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

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Short title: Treosulfan before HSCT in thalassemia

Key words: thalassemia major; hematopoietic stem cell transplantation (HSCT); treosulfan; conditioning regimen.

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Abstract

Sixty thalassemia patients (median age 7 years, range 1-37) underwent allogeneic hematopoietic stem cell transplantation (HSCT) after a preparation combining Thiotepa/Treosulfan/Fludarabine. Prior to HSCT, 27 children were assigned to class 1 of risk of the Pesaro classification, 17 to class 2, 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 Graft-versus-host-disease (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 employed influenced outcome. This treosulfan-based preparation proved to be safe and effective for thalassemia patients given allogeneic HSCT.
Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) still remains the only potentially curative treatment able to render patients with Thalassemia Major (TM) transfusion independent.\textsuperscript{1-5} However, the clinical outcome following 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, due to the high risk of developing life-threatening complications or graft failure.\textsuperscript{1-5} Treosulfan-based conditioning regimens have been initially shown to be safe and effective in adults with hematological malignancies not eligible to conventional preparation.\textsuperscript{6-9} These results have been recently confirmed in pediatric patients with high-risk hematological malignancies receiving a treosulfan-based preparative regimen before second or third HSCT.\textsuperscript{10} Moreover, a treosulfan-based conditioning proved to be valuable in children with primary immunodeficiencies.\textsuperscript{11} We previously reported preliminary, encouraging results on the use of a Thiotepa/Treosulfan/Fludarabine myeloablative regimen in a cohort of 20 patients with TM; in particular, in adults and in patients with poor performance status we found a high probability of cure of the disease in the absence of major transplantation-related complications.\textsuperscript{12} Here, we report the final results on the safety and efficacy of this regimen in a large cohort of TM patients.

Patients and methods

Patients

Sixty patients with TM (median age 7 years, range 1-37) were transplanted between November 2005 and August 2011 in 3 Italian Centres (Pavia, Roma, Cagliari). The trial was approved by the Institutional Review Boards (approval number 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 transplant characteristics are reported in Table 1. Prior to transplantation, pediatric patients were assigned to one of the 3 classes of risk of the Pesaro classification.\textsuperscript{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 (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 transplanted from an UD, high-resolution molecular typing was performed to characterize HLA class I and II loci (i.e 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 bone marrow (BM) cells was harvested and cryopreserved to be employed in case of graft failure.

All patients received the same conditioning regimen, which included intravenous thiotepa (8 mg/Kg on day -7), treosulfan (14 g/m²/day, from day -6 to -4), and fludarabine (40 mg/m²/day for 4 consecutive days, from day -6 to -3). Graft-versus-host disease (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. Pre-transplantation anti-thymocyte globulin (ATG Fresenius 10 mg/Kg/day for 3 consecutive days, from day -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 hematopoietic stem cells (HSCs), and the remaining 2 patients were transplanted with peripheral blood-mobilized HSCs.

Acute and chronic GvHD were diagnosed and graded according to the Seattle criteria. Patients surviving more than 14 and 100 days post-transplantation were evaluated for acute and chronic GvHD, respectively.

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 (OS) and thalassemia-free survival (TFS) were estimated by the Kaplan-Meier product-limit method and expressed as percentage and 95% confidence interval (95% 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 (TRM) were calculated as cumulative incidence curves, in order to adjust the analysis for competing risks. P values lower than 0.05 were considered statistically significant.

Results and Discussion
All patients but 1, who died on day +11, engrafted; the median time to neutrophil and platelet recovery was 20 (range, 11-30) and 20 (range, 11-36) days, respectively. Five patients (all 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 being 9% (95% CI, 3-19%; Figure 1a). Only 1 of these 5 patients belonged to class 3 of risk, whereas the others had been allocated to class 1. Three and 2 out of these 5 patients received the allograft from either an UD or a MFD, respectively. Four patients were successfully re-transplanted from the same donor (a MFD or an 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 out 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 out of the 56 patients at risk developed chronic GvHD (of limited extension), the cumulative incidence being 2% (95% CI, 0-8%).

Four patients died of transplantation-related complications (all within 4 months after HSCT): 3 patients because of severe acute GvHD, unresponsive to several lines of immunosuppressive therapy and 1 patient due to viral pneumonia. All dead patients had been transplanted from an UD and belonged to risk class 2 or 3. The cumulative incidence of TRM 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 OS 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=0.43; Figure 1c). Since 4 out 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 1/2 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=0.55, Figure 1d), respectively.

