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Brief Articles

Treosulfan-Thiotepa-Fludarabine–Based Conditioning Regimen for Allogeneic Transplantation in Patients with Thalassemia Major: A Single-Center Experience from North India

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A B S T R A C T

Hematopoietic stem cell transplantation (HSCT) is the definite treatment for patients with thalassemia major. A busulfan (Bu) and cyclophosphamide (Cy)–based regimen has been the standard myeloablative chemotherapy, but it is associated with higher treatment-related toxicity, particularly in patients classified as high risk by the Pesaro criteria. Treosulfan-based conditioning regimens have been found to be equally effective and less toxic. Consequently, we analyzed the safety and efficacy of treosulfan/thiotepa/fludarabine (treo/thio/flu)-based conditioning regimens for allogeneic HSCT in patients with thalassemia major between February 2010 and September 2012. We compared those results retrospectively with results in patients who underwent previous HSCT with a Bu/Cy/antithymocyte globulin (ATG)–based conditioning regimen. A treo/thio/flu-based conditioning regimen was used in 28 consecutive patients with thalassemia major. The median patient age was 9.7 years (range, 2-18 years), and the mean $CD34^+$ stem cell dose was 6.18×10^6/kg. Neutrophil and platelet engraftment occurred at a median of 15 days (range, 12-23 days) and 21 days (range, 14-34 days), respectively. Three patients developed veno-occlusive disease, 4 patients developed acute graft-versus-host disease (GVHD), and 2 patients had chronic GVHD. Treatment-related mortality (TRM) was 21.4%. Two patients experienced secondary graft rejection. We compared these results with results in patients who underwent previous HSCT using a Bu/Cy/ATG-based conditioning regimen. Twelve patients were treated with this protocol, at a median age of 7.2 years (range, 2-11 years). One patient had moderate veno-occlusive disease, 2 patients developed acute GVHD, 2 patients had chronic GVHD, and 2 patients experienced graft rejection. There was no TRM in this group. We found no significant differences between the 2 groups (treo/thio/flu vs Bu/Cy/ATG) in terms of the incidence of acute GVHD, chronic GVHD, TRM, and graft failure, although a trend toward higher TRM was seen with the treo/thio/flu regimen.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is the sole curative treatment for patients with thalassemia major (TM) [1-3]. However, the clinical outcome after HSCT in children with TM who are classified as Pesaro class 3 and in adults with poor performance status and/or organ dysfunction remains unsatisfactory, owing to the high risk of treatment-related complications or graft failure [2-5], and the event-free survival rate is only 60% [5,6]. Even class 3 represents a heterogenous group of patients with overall survival after HSCT, varying from 39.01% in high-risk class 3 patients to 78.3% in other class 3 patients [7]. The most commonly used conditioning regimens incorporate busulfan (Bu), cyclophosphamide (Cy), and antithymocyte globulin (ATG) and carry a high rate of regimen-related toxicity. New treatment strategies have been shown to improve outcomes in high-risk patients [5,6]. In patients with class 3 thalassemia aged <17 years, the protocol 26 regimen (with hydroxyurea, azathioprine, and fludarabine added to Bu and Cy) was well tolerated, with 93% survival, and the incidence of post-HSCT graft failure decreased from 30% to 8% [8]. Low liver toxicity has also been observed with the use of i.v. busulfan in children with thalassemia undergoing HSCT [9].

Bernardo et al. [10] reported that treosulfan-based conditioning regimens are safe and effective in patients with thalassemia major. To minimize regimen-related toxicity, we treated patients with thalassemia major with a treosulfan/thiotepa/fludarabine (treo/thio/flu)-based conditioning regimen in a prospective manner and compared the results retrospectively with those in the patients treated earlier with a Bu/Cy/ATG- based regimen at the same center.

