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Treosulfan-based conditioning regimen for allogeneic haematopoietic stem cell transplantation in patients with thalassaemia major

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Summary

The safety and efficacy of a preparation with treosulfan/thiotepa/fludarabine were explored in 20 thalassaemia patients given allogeneic marrow transplantation. Seventeen patients were transplanted from unrelated donors after receiving anti-thymocyte globulin. The regimen was well tolerated. Two patients experienced secondary graft failure; one died of acute graft-versus-host disease. Cumulative incidence (95% confidence interval, CI) of transplantation-related mortality and graft failure was 5% (95% CI, 0–34%) and 11% (95% CI, 3–43%), respectively. Two-year probability of survival and thalassaemia-free survival was 95% (95% CI, 85–100%) and 85% (95% CI, 66–100%), respectively. This regimen might find elective application in patients at high risk of developing life-threatening complications.

Keywords: haematopoietic stem cell transplantation, treosulfan, conditioning regimen, thalassaemia major, toxicity.

Allogeneic haematopoietic stem cell transplantation (HSCT) still remains the only potentially curative treatment for patients with thalassaemia major (Lucarelli *et al*, 1993, 1996). The best results have been reported in patients transplanted from a human leucocyte antigen (HLA)-identical sibling, in the presence of limited iron overload and in the absence of severe hepatic complications (Lucarelli *et al*, 1993, 1996; Locatelli *et al*, 2003). Busulfan has been largely employed as the myeloablative agent for these patients, usually combined with cyclophosphamide, and, more recently, with thiotepa and fludarabine (Lucarelli *et al*, 1993; Locatelli *et al*, 2003). Busulfan-based regimens, while unable to completely prevent the risk of both primary and secondary graft failure (Lucarelli *et al*, 1993; Locatelli *et al*, 2003), have been associated with relevant complications (in particular veno-occlusive disease, obliterans bronchiolitis and thrombotic thrombocytopenic purpura), which can significantly contribute to the occurrence of fatalities, especially in adults and in children belonging to the class 3 of the Pesaro classification (Lucarelli *et al*, 1996).

Treosulfan (L-treitol-1,4-bis-methanesulphonate) is a prodrug of a bifunctional alkylating agent, which differs from busulfan by the introduction of two hydroxyl groups in the molecule and the different mode of alkylation. Preclinical and clinical data have supported the use of treosulfan in regimens for autologous and allogeneic HSCT (Casper *et al*, 2004; Ploemacher *et al*, 2004). In particular, treosulfan, besides being effective against different types of malignant cells (Munkelt *et al*, 2008), displays pronounced killing of both committed and more immature haematopoietic progenitors (Casper *et al*, 2004; Ploemacher *et al*, 2004). Moreover, treosulfan is easily administered even in young children, has limited extra-medullary toxicity (Casper *et al*, 2004; Beelen *et al*, 2005) and its pharmacokinetics is linear, with reliable systemic exposure and low inter-patient variability (Hilger *et al*, 1998).

All these characteristics render the use of a treosulfan-based preparative regimen attractive in patients with thalassaemia eligible for an allograft, for reducing the risk of life-threatening complications and increasing the number of patients successfully cured. Here, we report the results of a phase I–II, prospective, non-randomized, clinical trial, aimed at assessing the safety, tolerability and efficacy of a treosulfan-based regimen in young patients with thalassaemia.

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Patients and methods

Patients

Twenty young (median age 13 years) patients with thalassaemia major, transplanted between November 2005 and September 2007 in two Centres (Oncoematologia Pediatrica, Pavia and Centro Trapianti Midollo Osseo, Cagliari) were enrolled in this trial, which was approved by the two Institutional Review Boards. Written informed consent was obtained from all patients, or from their parents or legal guardians.

Prior to transplantation, all patients were assigned to one of three risk classes according to the criteria proposed by Lucarelli *et al* (1993). Out of the 20 patients, seven were assigned to risk class 1, four to class 2, and nine to class 3. Three patients were transplanted from an HLA-identical sibling, whereas the remaining 17 received the allograft from an unrelated donor (UD). In all patients transplanted from a UD, high-resolution molecular typing was performed to characterize HLA class I and II loci (i.e. loci A, B, C, DRB1 and DQB1). All UD-HSCT recipients but two (with a single allelic disparity at locus B and C, respectively) were transplanted from an HLA-matched donor. An autologous rescue of bone marrow (BM) cells was harvested and cryopreserved in patients given HSCT from a UD to avoid complications related to persistent cytopenia in the case of either primary or secondary graft failure.

