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ORIGINAL ARTICLE

Second hematopoietic SCT in patients with thalassemia recurrence following rejection of the first graft

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There is a substantial incidence of graft failure in patients with thalassemia after myeloablative conditioning regimens especially in class 3 patients in whom its incidence could be as high as 8–38.5%. Most patients with graft failure have recurrence of thalassemic marrow. Historically, results of second transplants for thalassemia were poor because of a high rejection rate and/or increased TRM. Sixteen patients with thalassemia recurrence following rejection of the first graft and with a median age of 9 years (range, 4–20) were given second transplants using BM ($n = 7$) or PBSC ($n = 9$) after preparation with a new treatment protocol. All but two patients received stem cells from the same donor. The median interval between two transplants was 28 months (range, 8–204). The sustained engraftment rate was high (94%) with only one patient having primary graft failure. The probability of overall survival, event-free survival, TRM and graft failure were 79, 79, 16 and 6%, respectively. There were three transplant-related deaths. Thirteen patients are alive with Lansky/Karnofsky score of 100. This intensified treatment protocol was well tolerated with no significant increase in toxicity. The excellent results obtained with this new preparative regimen allow us to recommend it for second transplantation for patients with thalassemia recurrence.

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Introduction

Hematopoietic SCT (HSCT) is the only curative therapy for patients with thalassemia.[1,2] However, graft failure or rejection is one of the major obstacles to successful transplantation for thalassemia. There is a substantial incidence of graft rejection in thalassemia patients after most myeloablative preparatory regimens, and this seems to be related to the stage of disease at the time of transplant. In fact, the probability of graft rejection is low in class 1 patients (3%), whereas it occurs in 8–38.5% of class 3 younger patients (age <17 years).[3,4] In class 3 younger patients, conditioning regimens with less than 200 mg/kg CY and less than 100 RBC transfusions given before transplant were associated with a high rejection rate.[5] In most cases of graft failure, patients have recurrence of thalassemic marrow and subsequent survival is long albeit with thalassemia. Occasionally, patients reject grafts without the recurrence of thalassemia, and in this situation, second transplant attempts with intensive conditioning may provide the only treatment option to offer a chance of prolonged survival. Patients who reject their grafts and have a return of host hematopoiesis do not have an urgent need for second transplants, and such interventions can be delayed until the toxic effects of the conditioning regimen for the first transplant have resolved. At least a year should be allowed to elapse between the first and second transplants. Our historical experience of second BMT in patients with both thalassemia recurrence and irreversible marrow aplasia showed a low thalassemia-free survival rate and high graft failure probability.[6,7] Therefore, in 2003, we devised a new treatment protocol for second transplantation in patients with thalassemia recurrence in an attempt to decrease the high graft failure rate and increase thalassemia-free survival. Here, we report the results of the largest series of second transplant for thalassemia recurrence reported to date.

Materials and methods

All patients who had thalassemia recurrence following graft failure after the first graft, and have an HLA-matched

family donor, were eligible for a second transplantation. Patients with severe organ system dysfunction were not eligible for this study. The institutional review board approved the treatment protocol, and all parents or patients provided written informed consent, in accordance with the Declaration of Helsinki Principles.

Characteristics of the patients

Between the years 2003 and 2008, 16 patients who had thalassemia recurrence following graft failure after the first graft were prospectively enrolled in this study. The median patient age was 9 years (range, 4–20) and the median donor age was 16 years (range, 2–29). Patient characteristics at the time of second transplantation are summarized in Table 1. Most patients were in class 3 of risk. Five patients had splenectomy before the first transplantation, whereas two patients were splenectomized between two transplants. A vast majority of patients had severe iron overload at the time of the second transplant. Most patients showed moderate-to-severe fibrosis on liver biopsies. All but two patients received a second HSCT from the same HLA-matched sibling ($n=13$) or parent ($n=1$) donors. One of the two patients who received stem cells from different donors was given stem cells for the first transplantation (first BMT) from his haploidentical mother. Since the time of graft failure, an HLA identical sibling was born and this sibling served as a stem cell donor for the patient's second transplant. The second patient, who had two graft failure episodes from the same donor, was given a third transplant from another HLA identical sibling donor.

