Biol Blood Marrow Transplant 25 (2019) 1407–1415

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

Thiotepa, Busulfan, and Fludarabine Conditioning Regimen in T Cell-Replete HLA-Haploidentical Hematopoietic Stem Cell Transplantation

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Article history:
Received 25 January 2019
Accepted 28 February 2019

Key Words:
Antithymocyte globulin
Conditioning
Haploidentical transplantation
Graft-versus-host disease

A B S T R A C T

We report the outcomes of 51 patients who underwent unmanipulated haploidentical hematopoietic stem cell transplantation (haplo-HSCT) with post-transplantation cyclophosphamide (PT-Cy) and antithymocyte globulin (ATG), from peripheral blood stem cells (PBSCs) or bone marrow, after receipt of a TBF (thiotepa, busulfan, and fludarabine) conditioning regimen. Their median age was 55 years (range, 16 to 72 years). Hematologic diagnoses included acute leukemias (n = 31), lymphoid neoplasm (n = 12), myeloproliferative neoplasm (n = 5), and myelodysplastic syndromes (n = 3). Thirty-seven patients (73%) were in complete remission. Graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporine and mycophenolate for all patients, associated with ATG in 39 patients (76.5%). The median time to neutrophil engraftment was 17 days (range, 12 to 34 days). The cumulative incidences of grade II-IV and grade III-IV acute GVHD were 27.5% and 14%, respectively. In patients receiving a PBSC graft and ATG prophylaxis, grade II-IV aGVHD occurred in 16% of patients. The use of ATG and a lower thiotepa dose (5 mg/kg versus 10 mg/kg) were associated with a reduced cumulative incidence of grade II-IV acute GVHD (P = .03 and .005, respectively). The 2-year cumulative incidence of chronic GVHD was 29% and was significantly reduced to 13% with the lower thiotepa dose (P = .002). After a median follow-up of 25 months (range, 12 to 62 months), the cumulative incidences of nonrelapse mortality, relapse, overall survival (OS), disease-free survival (DFS), and GVHD-free, relapse-free survival (GFRFS) were 20%, 22.5%, 67%, 58%, and 51%, respectively. Pretransplantation disease status (complete remission versus others) was the main factor associated with OS, DFS, and GFRFS. In conclusion, the TBF conditioning regimen is an appealing platform in the haplo-HSCT setting with PT-Cy in terms of engraftment rate, toxicity, and disease control. We found no benefit of a thiotepa dose of 10 mg/kg compared with a dose of 5 mg/kg. ATG reduced the risk of acute GVHD without comprising outcomes.

INTRODUCTION

The widespread use of haploidentical (haplo) family donors allows for the rapid identification of an available donor for the majority of patients who need hematopoietic stem cell transplantation (HSCT). T cell-replete haplo-HSCT has now become feasible with the use of post-transplantation cyclophosphamide (PT-Cy) [1]. Recent retrospective studies suggest noninferior survival outcomes compared with HSCT with 10/10 matched unrelated donors (MUDs), 9/10 mismatched unrelated donors, or umbilical cord blood [2-10]. However, the optimal conditioning regimen in the setting of haplo-HSCT remains to be defined.

Thiotepa, an alkylating agent, has been used in HSCT with increasing frequency. Its antineoplastic, myelosuppressive, and immunosuppressive activities, associated with its ability to penetrate the blood-brain barrier and a good safety profile, make it an attractive agent in the setting of HSCT for hematologic malignancies [11-13]. A preparative regimen combining

Financial disclosure: See Acknowledgments on page 1413.
Conflict of interest statement: M.M., R.D., F.M., and M.T.R. received honoraria for lectures from Keocyt, whose drug was included in this study. The other authors have no conflicts of interest to report.
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https://doi.org/10.1016/j.bbmt.2019.02.025
1083-8791/

the alkylating agents thiotepa and busulfan with fludarabine (TBF) was initially reported by Sanz et al [14] as capable of improving engraftment and survival outcomes in umbilical cord blood HSCT. TBF with a myeloablative conditioning (MAC) regimen with busulfan has been compared with busulfan-fludarabine (BF)-MAC in patients with acute myelogenous leukemia (AML) in first remission who underwent transplantation with a matched-sibling donor (MSD) or an MUD. The rate of relapse was lower in patients who received TBF-MAC conditioning compared with those who received BF-MAC conditioning. However, nonrelapse mortality (NRM) was higher in the TBF-MAC recipients, which counterbalanced the stronger antileukemic effect, thus resulting in similar survival in the 2 groups [15].

This regimen was also applied in the haplo-HSCT setting by Bacigalupo et al [16,17] (thiotepa 10 mg/kg, busulfan 9.6 mg/kg or 6.4 mg/kg for patients age >60 years or in poor clinical condition, and fludarabine 150 mg/m^2), using unmanipulated bone marrow (BM) grafts and PT-Cy (50 mg/kg/day) on days +3 and +5 in association with cyclosporine (from day 0) and mycophenolate mofetil (MMF) (from day +6) as graft-versus-host disease (GVHD) prophylaxis. These authors reported lower rates of graft rejection than with the reduced-intensity conditioning (RIC) nonmyeloablative Baltimore regimen (.7% versus 13% [1]), acceptable NRM (18% at 1 year), low risk of acute GVHD (aGVHD) and chronic GVHD (cGVHD), and relapse rates comparable to those seen with MUD and MSD HSCT.

