

Hemoglobinopathy

Hematopoietic stem cell transplantation for multiply transfused patients with sickle cell disease and thalassemia after low-dose total body irradiation, fludarabine, and rabbit anti-thymocyte globulin

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Summary:

Patients with sickle cell disease ($N=3$) and thalassemia ($N=1$) with high-risk features received hematopoietic stem cell transplantations (HCT) to induce stable (full or partial) donor engraftment. Patients were 9–30 years of age. Fludarabine, rabbit anti-thymocyte globulin (ATG), and 200 cGy total body irradiation were administered pre-transplant. Patients received bone marrow ($N=3$) or peripheral blood stem cells ($N=1$) from HLA-identical siblings, followed by mycophenolate mofetil and cyclosporine for post-grafting immunosuppression. Significant lymphopenia, but only moderate neutropenia and thrombocytopenia developed post transplant. No grade IV nonhematological toxicities or acute graft-versus-host disease (GVHD) were observed. At 3 months after transplantation, three of four patients had evidence of donor myeloid chimerism (range, 15–100%). However, after post transplant immunosuppression was discontinued, graft rejection occurred in all but one patient. This patient is now doing well 27 months post transplant with full donor engraftment. One patient died after a second transplant, and another patient experienced a stroke as her graft was being rejected. These results suggest that stable donor engraftment after nonmyeloablative HCT is difficult to achieve among immunocompetent patients with hemoglobinopathies and that new approaches will need to be developed before wider application of this transplantation method for hemoglobinopathies.

Bone Marrow Transplantation (2005) 35, 171–177.

doi:10.1038/sj.bmt.1704745

Published online 8 November 2004

Keywords: sickle cell anemia; thalassemia; nonmyeloablative bone marrow transplantation

Since the first successful case report in a child with thalassemia in 1982, there has been ample evidence to show that bone marrow transplantation for clinically significant hemoglobinopathies can establish donor erythropoiesis and eliminate the underlying hereditary anemia. These transplants have, for the most part, combined myeloablative doses of busulfan and cyclophosphamide with HLA-identical sibling marrow transplantation.^{1–8} Overall, approximately 85% of children with severe sickle cell disease and β -thalassemia major were cured, and as a result of improved supportive care measures, the risk of transplant-related mortality was less than 10%.^{1,5,9} However, initial reports also suggested that a subset of patients had poorer outcomes, and were more likely to experience transplant-related mortality or disease recurrence. These were older patients who had evidence of organ damage from iron-overload or from vaso-occlusion, and investigations were initiated to explore alternative regimens that might reduce the toxicity of transplantation for those who had advanced disease⁹ (Walters MC, unpublished data).

One approach that has been tested in limited clinical series is nonmyeloablative allogeneic transplantation, which relies upon immunosuppressive rather than ablative pre-transplantation conditioning to facilitate engraftment of donor cells. A minimal toxicity regimen that utilized low dose total body irradiation (TBI) alone before transplantation was evaluated as a novel method to establish stable donor engraftment more safely in high-risk patients. It was tested initially in animal models,^{10–13} where the evolution of mixed donor–host chimerism was observed. This outcome, however, would not be problematic in hemoglobinopathies where sustained mixed chimerism is sufficient to eliminate most, if not all the clinical manifestations of sickle cell disease and thalassemia major.^{14,15} The minimal toxicity regimen has been applied most widely in adults with hematological malignancies who are either too old, or have significant comorbid conditions that preclude conventional allografting.¹⁶ When combined with post-grafting immunosuppression by mycophenolate mofetil (MMF) and cyclosporine (CSA), a single fraction of TBI (200 cGy) was sufficiently immunosuppressive to establish stable engraftment in most patients after HLA-identical sibling

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Received 22 July 2004; accepted 14 September 2004
Published online 8 November 2004

peripheral blood stem cell (PBSC) transplantation. However, it was noted that for some patients, such as those who had chronic myelogenous leukemia, a significant fraction developed graft rejection after nonmyeloablative transplantation. It was reasoned that since these patients did not receive intensive chemotherapy in the course of treating their underlying disorder, they were more likely to have graft rejection compared to their counterparts with acute leukemia. Thus, pre-grafting immunosuppression was expanded by the addition of fludarabine before transplantation to reduce the risk of graft rejection.¹⁶ Unfortunately, the addition of fludarabine alone did not reduce the risk of graft rejection in one series of patients with hemoglobinopathies.¹⁷

We reasoned that exposure to minor histocompatibility antigens contained in blood transfusions might increase the likelihood of an immunological rejection of donor cells after transplantation for sickle cell disease and thalassemia major. Thus, to mitigate the host-versus-graft reaction, we intensified the immunosuppression in the minimal toxicity regimen by including antithymocyte globulin (ATG), which has been shown to reduce the effect of transfusion exposures on the risk of graft rejection in patients with severe aplastic anemia.¹⁸ Here, we report the preliminary results of a trial in heavily transfused children and adults with advanced sickle cell disease and thalassemia.