Study Design

This study involved an analysis of 28 consecutive patients with thalassemia major who underwent HLA-matched allogeneic HSCT using a treo/thio/flu-based conditioning

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Table 1
Characteristics of Patients Undergoing HSCT with Bu/Cy/ATG-Based and Treo/Thio/Flu-Based Conditioning Regimens

Characteristic	Bu/Cy/ATG (n = 12)	Thio/Treo/Flu (n = 28)	*P* value
Sex, n			.68
Male	8	15	
Female	4	13	
Age, y, median (range)	7 (2-11)	9.6 (2-18)	
Pesaro class, n			.24
Class 1	1	0	
Class 2	4	7	
Class 3	7	21	
$CD34^+$ cells, $\times 10^6$/kg, mean	7.61	6.76	.32
VOD, n	1	3	.82
Neutrophil engraftment, days, mean	16	15	.87
Acute GVHD, n	2	4	.77
Chronc GVHD, n	1	2	.66
Graft failure, n	2	2	.73
Death, n	0	6	.07

regimen at the Bone Marrow Transplant Centre, BLK Superspeciality Hospital, New Delhi, between February 2010 and September 2012. Data for 12 patients who underwent HSCT using a Bu/Cy/ATG-based regimen were also analyzed retrospectively and compared with the patients receiving a treo/thio/flu-based protocol. Informed consent from parent/guardian was obtained before a patient was enrolled. The study was approved by the hospital's Institutional Review Board and Ethical Committee.

All patients in the treo/thio/flu group received the same conditioning regimen, comprising i.v. thiotepa 8 mg/kg on day -6, treosulfan 14 g/m^2/day on day -5 to day -3, and fludarabine 40 mg/m^2/day on day -5 to day -2. Patients in the Bu/Cy/ATG group received oral busulfan 3.5 mg/kg/day on day -9 to day -6, cyclophosphamide 50 mg/kg/day on day -5 to day -2, and ATG 30 mg/kg/day on day -4 to day -2. Graft-versus-host disease (GVHD) prophylaxis included cyclosporine (2.5 mg/kg i.v. twice daily) and methotrexate (10 mg/m^2 i.v. on day +1 and 7 mg/m^2 on days +3, +6, and +11). All patients received cyclosporine for 9 to 12 months post-HSCT, with trough plasma cyclosporine levels maintained at 200 to 350 ng/mL. Acute and chronic GVHD were diagnosed and graded according to the Seattle criteria. Veno-occlusive disease (VOD), also called *sinusoidal obstruction syndrome*, was diagnosed according to clinical criteria as the presence of 2 of the following before day 21 post-HSCT: (1) hyperbilirubinemia (bilirubin >2.0 mg/dL), (2) painful hepatomegaly, and (3) unexplained weight gain (>2% from baseline). Severity of VOD was classified as mild, moderate, or severe. Neutrophil recovery was defined as the first of 3 consecutive days with an absolute neutrophil count $\geq 0.5 \times 10^9$/L and a platelet count $\geq 20 \times 10^9$/L unsupported for 7 days. Chimerism was evaluated by fluorescein in situ hybridization in sex-mismatched transplantations and by PCR in others.

Statistical Analysis

The primary study endpoints were to determine the incidences of graft failure and treatment-related mortality (TRM) after HSCT. Secondary endpoints included the incidence and severity of VOD and acute and chronic GVHD. The incidences of relapse, TRM, and GVHD were calculated using cumulative incidence estimates. The differences in outcomes between the 2 groups (treo/thio/flu group and Bu/Cy/ATG group) were analyzed as well. Fisher's exact test was used for discrete variables, and the *t*-test was used for continuous variables. The log-rank test was used for the difference in survival outcome between the 2 groups. A *P* value $< .05$ was considered statistically significant.

