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ARTICLE

Treosulfan compared to busulfan in allogeneic haematopoietic stem cell transplantation for myelofibrosis: a registry-based study from the Chronic Malignancies Working Party of the EBMT

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We aimed to compare outcomes following treosulfan (TREO) or busulfan (BU) conditioning in a large cohort of myelofibrosis (MF) patients from the EBMT registry. A total of 530 patients were included; 73 received TREO and 457 BU (BU ≤ 6.4 mg/kg in 134, considered RIC, BU > 6.4 mg/kg in 323 considered higher dose (HD)). Groups were compared using adjusted Cox models. Cumulative incidences of engraftment and acute GVHD were similar across the 3 groups. The TREO group had significantly better OS than BU-HD (HR:0.61, 95% CI: 0.39–0.93) and a trend towards better OS over BU-RIC (HR: 0.66, 95% CI: 0.41–1.05). Moreover, the TREO cohort had a significantly better Progression-Free-Survival (PFS) than both the BU-HD (HR: 0.57, 95% CI: 0.38–0.84) and BU-RIC (HR: 0.60, 95% CI: 0.39–0.91) cohorts, which had similar PFS estimates. Non-relapse mortality (NRM) was reduced in the TREO and BU-RIC cohorts (HR: 0.44, 95% CI: 0.24–0.80 TREO vs BU-HD; HR: 0.54, 95% CI: 0.28–1.04 TREO vs BU-RIC). Of note, relapse risk did not significantly differ across the three groups. In summary, within the limits of a registry-based study, TREO conditioning may improve PFS in MF HSCT and have lower NRM than BU-HD with a similar relapse risk to BU-RIC. Prospective studies are needed to confirm these findings.

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INTRODUCTION

Myelofibrosis (MF) is a heterogeneous "Philadelphia Chromosome negative" myeloproliferative neoplasm characterized by marrow fibrosis, debilitating constitutional symptoms, bulky splenomegaly due to extramedullary hematopoiesis and frequent cytopenias. Weight loss, night sweats, significant fatigue, and bone pain are common disease-related symptoms that can considerably impact upon quality of life (QoL). Multiple risks are common throughout the disease course including impaired performance status due to disease-related symptoms, bleeding and thromboembolic complications, transfusion-related iron overload, infectious complications, shortened survival, and an inherent risk of leukemic transformation.

Prognostic scoring systems, such as the widely used Dynamic International Prognostic Scoring System (DIPSS) or mutation-enhanced IPSS-70 (MIPSS-70 scores) help guide therapy decisions and estimate survival in MF [1–3]. Median predicted survivals in primary MF ranged from more than 10 years in lower risk patients to less than 24 months in high risk patients [1, 3, 4]. Integration of both molecular and cytogenetic data has led to refinement of these scores. Regarding treatment options, most global experience to date has been with the JAK1/2 inhibitor ruxolitinib

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which can induce marked symptom improvement and spleen responses, with consequent improvements in QOL, but typically without significant effect on marrow fibrosis and, therefore are not curative. The median duration of response to ruxolitinib is 3.5–4 years and patients who have nonoptimal response at 6 months have median survival of <3 years [5, 6]. Although other JAK inhibitors and targeted therapies are increasingly available, only allogeneic hematopoietic stem cell transplantation (HSCT) can induce a sustained complete response [7]. However, only a select number of patients are suitable candidates and the non-relapse mortality (NRM) following HSCT remains considerable, often ≥20% at 3 years and hence HSCT is proposed only for those patients where the estimated risk of death directly due to MF is predicted higher than mortality related to the procedure [8, 9].