Patients, materials and methods

Patients and eligibility

This IRB-approved investigation was conducted at the University of Rochester Medical Center. Four consecutive patients were treated between August 7, 2001 and July 31, 2002. Written informed consent was obtained before enrollment. To be eligible for enrollment, patients were required to (1) have an HLA-identical sibling donor with serologic matching at HLA-A, B, C and allelic matching at HLA DRB1; (2) be between 2 and 40 years of age; (3) have received at least 5U RBC and/or platelet transfusion exposures before referral; (4) have experienced significant sickle cell-related complications (eg clinical stroke, recurrent acute chest syndrome, sickle nephropathy, etc.), have thalassemia major or another hereditary red blood cell disorder dependent upon regular RBC transfusions (eg Diamond-Blackfan anemia), or a platelet-transfusion-dependent disorder (eg amegakaryocytic thrombocytopenia). Before enrollment, all patients had their eligibility confirmed by an independent adjudication panel, which consisted of pediatric and adult hematologists from six separate institutions.

Treatment and evaluations

The preparative regimen consisted of a combination of TBI, fludarabine, and rabbit ATG. All patients received fludarabine 25 mg/m² on 5 consecutive days before transplantation (days -6, -5, -4, -3, -2) and rabbit ATG (Thymoglobulin[®]) on 4 consecutive days before transplantation (days -6, -5, -4, and -3). The regimen

included a limited dose escalation of rabbit ATG, which was based upon the CD3+ cell count 30 days after transplantation. This was employed to ensure that the dose of rabbit ATG administered did not cause excessive *in vivo* T-cell depletion of the graft, and therefore delay immune reconstitution. Anticipating that immune reconstitution would be slower in adult than in pediatric patients,^{19,20} we employed two separate age-dependent strata: in children between 2 and 15 years of age, the initial total dose was 12.0 mg/kg and in patients between 16 and 40 years of age, the initial total dose was 10.0 mg/kg. In each stratum, if the day 30 CD3+ count exceeded 100 cells/ μ l in at least 80% of patients (three of three, four of four, four of five, five of six, or six of seven), the dose was to be increased by 25% (total doses = 12.5 mg/kg, and 15.0 mg/kg, in the older and younger strata, respectively) in subsequent patients. Only two dose levels were tested in this trial. A single fraction of TBI (200 cGy delivered at a rate of 7 cGy/min) was administered in all patients on the day of transplantation. All males received testicular shielding. Patient 1 received donor G-CSF-mobilized PBSC and the remaining three patients received unmodified donor bone marrow. MMF and CSA were administered for post-grafting immune suppression. The MMF was administered for 3 months (15 mg/kg/dose p.o. b.i.d.) after transplantation and stopped without a taper, and CSA (1.5 mg/kg, i.v. q 12h) was administered for at least 6 months after transplantation. The CSA levels were targeted to the lower end of the therapeutic range (150–250 ng/ml through 90 days after transplantation, then 75–125 ng/ml; Abbott TDX, Abbott Park, IL, USA).

Prophylaxis against *Pneumocystis carinii*, fungal, and *herpes virus* infections was administered. Patients were monitored weekly for CMV reactivation by PCR of peripheral blood leukocytes, and either ganciclovir or foscarnet was administered preemptively if CMV was detected. Two patients with sickle cell disease had an automated red blood cell exchange transfusion performed before transplantation to achieve a hemoglobin S fraction of 30%. Red blood cell transfusions were administered after transplantation to maintain the Hb S fraction less than 30% and the plasma hemoglobin in a range of 9–11 gm/dl. To prevent neurological complications, sickle cell disease patients had standard guidelines followed, which included seizure prophylaxis by gabapentin, maintaining the platelet count > 50 000/mm³, aggressive antihypertensive therapy, and magnesium repletion as needed.