In this study, peripheral blood was the preferred stem cell source in our center, and we used antithymocyte globulin (ATG) in the conditioning regimen. ATG can reduce the risk of GVHD risk in conventional HSCT settings with MSD and MUD [18-20]; however, data are scarce on the use of ATG in a haplo-HSCT setting with PT-Cy. Thus, we aimed to evaluate the clinical outcomes of the patients undergoing unmanipulated haplo-HSCT in our center with TBF conditioning.

METHODS

Patient Selection

This study is a single-center retrospective analysis of all consecutive patients undergoing haplo-HSCT after a TBF conditioning regimen between August 2013 and December 2017 at Saint Antoine Hospital, Paris. Fifty-one patients age ≥16 years were included. All patients who proceeded to transplantation provided written informed consent for the use of their data for clinical research, in accordance with the modified Declaration of Helsinki and with approval of the local Ethical Committee. Patients were stratified according to the Disease Risk Index (DRI) [21].

Conditioning Regimens and GVHD Prophylaxis

All patients received a TBF conditioning regimen (Table 2). In accordance with our center's policy, all but 6 patients who underwent transplantation in 2013 and 2014 received a total dose of 10 mg/kg of thiotepa (5 mg/kg on days -7 and −6), which was reduced to 5 mg/kg at day -6 in those who underwent transplantation between 2015 and 2017. A total fludarabine dose of 150 mg/m^2 was administered to all patients (30 mg/m^2 from day -5 to day -1), and the total dose of i.v. busulfan was 6.4 or 9.6 mg/kg (3.2 mg/kg/day on days -4 and -3 or on days -4, -3, and -2) according to the patient's age, comorbidities, and clinical condition. The stem cell source was peripheral blood stem cells (PBSCs) or BM, with no ex vivo T cell depletion.

The PT-Cy dose was adjusted according to the stem cell source. BM recipients were scheduled to receive 1 dose of PT-Cy (50 mg/kg/day) on day +3. PBSC recipients were given 2 doses of PT-Cy on days +3 and +5. In all patients, GVHD prophylaxis consisted of cyclosporine A starting on day -3 at 3 mg/kg/day as a continuous i.v. infusion, with adjustment based on blood levels, and MMF i.v. from day +6 to day +35 at 15 mg/kg every 12 hours. In the absence of GVHD, cyclosporine A was tapered over 4 to 8 weeks from day +60 in patients with high-risk disease and from day +90 in those with intermediate-risk disease. Patients could receive rabbit ATG (Thymoglobulin; Genzyme) at a total dose of 5 mg/kg (between 2013 and 2014) or 2.5 mg/kg (between 2015 and 2017). aGVHD and cGVHD were diagnosed and graded according to standard criteria [22,23].

Supportive Care

Granulocyte colony-stimulating factor was given from day +6 until neutrophil engraftment. Mesna and hyperhydration were administered for prevention of the uroepithelial damage of Cy, and prophylactic clonazepam was given in association with busulfan. Prophylaxis of hepatic veno-occlusive disease (VOD) was provided with orally applied ursodeoxycholic acid and low-dose unfractionated heparin. Patients at high-risk of VOD according to the European Society for Blood and Marrow Transplantation criteria received defibrotide instead of heparin [24,25].

Antimicrobial prophylaxis consisted of valacyclovir or acyclovir administered at the start of the conditioning regimen. After engraftment, patients received amoxicillin and atovaquone or trimethoprim-sulfamethoxazole. Primary antifungal prophylaxis was not administered routinely. Patients were monitored weekly by real-time polymerase chain reaction (PCR) for cytomegalovirus (CMV), human herpesvirus 6 (HHV6), Epstein-Barr virus (EBV), and toxoplasma until day +100. Monitoring of BK virus in blood and urine was done according to clinical signs, as reported previously [26].

Statistical Analysis

Continuous variables were recorded as median and range and compared using the Mann-Whitney *U* test. Qualitative variables were recorded as frequency and percent and compared using the chi-square test. Overall survival (OS), defined as the time from transplantation to death from any cause; disease-free survival (DFS), defined as the time from transplantation to relapse or progression or death from any cause, whichever came first; and GVHD-free, relapse-free survival (GFRFS) [27], defined as being alive with neither grade III-IV aGVHD, severe cGVHD nor disease relapse, were calculated by the Kaplan-Meier method, and the differences between groups were compared using the log-rank test. Neutrophil engraftment was defined as an absolute neutrophil count (ANC) $\geq .5 \times 10^9$/L for 3 consecutive days, and platelet engraftment was defined as achievement of a platelet count $\geq 20 \times 10^9$/L unsupported by platelet transfusions for 7 days. Full donor chimerism was measured by PCR and defined as ≥95% leukocytes of donor origin in the peripheral blood. Engraftment, GVHD, relapse, and NRM were calculated using the cumulative incidence method and analyzed in a time-dependent fashion. For aGVHD and cGVHD or relapse, death was considered a competing risk of the event. For NRM, the competing event was relapse.

