7 mg/dl [221–239 μmol/liter]), elevated TRV, acute chest syndrome, frequent VOC, red-cell alloimmunization, hepatitis C	Hydroxyurea, simple and exchange red-cell transfusions, darbepoetin
10	26	M	SS	Sickle-cell–related nephrotic syndrome, elevated TRV, acute chest syndrome	Hydroxyurea, simple red-cell transfusions, prednisone

*CNS denotes central nervous system, FSGS focal segmental glomerulosclerosis, MRA magnetic resonance arteriography, TIA transient ischemic attack, TRV tricuspid regurgitant jet velocity, and VOC vaso-occlusive crises.

Table 2

Hematopoietic-Graft Composition and Outcome after Hematopoietic Stem-Cell Transplantation (HSCT).*

Patient No.	Composition of Infused Graft		Months after Transplantation	Duration of ANC <0.50×10^{-9}/liter	Duration of ALC <0.75×10^{-9}/liter	Donor CD3+ Cells	Donor CD14+15+ Cells	Hemoglobin	Hemoglobin S	
									Donor	Recipient
	CD34+ Cells ×10^{-6}†	CD3+ Cells ×10^{-8}†		*days*	*months*	*%*		*g/dl*	*%*	
1	5.72	3.21	54	21	3.5	7	48	12.0	0	0
2‡	7.56	2.27	36	18	2.5	63	19	11.1	40.5	51.6
3	10.0	3.42	42	12	6	61	100	14.8	35.2	35.2
4‡	8.3	5.35	33	29	6	0	0	11.4	0	45.9§
5	5.51	3.71	30	10	4	72	100	14.3	0	0
6	23.8	2.81	32	10	6	35	97	14.7	38.2	37.0
7	18.8	3.32	29	19	8	62	100	12.2	36.6	35.4
8	20.1	3.04	30	11	1.5	63	100	12.1	0	0
9	16.6	3.7	16	15	3.5	23	97	11.7¶	0	0
10	15.1	3.64	15	18	4	75	100	10.5¶	35	34.6

*Results are from the most recent follow-up assessment. ALC denotes absolute lymphocyte count, and ANC absolute neutrophil count.

†Values are per kilogram of the recipient's body weight.

‡The results shown are from a second transplantation.

§The patient had received an exchange transfusion within the previous 2 months.

¶The patient was receiving supportive treatment with erythropoietin owing to renal insufficiency.

Table 3

Summary of Serious Adverse Events.*

Event	Interval between HSCT and Event	No. of Patients	Treatment and Outcome
CMV reactivation	14 days	1	300 copies of CMV genome; treated with 1 wk of intravenous foscarnet, followed by maintenance therapy with oral valacyclovir
Narcotic-withdrawal syndrome	Various times during the first 6 mo	3	Hospital admission for tapering of narcotics; in all 3 patients, narcotics have been withdrawn
Abdominal pain			
Gastric ulcer on gastroscopy	3 mo	1	Proton-pump–inhibitor therapy for 1 mo
Unknown cause	12 mo	1	Self-limited; no treatment
Sirolimus-associated complications			
Pneumonitis			
Patient 3	16 mo	1	When sirolimus was switched to cyclosporine, the pneumonitis completely resolved
Patient 9	4 mo	1	When the sirolimus dose was decreased to achieve a trough level of 5 ng/ml, the pneumonitis completely resolved
Arthralgias			
Patient 9	3 mo	1	Supportive care; target trough level reduced to 5 ng/ml; patient was admitted for arthralgia workup
Patient 1	4 mo	1	Supportive care; target trough level reduced to 5 ng/ml; patient remained an outpatient
Transfusion-associated babesiosis	8 mo	1	Azithromycin and atovaquone; condition resolved completely
Exercise-related rhabdomyolysis	3 mo	1	Hydration and switch from oral sulfamethoxazole–trimethoprim to inhaled pentamidine
Ventricular tachycardia	1 mo before transplantation and during conditioning regimen	1	Due to iron overload and previous cardiac ischemia; rate controlled with amiodarone; patient is currently receiving carvedilol
Clostridium difficile colitis	4 mo	1	Supportive care; antibiotics
Cholelithiasis-induced acute pancreatitis	15 mo	1	Supportive care
Fever	1 mo	1	Supportive care

*CMV denotes cytomegalovirus, GVHD graft-versus-host disease, and HSCT hematopoietic stem-cell transplantation.