Determinations of donor chimerism in peripheral blood T-lymphocyte (CD3+) and myeloid (CD33+) compartments were performed 30, 90, 180, 270, 365 days after transplantation. In some cases, the monitoring was performed more frequently. The methodology for these determinations was Y-chromosome fluorescent *in situ* hybridization in sex-mismatched donor–host pairs or analysis of the variable number tandem repeats in sex-matched pairs. Serial complete blood counts, absolute neutrophil and lymphocyte counts, quantitative immunoglobulin levels, and lymphocyte subsets were monitored in all patients at 30, 60, 90, 180, and 365 days post transplant. Patients with sickle cell disease were assessed regularly for

disease manifestations and had serial monitoring of quantitative hemoglobin S levels.

Treatment aimed at reversing graft rejection was permitted. Glucocorticoids and other immunosuppressive agents were administered, but ATG (to avoid prolonged severe immune deficiency) or the administration of a donor lymphocyte infusion (DLI) was not permitted in this trial. However, an exception was made in Patient 2 who received DLI and graft-versus-host disease (GVHD) prophylaxis to treat prolonged, life-threatening red cell aplasia. This protocol exception first was reviewed and subsequently approved by the IRB.

Results

Patient characteristics

The patient characteristics are summarized in Table 1. Patient 1 (22 years) had hemoglobin E/ β -thalassemia with a thalassemia major phenotype. Although he complied with iron chelation therapy in the year before transplantation, he had a severe cardiomyopathy due to iron overload that was complicated by ventricular and atrial dysrhythmias. His cardiac disease was managed by a combination of metoprolol, enalapril, digoxin, and furosemide to enhance ventricular function and control dysrhythmias. An implantable defibrillator/pacemaker was inserted soon after transplantation to ensure control of dysrhythmias. He also had moderate hepatic fibrosis due to iron overload and a chronic active hepatitis C infection. He had multiple endocrinopathies that included insulin-dependent diabetes mellitus.

Patient 2 had sickle cell anemia (Hb SS, 28 years) and a history of recurrent episodes of acute chest syndrome and painful vaso-occlusive crises. To reduce the frequency of painful events, he received hydroxyurea but had only a modest response. He had moderately severe sickle cell chronic lung disease that was characterized by restrictive abnormalities on pulmonary function testing (forced vital capacity 61% predicted, DLCO 59% predicted after correction for anemia) and borderline pulmonary hypertension as estimated by echocardiography (trace tricuspid regurgitation, systolic pulmonary pressure was 36 mmHg, right atrial pressure was 7 mmHg, tricuspid gradient was 29 mmHg). Although no pulmonary fibrosis was apparent by chest radiograph, fibrosis was demonstrated by high-resolution computed tomography of the chest.

Patient 3 had sickle cell anemia (Hb SS, 9 years), and had an infarctive cerebrovascular accident in the distribution of the left middle cerebral artery 9 months before transplantation. Cerebral magnetic resonance imaging and angiography demonstrated a moyamoya pattern. She was treated by regular RBC transfusions before transplantation. She never suffered any other acute or any chronic sickle cell-associated complications.

Patient 4 had sickle cell disease (Hb SC, 30 years) that was complicated by frequent painful vaso-occlusive crises that required intermittent hospitalizations. A trial of hydroxyurea had little impact of the frequency of painful events. He had a previous history of acute chest syndrome,

Table 1 Patient, donor, and graft characteristics

Pt no.	Age (years)	Hb genotype	Major complications	No. transfusions pre-HCT (1,6-20, 2; 21-50, 3; >50)	Donor		Graft composition			
					Recipient/donor gender	Recipient/donor ABO	Stem cell source	CD34+ ($\times 10^6$ /kg)	TNC ($\times 10^8$ /kg)	CD3+ ($\times 10^6$ /kg)
1	22	HbE/Thal	Transfusion dependent, cardiomyopathy from iron overload, hepatic fibrosis	3	M/F	O+/O+	PBSC	3.7	7.9	33.3
2	28	Hb SS	Sickle cell chronic lung disease	2	M/M	O+/A+	BM	1.5	2.7	1.4
3	9	Hb SS	Infarctive stroke/moyamoya syndrome	1	F/M	A+/O+	BM	2.8	3.3	1.9
4	30	Hb SC	Frequent painful VOCs unresponsive to hydroxyurea	1	M/M	O+/A+	BM	0.9	1.3	1.2

but had experienced no episodes in the 2 years before transplantation. He had no chronic complications.