All variables with a significance level of $P < .10$ in univariate analysis were introduced in a multivariable Cox regression model, with backward selection [28]. Interactions between main effect and adjusting were tested, and variables with significant interaction were excluded from the final model. Adjusted hazard ratios (HRs) and 95% confidence intervals (CIs) were computed, and $P < .05$ was considered statistically significant. Statistical analyses were performed with SPSS version 22 (IBM, Armonk, NY) and R version 3.2 (R Development Core Team, Vienna, Austria) software packages.

RESULTS

Patient, Disease, and Transplantation Characteristics

Patients and disease characteristics are summarized in Table 1. The median age at HSCT was 55 years (range, 16 to 72 years). Patients had AML (n = 23), acute lymphoblastic leukemia (ALL; n = 8), lymphoid neoplasms (n = 12), myeloproliferative neoplasms (n = 5), and myelodysplastic syndrome (n = 3). Thirty-seven patients were in complete remission (CR) at transplantation (27 in first CR [CR1]), 6 were in partial remission, and 8 had progressive disease. DRI stratified the patients as intermediate risk (n = 35), high risk (n = 14), and very high risk (n = 2). Nine patients had failed a previous allogeneic (n = 3) or autologous (n = 6) HSCT. Twenty-seven patients received a busulfan dose of 6.4 mg/kg (Table 2). Thirty-nine patients received ATG associated with the conditioning regimen, at a dose of 2.5 mg/kg (n = 29) or 5 mg/kg (n = 10). BM recipients (n = 9) received mainly (n = 8) 1 dose of PT-Cy (50 mg/kg/day) on day +3. PBSC recipients (n = 42) received 2 doses of PT-Cy on days +3 and +5, except for 2 patients, including 1 patient with pretransplantation left ventricular ejection fraction of 40% and another who received a graft with a low CD34$^+$ cell count (Table 2). Nine patients received defibrotide prophylaxis for VOD.

Engraftment

All 51 evaluable patients achieved neutrophil engraftment, within a median of 17 days (range, 12 to 34 days). A platelet

Table 1
Patient and Donor Characteristics at Transplantation

Characteristic	Value
Recipient age, yr, median (range)	55 (16-72)
Sex, male/female, n (%)	32 (63)/19 (38)
Diagnosis, n (%)	
Acute leukemia	31 (61)
AML	23 (45)
ALL	8 (16)
Lymphoid neoplasm	12 (24)
NHL (DLBCL, MCL, T NOS, ATLL)	7 (14)
HL	3 (6)
Prolymphocytic leukemia	2 (4)
Myeloproliferative neoplasm	5 (10)
Myelodysplastic syndromes	3 (6)
Disease status at transplantation, n (%)	
Complete remission	37 (72.5)
CR1	27 (53)
CR≥2	10 (19.5)
Partial remission	6 (12)
Active/progressive disease	8 (15.5)
Disease risk index, n (%)	
Intermediate	35 (68.5)
High	14 (27.5)
Very high	2 (4)
Previous lines of therapy, n (%)	
0	2 (4)
1	28 (55)
2	10 (19.5)
≥3	11 (21.5)
Previous autologous HSCT, n (%)	6 (12)
Previous allogeneic HSCT, n (%)	3 (6)
Donor-recipient CMV serostatus match, n (%)	
Donor negative/recipient negative	13 (25.5)
Donor negative/recipient positive	11 (21.5)
Donor positive/recipient negative	1 (2)
Donor positive/recipient positive	26 (51)
Stem cell source, n (%)	
PBSCs	42 (82)
BM	9 (18)
$CD34^+$ cells/kg, median (range)	5 (1.02-9.74)

NHL indicates non-Hodgkin lymphoma; DLBCL, diffuse large B cell lymphoma; MCL, mantle cell lymphoma; T NOS, lymphoma T not otherwise specified; ATLL, acute T cell leukemia/lymphoma.

count $>20 \times 10^9$/L was achieved in 48 patients, within a median of 18 days (range, 7 to 130 days), with 7 nonevaluable patients due to transfusion support at superior platelet number cutoffs (Table 3). Forty-five patients (88%) had a platelet count recovery to $>50 \times 10^9$/L within a median of 28 days (range, 9 to 406 days). All patients had full donor chimerism at days +30, +60, and +90, except for 1 patient presenting with 94% donor chimerism at day +30 and full donor chimerism at days +60 and +90. No graft rejection was observed. Four patients received a successful $CD34^+$ cell boost from the same donor for poor graft function after initial engraftment and achieved neutrophil recovery within 4, 5, 7, and 11 months post-HSCT, respectively.

