Brief report

Allogeneic hematopoietic stem cell transplantation in thalassemia major: results of a reduced-toxicity conditioning regimen based on the use of treosulfan

Maria Ester Bernardo,[1] Eugenia Piras,[2] Adriana Vacca,[2] Giovanna Giorgiani,[3] Marco Zecca,[3] Alice Bertaina,[1] Daria Pagliara,[1] Benedetta Contoli,[1] Rita Maria Pinto,[1] Giovanni Caocci,[2] Angela Mastronuzzi,[1] Giorgio La Nasa,[2] and Franco Locatelli[1,4]

[1]Department of Pediatric Hematology-Oncology, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS), Bambino Gesù Children's Hospital, Rome, Italy; [2]Hematology Unit, Department of Internal Medical Sciences, University of Cagliari, Cagliari, Italy; [3]Department of Pediatric Onco-Hematology, IRCCS, Policlinico San Matteo Foundation, Pavia, Italy; and [4]University of Pavia, Pavia, Italy

Sixty thalassemia patients (median age, 7 years; range, 1-37) underwent allogeneic hematopoietic stem cell transplantation (HSCT) after a preparation combining thiotepa, treosulfan, and fludarabine. Before HSCT, 27 children were assigned to risk class 1 of the Pesaro classification, 17 to class 2, and 4 to class 3; 12 patients were adults. Twenty patients were transplanted from an HLA-identical sibling and 40 from an unrelated donor. The cumulative incidence of graft failure and transplantation-related mortality was 9% and 7%, respectively. Eight patients experienced grade II-IV acute GVHD, the cumulative incidence being 14%. Among 56 patients at risk, 1 developed limited chronic GVHD. With a median follow-up of 36 months (range, 4-72), the 5-year probability of survival and thalassemia-free survival are 93% and 84%, respectively. Neither the class of risk nor the donor used influenced outcome. This treosulfan-based preparation proved to be safe and effective for thalassemia patients given allogeneic HSCT. (*Blood*. 2012;120(2):473-476)

Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) remains the only potentially curative treatment able to render patients with thalassemia major (TM) transfusion independent.[1-5] However, the clinical outcome after HSCT in children with TM who belong to class 3 of the Pesaro classification and in adults with poor performance status and/or organ dysfunction is still unsatisfactory because of the high risk of life-threatening complications or graft failure.[1-5]

Treosulfan-based conditioning regimens have been shown initially to be safe and effective in adults with hematologic malignancies not eligible for conventional preparations.[6-9] These results have been confirmed recently in pediatric patients with high-risk hematologic malignancies receiving a treosulfan-based preparative regimen before second or third HSCT.[10] Moreover, a treosulfan-based conditioning regimen proved to be valuable in children with primary immunodeficiencies.[11]

We reported recently preliminary, encouraging results on the use of a thiotepa/treosulfan/fludarabine myeloablative regimen in a cohort of 20 patients with TM. In particular, we found a high probability of cure of the disease in the absence of major transplantation-related complications in adults and in patients with poor performance status.[12] In the present study, we report the final results on the safety and efficacy of this regimen in a large cohort of TM patients.

Methods

Patients

Sixty patients with TM (median age, 7 years; range, 1-37) received transplantations between November 2005 and August 2011 in 3 Italian centers (Pavia, Roma, and Cagliari). The trial was approved by the institutional review boards (approval no. 2005-005182-11) of all participating institutions. Written informed consent was obtained from all patients or from their parents/legal guardians in accordance with the Declaration of Helsinki.

Details on patient, donor, and transplantation characteristics are reported in Table 1. Before transplantation, pediatric patients were assigned to 1 of the 3 classes of risk using the Pesaro classification.[1] Among 48 children, 27 were assigned to class 1, 17 to class 2, and 4 to class 3; the remaining 12 patients were adults. Twenty patients were transplanted from an HLA-identical sibling (matched family donor [MFD]) and the remaining 40 from an unrelated donor (UD). In the MFD cohort, the donor was always an HLA-identical sibling, and 11 patients received cord blood cells. In all patients receiving transplantations from a UD, high-resolution molecular typing was performed to characterize HLA class I and II loci (ie, loci A, B, C, DRB1, and DQB1); only donors fully matched or with a single class I allelic disparity were selected. In UD recipients, an autologous rescue of BM cells was harvested and cryopreserved to be used in case of graft failure.