All patients were transplanted after having received the same conditioning regimen, which included intravenous thiotepa (8 mg/kg on day −7), treosulfan (14 g/m^2 for three consecutive days, from day −6 to −4), and fludarabine (40 mg/m^2 for four consecutive days, from day −6 to −3). Graft-versus-host disease (GvHD) prophylaxis was based on the use of cyclosporine (given for 12 months after transplantation), together with short-term methotrexate (10 mg/m^2 on day +1, +3, +6 and +11). Pretransplantation anti-thymocyte globulin (ATG Fresenius 10 mg/kg/d for three consecutive days, from day −5 to −3) was administered in all UD-HSCT recipients, with the aim of modulating the alloreactivity of both donor and recipient lymphocytes, thus contributing to prevent both GvHD and graft failure. BM was used as the stem cell source in all patients. Details on patient and donor characteristics, GvHD prophylaxis, as well as the median number of nucleated BM cells and CD34+ cells infused are reported in Table I.

Acute and chronic GvHD were diagnosed and graded according to the Seattle criteria (Glucksberg *et al*, 1974). Patients surviving more than 14 and 100 d post-transplantation were evaluated for acute and chronic GvHD, respectively. Primary graft rejection was defined as the absence of haematopoietic reconstitution of donor origin on day +45 after HSCT, while secondary graft rejection was considered as loss of donor cells after a transient engraftment of donor-origin haematopoiesis, with return to erythrocyte transfusion dependence (see also Table I). Organ toxicity was scored according to the Bearman criteria (Bearman *et al*, 1988).

Table I. Patient, donor and transplantation characteristics.

Number of patients (%)	20 (100)
Gender (%)	
Males	14 (70)
Females	6 (30)
Age at HSCT [years (median, range)]	13 (1–28)
Pesaro class at time of HSCT (%)	
Class 1	7 (35)
Class 2	4 (20)
Class 3	9 (45)
Type of donor employed (%)	
Matched family donor	3 (15)
Matched unrelated donor	17 (85)
HCMV serology (%)	
Negative donor/negative recipient	1 (5)
Positive donor/negative recipient	4 (20)
Negative donor/positive recipient	2 (10)
Positive donor/positive recipient	13 (65)
Number of BM cells infused (nucleated cells × 10^8/kg; median, range)	4 (2–9)
Number of BM CD34+ cells infused (×10^6/kg; median, range)	3·5 (1·9–6·8)
Conditioning regimen (%)	
Thiotepa + Treo + Flu	3 (15)
Thiotepa + Treo + Flu + ATG	17 (85)
GvHD prophylaxis (%)	
CsA + MTX†	3 (15)
CsA + MTX + ATG‡	17 (85)
Number of days to PMN recovery§ (median, range)	23 (14–30)
Number of days to PLT recovery¶ (median, range)	24 (15–36)
Graft Failure* (%)	2 (10)
Chimerism** (median, range)	100% (40–100)

HSCT, haematopoietic stem cell transplantation; HCMV, human cytomegalovirus; BM, bone marrow; Treo, treosulfan; Flu, fludarabine; ATG, anti-thymocyte globulin; GvHD, graft-versus-host disease; CsA, cyclosporine; MTX, methotrexate; PMN, polymorphonuclear neutrophil; PLT, platelet.

†Recipients of HLA-identical sibling HSCT.

‡Recipients of unrelated donor HSCT.

§Defined as the first of three consecutive days with an absolute neutrophil count ≥0·5 × 10^9/l.

¶Defined as the first of seven consecutive days with an unsupported platelet count ≥20 × 10^9/l.

*Defined according the criteria reported in the text.

**Haematopoietic chimerism was evaluated, starting from DNA obtained either from peripheral blood and/or BM mononuclear cells and T-cell subsets, by microsatellite analysis.

Statistical analysis

Analysis used March 31, 2008 as the report date, i.e. the day at which the two centres locked data on patient outcomes. Patients were censored at time of death or last follow-up. Probability of survival (OS) and of thalassaemia-free survival (TFS) were estimated by the Kaplan–Meier product-limit

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method and expressed as percentage and 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 (TRM), were calculated as cumulative incidence curves, in order to adjust the analysis for competing risks.

Results and discussion

All patients engrafted; details on neutrophil and platelet recovery are reported in Table I. Two patients (both transplanted from a UD) experienced secondary graft failure, after transient engraftment of donor cells (with a maximum percentage of donor cells of 40% and 90%) at day 36 and 270 after HSCT, respectively. The first child was a 15-year-old boy belonging to risk class 3, whereas the second was a class 1 male, 3 years old at time of HSCT. Graft failure was followed by spontaneous recovery of autologous haematopoiesis in the child with the late event and by persistent cytopenia in the other, who was rescued by infusion of the autologous, cryopreserved BM cells. Both patients are currently alive; and transfusion dependent. The overall cumulative incidence of graft failure was 11% (95% CI, 3–43%; Fig 1).

Three patients developed grade II–IV acute GvHD, the cumulative incidence being 15% (95% CI, 5–43%). Two of them, with grade II acute GvHD, responded to steroid treatment, whereas the remaining patient died on day 69 after HSCT because of the grade IV acute GvHD, unresponsive to several immunosuppressive therapies. Limited chronic GvHD developed in only one of the 18 patients at risk; this patient had previously had grade II acute GvHD. The cumulative incidence of chronic GvHD was 7% (95% CI, 1–47%).