Transplantation-Related Complications and Infections

Transplantation-related complications and infections are outlined in Table 4. VOD developed in 2 patients (4%) and was fatal in 1 of them. Signs of transplantation-associated microangiopathy were observed in 8 patients (16%), requiring plasma exchanges in 1 patient and responding to cyclosporine withdrawal alone in the other patients. Grade 3-4 mucositis occurred in 16 patients (31%) and hepatic, cardiac, or neurologic toxicities of grade ≥3 after the conditioning chemotherapy were observed in 5 patients, 4 patients, and 1 patient, respectively. At last follow-up, hemorrhagic cystitis associated with BK virus reactivation occurred in 24 patients (47%), necessitating bladder irrigation in 13 (26%). Four patients received cidofovir and 9 received polyvalent i.v. immunoglobulins. CMV reactivation occurred in 34 patients (67%). All patients received

Table 2
Conditioning Regimens

Regimen	n (%)
Thiotepa and busulfan	
T1B2F	26 (51)
T1B3F	12 (23.5)
T2B2F	1 (2)
T2B3F	12 (23.5)
ATG	
No	12 (23.5)
Yes	39 (76.5)
2.5 mg/kg	29 (57)
5 mg/kg	10 (19.5)
PT-Cy	
No	0 (0)
Yes	51 (100)
Day +3 only	10 (19.5)
Days +3 and +5	41 (80.5)

T1B2F indicates thiotepa 5 mg/kg/day for 1 day, busulfan 3.2 mg/kg/day for 2 days, and fludarabine 150 mg/m^2; T1B3F, thiotepa 5 mg/kg/day for 1 day, busulfan 3.2 mg/kg/day for 3 days, and fludarabine 150 mg/m^2; T2B2F, thiotepa 5 mg/kg/day for 2 days, busulfan 3.2 mg/kg/day for 2 days, and fludarabine 150 mg/m^2; T2B3F, thiotepa 5 mg/kg/day for 2 days, busulfan 3.2 mg/kg/day for 3 days, and fludarabine 150 mg/m^2.

Table 3
Engraftment and Clinical Outcomes (N = 51)

Outcome	Value
Time to neutrophils $>.5 \times 10^9$/L, d, median (range)	17 (12-34)
Time to platelets $>20 \times 10^9$/L, d, median (range)	18 (7-130)
Platelets $>50 \times 10^9$/L, n (%)	45 (88)
Time to platelets $>50 \times 10^9$/L, d, median (range)	28 (9-406)
Acute GVHD, n (%)	
Grade II-IV	14 (27.5)
Grade III-IV	7 (13.5)
Systemic steroid treatment for acute GVHD, n (%)	14 (27.5)
Steroid-refractory acute GVHD, n (%)	8 (16)
2-yr cumulative incidence of chronic GVHD, n (%)	15 (29)
Mild	9 (17.5)
Moderate	2 (4)
Severe	4 (7.5)
Cumulative incidence of NRM, % (95% CI)	
At day +100	6 (1.9-17.8)
At 2 yr	20 (11.2-34.4)
Cumulative incidence of relapse, % (95% CI)	
At day +100	4 (1.0-15.5)
At 2 yr	22.5 (13.2-38.5)
2-yr OS, % (95% CI)	67 (60-74)
2-yr DFS, % (95% CI)	58 (51-65)
Follow-up, mo, median (range)	25 (12-62)

Table 4
Transplantation-Related Complications and Infections

Complication/Infection	n (%)
Mucositis, n (%)	
Grade 1-2	28 (55)
Grade 3-4	16 (31)
Organ toxicity grade ≥3, n (%)	
Hepatic	5 (10)
Cardiac	4 (8)
Neurologic	1 (2)
Pulmonary or renal	0
VOD	2 (4)
TMA	8 (16)
Viral infections	
BK virus hemorrhagic cystitis	24 (47)
Grade 2	11 (21.5)
Grade 3	11 (21.5)
Grade 4	2 (4)
CMV reactivation	27 (73)
EBV reactivation > 5000 UI/mL	30 (59)
EBV-related lymphoproliferative disease	2 (4)
HHV6 reactivation (46 evaluable patients)	31 (67)
Adenovirus infection	2 (4)
Varicella zoster reactivation	5 (10)
Bacteremia	33 (64)
Gram positive	23 (45)
Gram negative	10 (20)
Severe sepsis during aplasia	9 (18)
Sepsis requiring ICU admission	8 (16)
Invasive fungal infections	5 (10)
Aspergillus spp	2 (4)
Candida spp	1 (2)
Fusarium spp	1 (2)
Mucor spp	1 (2)
Pneumocystis pneumonia	2 (4)
Toxoplasmosis	2 (4)

TMA indicates thrombotic microangiopathy; ICU, intensive care unit.

preemptive therapy with foscarnet or gancyclovir (according to hematopoietic recovery and renal function). One patient developed CMV retinitis at 9 months after HSCT, which resolved with intraocular injections of ganciclovir. EBV reactivation (viral load >5000 UI/mL) occurred in 30 patients (59%) and was preemptively treated with rituximab. Two patients (4%) had an EBV-related post-transplant lymphoproliferative disease (PTLD), which was treated successfully by rituximab alone in 1 patient and chemotherapy in the other. HHV6 reactivation occurred in 31 of 46 evaluable patients (67%). Two patients developed symptomatic adenovirus infection, and 5 had varicella zoster virus reactivation. Bacteremia occurred in 33 patients (64%), including 16 (31%) *Staphylococcus*-positive blood cultures and 19 (37%) catheter-related bloodstream infections. Invasive fungal infection was documented in 5 patients (10%), including possible or probable invasive aspergillosis in 2 patients (4%).

The use of ATG and ATG doses were not significantly associated with an increased incidence of infection. In patients receiving ATG, CMV, EBV, and HHV6 reactivations occurred in 67%, 59%, and 62% of cases, respectively. BK virus hemorrhagic cystitis developed in 38% of patients and necessitated irrigation in 23%. Bacteremia and invasive fungal infections occurred in 64% and 8% of patients, respectively.