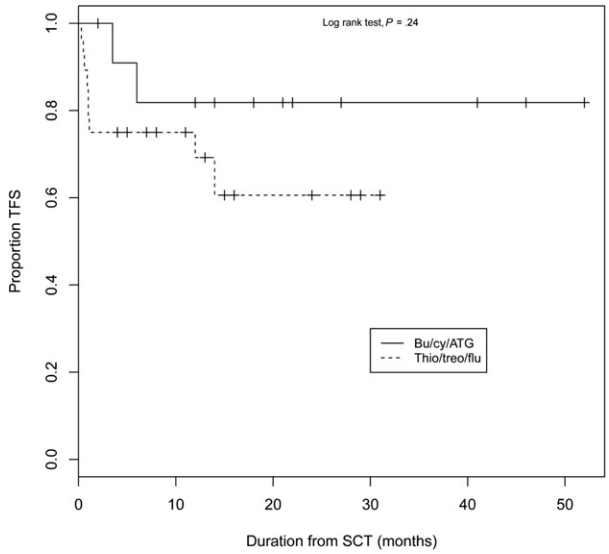

Figure 1. Thalassemia-free survival by conditioning regimen.

RESULTS

Twenty-eight consecutive patients with thalassemia major who underwent allogeneic HSCT using a treo/thio/flu-based conditioning regimen were evaluated. The median patient age was 9.6 years (range, 2-18 years). The group included 15 males and 13 females. Patients were classifed according to the Pesaro classification scheme based on liver size, adequacy of chelation, and hepatic fibrosis. Seven patients were in Pesaro class 2, and 21 patients were in Pesaro class 3. When stratified according to age (≥7 years) and liver size (≥5 cm) [7], 11 of 21 class 3 patients (52.4%) were considered high risk. The stem cell source was filgrastim (granulocyte colony-stimulating factor)-mobilized peripheral blood in 2 patients, bone marrow in 21 patients, and bone marrow plus cord blood in 5 patients. The mean CD 34^+ stem cell dose was 6.18×10^6/kg. Neutrophils and platelets engrafted at a median of 15 days (range, 12-23 days) and 21 days (range, 14-34 days), respectively. Three patients had a major blood group mismatch, 2 patients had a bidirectional blood group mismatch, and 4 patients had a minor blood group mismatch. The median duration of follow-up was 387 days (range, 37-930 days). Nineteen patients developed World Health Organization stage 1-3 oral mucositis (stage 3 in 2 patients). Only 3 patients required total parenteral nutrition, 2 with stage IV gut GVHD and 1 with stage 3 oral mucositis. No renal, pulmonary, cardiac, or central nervous system toxicities were observed. Four patients developed grade II-IV acute GVHD, for a cumulative incidence of 14.3% (95% confidence interval [CI], 3.6%-26.2%). Two patients had grade II-IV skin GVHD, and 2 patients had grade II-IV gut GVHD. Three patients developed severe VOD. Six deaths occurred in the treo/thio/flu group, including 3 due to VOD (on days +17, +27, and +29), 2 due to acute gut GVHD (on days +37 and +60), and 1 due to sepsis, for a cumulative incidence of TRM of 21.4% (95% CI, 4%-35.8%). The cumulative incidence of limited chronic GVHD was 10% (95% CI, 1%-16%). The median hospital stay was 37 days

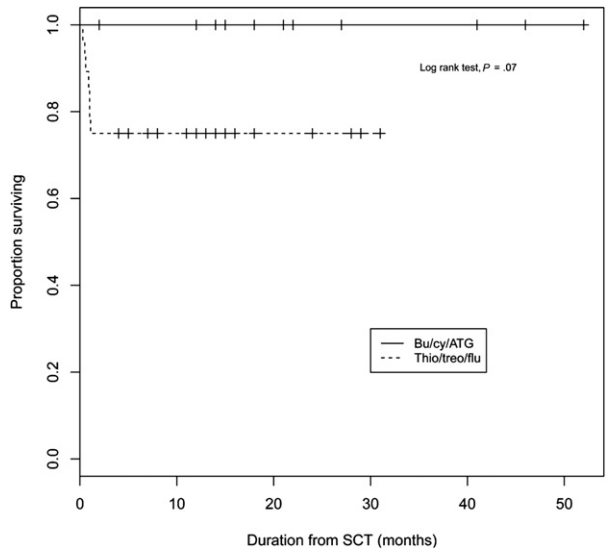

Figure 2. Overall survival by type of conditioning regimen.