Regimen-related toxicity, infections, and graft-versus-host disease

One child was assigned to rabbit ATG stratum 1, and three young adults were assigned to stratum 2 before enrollment. The conditioning regimen caused significant lymphopenia after transplantation, but moderate myelosuppression (Figure 1). The neutrophil nadir was 1532, 1368, 546, and 192/mm³ in the four patients. The patient with thalassemia had a platelet nadir of 196 000/mm³ after PBCT, and the three patients with sickle cell disease had a platelet nadir of 49 000, 52 000, and 44 000. Applying a threshold of 50 000/mm³ before platelet transfusions in the sickle cell disease patients, the median number of platelet transfusions administered was 1 (range, 0–3). Three of the four patients had CD3+ cell enumerations that exceeded 100/mm³ 30 days after transplantation. The rabbit ATG did not significantly delay T-lymphocyte reconstitution (Table 2). Immunoglobulin levels were adequate after transplantation. Using a threshold serum level of 500 mg/dl before repletion, no intravenous immunoglobulin (IVIg) was administered.

There was moderate toxicity associated with the conditioning regimen in one patient. Patient 2 developed serum sickness, which was associated with toxic megacolon, mild renal insufficiency, and mild congestive heart failure. He was treated by methylprednisolone and recovered uneventfully. After transplantation, three of the four patients developed bacteremia that was associated with the central venous catheter, and which was successfully treated with antibiotics in each case. Two of the three patients at risk for CMV infection had reactivation after transplantation, as detected by PCR. After institution of preemptive antiviral therapy, no evidence of CMV disease was observed. Patient 2 developed a localized herpes zoster dermal reactivation.

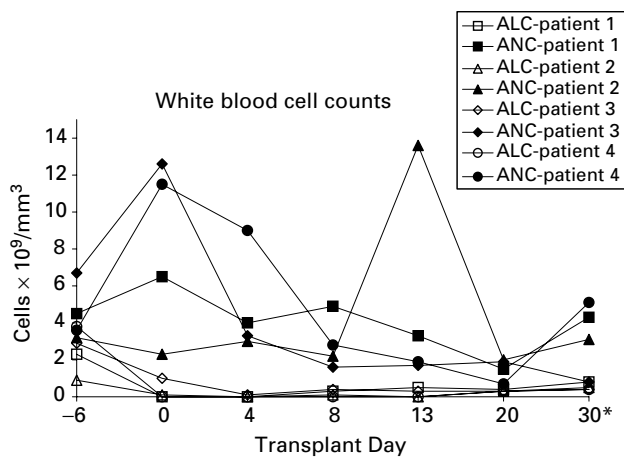


Figure 1 A highly immunosuppressive, but minimally myelosuppressive conditioning regimen. The preparative regimen produced profound peripheral blood lymphopenia in all patients. In contrast, the approach produced more modest neutropenia. *Days 28 and 29 were used for patients 2 and 1, respectively.

None of the four patients developed acute GVHD after transplantation.

Engraftment, graft rejection, and disease manifestations

The PBSC graft contained more nucleated cells, CD34+ cells, and CD3+ cells than the marrow grafts (Table 1). Overall, the total nucleated cell count ranged from 1.3 to 7.9 ($\times 10^8$ /kg), while the CD34+ and CD3+ cell doses, ranged from 0.9 to 3.7 ($\times 10^6$ /kg) and 1.2 to 33.3 ($\times 10^7$ /kg), respectively. Initially, there was engraftment of donor cells observed in all four patients (Table 3). Patient 1 had a majority of donor lymphoid and myeloid representation by chimerism studies at 6 months after transplantation. Thus, CSA was discontinued 7 months after transplantation. However, the fraction of donor lymphoid chimerism declined by 9 months after transplantation, and thereafter an apparent graft failure occurred. Immunosuppressive therapy was reinstated and included CSA, MMF, and daclizumab. However, these interventions failed to prevent graft rejection and reconstitution of autologous hematopoiesis. Thus, the patient received a second transplantation 15 months after the initial transplant according to an IRB-approved second transplantation protocol. He received three courses of fludarabine (25 mg/m² for 4 days every 4 weeks) prior to enrollment to decrease the host-versus-graft response. Prophylaxis was administered against *Pneumocystis carinii*, fungal, and *herpes simplex* infections during fludarabine therapy. After the second cycle of