GVHD

aGVHD

At day +100, the cumulative incidence of grade II-IV aGVHD was 27.5%. Grade III-IV aGVHD occurred in 7 patients (14%) (Table 3). Among the 31 patients who received a PBSC graft and ATG prophylaxis, 5 (16%) developed grade II-IV aGVHD and 3 (10%) developed grade III-IV aGVHD. All patients with grade ≥II aGVHD were treated with methylprednisolone 1 to 2 mg/kg/day as standard first-line therapy. Eight patients (16%) were steroid-refractory and received second-line treatment consisting of weekly low-dose methotrexate (n = 6) and/or extracorporeal photopheresis (n = 3) according to the GVHD organ involvement. A third line of treatment with anti-CD25 monoclonal antibody (inolimomab) was administered to 2 patients with refractory GVHD.

In univariate analysis, the cumulative incidence of grade II-IV aGVHD was significantly lower in patients receiving ATG (21% versus 50%; $P = .03$) (Table 5). There was with no significant difference according to ATG dose ($P = .09$). The cumulative incidence of grade II-IV aGVHD was significantly lower with 5 mg thiotepa compared with 10 mg thiotepa (16% versus 57%; $P = .005$). Patients who underwent transplantation after 2015 also had a lower cumulative incidence of aGVHD ($P = .02$). No factor significantly influenced aGVHD in multivariate analysis (Table 6).

cGVHD

The overall cumulative incidence of cGVHD was 29% at 2 years. Six patients (12%) had moderate to severe cGVHD (Table 3). The organs affected were skin (n = 12), mucosa (n = 11), eyes (n = 6), lungs (n = 4), genitals (n = 3), gastrointestinal tract (n = 2), and liver (n = 1). Four patients died during the follow-up period, including 2 from relapse and 2 from infection. No significant benefit of ATG in reducing the cumulative incidence of cGVHD was observed. The cumulative incidence of cGVHD was significantly reduced in intermediate-risk DRI patients compared with high-risk and very-high-risk patients (18% versus 47%; $P = .02$) and in patients who received 5 mg/kg thiotepa compared with 10 mg/kg thiotepa (13% versus 57%; $P = 002$). However, these factors were not significant in multivariate analysis (Table 6).

Relapse and NRM

The relapse incidence (RI) was 4% at day +100 and 23% at 2 years post-transplantation. Relapse or disease progression occurred in 11 patients, within a median of 171 days (range, 20 to 559 days), including 5 patients with AML with high risk features, 2 patients with ALL, 1 patient with human T cell leukemia virus , type 1-positive acute T cell lymphoma, 1 patient with Hodgkin lymphoma in third partial remission, 1 patient with Sezary syndrome with progressive disease at transplantation, and 1 patient with refractory double-hit diffuse large B-cell lymphoma.

At last follow-up, 4 of the 11 patients experiencing relapse were in remission, and 7 had died. Ten patients received prophylactic therapy to prevent relapse: azacytidine in 2 patients with AML, sorafenib in 4 patients with AML with internal tandem duplications of the Fms-like tyrosine kinase 3, dasatinib in 1 patient with AML secondary to chronic myelogenous leukemia, nilotinib in 1 patient with Philadelphia chromosome-positive ALL, and donor lymphocyte infusion in 1 patient each with refractory Hodgkin lymphoma and biclonal ALL. One of these 10 patients experienced further relapse during the follow-up period after sorafenib withdrawal.

Table 5
Univariate Analysis

Variable		DFS at 24 mo			OS at 24 mo			2-yr RI		
		%	Error +/-	*P*	%	Error +/-	*P*	%	95% CI	*P*
Recipient age										
	≤54.9 yr	49	10	.20	65	10	.57	28	14.5-53.8	.35
	>54.9 yr	68	9		68	10		16	6.4-40.2	
Recipient sex										
	Male	57	9	.93	72	8	.85	24	12-47.7	.86
	Female	58	11		62	11		21	8.5-52	
Diagnosis										
	Other	47	12	.09	53	12	.06	24	9.6-57.5	.72
	Acute leukemia	63	9		74	8		22	11.2-44	
Disease status										
	Other	43	13	.04	43	13	.003	21	7.5-61.5	.96
	CR	63	8		76	8		23	12.2-43.7	
DRI										
	Intermediate	63	8	.37	70	8	.30	20	10.2-39.3	.71
	High/very high	48	13		61	13		27	10.9-65.8	
ATG use										
	No	50	14	.67	56	15	.57	25	8.8-71.2	.84
	Yes	60	8		71	8		22	11.5-41.1	
ATG dose, mg/kg										
	0	50	14	.34	56	15	.46	25	8.8-71.2	.74
	2.5	67	9		79	8		19	8.4-44.8	
	5	40	15		50	16		30	10.7-83.9	
Thiotepa dose, mg/kg										
	5	65	8	.37	76	7	.11	19	9.6-37.3	.62
	10	43	13		50	13		29	11.8-68.9	
Graft source										
	BM	56	17	.97	56	17	.73	22	5.9-83.2	.97
	PBSC	58	8		71	7		23	12.6-41.8	
Year of HSCT										
	≤2015	44	10	.05	56	10	.07	29	16.3-53.9	.19
	>2015	75	8		83	8		13	4.2-37	