The median interval between the first BMT and graft failure was 1.5 months (range, 0–36). The median interval between graft failure and second HSCT was 19.5 months (range, 5–204). The median interval between two transplants was 28 months (range, 8–204). Nine patients received unmanipulated PBSC and the remaining seven patients were given BM as a stem cell source. PBSC donors were given G-CSF (6 μg/kg twice daily for 5 days, Neupogen; Dompè Biotec, Milan, Italy) and mobilized PBSC were collected on days 5 and 6.

Transplantation procedure

The primary conditioning regimens used for the first transplant are given in Table 1.

All patients were prepared for the second HSCT according to Protocol 26.1 (Figure 1). The treatment protocol consisted of a preconditioning phase with an intensified preparation with 3 mg/kg of azathioprine and 30 mg/kg of hydroxyurea daily from day −45 pretransplant, fludarabine 30 mg/m^2 from day −16 through day −12, followed by conditioning with BU 14 mg/kg total dose, thiotepa 10 mg/kg total dose, CY 200 mg/kg total dose and rabbit antithymocyte globulin (Thymoglobulin; Genzyme-Sangstat, Lyon, France) 12.5 or 10 mg/kg total dose. The last four patients were given weight-based i.v. Busilvex with targeted dose adjustment (target AUC range, 900–1350 μM/min) instead of oral BU. Continuous 24 h infusions of 40 mg/kg of deferoxamine via central venous catheter were initiated on day −45 and a regimen of hypertransfusion with RBCs was used to keep the level of

Table 1 Patient, transplant and graft characteristics before second transplant

Characteristics	N
No. of patients	16
Patient gender	
Male	9
Female	7
Patient age (years)	
Median	9
Range	4–20
Donor age (years)	
Median	16
Range	2–29
Risk classes	
Class 1	1
Class 2	5
Class 3	10
Splenectomy	7
No. of pretransplant RBC units	
Median	147
Range	20–600
Serum ferritin (ng per 100 ml)	
Median	3043
Range	590–4670
Liver fibrosis	
Mild	6
Moderate	9
Severe	1
Donor	
Same	14
Different	2
Conditioning regimen for first BMT	
BU14CY200 (Protocol 6)	6
BU14CY160 preceded by preconditioning with azathioprin, hydroxyurea and fludarabine (Protocol 26)	9
BU14CY200 ATG preceded by preconditioning with azathioprin, hydroxyurea and fludarabine	1
Interval: first BMT to graft failure (months)	
Median	1.5
Range	0–36
Interval: graft failure to second HSCT (months)	
Median	19.5
Range	5–204
Interval: first BMT to second HSCT (months)	
Median	28
Range	8–204
Nucleated cell dose ($\times 10^8$/kg)	
Median	8.9
Range	2.4–22.4
CD34+ cell dose ($\times 10^6$/kg)	
Median	7.5
Range	3.8–15.7
Patient/donor CMV serology	
+/+	14
+/−	1
−/−	1
Patient/donor EBV serology	
+/+	12
+/−	1
−/−	2

Abbreviation: HSCT = hematopoietic SCT.