NRM is particularly high in MF patients due to the relatively older recipient age at time of transplantation, the frequent co-existence of bulky splenomegaly and hepatic disease, and their propensity to have higher rates of graft rejection and prolonged poor graft function post-transplant. In this regard, many different conditioning regimens have been trialed in attempts to limit toxicity and risks of severe acute graft-versus-host-disease (GVHD) without an increased risk of primary/secondary rejection or relapse. However, even in 2023, we cannot confidently recommend one regimen as demonstrating clear superiority. Busulfan (BU)- or melphalan-based conditioning regimens have been the most frequently utilized, particularly in the limited number of prospective studies [7, 10, 11]. A large retrospective study from the CMWP of the EBMT including 2224 MF patients who underwent HSCT found no significant differences in survival outcomes when comparing myeloablative (MAC) and reduced intensity (RIC) regimens [12]. Previous studies have reported that the intensity of the conditioning regimen is correlated with donor chimerism i.e., higher full donor chimerism was observed in patients receiving higher-intensity conditioning regimens [13]. In this regard, melphalan-based regimens tend to demonstrate better donor chimerism rates and less relapse than RIC busulfan-based regimens but are associated with higher NRM rates [14–16]. Thiotepa has been also proposed as part of the conditioning regimens for MF HSCT but there remains a lack of robust evidence demonstrating superiority when compared to busulfan [17, 18]. Encouraging results have been reported through retrospective studies with the addition of thiotepa to busulfan and fludarabine, demonstrating good engraftment and event-free survival (EFS) rates [19, 20]. The use of treosulfan (TREO)-based conditioning has rarely been reported in HSCT for MF [21] but several phase 2 studies have reported low NRM rates when incorporated into HSCT conditioning for other disorders [22–25]. Furthermore, remarkable results in cases of second HSCT for MF have been reported [26]. The EBMT Chronic Malignancies Working Party (CMWP) has previously reported that fludarabine plus treosulfan conditioning is associated with a better overall outcome than RIC or MAC in myelodysplastic syndrome (MDS), due to a lower risk of relapse compared to RIC and a lower risk of NRM than MAC [27]. Recently, a phase 3 randomized trial comparing treosulfan or busulfan plus fludarabine as conditioning for MDS or acute myeloblastic leukemia (AML) reported a better event-free survival (EFS) in the treosulfan cohort; 2-year EFS was 64% vs 50% and 2-year NRM was 12% and 28%, respectively [28]. In the current EBMT study, we aimed to explore if treosulfan-based regimens are superior to busulfan-based conditioning in HSCT in patients with MF.

METHODS

This was a retrospective, multicenter, registry-based analysis approved by the CMWP of the EBMT. The EBMT is a non-profit, scientific society representing more than 600 transplant centers mainly in Europe. Data are entered, managed, and maintained in a central database with internet access; each EBMT center is represented in this database. Patient selection included adult patients transplanted between 2010 and 2018 for primary MF or post-polycythemia vera or essential thrombocythemia MF who received a conditioning regimen containing busulfan or treosulfan. Patients who had MF transformed into acute myeloblastic leukemia were excluded. RIC or MAC were defined by standard EBMT criteria (>6.4 mg/kg IV busulfan for MAC) as previously recommended [29, 30]. During the study period, 215 centers with 1589 patients (173 treosulfan) met the original inclusion criteria. Only centers who confirmed their data and were able to confirm the busulfan dosage administered intravenously participated in the study leading to 63 centers and 536 patients. A total of 51 centers used busulfan only, three used treosulfan only, and nine used both. From an initial total of 536 patients, six patients were excluded because of insufficient available data (on either regimen or disease status after transplantation). A final cohort of 530 patients were thus analyzed within this study. Centers were asked to complete disease status at transplantation: partial remission, stable, clinical improvement including spleen response or progression or relapse according to the International Working Group consensus criteria [31]. Patients with clinical improvement were further considered in the same group same stable patients for the analysis.

Statistical methods

The main endpoints of interest were Overall Survival (OS), Progression-Free Survival (PFS), and the cumulative incidence of Relapse and NRM (mutually competing), all measured since transplantation. Other endpoints were the cumulative incidence of engraftment and of acute and chronic GVHD (competing events for GVHD were death, primary or secondary graft failure, relapse, second HSCT; cGVHD from day 60). Standard methods were used for descriptive statistics and unadjusted comparisons [32]. For the adjusted analysis of the (cause-specific) hazards of the main endpoints, we applied Cox regression. Variables considered for inclusion were patient sex, calendar year of HSCT, type of donor, donor age, patient-donor gender mismatch, patient-donor CMV combination, splenomegaly and splenectomy at HSCT, HCT-CI at HSCT, Karnofsky score at HSCT, interval diagnostic to transplantation, use of ATG, use of JAK-2 inhibitor, use of immunomodulatory drugs. Due to the presence of missing values, we performed both the Complete Case analysis, the missing indicator method, and MICE multiple imputation, reaching consistent conclusions [32].