Table 2 Results of peripheral blood donor chimerism

Patient no.	Marker	Months post transplant (%)					
		1	3	6	9	12	24
1	CD3+	51	48	83	9	0	—
	CD33+	97	87	97	86	20	—
2	CD3+	35	50	65	70	65	100
	CD33+	95	100	100	100	100	100
3	CD3+	5	5	5	5	—	—
	CD33+	5	40	15	10	—	—
4	CD3+	5	0	0	—	—	—
	CD33+	50	15	0	—	—	—

Table 3 CD3+ and CD4+ cell counts post transplant

Patient	CD3 (cells/mm ³) ^a			CD4 (cells/mm ³) ^a		
	Day 30	Day 90	Day 180	Day 30	Day 90	Day 180
1	1049	559	776	570	260	425
2	74	233	685	43	129	300
3	579	655	N/A	270	290	N/A
4	213	975	N/A	54	202	N/A

^aThese values were obtained just prior to the start of the 3rd cycle (4 weeks after the start of the second cycle). Since ATG was started 2 weeks after the start of the third cycle, lymphocyte subset testing was not performed.

fludarabine, there was a significant decrease in all T-lymphocyte subsets that included memory T (CD45RO+) cells (Table 4). Preparation for the second transplantation commenced 2 weeks after the fourth cycle of fludarabine, and consisted of equine ATG (ATGAM®) 45 mg/kg for 3 days (-16, -15, and -14), fludarabine 25 mg/m² for 5 days (-6 through -2), and 2 fractions of TBI (250 cGy doses on days -1 and 0). There was shielding of the testes and liver. After infusion of unmodified bone marrow from a second HLA-identical sibling donor, he received a combination of CSA and MMF for post-grafting immunosuppression. There was no unexpected or serious toxicity and there was full donor engraftment in both T lymphocyte and myeloid cell populations by 30 days after transplantation. Although T-cell reconstitution was rapid post transplant (day 30 CD3+ 1005/mm³ and CD4+ 595/mm³), unfortunately, the patient subsequently developed an RSV infection, and died 52 days after transplantation of pneumonitis and intractable congestive heart failure.

The level of donor chimerism in Patient 2 increased slowly, and by 24 months after transplantation there was full donor lymphoid and myeloid chimerism. In addition, by hemoglobin electrophoresis, there was no Hb S detected. However, due to a major ABO incompatibility (donor A, recipient O), the patient developed immune-mediated red cell aplasia, which persisted until 21 months after transplantation. The red cell aplasia was treated sequentially (at intervals of 3–6 months) by prednisone, dexamethasone, anti-CD 20 monoclonal antibody, and a donor lymphocyte infusion (coupled with an abbreviated course of CSA/MMF). There was a gradual decline in the anti-A immunoglobulin titer level, but the impact of these interventions was uncertain (Figure 2). He is no longer receiving RBC transfusions and the hemoglobin level is 15.5 g/dl. There have been no sickle-related complications and his pulmonary function was also improved. A program of phlebotomy for iron overload has been instituted.

Patient 3 had low-level donor chimerism 1 month after transplantation. However, the donor myeloid chimerism increased transiently, but donor lymphoid chimerism never exceeded 5%. At 4 months after transplantation, prednisone was administered in combination with MMF to suppress the host-versus-graft reaction. Initially, there was a response with increased donor erythropoiesis (evidenced by a transition to the donor RBC phenotype and a hemoglobin S fraction identical to the donor trait level of

40%) 5 months after transplantation. In preparation for removal of a central venous catheter 5 1/2 months after transplantation, a simple transfusion was administered to reduce the pre-operative Hb S fraction of 53%. Unfortunately, the patient had a second stroke in the post-operative period when the hgb level was 13.0 gm/dl and the Hb S fraction was 36%. She was treated acutely by an exchange transfusion, and resumed regular RBC transfusions to prevent stroke. When immunosuppression was withdrawn, there was graft rejection/disease recurrence.

Patient 4 had little evidence of donor T-cell engraftment initially, and the host-versus-graft reaction was treated by a combination of MMF, CSA, and prednisone. Unfortunately, this intervention was unsuccessful in preventing graft rejection, and by 3 months after transplantation, there was no detectable evidence of donor T-lymphocyte engraftment.

Discussion

In this report, we describe our attempt to overcome the barrier to engraftment in patients with sickle cell anemia and thalassemia major who had significant transfusion exposures before transplantation. Unfortunately, we failed to identify a regimen that was both minimally toxic and effective. In this limited series, four patients underwent HLA-identical sibling allografting, and three of the four had graft rejection that was accompanied by disease recurrence. One of the patients developed a fatal infection after a second transplantation procedure. While the motivation for conducting this trial was to identify a novel transplantation regimen that might improve the safety profile of this treatment in high-risk patients, this objective remained elusive.