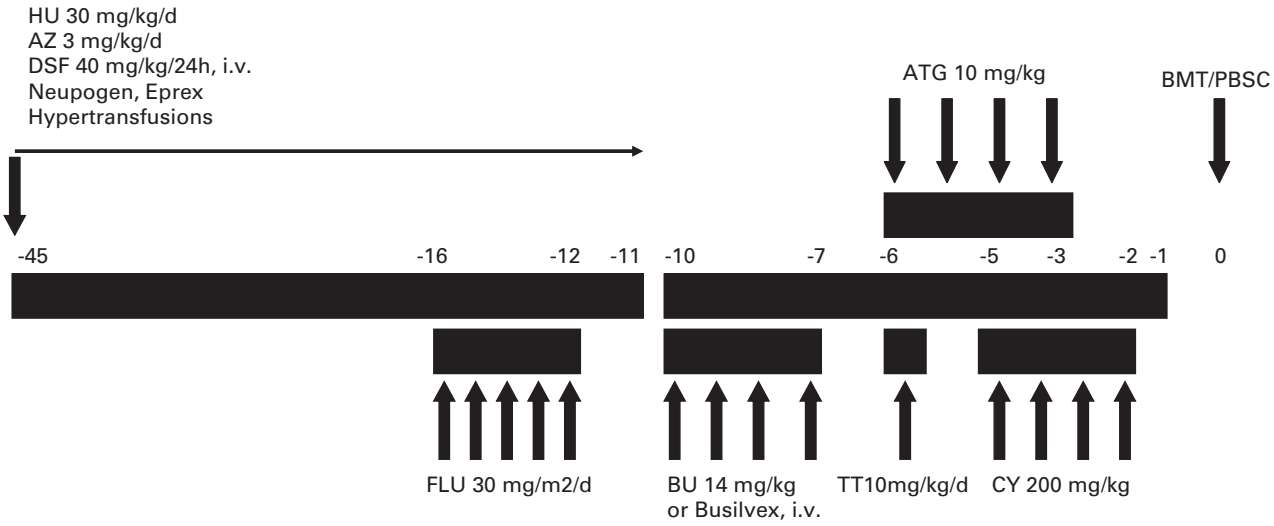

Figure 1 Treatment protocol (Protocol 26.1) developed for a second transplantation in thalassemia. The preconditioning phase includes administration of hydroxyurea, azathioprine from day −45 through day −12 and fludarabine from day −16 through day −12. During this phase, patients receive desferrioxamine, Neupogen, Eprex and hypertransfusion regimen in outpatient. Conditioning regimen includes BU/Busilvex, thiotepa, CY and thymoglobulin. GVHD prophylaxis consisted of CYA, a short course of MTX and methylprednisolone. ATG, thymoglobulin; AZ, azathioprine; DSF, desferrioxamine; FLU, fludarabine; HU, hydroxyurea; TT, thiotepa.

hemoglobin between 14 and 15 g per 100 ml. During this time interval, patients received growth factors (Neupogen (Dompè Biotec) and Eprex (Janssen-Cilag, Milan, Italy)) twice weekly to maintain stem cell proliferation in the face of hypertransfusion, thereby facilitating the effect of hydroxyurea.

Prophylaxis against GVHD for the first nine patients consisted of CY (7.5 mg/kg i.v.) on day +1, MTX (10 mg/m^2 i.v.) on days +3 and +6 and CYA (5 mg/kg i.v.) daily from day −2 through day +5, reduced to 3 mg/kg per day i.v. until oral administration of Sandimmun-Neoral (9 mg/kg per day) could be tolerated. The last seven patients were given CYA and a short course of MTX 10 mg/m^2 (on days +1, +3 and +6) as GVHD prophylaxis. Protocol 26.1 was devised on the assumption that preparation with Protocol 26 (preconditioning with azathioprine, hydroxyurea and fludarabine, and conditioning with BU 14 mg/kg and CY 160 or 200 mg/kg) was inadequate[7] in ensuring sufficient myeloablation and immunosuppression for sustained engraftment following the second transplantation in patients with thalassemia.