RESULTS

Population

Patient and transplant characteristics are detailed in Table 1. Patients were categorized according to the regimen they received: treosulfan (TREO, $n = 73$) based regimen or busulfan ($n = 457$). Most patients received fludarabine as a single additional drug: 69/73 (95%) TREO and 375/457 (82%) BU. Thirty three patients treated by BU did not receive fludarabine and they received the combination of cyclophosphamide–busuflan for 31 of them. among them According to a recent EBMT classification on regimen intensity [33], TREO patients received low dose (30 g/m^2) ($n = 19$, 26.0%), intermediate dose (36 g/m^2) ($n = 3$, 4.1%) or (42 g/m^2) ($n = 51$, 69.9%) with the median dose for the whole cohort being 42 g/m^2. Regarding patients who received BU, all received intravenous BU, 134 received low dose of bu ≤6.4 mg/kg and were considered RIC [29, 30]. Among patients who received higher doses of busulfan (BU-HD), only 57 (18%) received high doses (≥12.8 mg/kg), all other patients received intermediate doses (82%) [33]. The majority of patients received HSCT after 2014. Median follow-up of alive patients was 43, 51, and 57 months in the TREO, BU-RIC, and BU-HD groups, respectively. The main differences between the three groups were as follows: (1) Recipient age; patients were older in the BU-RIC group (median age 61 years, (IQR: 55–65)), the youngest being the BU-HD group (56 years, (IQR: 51–62)), and intermediate in the TREO group (59 years, (IQR: 54–64)); (2) Performance status; Karnofsky score was more frequently ≤80 in BU-RIC, (3) Comorbidity score (HCT-CI) was the lowest among the BU-HD group (4) Disease status; patients in the TREO group were more often transplanted with progressive disease; most patients were stable or with clinical response (only 27 isolated

Table 1. Patient and transplantation characteristics.

Variables	Busulfan >6.4 mg/kg	Busulfan RIC ≤6.4 mg/kg	Treosulfan	*P* value
Total number	323	134	73	
Median age (IQR)	56 (51–62)	61 (55–65)	59 (54–64)	<0.001
Period of HSCT				
2010–2014	128 (39.6)	47 (35.1)	28 (38.4)	0.158
2015–2018	195 (60.4)	87 (64.9)	45 (61.6)	
Recipient sex at birth				
Male (%)	209 (64.7)	86 (64.2)	38 (52.1)	0.121
Female (%)	114 (35.3)	48 (35.8)	35 (47.9)	
MF classification				
Primary myelofibrosis	226 (70.0)	91 (67.9)	50 (68.5)	0.900
Secondary myelofibrosis	97 (30.0)	43 (32.1)	23 (31.5)	
Disease-related symptoms				
No	112 (36.8)	55 (43.3)	25 (35.7)	<0.001
Yes	192 (63.2)	72 (56.7)	45 (64.3)	
Spleen				
No splenomegaly	49 (17.4)	29 (23.4)	16 (24.6)	0.176
Palpable splenomegaly	209 (74.1)	79 (63.7)	45 (69.2)	
Splenectomy	24 (8.5)	16 (12.9)	4 (6.2)	
Karnofsky score				
>80	219 (69.7)	74 (57.8)	56 (80.0)	0.004
≤80	95 (30.3)	54 (42.2)	14 (20.0)	
HCT-CI score				
Low risk	178 (55.6)	59 (44.7)	27 (37.5)	0.031
Intermediate risk	74 (23.1)	41 (31.1)	26 (36.1)	
High risk	68 (21.3)	32 (24.2)	19 (26.4)	
JAK2 inhibitor before HSCT				
No	152 (49.7)	72 (57.6)	27 (38.6)	0.038
Yes	154 (50.3)	53 (42.4)	43 (61.4)	
Disease status at HSCT				
Partial remission	43 (14.7)	10 (8.2)	2 (2.8)	<0.001
Stable disease[a]	162 (55.7)	70 (57.4)	28 (38.9)	
Progressive disease	86 (29.6)	42 (34.4)	42 (58.3)	
Driver mutation				
JAK2 mutation	186 (65.3)	84 (70)	44 (64.7)	0.622
MPL mutation	10 (3.5)	5 (4.2)	4 (5.9)	0.666
CALR mutation	43 (15.1)	18 (15.0)	8 (11.8)	0.776
Triple negative	52 (18.2)	16 (13.3)	13 (19.1)	
Unknown	38 (11.8)	14 (10.4)	5 (6.8)	
Chromosome abnormalities				
Yes	119 (52.7)	53 (50.0)	27 (46.6)	0.688
No	107 (47.3)	53 (50.0)	31 (53.4)	
DIPSS				
Low	11 (4.7)	4 (4.1)	3 (4.6)	0.948
Intermediate-1	47 (20.2)	20 (20.4)	13 (20.0)	
Intermediate-2	87 (37.3)	36 (36.7)	29 (44.6)	
High	88 (37.8)	38 (38.8)	20 (30.8)	
CIBMTR score				
Low	98 (31.5)	44 (34.9)	22 (30.1)	0.595
Intermediate	185 (59.5)	72 (57.1)	48 (65.8)	
High	28 (9)	10 (7.9)	3 (4.1)	