Sustained engraftment was achieved in only one of four patients, while a predominance of donor T-lymphocyte

Table 4 Effect of sequential cycle fludarabine on absolute lymphocyte subset values (Patient 1)

Subset	Pre-therapy value (cells/ μ l)	After 2nd course (cells/ μ l) ^a
CD3+	947	71
CD4+	181	19
CD8+	638	50
CD19+	14	0
CD4+/CD45RO+ (memory)	104	9

^aThese values were obtained just prior to the start of the 3rd cycle (4 weeks after the start of the second cycle). Since ATG was started 2 weeks after the start of the third cycle, lymphocyte subset testing was not performed.

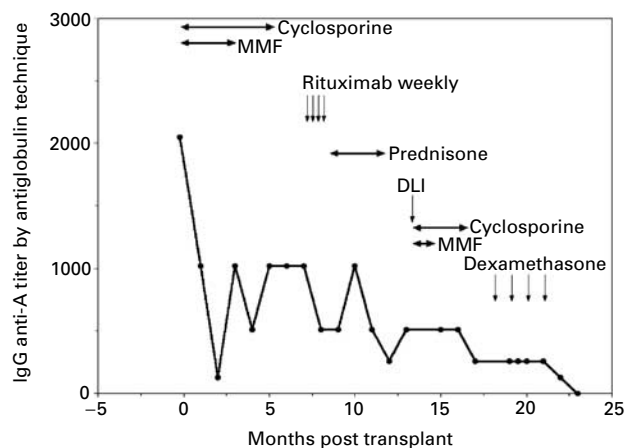


Figure 2 Owing to a major ABO incompatibility (donor A, recipient O), patient two developed immune-mediated red cell aplasia, which persisted until 21 months after transplantation. The IgG anti-A titer is plotted as a function of time. Various treatments were administered, which are displayed above. There was a sharp drop in the titer in the early post transplant period, followed by a rebound, and then a more gradual and stuttering decline.

representation, which is an important predictor of sustained engraftment after nonmyeloablative transplantation,¹⁶ was demonstrated in two. The findings in this study confirm the observations of another small series of patients with hemoglobinopathies who underwent nonmyeloablative HCT, where low-level donor T-lymphocyte chimerism appeared to be a harbinger of graft rejection.¹⁷ In older patients with hematological malignancies who received intensive chemotherapy before nonmyeloablative HCT (eg those who had acute leukemia), a minimal toxicity pre-transplantation regimen containing low-dose TBI appeared to be sufficient for donor T-lymphocyte engraftment.¹⁶ To recapitulate this effect of having a relative state of immunosuppression in patients with hemoglobinopathies, intensification of the regimen will be required. The challenge is how to accomplish dose intensity without significantly altering the safety profile of the regimen.

It is possible that the dose and timing of rabbit ATG administration might be optimized for use in this setting. One recent study in primates demonstrated that depletion of T-lymphocyte populations in lymph nodes, where most memory T cells reside, required a relatively high dose of rabbit ATG.²¹ In a clinical trial of conventional myeloablative transplantation for sickle cell anemia, the frequency of graft rejection was significantly reduced after rabbit ATG at a high dose (20 mg/kg) was employed in the preparative regimen.⁵ Unfortunately, one consequence of intensifying the rabbit ATG dose is an increased risk of opportunistic infections as illustrated by our observation of CMV reactivation in two of three patients in our trial. An alternative that might be considered is to extend the duration of pre-transplantation immunosuppression. This has been applied successfully in thalassemia major among patients with high-risk, Lucarelli Class 3 features.²² We used a related approach before the second transplantation in patient 1. Transplantation preparation followed sequential courses of fludarabine as a means to abrogate the host-versus-graft reaction. The fludarabine exposure before transplantation elicited a significant reduction in both memory and naïve T lymphocytes in this patient (see Table 4), and was followed by prompt engraftment of donor cells. A similar approach before nonmyeloablative transplantation was used in children with hematological malignancies, where 1–3 cycles of pre-transplant fludarabine were administered, and was associated with rapid and complete engraftment in most patients.²³ With supportive measures in place to prevent and treat opportunistic infections, intensification of pre-transplantation immunosuppression might represent a suitable alternative for selected high-risk patients.