Diagnosis and degree of acute and chronic GVHD was assessed according to consensus criteria.[8] Patients received stem cell infusion 36 h after the last dose of CY. Patients were given prophylactic broad-spectrum antibiotics and antifungal drugs (amphotericin B preparations) until the neutrophil level exceeded 1.0×10^9/l. They also received acyclovir as herpes virus prophylaxis and trimethoprim/sulfamethoxazole for *Pneumocystis jiroveci* prophylaxis. Patients were monitored weekly for EBV, CMV, Adenovirus and BK virus in the blood and/or urine using sensitive reverse transcriptase-PCR starting before conditioning until at least 100 days post transplant. CMV antigenemia monitoring was done twice weekly at hematological recovery.

Graft definitions

Primary graft failure was defined by persistent pancytopenia with no evidence of hematological recovery of donor cells beyond 21 days after transplant, and secondary graft failure by a rapid decrease in neutrophil count after successful engraftment.[6] Five patients had primary graft failure and 11 patients secondary graft failure following the first transplant. None of these patients received an autologous backup, and all patients had spontaneous thalassemia recurrence.

Immune reconstitution

Immune reconstitution was prospectively measured in 10 patients. Peripheral blood samples for lymphocyte subset analysis were obtained at 1, 3, 6, 12 and 18–24 months after transplantation.

Briefly, whole-blood phenotype analysis consisted of lysing 500 µl blood with 10 ml of Ortho-mune lysing reagent (Ortho Diagnostic Systems Inc., Raritan, NJ, USA) at room temperature, washing and labeling with a cocktail of four MoAbs for 30 min at 4 °C. Anti-CD3-FITC, anti-CD4-allophycocyanin, anti-CD8-peridinin chlorophyll protein and anti-CD19-phycoerythrin were purchased from Becton Dickinson (San Diego, CA, USA). The NK phenotype of PBMCs was assessed by immunofluorescence and flow cytometry, with the use of FITC-conjugated anti-CD3 and phycoerythrin-conjugated anti-CD56 MoAbs (Becton Dickinson). After staining, cells were washed once in PBS containing 2% fetal bovine serum and analyzed on a FACSCalibur cytofluorometer (Becton Dickinson, Mountain View, CA, USA) using the Cell Quest software. Absolute lymphocyte counts were calculated by the standard hemocytometric technique. To determine marker expression on CD4+ and CD8+ cells, total lymphocytes

were first identified and gated by forward and side scatter. The cells were then additionally gated for CD4 or CD8 expression. Normalization of the absolute counts of lymphocyte subpopulation in the peripheral blood was defined as reaching the fifth percentile (p5) of age-matched reference values.[9]

Assessment of chimerism

The first chimerism analysis was performed on BM samples obtained at 20 days after transplant for the percentage of donor/recipient DNA using PCR-based analysis of short tandem repeats. Subsequently at 60, 90, 180 and 365 days post transplant, lineage-specific chimerism was performed by PCR using fluorescent primers flanking a single informative short tandem repeat (Profiler Plus Applera, Foster City, CA, USA), previously identified to be polymorphic between the patient and donor. In sex-mismatched donor/recipient pairs, dual-color fluorescence *in situ* hybridization was also performed according to standard procedures with commercially available probes specific for the centromeric and heterochromic regions of the X and Y chromosomes, respectively.

Statistical analysis

The primary end point was thalassemia-free survival. Secondary end points were graft failure and acute and chronic GVHD. The probabilities of survival and mortality were calculated using the Kaplan–Meier estimator.[10] The probabilities of acute and chronic GVHD were calculated using the cumulative incidence estimator. All *P*-values are two-sided. All analyses were performed with StatView (Version 5.0).

Results

Engraftment

One patient had primary graft failure and the remaining 15 patients had sustained engraftment with donor chimerism of 95–100% (Table 2). Lineage-specific chimerism in all but one patient showed 100% donor origin DNA in lymphoid and myeloid lineages. The only patient who had 95% donor chimerism showed 92% lymphoid and 95% myeloid chimerism. This patient has stable mixed chimerism more than 3 years after transplantation. The median time to neutrophil recovery (ANC $\geqslant 500 \times 10^9$/l) was 15 days (range, 10–24) and median time to a platelet count $\geqslant 20 \times 10^9$/l was 18 days (range, 12–60). Patients given PBSC had a significantly short time to neutrophil and platelet recovery than patients given BM (14 vs 19 days and 17 vs 22 days, respectively; $P = 0.01$). The median nucleated cell dose infused was significantly higher in patients who received PBSC, whereas the median CD34+ cell dose did not differ by stem cell source (data not shown). The only patient who had primary graft failure was given PBSC.