100-day Cumulative Incidence of aGVHD			2-year Cumulative Incidence of cGVHD			2-year Cumulative Incidence of NRM		
%	95% CI	*P*	%	95% CI	*P*	%	95% CI	*P*
35	20.1-59.5	.21	39	22.6-66.6	.08	23	11.2 - 47.5	.57
20	9-44.6		13	4.33-8.3		16	6.4-40.1	
25	13.6-46.1	.71	26	13.2-49.7	.88	19	9-39.1	.75
32	15.9-62.5		30	13.3-66.3		21	8.6-51.7	
29	13.7-63.1	.83	26	10.5-65.9	.94	29	13.6-63.5	.19
27	15-46.8		27	14.6-50.1		15	6.5-33.5	
36	17.1-74.5	.51	NA	NA	.88	36	17-75.2	.06
24	13.7-43.3		28	15.6-49.7		14	5.9-30.9	
31	19.1-51.7	.39	18	7.8-41.7	.02	17	8.2-36	.48
19	6.5-53.9		47	26.2-83.8		25	10.3-60.5	
50	27.4-91.3	.03	31	10.7-90.9	.88	25	8.9-70.1	.65
21	11-38.4		25	14-44.8		18	9.1-35.5	
50	27.4-91.3	.09	31	10.7-90.9	.44	25	8.9-70.1	.52
17	7.6-38.9		20	8.7-44.5		14	5.4-34.9	
30	11-82		40	17.5-91.5		30	10.8-83.3	
16	7.7-34.1	.005	13	4.9-32.8	.002	16	7.7-34.1	.38
57	35.3-92.4		57	35.1-93.2		29	12-68	
33	12.4-89.7	.81	44	20-98.7	.15	22	6-82.7	.90
26	15.7-43.8		23	12.4-44.4		19	10.1-35.9	
41	25.6-64.9	.02	33	19.2-57.8	.22	26	13.5-49.9	.25
13	4.2-36.9		NA	NA		13	4.2-37	

NA indicates not applicable.

Table 6
Multivariate Analysis

Variable	DFS			OS			Relapse		
	P	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI
Disease status (CR versus others)	.011	3.324	1.312-8.418	.001	5.185	1.897-14.173	.344	1.957	.487-7.869
ATG (yes versus no)	.733	1.189	.439-3.224	.581	1.370	.448-4.190	.960	1.036	.261-4.105
Thiotepa dose (10 versus 5 mg/kg)	.535	1.384	.495-3.872	.697	.799	.258-2.476	.608	1.443	.356-5.852
Year of HSCT (>2015 versus ≤2015)	.029	3.639	1.139-11.626	.121	3.022	.747-12.222	.104	3.675	.764-17.672

Acute GVHD			Chronic GVHD			NRM		
P	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI
.236	2.052	.625-6.732	.400	1.676	.503-5.584	.010	5.669	1.519-21.157
.058	3.086	.962-9.898	.752	.821	.241-2.795	.608	1.473	.335-6.476
.133	.357	.093-1.368	.080	.246	.051-1.181	.750	1.286	.273-6.064
.479	1.806	.352-9.278	.957	.955	.182-5.023	.171	3.388	.591-19.437

The cumulative incidence of NRM was 6% at day +100 and 20% at 2 years post-HSCT. At last-follow-up, 17 patients had died. The main causes of death were disease relapse (n = 7), GVHD (n = 7), infection (n = 2), and VOD (n = 1). ATG was not significantly associated with RI or NRM in both univariate and multivariate analyses. Patients in CR had a lower NRM (HR, 5.67; 95% CI, 1.52 to 21.16; $P = .01$) in multivariate analysis (Table 6).

OS, DFS, and GRFS

With a median follow-up of 25 months (range, 12 to 62 months), the GRFS, DFS, and OS were 51%, 58%, and 67%, respectively (Figure 1). In univariate analysis, the DFS and OS were 63% and 74%, respectively, in patients with acute leukemia and 47% and 53%, respectively, in those with other diseases ($P = .09$ and .06, respectively). OS and DFS were significantly higher in patients in CR at transplantation compared with those who were not (76% versus 43%; $P = .003$ and 63% versus 43%; $P = .04$, respectively) (Figure 2). In multivariate analysis (Table 6), CR disease status was independently associated with a better GRFS (HR, 3.04; 95% CI, 1.27 to 7.26; $P = .01$), DFS (HR, 3.32; 95% CI, 1.31 to 8.42; $P = .01$) and OS (HR, 5.19; 95% CI, 1.90 to 14.17; $P = .001$). Patients who underwent transplantation after 2015 had also a higher DFS (HR, 3.64; 95% CI, 1.14 to 11.63; $P = .003$).

DISCUSSION

The findings from this study confirm that the TBF conditioning regimen is feasible in a haplo-HSCT setting with PT-Cy and allows for sustained engraftment, manageable toxicity, and efficient disease control. The use of ATG reduced the cumulative incidence of aGVHD and did not seem to increase the incidence of infectious complications or relapse. Disease status before HSCT remained the most significant factor associated with GRFS, DFS, and OS.