Coupled with dose intensity, it might also be important to avoid strategies that diminish the allogeneic effect of transplantation, as the graft-versus-host reaction appears to be an important factor in ensuring engraftment of donor cells. In our study, rabbit ATG was administered in the week before the bone marrow infusion. It is quite likely that the long-lived presence of rabbit ATG reduced donor and host T-lymphocyte populations as a consequence of this dosing schedule.^{24,25} This consideration is supported by observations of the prophylactic effect of rabbit ATG on GVHD after allogeneic transplantation.²⁶ Unlike hemato-

logical malignancies, the avoidance of GVHD is particularly desirable after HCT for nonmalignant conditions, as it confers no obvious benefit for these disorders. However, abrogation of the allogeneic effect may outweigh any benefit of GVHD prevention if it also interferes with engraftment.²⁷ In our hands, the combination of cyclosporine, mycophenolate mofetil, and rabbit ATG was a very effective regimen for GVHD prevention.^{16,28} To retain the allogeneic effect of donor T lymphocytes, the optimal timing of rabbit ATG may occur earlier before transplantation when its effects might target only host lymphocytes. In addition, choosing PBSC in lieu of bone marrow might also optimize the allogeneic effect and promote enhanced donor T-lymphocyte engraftment. Of interest, PBSC were utilized in most patients with hematologic malignancies who underwent nonmyeloablative allografting and were superior to marrow with regard to overall outcome.^{16,28,29}

In summary, the safest and most effective means of allogeneic transplantation is evolving for patients with clinically significant hemoglobinopathies who have high-risk features. To date, the use of a minimally toxic regimen has not been sufficient to ensure engraftment of donor cells in a majority of patients. In the future, it is likely that more intensive regimens will be tested, but these must be designed in a manner such that patient safety is maintained. Appropriately, these efforts remain an intense focus of research as transplantation remains the only curative therapy for individuals who inherit these disorders.

Acknowledgements

We are grateful for the kind and thoughtful advice provided by the advisory panel members Dorothy Moore (New Jersey Medical School) and Phyllis Bazen (Upstate Medical University and Executive Director of the Syracuse Sickle Cell Association). We also thank those advisory panel members who served on the adjudication committee: Mary Jane Petruzzi, Children's Hospital of Buffalo; Stephan Dubansky, Upstate Medical University; Norma Lerner, University of Rochester; Sharon Space, Boston University; Charles Packman, Carolinas Medical Center; Maala Varma, New Jersey Medical School. This work was supported by grants from the Genzyme Corporation, the Strong Children's Research Center and the National Institute's of Health grant no. HL 68091.

References

- 1 Walters MC, Storb R, Patience M *et al*. Impact of bone marrow transplantation for symptomatic sickle cell disease: an interim report. *Blood* 2000; **95**: 1918–1924.
- 2 Vermylen C, Cornu G, Ferster A *et al*. Haematopoietic stem cell transplantation for sickle cell anaemia: the first 50 patients transplanted in Belgium. *Bone Marrow Transplant* 1998; **22**: 1–6.
- 3 Bernaudin F, Souillet G, Vannier J. Report of French experience concerning 26 children transplanted for severe sickle cell disease. *Bone Marrow Transplant* 1997; **19** (Suppl 2): 112–115.
- 4 Bernaudin F. Results and current indications of bone marrow allograft in sickle cell disease. *Pathol Biol (Paris)* 1999; **47**: 59–64.