Table 1. continued

Variables	Busulfan >6.4 mg/kg	Busulfan RIC ≤6.4 mg/kg	Treosulfan	*P* value
GVHD prophylaxis				
CNI + methotrexate	150 (47.2)	65 (53.3)	34 (46.6)	
CNI + MMF	92 (28.9)	38 (31.1)	31 (42.5)	
CNI alone	43 (13.5)	12 (9.8)	2 (2.7)	
PTCY ± other	24 (7.5)	4 (3.3)	1 (1.4)	
Other	9 (2.8)	3 (2.5)	5 (6.8)	
T cell depletion				
Antithymoglobulin	242 (74.9)	90 (67.2)	62 (84.9)	0.572*
Alemtuzumab	15 (4.6)	17 (12.7)	0	
No TCD	66 (20.4)	27 (20.1)	11 (15.1)	
Donor type				
HLA matched sibling	115 (35.6)	38 (28.3)	13 (17.8)	<0.001
Matched unrelated donor	149 (46.1)	71 (53.0)	55 (75.3)	
HLA mismatched related	10 (3.1)	4 (3.0)	0	
HLA mismatched unrelated	49 (15.2)	21 (15.7)	5 (6.9)	
Recipient male/donor female				
No	271 (83.9)	113 (84.3)	68 (93.2)	0.123
Yes	52 (16.1)	21 (15.7)	5 (6.8)	
CMV serology recipient/donor				
−/−	88 (27.7)	47 (35.6)	30 (41.1)	0.031
−/+	25 (7.9)	15 (11.4)	2 (2.7)	
+/−	56 (17.6)	27 (20.5)	13 (17.8)	
+/+	149 (46.9)	43 (32.6)	28 (38.4)	

RIC reduced intensity conditioning regimen, *HSCT* allogeneic hematopoietic stem cell transplantation, *MF* myelofibrosis, *HCT-CI* hematopoietic stem cell transplantation comorbidity index, *CNI* calcineurin inhibitor, *MMF* Mycophenolate mofetil, *PTCY* post-transplantation cyclophosphamide.
**p* value for the comparison TCD vs no TCD.
[a]Stable or with clinical improvement (including spleen response), secondary myelofibrosis are post polycythemia Vera or post essential thrombocythemia myelofibrosis.

spleen response) but TREO patients were less often stable or in PR; (5) JAK inhibitor prior to HSCT; a higher proportion of patients in the TREO group were treated with a JAK inhibitor before HSCT (6) Cytomegalovirus (CMV) donor/recipient status; CMV combinations were more frequently donor recipient −/− in the TREO cohort (7) an HLA matched sibling donor was less frequently used in TREO group. The 3 groups were well balanced regarding period of transplantation, MF classification (primary versus secondary), splenomegaly at time of transplantation, chromosome abnormalities, MF-related symptoms, DIPSS score, CIBMTR pre HSCT score [34], GVHD prophylaxis and use of in vivo T-cell depletion (a majority of antithymoglobulin).