Neutrophil engraftment occurred in all patients, which compares favorably to the reported 13% graft failure rate with the Baltimore RIC regimen [1]. The median time to neutrophil recovery was similar to that reported by Bacigalupo et al [16] with a TBF conditioning regimen in haplo-HSCT using BM grafts (median, 17 days; range, 13 to 32 days) [16], and shorter than that with the Baltimore RIC regimen using PBSC grafts (median, 20 days; range, 14 to 27 days) [29].

Our results regarding bacterial and fungal infections are in line with previous reports in a haplo-HSCT setting with PT-Cy [1,16,17,29-33]. However, viral reactivations seemed to have occurred more frequently in our cohort, with CMV and EBV reactivations necessitating preemptive therapy in 67% and 59% of patients, respectively. The incidence of PTLD in our series was 4%. Previous studies reported incidences of CMV reactivation of 38% to 81%, EBV reactivation of 0% to 34%, and PTLD of 0% to 3% [1,16,17,29-33]. The 47% incidence of BK virus hemorrhagic cystitis, which necessitated bladder irrigation in one-half of affected patients, was in line with the incidence of 11% to 75% reported in other studies [26,29,30,33]. The use of ATG in our study may explain the higher incidence of viral reactivations found in this population. Indeed, ATG is known to increase the risk of infection after HSCT, especially EBV reactivation [18-20]. Nevertheless, we observed no significant difference between patients receiving ATG and those not receiving ATG in terms of the incidence of viral reactivations.

The cumulative incidence of cGVHD in our study was in line with previous studies, which reported a cumulative incidence ranging from 7% to 56% [1,2,9,16,17,29,31,32,34-36]. However, the cumulative incidence of grade II-IV aGVHD was higher in our patients compared with that reported by Bacigalupo et al [16] in patients who received MAC in a haplo-HSCT setting with PT-Cy (27.5% versus 18%). Possible explanations for this difference include the use of PBSCs in the majority of our patients and our sole use of a TBF conditioning regimen, in contrast to the fludarabine-TBI used in 38% of patients in the study of Bacigalupo et al [16]. Indeed, our results are in line with the reported cumulative incidence of grade II-IV aGVHD in MAC haplo-HSCT with PBSC, which ranges from 15% to 43% [2,31,33,34]. Three retrospective studies have confirmed that the use of PBSCs increases the risk of aGVHD in haplo-HSCT with PT-Cy [35-37], and the cumulative incidence was higher with PBSCs but not significantly different than with BM in another study [29]. Interestingly, a low thiotepa dose of 5 mg/kg significantly reduced the risk of aGVHD and cGVHD without compromising outcomes. Based on these data, we found no benefit of a thiotepa dose of 10 mg/kg compared with 5 mg/kg. Our results are in line with previous studies using a melphalan-based conditioning regimen with thiotepa at 5 mg/kg, which found that a thiotepa dose of 10 mg/kg was not necessary to provide disease control [38,39].

Our data show for the first time the benefit of ATG on aGVHD in a haplo-HSCT setting with PT-Cy. Patients in our cohort who received both ATG and PT-Cy had a similar cumulative incidence of aGVHD with PBSCs as in studies reporting BM haplo-HSCT without ATG. In contrast to our present findings, ATG had no impact on GVHD and had detrimental effects

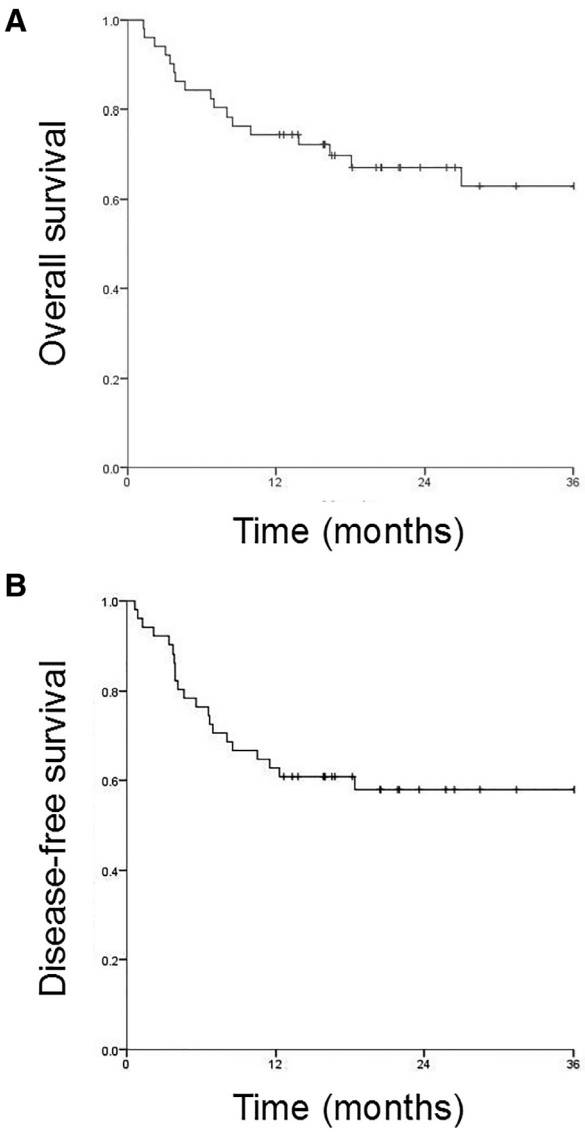

Figure 1. Kaplan-Meier estimates of OS (A) and DFS (B).