Acute and chronic GVHD

Five out of 15 evaluable patients developed grades 2–4 acute GVHD. The cumulative incidence of grades 2–4 acute GVHD was 33%. Three patients had grades 2–3 and two

Table 2 Second hematopoietic SCT

UPN	*Age (years)*	*BMT1 to HSCT2 (months)*	*Risk classes*	*Donor*	*Stem cell source*	*TNC dose ($\times 10^8$/kg)*	*CD34+ ($\times 10^6$/kg)*	*ANC>500 days*	*aGVHD grades 2–4*	*cGVHD*	*Chimerism*	*Survival (months)*	*Cause of death*	*Outcome*	*LS/KS*
1601	10	8	3	Same	BM	9.36	6.3	15	0	0	100%D	55		Alive	100
1602	13	31	3	Different	PBSC	22.4	11.2	11	2	Extensive	100%D	55		Alive	100
1603	9	15	3	Same	PBSC	9	4.7	13	0	0	100%D	51		Alive	100
1606	18	24	3	Same	PBSC	17.7	15.7	10	4	0	100%D	6	Pneumonia	Dead	0
1608	8	55	2	Same	BM	6.5	7	14	0	0	95%D	38		Alive	100
1614	4.3	29	2	Same	PBSC	15.4	12	14	0	0	100%D	37		Alive	100
1618	8	41	2	Different	BM	2.4	7.2	24	0	0	100%D	32		Alive	100
1621	9	29	2	Same	PBSC	2.6	6.9	14	3	0	100%D	31		Alive	100
1634	4	24	1	Same	PBSC	28.2	7.8	19	2	Extensive	100%D	12	Pneumonia	Dead	0
1637	10	55	3	Same	BM	7	3.8	17	0	0	100%D	26		Alive	100
1647	15.7	68	3	Same	PBSC	10.3	5.3	0	0	0	100%R	3	Bleeding	Dead	0
1679	8	28	3	Same	PBSC	22.1	8.4	16	0	0	100%D	24		Alive	100
1680	20	204	2	Same	PBSC	2.8	10	14	2	Extensive	100%D	13		Alive	100
1681	11	13	3	Same	BM	4.6	11	19	0	0	100%D	14		Alive	100
1682	6	12	3	Same	BM	8.8	15.1	20	0	0	100%D	9		Alive	100
1683	7	8	3	Same	BM	9	6.2	20	0	0	100%D	8		Alive	100

Abbreviations: aGVHD = acute GVHD; cGVHD = chronic GVHD; LS/KS = Lansky score/Karnofsky score.

patients had grade 4 acute GVHD (with skin and gastrointestinal involvement). All these patients had steroid responsive acute GVHD. Three out of 15 evaluable patients developed extensive chronic GVHD (20%). One of them died and the remaining two patients have mild skin or oral mucosa involvement and are on tapering immunosuppressive treatment. Both acute or chronic GVHD occurred in patients who received PBSC.

Toxic and infectious complications

The patient who had graft failure developed moderate hepatic sinusoidal obstructive syndrome, which was resolved with supportive care. Two patients had grade 2 mucositis. Three patients had CsA-related neurotoxicity with seizures. No other toxic complications were observed in these patients.