All patients received the same conditioning regimen, which included IV thiotepa (8 mg/kg on day −7), treosulfan (14 g/m^2/d from days −6 to −4), and fludarabine (40 mg/m^2/d for 4 consecutive days from days −6 to −3). GVHD prophylaxis varied according to the stem cell source and type of donor (see Table 1 for details). In all patients, cyclosporin A was given for 12 months after transplantation. Pretransplantation antithymocyte globulin (Fresenius; 10 mg/kg/d for 3 consecutive days from days −5 to −3) was administered in all recipients of UD-HSCT. BM was used as stem cell source in 47 patients, 11 patients received MFD cord blood–derived HSCs, and the remaining 2 patients were given transplantations with peripheral blood–mobilized HSCs.

Acute and chronic GVHD were diagnosed and graded according to the Seattle criteria.[13] Patients surviving more than 14 and 100 days after transplantation were evaluated for acute and chronic GVHD, respectively.

Submitted April 16, 2012; accepted May 18, 2012. Prepublished online as *Blood* First Edition paper, May 29, 2012; DOI 10.1182/blood-2012-04-423822.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

Table 1. Patient, donor, and transplantation characteristics

No. of patients (%)			60 (100%)
Sex, n (%)			
Males			32 (53%)
Females			28 (47%)
Age at HSCT, y, median (range)			7 (1-37)
Type of donor, n (%)			
MFD			20 (33%)
UD			40 (67%)
Pesaro class at time of HSCT, n (%)	**Whole cohort**	**MFD**	**UD**
Class 1	27 (45%)	12 (60%)	15 (38%)
Class 2	17 (28%)	4 (20%)	13 (32%)
Class 3	4 (7%)	1 (5%)	3 (7%)
Adults	12 (20%)	3 (15%)	9 (23%)
Human CMV serology, n (%)			
Negative donor/negative recipient			4 (7%)
Positive donor/negative recipient			18 (30%)
Negative donor/positive recipient			12 (20%)
Positive donor/positive recipient			26 (43%)
Stem cell source, n (%)			
BM			47 (79%)
Umbilical cord blood			11 (18%)
Peripheral blood stem cells			2 (3%)
No. of cells infused			
BM, $\times 10^8$/kg, median (range)			3.9 (0.5-13)
UCB, $\times 10^7$/kg, median (range)			4.2 (1.8-6)
$CD34^+$ PBSCs, $\times 10^6$/kg, median (range)			4.8 and 6.5
GVHD prophylaxis, n (%)			
CsA*			11 (18%)
CsA + MTX†			9 (15%)
CsA + MTX + ATG‡			40 (67%)
No. of days to PMN recovery, median (range)§			20 (11-30)
No. of days to PLT recovery, median (range)¶			20 (11-36)
Graft failure, n (%)#			5 (8%)
Chimerism at time of last follow-up, median (range)**			100% (80-100)

ATG indicates antithymocyte globulin; CsA, cyclosporine; MTX, Methotrexate; PMN, polymorphonuclear neutrophils; and PLT, platelets.

*Recipients of HLA-identical sibling umbilical cord blood transplantation.

†Recipients of HLA-identical sibling BM transplantation.

‡Recipients of UD HSCT.

§Defined as the time needed to reach an absolute neutrophil count $\geq 0.5 \times 10^9$/L.

¶Defined as the time needed to reach an unsupported platelet count $\geq 20 \times 10^9$/L.

#Defined as either the absence of hematopoietic reconstitution of donor origin on day +45 after the allograft (primary graft rejection) or as loss of donor cells after a transient engraftment of donor-origin hematopoiesis, with return to erythrocyte transfusion dependence (secondary graft rejection).

**Hematopoietic chimerism was evaluated, starting from DNA obtained either from peripheral blood and/or BM mononuclear cells and cell subsets, by microsatellite analysis. Chimerism was analyzed at time of engraftment and at days +45, +60, +90, and +180. After these time points, chimerism analysis was performed at the time of each clinical control until 5 years after the allograft.