- 5 Bernaudin F, Vernant J, Vilmer E *et al*. Results of myeloablative allogeneic stem cell transplant for severe sickle cell disease in France. *Blood* 2002; **100**: 1a.
- 6 Lucarelli G, Galimberti M, Polchi P *et al*. Bone marrow transplantation in patients with thalassemia. *N Engl J Med* 1990; **322**: 417–421.
- 7 Lucarelli G, Clift RA, Galimberti M *et al*. Marrow transplantation for patients with thalassemia: results in class 3 patients. *Blood* 1996; **87**: 2082–2088.
- 8 Lucarelli G, Clift RA, Galimberti M *et al*. Bone marrow transplantation in adult thalassemic patients. *Blood* 1999; **93**: 1164–1167.
- 9 Lucarelli G, Clift R. *Marrow Transplantation in Thalassemia*, 3rd edn. Blackwell Scientific: Malden, MA, 2004, p 1412.
- 10 Yu C, Storb R, Mathey B *et al*. DLA-identical bone marrow grafts after low-dose total body irradiation: effects of high-dose corticosteroids and cyclosporine on engraftment. *Blood* 1995; **86**: 4376–4381.
- 11 Storb R, Yu C, Wagner JL *et al*. Stable mixed hematopoietic chimerism in DLA-identical littermate dogs given sublethal total body irradiation before and pharmacological immunosuppression after marrow transplantation. *Blood* 1997; **89**: 3048–3054.
- 12 Sharabi Y, Sachs DH. Mixed chimerism and permanent specific transplantation tolerance induced by a nonlethal preparative regimen. *J Exp Med* 1989; **169**: 493–502.
- 13 Huang CA, Fuchimoto Y, Scheier-Dolberg R *et al*. Stable mixed chimerism and tolerance using a nonmyeloablative preparative regimen in a large-animal model. *J Clin Invest* 2000; **105**: 173–181.
- 14 Andreani M, Manna M, Lucarelli G *et al*. Persistence of mixed chimerism in patients transplanted for the treatment of thalassemia. *Blood* 1996; **87**: 3494–3499.
- 15 Walters MC, Blume K, Foreman S, Applebaum F (eds). *Hematopoietic Cell Transplantation for Sickle Cell Disease*. Blackwell Scientific: Malden, MA, 2004.
- 16 McSweeney PA, Niederwieser D, Shizuru JA *et al*. Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects. *Blood* 2001; **97**: 3390–3400.
- 17 Iannone R, Casella JF, Fuchs EJ *et al*. Results of minimally toxic nonmyeloablative transplantation in patients with sickle cell anemia and beta-thalassemia. *Biol Blood Marrow Transplant* 2003; **9**: 519–528.
- 18 Storb R, Etzioni R, Anasetti C *et al*. Cyclophosphamide combined with antithymocyte globulin in preparation for allogeneic marrow transplants in patients with aplastic anemia. *Blood* 1994; **84**: 941–949.
- 19 Weinberg K, Annett G, Kashyap A *et al*. The effect of thymic function on immunocompetence following bone marrow transplantation. *Biol Blood Marrow Transplant* 1995; **1**: 18–23.
- 20 Storek J, Witherspoon RP, Storb R. T cell reconstitution after bone marrow transplantation into adult patients does not resemble T cell development in early life. *Bone Marrow Transplant* 1995; **16**: 413–425.
- 21 Preville X, Flacher M, LeMauff B *et al*. Mechanisms involved in antithymocyte globulin immunosuppressive activity in a nonhuman primate model. *Transplantation* 2001; **71**: 460–468.
- 22 Sodani P, Gaziev D, Polchi P *et al*. New approach for bone marrow transplantation in patients with class 3 thalassemia aged younger than 17 years. *Blood* 2004; **104**: 1201–1203.
- 23 Fry TJ, Wayne A, Fowler D *et al*. Non-myeloablative allogeneic hematopoietic stem cell transplantation with pre-transplant immune depletion results in rapid full donor engraftment in pediatric patients with malignancy. *Biol Blood Marrow Transplant* 2004; **10** (Suppl 1): 82a.
- 24 Waller N, Langston A, Lonial S *et al*. Thymoglobulin pharmacokinetics and immune reconstitution in recipients of T-cell depleted CD34+ transplants from HLA mis-matched donors. *Blood* 2001; **98**: 2807a.
- 25 Regan JF, Lyonais C, Campbell K *et al*. Total and active thymoglobulin levels: effects of dose and sensitization on serum concentrations. *Transpl Immunol* 2001; **9**: 29–36.
- 26 Bacigalupo A, Lamparelli T, Bruzzi P *et al*. Antithymocyte globulin for graft-versus-host disease prophylaxis in transplants from unrelated donors: 2 randomized studies from Gruppo Italiano Trapianti Midollo Osseo (GITMO). *Blood* 2001; **98**: 2942–2947.
- 27 Storb R, Yu C, Barnett T *et al*. Stable mixed hematopoietic chimerism in dog leukocyte antigen-identical littermate dogs given lymph node irradiation before and pharmacologic immunosuppression after marrow transplantation. *Blood* 1999; **94**: 1131–1136.
- 28 Maris MB, Niederwieser D, Sandmaier BM *et al*. HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative conditioning for patients with hematologic malignancies. *Blood* 2003; **102**: 2021–2030.
- 29 Yu C, Sandmaier B, Seidel K *et al*. Peripheral blood stem cell grafts from DLA-identical littermates result in enhanced mixed hematopoietic chimerism after non-myeloablative total body irradiation when compared to marrow grafts. *Blood* 1998; **92**: 318b.