The treosulfan-based preparative regimen was well tolerated, with limited organ toxicity. In detail, only two patients (10%) developed grade I and II liver toxicity (i.e. transient elevation of liver enzymes), respectively; no case of veno-occlusive disease was diagnosed. No lung, heart and central nervous system toxicity was recorded. The only toxic effect frequently observed was mucositis, occurring in six (30%) of the 20 enrolled patients; it was of grade I and II, in three cases each. As only one patient died of transplantation-related complications (namely acute GvHD), the cumulative incidence of TRM was 5% (95% CI, 0–34%; Fig 1).

Nineteen patients were alive, at a median follow-up of 20 months (range 8–28). Seventeen were transfusion-independent with sustained donor engraftment. The 2-year Kaplan–Meier estimates of OS and TFS were 95% (95% CI, 85–100%) and 85% (95% CI, 66–100%), respectively (Fig 1). All surviving patients had a Karnofsky/Lansky score of 100%. Fourteen of the 17 evaluable patients achieved sustained full donor chimerism (see also Table I).

We did not observe any difference in terms of outcome between patients belonging to class 1/2 of the Pesaro classification and those allocated to class 3, the probability of TFS being 86% (95% CI, 60–100%) and 78% (95% CI, 51–100%), respectively. The TFS probability of patients given a UD-HSCT was 78% (81% CI, 61–100%).

Our results indicated that, in addition to mild extramedullary toxicity, the combination thiotepa/treosulfan/fludarabine is an effective myeloablative regimen for allogeneic HSCT. It is reasonable to speculate that the limited non-haematopoietic toxicity, with the associated cytokine storm, also contributed to the low incidence of acute and chronic GvHD observed. Remarkably, the outcome of our patients transplanted from UDs was at least as good as that of patients

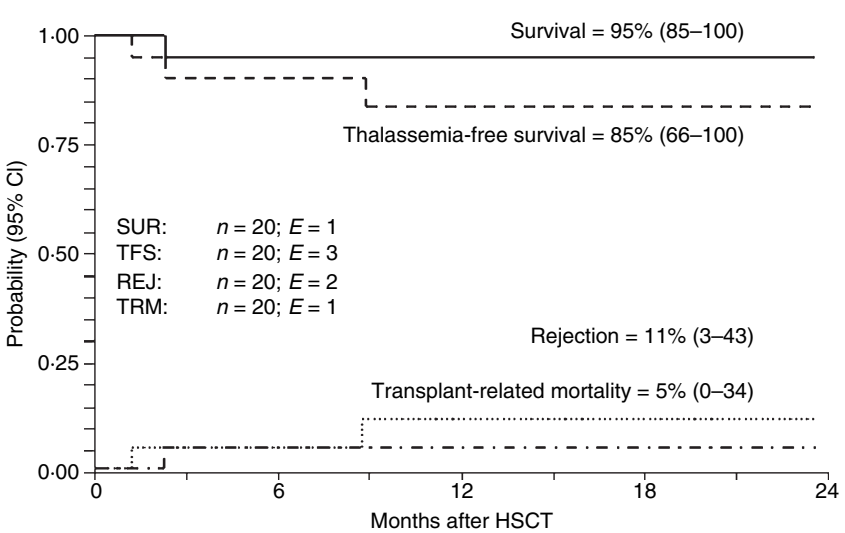

Fig 1. Two-year Kaplan–Meyer estimate of survival and thalassaemia-free survival (TFS), as well as 2-year cumulative incidence of transplantation-related mortality (TRM) and graft rejection for the whole cohort of patients. In the calculation of TFS, both death and graft failure were considered as events. SUR, survival; REJ, rejection; N, number of patients at risk; E, number of events observed.

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previously reported and prepared with a busulfan-based regimen (La Nasa *et al*, 2005). Although the number of patients investigated was small, our data also suggest that this regimen might abolish the difference usually observed among thalassaemia patients of different risk classes (Lucarelli *et al*, 1996).

While the outcome of thalassaemia patients given reduced-intensity preparative regimens has been reported to be dismal (Iannone *et al*, 2003), we demonstrated that, although this preparation was associated with limited extra-medullary toxicity, the majority of patients benefited from sustained donor engraftment. Treosulfan-based myeloablation might be a suitable elective application in adult patients or in those with poor performance status and/or organ dysfunction, who have a high risk of life-threatening complications if busulfan is employed. Future prospective, randomized trials comparing this treosulfan-based and the busulfan-based regimens are warranted.

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Author contributions

M.E.B. performed transplantations, collected and analyzed data and wrote the paper; M.Z. performed transplantations, analyzed data and performed the statistical analysis; G.G., E.P., C.C., P.C., P.M. and A.M. performed transplantations, collected and analyzed data; A.V. and G.C. 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

The authors declare no competing financial interests.

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