Statistical analysis

Analysis used December 31, 2011 as the report date at which the centers locked data on patient outcome. Patients were censored at time of death or last follow-up. Probability of overall survival and thalassemia-free survival (TFS) were estimated by the Kaplan-Meier product-limit method and expressed as a percentage with 95% confidence interval (CI). For calculation of TFS, data on patients were recorded at time of death, graft failure, or last follow-up. Probabilities of acute and chronic GVHD, graft failure, and transplantation-related mortality were calculated as cumulative incidence curves, to adjust the analysis for competing risks. $P < .05$ was considered statistically significant.

Results and discussion

All patients engrafted except one, who died on day +11; the median time to neutrophil and platelet recovery was 20 days (range, 11-30 and 11-36, respectively). Five patients (all of whom were given BM cells) experienced secondary graft failure at a median of 9 months (range, 1.5-18) after HSCT; the cumulative incidence of graft failure was 9% (95% CI, 3%-19%; Figure 1A). Only 1 of these 5 patients belonged to risk class 3; all of the others had been allocated to class 1. A total of 3 and 2 of these 5 patients received the allograft from either a UD or an MFD, respectively. Four patients were successfully retransplanted from the same donor (an MFD or a UD in 2 cases each) using a preparative regimen combining busulfan/thiotepa/fludarabine and are currently alive and transfusion independent without any sign of GVHD.

Eight of the 59 patients at risk experienced grade II-IV acute GVHD, which was severe in 4 cases; the cumulative incidence of grade II-IV and grade III-IV acute GVHD was 14% (95% CI, 6%-24%) and 7% (95% CI, 2%-15%), respectively. Only 1 child of the 56 patients at risk developed chronic GVHD (of limited extension); the cumulative incidence was 2% (95% CI, 0%-8%).

Four patients died of transplantation-related complications (all within 4 months after HSCT): 3 patients due to severe acute GVHD