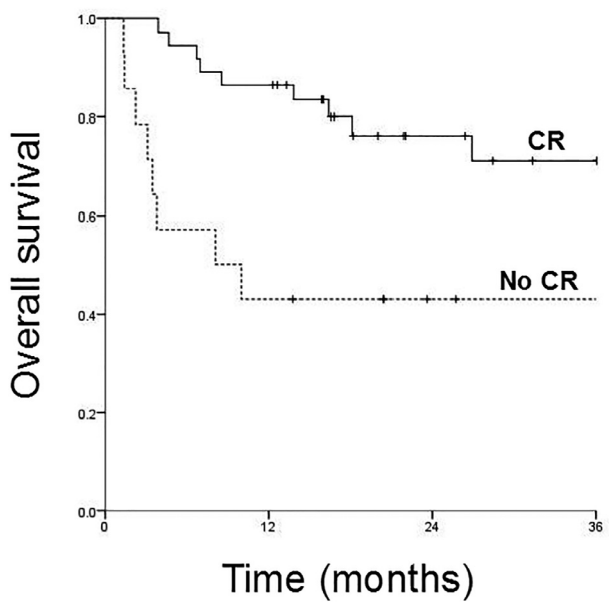

Figure 2. Kaplan-Meier estimates of OS according to disease status at transplantation.

on NRM, leukemia-free survival, and OS in a trial including patients with secondary AML undergoing unmanipulated haplo-HSCT compared with patients receiving PT-Cy without ATG; however, patients receiving both ATG and PT-Cy were excluded from that study [40]. We recently published a series of patients undergoing HSCT for the treatment of refractory hematologic malignancies after sequential conditioning with thiotepa and ATG [10], reporting no significant difference in terms of toxicity and outcomes between haplo-HSCT with PT-Cy and MRD. Compared with MUD, the outcomes were improved in haplo-HSCT in terms of the incidence of aGVHD and GFRFS. Despite the use of ATG in patients with progressive disease, the 2-year RI was 36% in haplo-HSCT recipients. This rate is comparable to the reported RIs of 33% in patients with active disease who underwent MAC haplo-HSCT without ATG and 37% in those treated with the FLAMSA regimen [17,41]. Overall, ATG in haplo-HSCT with PT-Cy seems to reduce the risk of aGVHD without compromising outcomes. Owing to the limited number of patients who did not receive ATG in our study, these results need to be validated in larger cohorts and in well-designed prospective randomized trials.

The NRM in our study is in the higher range of the reported NRM in previous studies on haplo-HSCT with PT-Cy, in which 1-year NRM ranged from 10% to 22% [1-3,9,16,17,29,31,32,37,42]. However, our study included only 53% patients in CR1. The NRM was significantly reduced to 14% in patients in CR. Our results are in line with the 26% incidence of NRM at 6 months reported with TBF haplo-HSCT in patients not in CR [17]. For the entire patient group, the outcomes compare favorably to the results of haplo-HSCT with PT-Cy using the Baltimore RIC regimen [1]. The TBF conditioning regimen allowed for favorable outcomes in patients in CR, in line with previous studies on MAC haplo-HSCT. However, our data in patients in partial response or with progressive disease and the results of recent studies suggest that those patients would benefit more from a sequential approach including early post-transplantation immune intervention combining prophylactic donor lymphocyte infusion with preventive targeted therapies [10,43,44].

In conclusion, our study confirms that the TBF conditioning regimen is an appealing platform in a haplo-HSCT setting with PT-Cy. Engraftment was sustained in all patients, and toxicities were manageable and similar to those reported with other MAC in haplo-HSCT. A thiotepa dose of 10 mg/kg was more toxic than a dose of 5 mg/kg, mainly because of the induction of aGVHD, with no apparent benefit in terms of disease control. Although the use of PBSCs might have increased the cumulative incidence of aGVHD, the cumulative incidence of cGVHD and the outcomes were similar to those with BM. Most notably, the addition of ATG appeared to be effective in reducing the cumulative incidence of aGVHD without increasing the RI or the risk of infectious complications.

ACKNOWLEDGMENTS

We thank the nursing staff for providing excellent care for our patients.

Financial disclosure: The study was supported by the Association for Training, Education and Research in Hematology, Immunology, and Transplantation.

Authorship statement: M.M. designed the study, supervised research, analyzed data, and wrote the manuscript.

J.B. and R.D. designed the study; collected, assembled, and analyzed data; performed statistical analysis; and wrote the manuscript.

A.R. and A.P. analyzed data, performed statistical analysis, and commented on the manuscript.

F.M., E.B., C.M., F.G, G.B., A.B., Z.V.d.W., T.L., R.B., S.S., A.V., R. A., F.I., O.L., and M.T.R. recruited patients and wrote and commented on the manuscript;

All authors approved submission of the final manuscript for publication purposes.

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