All but one patient in the present study had positive serology for CMV. Asymptomatic CMV reactivation occurred in 11 (68%) patients. One patient developed encephalitis on day 55. At diagnosis, the CMV antigenemia value was 500 cells per 50 000 cells. His cerebrospinal fluid was negative for qualitative CMV-PCR but was positive for qualitative EBV-PCR. The patient was successfully treated with Ganciclovir and Foscarnet. The median time to CMV reactivation was 29 days (range, 19–56). Five out of 13 patients (38%) had EBV reactivation in blood without developing lymphoproliferative disorders. All these patients had spontaneous resolution of EBV reactivation in their blood 3–5 months after transplantation. None of the patients developed adenovirus reactivation. Seven patients had BK virus-related late hemorrhagic cystitis: three moderate, two severe and two mild cystitis. Moderate-to-severe hemorrhagic cystitis responded to cidofovir in three out of four patients. Hemorrhagic cystitis of the patient who had primary graft failure with severe refractory thrombocytopenia did not respond to cidofovir.

In one patient, *Aspergillus fumigatus* and *Scedosporium prolificans* were isolated from sputum specimens without any organ manifestation. Two patients had bacteremia due to *Staphylococcus haemolyticus* or *Staphylococcus saprophyticus* and one patient had sepsis due to *Sphingomonas paucimobilis*. Two other patients had bacterial pneumonia successfully treated with antibiotics. One patient had localized skin *Varicella zoster* infection at 6 months following transplantation.

Survival

Thirteen out of 16 patients are alive without thalassemia with Lansky/Karnofsky score of 100. Median follow-up for survivors was 31 months (range, 8–55). There were three transplant-related deaths, two of them being in class 3 patients. The probability of TRM was 16%. The patient who had primary graft failure with prolonged severe aplasia and refractory thrombocytopenia died at 3 months from cerebral bleeding despite an autologous backup. The second patient who had steroid responsive grade 4 acute GVHD died 6 months after transplant in his home country probably from pneumonia. The third patient was on a tapering low dosage of mycophenolate mofetil and methylprednisolone for treatment of chronic GVHD and developed sepsis at 1 year after transplant due to *S. paucimobilis* while in her home country, which led to multiorgan failure and death. All three deaths occurred in patients given PBSC. The probability of overall survival, event-free survival and rejection at 3 years after transplant were 79, 79 and 6%, respectively (Figure 2). Event-free survival was better in patients given BM (100%) than in patients receiving PBSC (65%) as a stem cell source, although the difference was not statistically significant, probably because of the small number of patients.

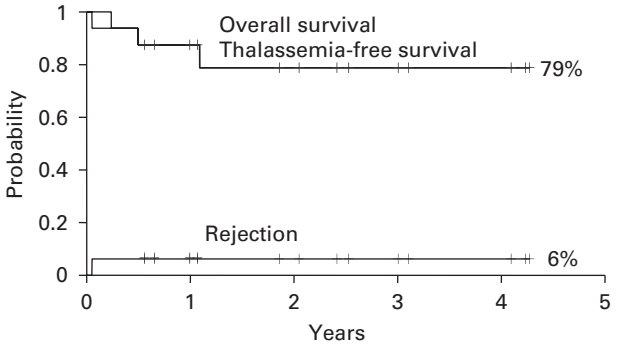

Figure 2 Estimates of overall survival, thalassemia-free survival and graft failure/rejection for 16 patients with thalassemia recurrence who received second HSCT.

Immune reconstitution

Ten pediatric patients who had at least 1 year follow-up and a complete lymphocyte subset data were included in this analysis (Table 3). Peripheral NK cells (CD16/CD56+) and cytotoxic/suppressor T cells (CD3+CD8+) were the first lymphoid cells to emerge, with most patients reaching p5 of age-matched reference value within 6 months. Only six patients reached p5 of age-matched reference value of helper cells (CD3+CD4+) within 12 months after transplant, whereas the two patients who developed chronic GVHD and one patient who had BK virus-related severe hemorrhagic cystitis did not reach it even at 1 year after transplant. A marked decrease in the number of CD19+ cells persisted beyond 12 months after transplant in three patients who had acute GVHD or were on steroid treatment for chronic GVHD. The CD4+/CD8+ ratio remained inverted until 1 year after transplant in seven patients, and in three patients it reached the p5 of age-matched reference value at 1, 6 and 12 months. There was no difference in lymphocyte subset recovery in patients receiving BM or PBSC (data not shown).