(range, 18-51 days). Twenty-two patients were alive at a median follow-up of 387 days (range, 37-930 days), and all but 2 patients were transfusion-independent with sustained donor engraftment. Graft rejection occurred in 2 patients, on days +369 and +414. Twelve patients were prescribed regular phlebotomy therapy. Thalassemia-free survival and overall survival were 71.4% and 78.5%, respectively. Median chimerism in 18 of the 20 evaluable patients in the treo/thio/flu group at last follow-up was 100% (range, 31%-100%). Fourteen of these patients achieved sustained full-donor chimerism, and 4 patients had mixed chimerism.

We also retrospectively analyzed our data on patients who had undergone previous HSCT at our center with a Bu/Cy/ATG-based conditioning regimen. Twelve patients, median age 7.2 years (range, 2-11 years), were treated with this protocol. One patient had moderate VOD, and 2 patients developed acute gut GVHD; all 3 patients recovered. Two patients developed limited chronic GVHD that responded to steroids. Rejection occurred in 2 patients in the Bu/Cy/ATG group (on days +154 and +210). There was no TRM in this group. The thalassemia-free survival and overall survival were 83.3% and 100%, respectively. Median chimerism in 8 of the 10 evaluable patients in the Bu/Cy/ATG group at last follow-up was 100% (range, 61%-100%). Six of these patients achieved sustained full-donor chimerism, and 2 patients had mixed chimerism.

There were no significant differences in the incidence of acute GVHD, chronic GVHD, TRM, and graft failure between the treo/thio/flu and Bu/Cy/ATG groups (Table 1 and Figure 1), although a trend toward higher TRM was seen in the treo/thio/flu group (Figure 2). Two patients in each group had an HLA 5/6-matched donor; these patients demonstrated no difference in morbidity or mortality compared with the total cohort.

DISCUSSION

Allogeneic HSCT remains the sole available curative treatment for patients with thalassemia major [1]. The best candidates for HSCT are patients with an HLA-identical sibling donor, limited iron overload, and an absence of severe hepatic complications [1,2,4]. Bu/Cy/ATG-based conditioning regimens are most commonly used in patients with thalassemia major. High-risk patients are known to be at increased risk for treatment-related morbidity and TRM with Bu/Cy/ATG-based regimens. Lower-toxicity treo/thio/flu-based regimens have been found to be equally effective, with less TRM [10]. Bu-based regimens have been associated with increased treatment-related morbidity (ie, VOD and obliterans bronchiolitis), particularly in Pesaro class 3 patients [4]. The probability of overall event-free survival with this regimen is 89% to 97% in patients with low-risk disease and 80% to 87% in those with high-risk disease [11]. In a study of 179 patients treated with a Bu/Cy-based regimen [12], 17 patients (9.5%) experienced graft failure, and the day +100 probability of acute GVHD and 5-year probability of chronic GVHD were 38% and 13%, respectively. The 5-year probabilities of overall and disease-free survival were 64% and 62%, respectively, for Pesaro risk class 3. The protocol 26 regimen and i.v. Bu-based regimens have also been used in an attempt to reduce graft failure and liver toxicity [8,9], with encouraging results. Treosulfan has been used as a conditioning regimen in patients with thalassemia major based on its significant lytic action in hematopoietic progenitors [13]. Bernardo et al. [10] reported the results of 60 patients with thalassemia (median age, 7 years) undergoing allogeneic HSCT with a treo/thio/flu-based conditioning regimen. They found cumulative incidences of acute GVHD, graft failure, and TRM of 14%, 9%, and 7%, respectively, compared with our values of 14%, 7.14%, and 21.4%. Although other studies [14,15] have also reported reduced TRM owing to treo/flu-based conditioning, the higher TRM in our study was likely related to the fact that 52.4% of our patients in class 3 were considered high risk.