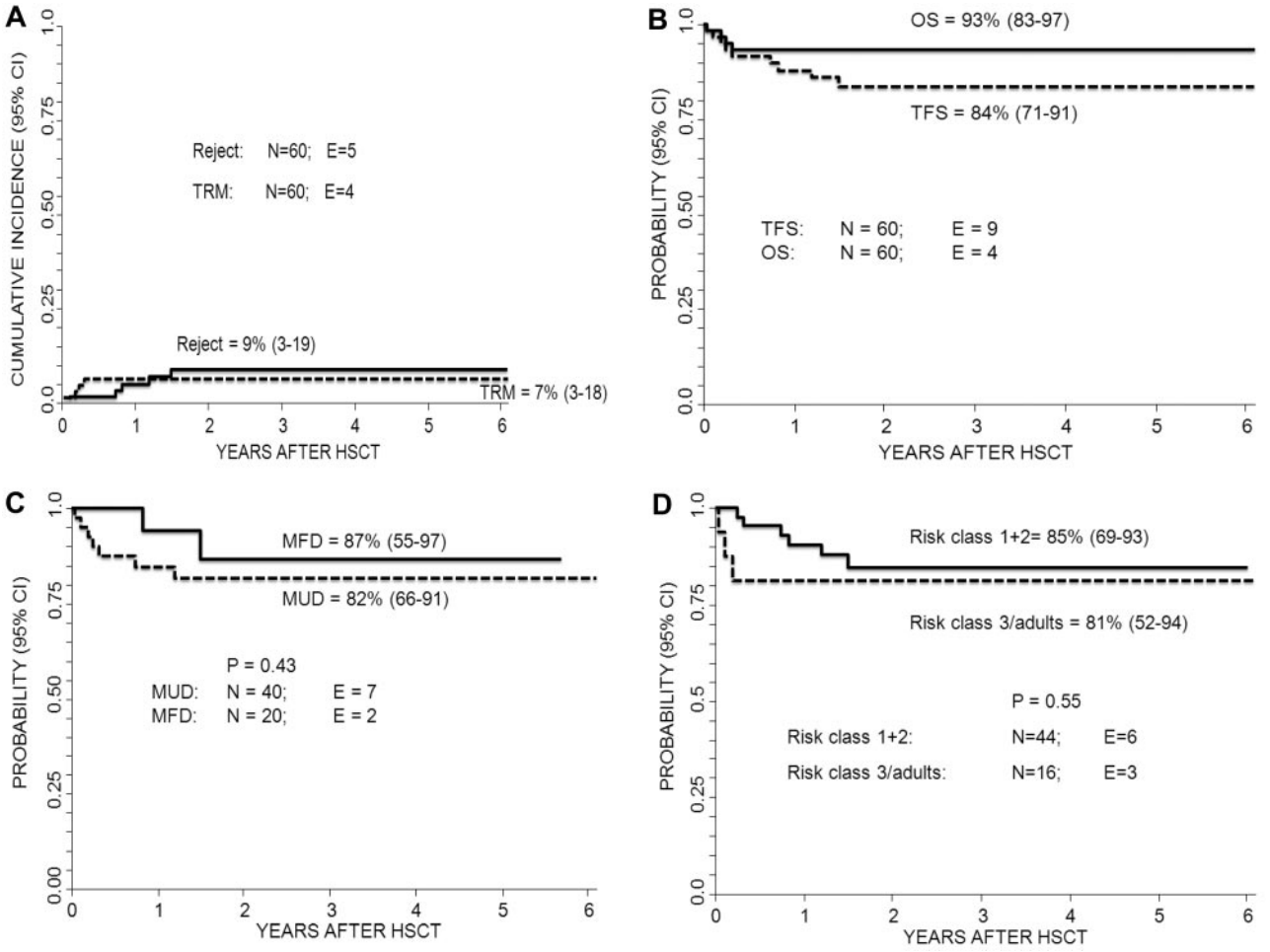

Figure 1. Outcomes of the study population. (A) Cumulative incidence of transplantation-related mortality (TRM) and graft rejection (Reject). (B) Five-year Kaplan-Meyer estimate of overall survival (OS) and TFS for the whole cohort of patients. (C) Five-year Kaplan-Meyer estimate of TFS according to the type of donor used (MFD indicates matched family donor; and MUD, matched unrelated donor). (D) Five-year Kaplan-Meyer estimate of TFS according to the patient's class of risk.

unresponsive to several lines of immunosuppressive therapy and 1 patient due to viral pneumonia. All patients who died had received transplantations from a UD and belonged to risk class 2 or 3. The cumulative incidence of transplantation-related mortality was 7% (95% CI, 3%-18%; Figure 1A). No case of veno-occlusive disease was recorded. With a median follow-up of 36 months (range, 4-73), the 5-year overall survival probability is 93% (95% CI, 83%-97%; Figure 1B), with no difference between MFD and UD recipients (data not shown). The probability of TFS after first HSCT for the whole cohort is 84% (95% CI, 71%-91%); it was 87% (95% CI, 55%-97%) and 82% (95% CI, 66%-91%) for patients receiving either MFD or UD HSCT, respectively ($P = .43$; Figure 1C). Because 4 of the 5 patients experiencing secondary graft failure were rescued by a second allograft, 55 patients are currently alive and transfusion independent with sustained donor engraftment. We did not observe any difference in terms of outcome between patients belonging to class 1 or 2 of the Pesaro classification and class 3 adult patients, the probability of TFS being 85% (95% CI, 69%-93%) and 81% (95% CI, 52%-94%; $P = .55$¸ Figure 1D), respectively.

These results confirm in a large cohort of TM patients the safety and efficacy of the thiotepa/treosulfan/fludarabine combination as a preparative regimen. This regimen was also able to abolish the difference usually observed among patients of different classes of risk.[1-5] The outcome of class 3 adult patients was comparable with that reported by Sodani et al in class 3 children receiving transplantations from an MFD using a novel approach aimed at enhancing both immune suppression and eradication of the thalassemic erythropoiesis.[14] High-resolution typing and the stringent criteria of donor compatibility contributed to the excellent probability of TFS observed in our patients receiving transplantations from UDs. We propose this preparation as a suitable and appropriate option for minimizing the risk of life-threatening complications in adult poor-performance status TM patients, although it is also of value for patients with good prognostic characteristics. The incidence and severity of late effects after this regimen is a subject for future investigation. Randomized trials comparing treosulfan-based and busulfan-based regimens are warranted.