Discussion

BMT, which is increasingly being used worldwide, is the only treatment currently available to cure thalassemia. However, despite potent myeloablative conditioning regimens, the incidence of graft failure or rejection still remains high in these patients probably due to an intact immune system. The incidence of graft failure is particularly high in class 3 younger patients who have a large disease burden

Table 3 Immune reconstitution ($n = 10$)

	Number of patients who reached the 5th percentile[a] of age-matched reference value					
	1–3 months	*3–6 months*	*6–9 months*	*9–12 months*	*12–18 months*	*18–24 months*
Absolute lymphocyte count	7	1	1	1		
CD3+	6	2	2			
CD3+CD8+	7	3				
CD3+CD4+		4		2	1	0
CD16+CD56+	8	1	1			
CD19+	1	4		2	0	0

[a]5th percentile (p5) = normalization of the absolute counts of lymphocyte or lymphocyte subpopulations in the peripheral blood.

and organ damage due to inadequate transfusion and chelation treatment.[4,11,12] In the developing world where thalassemia is more common, most children belong to class 3 risk; therefore, we could have a substantial patient population with graft failure in the future. The management of patients with thalassemia recurrence following the first transplant was a dilemma because results of a second transplant in thalassemia were unsatisfactory until the present study. In 1999, we retrospectively analyzed the results of second transplant in 21 patients with thalassemia recurrence. This study showed a high graft failure rate (63%) and a low overall (55%) and event-free (29%) survival rates.[6] The higher graft failure rate in this study was probably from inadequate myeloablation and immunosuppression of a large disease burden characteristic of these patients. In 2003, we analyzed the results of a prospective study of second transplants for thalassemia recurrence in 16 patients who were treated according to the new treatment protocol (Protocol 26) initially designed for class 3 patients,[3] with the exception that all but 5 patients were given CY 200 mg/kg total dose to increase the immunosuppressive capacity of the conditioning regimen. The intention was to reduce a large disease burden and to increase immunosuppression before the conditioning regimen to avoid peritransplant drug toxicity. Results of this study showed a high graft failure rate (69%), although the overall survival was significantly improved (81%) as compared with the previous retrospective study.[6] This study clearly showed that the preparative regimen used was not adequately myeloablative and immunosuppressive to ensure a high sustained engraftment rate after the second transplants for thalassemia. Therefore, in 2003, we decided to increase both the myeloablative and the immunosuppressive capacities of this treatment protocol by increasing the dose of fludarabine from 25 to 30 mg/m^2 and adding thiotepa and thymoglobulin to the conditioning regimen. In fact, these modifications were successful and ensured a higher sustained engraftment rate in our patients receiving second transplants. After having seen these promising data, we are applying the same treatment protocol for high-risk transplants as well, such as phenoidentical and/or one antigen-mismatched transplants for thalassemia, with very encouraging results.

The optimal conditioning regimen for second transplants in patients with thalassemia at increased risk for graft failure is unknown. Although addition of ATG (horse) to CY conditioning was effective in increasing the engraftment rate after a second transplant for severe aplastic anemia,[13,14] the true contribution of ATG in reducing the risk of graft failure is unclear. Thymoglobulin is known to be more potent than other anti-thymocyte globulin preparations in preventing GVHD and rejection of organ transplants;[15] however, we suppose that by itself it is not sufficient to ensure sustained engraftment in most patients following a second transplantation for thalassemia. The potent myeloablative potential of thiotepa along with its immunosuppressive effect[16] probably enhanced engraftment in our patients, which is supported by the observation that adding thiotepa to BUCY regimen resulted in promoting engraftment in genetic diseases.[17] In fact, in a group of patients with characteristics similar to those reported in the previous studies,[6,7] the present conditioning regimen reduced the probability of graft failure from 69 to 6%.