Outcomes after transplantation

Median time to neutrophil recovery was 17 days (IQR: 15–21) for the whole group, and 14 (IQR: 14–20), 15 (IQR: 15–21) and 16 days (IQR:16–24) in the BU-HD, BU-RIC, and TREO cohorts, respectively. Median time to reach platelet $>50 \times 10^9$ /L was 27 (IQR: 19–72), 26 (IQR: 18–44.5) and 34.5 days (IQR: 19.5–88) in the BU-HD, BU-RIC, and TREO cohorts, respectively. Cumulative incidence of engraftment at day 30 was 91.5% (95% CI: 89.1–93.9) for the entire group and 93.1% (90.4–95.9), 89% (83.5–94.4), 88.9% (81.6–96.1) in BU-HD, BU-RIC and TREO groups. Grade 2–4 acute GVHD occurred in 117 patients in the entire cohort, at a median of 31 days (range, 7–117) after transplantation. Cumulative incidence of grade 2–4 acute GVHD at 120 days for the entire group was 23% (95% CI: 19–26%) without significant difference between the three conditioning cohorts (Table 2). Cumulative incidence of chronic GVHD at 3 years was 43% (38–47%), again with no significant difference between the three cohorts.

A total of 233 deaths occurred during the follow-up, most of them attributed to NRM ($n = 199$). Causes of death are available in Table 1S. GVHD was more frequently the cause of death in BU-HD (32.9%) than in BU-RIC (21.7%) or TREO (22.2%). Three-year OS estimate was 62% (95% CI 58–66) for the whole group and 61% (55–66), 60% (51–68) and 71% (61–82) in BU-HD, BU-RIC and TREO cohorts, respectively (Fig. 1). Median survival was 104 months in the TREO cohort, 91 months in the BU-HD cohort and 82 months in the BU-RIC cohort. Three-year PFS was 49% (45–53) in the whole group and 47% (42–53), 47% (38–56) and 62% (50–73) in BU-HD, BU-RIC and TREO cohorts. Median time to relapse was 6 months (0.36–126.32). Cumulative incidence of relapse at 3 years were 26% (22–29) in the whole group and 26% (21–30), 29% (21–37), 20% (11–29) in the BU-HD, BU-RIC and TREO cohorts, respectively. NRM estimate at 3 years was 25% (21–29) in the whole group and 27% (22–32), 24% (17–31), and 18% (9–27) in the BU-HD, BU-RIC, and TREO, cohorts, respectively. A total of 48 patients received a second HSCT, mainly for relapse ($n = 33$). Cumulative incidence of patients requiring a second HSCT was similar across the 3 groups: 9% (2–16%) in TREO, 9% (6–12%) in BU-HD, and 11% (5–16%) in BU-RIC.

Adjusted analysis of the role of conditioning regimen

Multiple variables models adjusted on regimens, recipient age, disease stage at transplantation, *CALR* mutation status, karyotype, and DIPSS score are available in the Supplementary Data. Doing the same analyses only in patients who did not receive alemtuzumab gave same results (data not shown). The model with multiple imputations showed that the TREO cohort had a significant better OS than BU-HD (HR: 0.61, 95% CI: 0.39–0.93) and

Table 2. Outcome in the 3 different groups.

	3-year Overall survival	3-year PFS	3-year Relapse	3-year Non-Relapse Mortality	30-day Neutrophil recovery	120-day Acute GVHD	3-year Chronic GVHD
All patients	62% (58–66)	49% (45–53)	26% (22–29)	25% (21–29)	91% (89–94)	23% (19–26)	42% (37–46)
Treosulfan	71% (61–82)	62% (50–73)	20% (11–29)	18% (9–27)	89% (82–96)	25% (14–35)	40% (28–52)
Busulfan HD[a]	61% (55–66)	47% (42–53)	26% (21–30)	27% (22–32)	93% (90–96)	23% (19–28)	43% (37–49)
Busulfan RIC	60% (51–68)	47% (38–56)	29% (21–37)	24% (17–31)	89% (83–94)	20 (13–27)	39% (30–48)

OS, PFS, relapse, NRM estimates are given at 3 years; neutrophil recovery is given at 30 days, grade 2–4 acute GVHD in given at 120 days, while chronic GVHD incidence is given at 3 years. 95% confidence intervals are added in parentheses.
[a]HD: busulfan doses >6.4 mg/kg.