Acknowledgments

This study was supported in part by grants from Istituto Superiore di Sanità (National Program on Stem Cells), Ministero dell'Istruzione, dell'Università e della Ricerca, Progetti di Rilevante Interesse Nazionale, Associazione Italiana per la Ricerca sul Cancro (IG9062 to M.E.B.), and by the special grant "5 × 1000" from Associazione Italiana per la Ricerca sul Cancro (to F.L.).

Authorship

Contribution: M.E.B. performed the transplantations, collected and analyzed the data, and wrote the manuscript; G.G., A.B., D.P., E.P., A.V., G.C., A.M. and R.M.P. performed the transplantations, collected and analyzed the data; M.Z. performed the transplantations; B.C. performed the statistical analysis; G.L.N. performed the transplantations, collected and analyzed the data, and contributed to the design of the study; and F.L. designed the study, performed the transplantations, analyzed the data, and contributed to the final writing of the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Maria Ester Bernardo, MD, PhD, Dipartimento di Ematologia ed Oncologia Pediatrica, IRCCS Ospedale Pediatrico Bambino Gesù, P le S Onofrio, 00165 Roma, Italy; e-mail: mariaester.bernardo@opbg.net.

References

1. Lucarelli G, Galimberti M, Polchi P, et al. Marrow transplantation in patients with thalassemia responsive to iron chelation therapy. *N Engl J Med.* 1993;329(12):840-844.
2. Lucarelli G, Clift RA, Galimberti M, et al. Marrow transplantation for patients with thalassemia: results in class 3 patients. *Blood.* 1996;87(5):2082-2088.
3. Locatelli F, De Stefano P. Innovative approaches to hematopoietic stem cell transplantation for patients with thalassemia. *Haematologica.* 2005; 90(12):1592-1594.
4. La Nasa G, Giardini C, Argiolu F, et al. Unrelated donor bone marrow transplantation for thalassemia: the effect of extended haplotypes. *Blood.* 2002;99(12):4350-4356.
5. Locatelli F, Rocha V, Reed W, et al; for the Eurocord Transplant Group CBT. Related umbilical cord blood transplantation in patients with thalassemia and sickle cell disease. *Blood.* 2003; 101(6):2137-2143.
6. Casper J, Knauf W, Kiefer T, et al. Treosulfan and Fludarabine: a new toxicity-reduced conditioning regimen for allogeneic haematopoietic stem cell transplantation. *Blood.* 2004;103(2):725-731.
7. Beelen DW, Trenschel R, Casper J, et al. Dose-escalated treosulfan in combination with cyclophosphamide as a new preparative regimen for allogeneic haematopoietic stem cell transplantation in patients with an increased risk for regimen-related complications. *Bone Marrow Transplant.* 2005;35(3):233-241.
8. Schmidt-Hieber M, Blau IW, Trenschel R, et al. Reduced-toxicity conditioning with fludarabine and treosulfan prior to allogeneic stem cell transplantation in multiple myeloma. *Bone Marrow Transplant.* 2007;39(7):389-396.
9. Ruutu T, Volin L, Beelen DW, et al. Reduced-toxicity conditioning with treosulfan and fludarabine in allogeneic hematopoietic stem cell transplantation for myelodysplastic syndromes: final results of an international prospective phase II trial. *Haematologica.* 2011;96(9):1344-1350.
10. Wachowiak J, Sykora KW, Cornish J, et al. Treosulfan-based preparative regimens for allo-HSCT in childhood hematological malignancies: a retrospective study on behalf of the EBMT pediatric diseases working party. *Bone Marrow Transplant.* 2011;46(12):1510-1518.
11. Slatter MA, Rao K, Amrolia P, et al. Treosulfan-based conditioning regimens for hematopoietic stem cell transplantation in children with primary immunodeficiency: United Kingdom experience. *Blood.* 2011;117(16):4367-4375.
12. Bernardo ME, Zecca M, Piras E, et al. Treosulfan-based conditioning regimen for allogeneic haematopoietic stem cell transplantation in patients with thalassaemia major. *Br J Haematol.* 2008;143(4):548-551.
13. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HLA-matched sibling donors. *Transplantation.* 1974;18(4):295-304.
14. 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(4):1201-1203.