Although most patients had heavy iron overload and moderate liver fibrosis, this intensified conditioning did not increase drug-related toxicity in our patients. The hematological recovery was fast and similar to those observed after the first transplantation in patients without graft failure (data not shown). Our previous study showed that the occurrence of graft failure later than 60 days after the first transplant had a positive influence on both the engraftment rate and survival following the second transplant.[6] We could not confirm these data because all five patients with primary graft failure after the first BMT had sustained engraftment following the second transplant in the present study. Probably the potent suppression of host immunity by our conditioning regimen has eliminated the value of the time elapsed from transplant to graft failure on the second transplant outcome. An interesting point is that all but two patients were given stem cells from the same donor, which resulted in an excellent engraftment rate. This clearly indicates that the difference in conditioning regimen used for the second transplantation plays a major role than donor change. Some studies showed the importance of using PBSC as a source of transplantable stem cells for second transplants.[18,19] However, a recent study did not find any difference in primary and secondary graft failure rates after PBSC or BMT in patients with aplastic anemia.[20] In our study, all patients given BM had sustained engraftment and the only case of graft failure occurred in patient who received PBSC, showing the importance of conditioning regimen for successful engraftment. Furthermore, a shorter duration of the neutropenic phase in

patients who received PBSC did not translate into a survival advantage in the present study.

Both acute and chronic GVHD developed in patients who received PBSC, and two out of three deaths that occurred in our patient group were related to these complications. Most published data support a higher risk of chronic GVHD with PBSC than with BM,[21] which result in inferior survival in patients with aplastic anemia treated with PBSC.[20] Unlike hematological malignancies where the high rate of chronic GVHD may be offset by lower relapse rates, there is no benefit of chronic GVHD in non-malignant diseases.

Thalassemia-free survival in the present study was higher (79%) than in the previous studies (25–29%)[6,7] and was similar to that obtained following the first transplant without graft rejection. Although limited in number, patients who received BM had better thalassemia-free survival than patients given PBSC, indicating that BM grafts are preferred to PBSC grafts for the second transplantation in patients with thalassemia recurrence.

The incidence of CMV reactivation was high in the present study. Most patients had asymptomatic CMV reactivation treated with pre-emptive therapy and only one patient had probable CMV encephalitis. One-third of the patients had EBV reactivation without developing lymphoproliferative disorders. Interestingly, the incidence of BK virus-related late hemorrhagic cystitis was high in the present study. Probably the use of thymoglobulin in the conditioning regimen increased viral reactivation in our patients. An association between high dose of thymoglobulin and increased infections has been reported.[22] In that study, median doses of 6–8 mg/kg of thymoglobulin resulted in lower TRM and better survival. We initially used 12.5 mg/kg of thymoglobulin, and after we saw a high rate of viral reactivation, we reduced the dose to 10 mg/kg in the last five patients. We recommend to perform careful viral monitoring and to start pre-emptive antiviral therapy in patients undergoing a second transplantation.

Despite the use of thymoglobulin in the conditioning regimen, immune recovery in our patients was similar to those reported in the literature after allogeneic HSCT in children. This probably related to the timing of thymoglobulin (the last dose of the drug was given on day −3), which led to less pronounced *in vivo* T-cell depletion, although no relationship between thymoglobulin use and delayed immune reconstitution has been shown.[23]

In conclusion, this new preparative regimen for second transplants in patients with thalassemia recurrence is highly effective in terms of sustained engraftment and event-free survival. Based on the excellent outcome seen in these patients, we recommend using the initial donor for second transplants for patients with thalassemia recurrence following the first graft. These results will help both patients and the physicians treating them to face the dilemma of second transplantation for thalassemia recurrence.

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