In this study, the cumulative incidences of acute GVHD, chronic GVHD, and TRM were 14.3%, 7%, and 21.4% respectively, in the treo/thio/flu group, compared with 16.6%, 8.3%, and 0% in the Bu/Cy/ATG group (Table 1 and Figure 1). The incidence of VOD did not differ in the 2 groups ($P = .82$). We noted a trend toward increasing TRM in the treo/thio/flu group compared with the Bu/Cy/ATG group ($P = .07$) (Figure 2). We conclude that there is no significant difference between the 2 groups in the incidence of acute GVHD, chronic GVHD, TRM and graft failure, although the treo/thio/flu demonstrated a trend toward a higher TRM. Our sample size was small; however, randomized trials are needed to identify statistically significant differences between the 2 conditioning regimens.

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Outcome of Allogeneic Peripheral Blood Stem Cell Transplantation by Donor Graft CD3$^+$/Tregs Ratio: A Single-Center Experience

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The therapeutic efficacy of allogeneic peripheral blood stem cell transplantation (PBSCT) for hematological malignancies relies largely on the graft-versus-leukemia (GVL) effects exerted by the donor CD3 cells, but there is a risk of onset of uncontrolled graft-versus-host disease (GVHD). Regulatory T cells (Tregs) (CD4+CD25^{high} Foxp3+) are believed to maintain tolerance and to inhibit acute GVHD (aGVHD) after allogeneic PBSCT. Nevertheless, when looking at post-allotransplantation patient outcomes, although the impact of aGVHD on survival is amply documented, so far there is no evidence that the donor graft CD3/Tregs ratio may affect overall survival (OS), nonrelapse mortality (NRM), disease-free survival (DFS), and relapse rates. Our aim was to study the possible impact of the gCD3/Tregs ratio on survival after myeloablative allogeneic PBSCT. We analyzed 74 consecutive patients diagnosed with acute myeloid leukemia (n = 62), acute lymphoblastic leukemia (n = 10), and chronic myeloid leukemia (n = 2) who underwent transplantation with unmanipulated PBSCs from a human leukocyte antigen–identical related donor (n = 48) or a human leukocyte antigen–identical unrelated donor (n = 26). Patients were subdivided into a high gCD3/Tregs ratio (≥36) group (HR group, n = 30) and a low gCD3/Tregs ratio (<36) group (LR group, n = 44). The OS, DFS, NRM, and relapse rates at 3 years were 53%, 51%, 29%, and 34%, respectively. Comparing the LR and HR groups, a statistically significant difference was demonstrated for the 3-year OS, DFS, and NRM rates (65% vs 31%, $P = .0001$; 67 versus 26%, $P = .0001$; 5% versus 71%, $P < .0001$, respectively) but not for relapse (30% vs 25%, $P =$ ns). By multivariate analysis, LR significantly predicted better OS ($P = .019$), DFS ($P = .003$), and NRM ($P = .05$), whereas there was no statistically significant association between LR and relapse ($P = .155$). Overall, our data may suggest that LR preserves GVL effects but is also protective against aGVHD in allotransplantation patients.

INTRODUCTION

The contribution of regulatory T cells (Tregs) to post-transplantation immunological reconstitution has been clearly established, given their impact on T cell immunity [1] and on modulating graft-versus-host disease (GVHD) while preserving graft-versus-leukemia (GVL) effects in mouse models [2]. The pathophysiological link between GVHD and immune reconstitution is well defined [3,4]. Therefore, given that acute GVHD (aGVHD) is triggered by alloreactive mature donor CD3 T cells [5,6] and antagonized by Tregs [7,8], the immunity of patients undergoing allotransplantation might depend on the ratio between these two cellular populations, as we have already suggested in humans [9], in line with murine experimental models of aGVHD [10,11].

Moreover, it is well known that, apart from inducing prolonged immunosuppression [12] and a graft failure risk [13], T cell depletion of the donor graft results in a higher leukemia relapse rate [14]. Nevertheless, when looking at the outcomes of patients undergoing allotransplantation, although the impact of human leukocyte antigen (HLA) incompatibility (and of the consequent aGVHD) on patient

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