These results confirm in a large cohort of TM patients the safety and efficacy of the thiotepa/treosulfan/fludarabine combination as preparative regimen; it also proved to be able to abolish the difference usually observed among patients of different classes of risk.\textsuperscript{1-5} The outcome of class 3/adult patients is comparable to that reported by Sodani et al. in class 3 children transplanted from a MFD using a novel approach aimed at enhancing both immune suppression and eradication of the thalassemic erythropoiesis.\textsuperscript{14} High-resolution typing and the stringent criteria of donor compatibility adopted contributed to the excellent probability of TFS observed in our patients transplanted 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 of value also for patients with good prognostic characteristics. The incidence and severity of late effects after this regimen remains a field of future investigation. Randomized trials comparing this treosulfan-based and busulfan-based regimens are warranted.
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Authorship
Contribution: M.E.B. performed transplantations, collected and analyzed data and wrote the paper; G.G., A.B., D.P., E.P., A.V., G.C. and A.M. performed transplantations, collected and analyzed data; M.Z. performed transplantations; G.L.N. performed transplantations, collected and analyzed data and contributed to the design of the study; F.L. designed the study, performed transplantations, analyzed data and contributed to the final writing of the paper.

Conflict of interest disclosure: the authors declare no competing financial interests.
References


Table 1. Patient, donor and transplantation characteristics

- **Number of Patients**: 60 (100%)
- **Gender**
  - Males: 32 (53%)
  - Females: 28 (47%)
- **Age at HSCT** (years, median, range): 7 (1-37)
- **Type of donor employed**
  - Matched family donor (MFD): 20 (33%)
  - Unrelated donor (UD): 40 (67%)
- **Pesaro class at time of HSCT**
  - Whole cohort
    - Class 1: 27 (45%)
    - Class 2: 17 (28%)
    - Class 3: 4 (7%)
    - Adults: 12 (20%)
  - MFD
    - Class 1: 12 (60%)
    - Class 2: 4 (20%)
    - Class 3: 1 (5%)
    - Adults: 3 (15%)
  - UD
    - Class 1: 15 (38%)
    - Class 2: 13 (32%)
    - Class 3: 3 (7%)
    - Adults: 9 (23%)
- **HCMV serology**
  - 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**
  - BM: 47 (79%)
  - UCB: 11 (18%)
  - PBSC: 2 (3%)
- **Number of cells infused**
  - BM x10⁹/Kg; median, range: 3.9 (0.5-13)
  - UCB x10⁷/Kg; median, range: 4.2 (1.8-6)
  - PBSC CD34+ x10⁶/Kg; median, range: 4.8 and 6.5
- **GvHD prophylaxis**
  - CsA§: 11 (18%)
  - CsA+MTX§§: 9 (15%)
  - CsA+MTX+ATG§§§: 40 (67%)
- **Number of days to PMN recovery*** (median, range): 20 (11-30)
- **Number of days to PLT recovery**** (median, range): 20 (11-36)
- **Graft Failure***: 5 (8%)
- **Chimerism at time of last follow-up**** (median, range): 100% (80-100)

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HSCT: hematopoietic stem cell transplantation; HCMV: human cytomegalovirus; BM: bone marrow; UCB: umbilical cord blood; PBSC: peripheral blood stem cells; Treo: Treosulfan; Flu: Fludarabine; ATG: anti-thymocyte globulin; GvHD: graft-versus-host disease; CsA: cyclosporine; MTX: Methotrexate; PMN: polymorphonuclear neutrophils; PLT = platelets

§ recipients of HLA-identical sibling UCB transplantation

§§ recipients of HLA-identical sibling BM transplantation

* median, range

** median, range

*** median, range

**** median, range
recipients of unrelated donor HSCT
* defined as time needed to reach an absolute neutrophil count equal to or greater than $0.5 \times 10^9/l$.
** defined as time needed to reach an unsupported platelet count equal to or greater than $20 \times 10^9/l$.
*** 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; +180. After these time-points, chimerism analysis was performed at time of each clinical control till 5 years after the allograft.
Figure 1. A: Cumulative incidence of transplantation-related mortality (TRM) and graft rejection (Reject); B: Five-year Kaplan-Meyer estimate of overall survival (OS) and Thalassemia-free survival (TFS) for the whole cohort of patients; C: Five-year Kaplan-Meyer estimate of TFS according to the type of donor employed (MFD: matched family donor; MUD: matched unrelated donor); D: Five-year Kaplan-Meyer estimate of TFS according to the patient’s class of risk
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Figure 1

A

CUMULATIVE INCIDENCE (95% CI)

Reject: N=60; E=5
TRM: N=60; E=4

Reject = 9% (3-19)

TRM = 7% (3-18)

YEARS AFTER HSCT

B

PROBABILITY (95% CI)

OS = 93% (83-97)
TFS = 84% (71-91)

OS: N = 60; E = 4
TFS: N = 60; E = 9

YEARS AFTER HSCT

C

PROBABILITY (95% CI)

P = 0.43
MUD: N = 40; E = 7
MFD: N = 20; E = 2

MFD = 87% (55-97)
MUD = 82% (66-91)

YEARS AFTER HSCT

D

PROBABILITY (95% CI)

Risk class 1+2 = 85% (69-93)
Risk class 3/Adults = 81% (52-94)

Risk class 1+2:
N = 44; E = 6
Risk class 3/Adults:
N = 16; E = 3

P = 0.55

YEARS AFTER HSCT
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