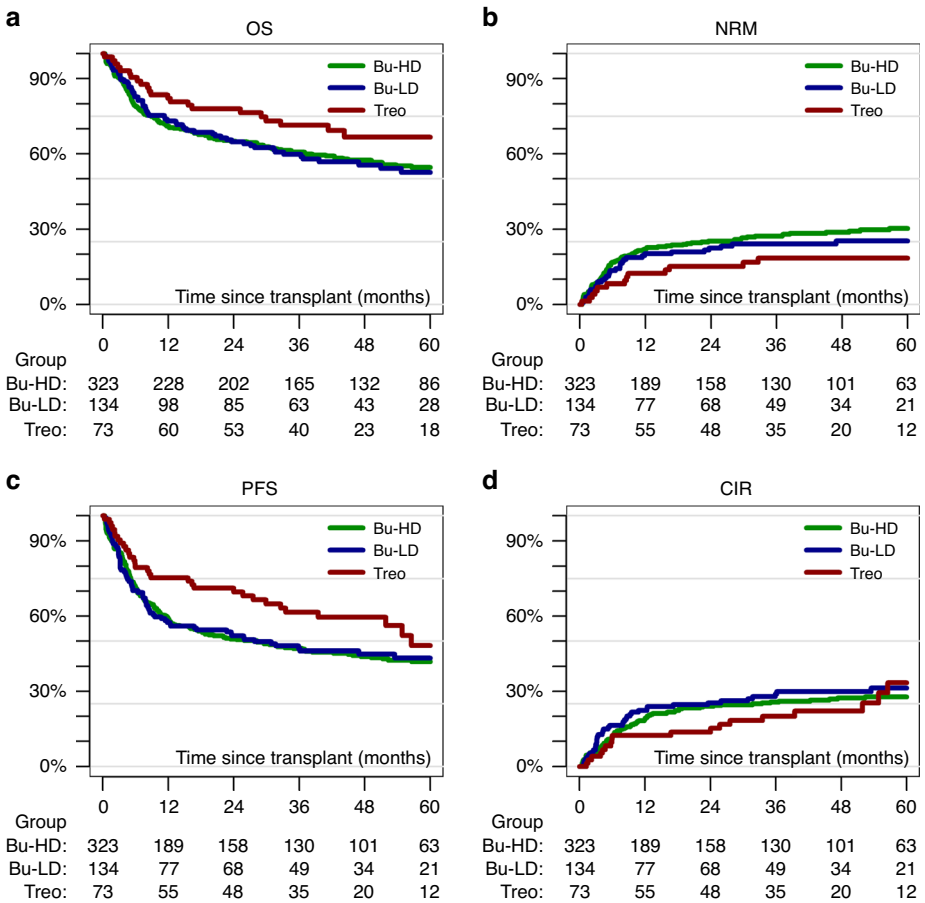

Fig. 1 Outcome according to conditioning regimen. Figure shows overall survival (**a**), non-relapse mortality (**b**), progression-free survival (**c**), and cumulative incidence of relapse (**d**) according to BU doses >6.4 mg/kg (green), BU <or = 6.4 mg/kg (blue) and TREO (red).

a trend for a better OS than BU-RIC (HR: 0.66, 95% CI: 0.41–1.05) (Table 3). BU-RIC had similar risk of mortality than BU-HD (HR: 0.92, 95% CI: 0.68–1.26). The same trend was also observed for PFS with a significant advantage of TREO over BU-HD (HR: 0.57, 95% CI: 0.38–0.84) and BU-RIC (HR: 0.60, 95% CI: 0.39–0.91). PFS was not significantly different between BU-RIC and BU-HD (HR: 0.95, 95% CI: 0.72–1.26) (Table 3). TREO had a significant lower NRM than BU-HD (HR: 0.44, 95% CI: 0.24–0.80) and there was a trend for a lower NRM when compared to BU-RIC (HR: 0.54, 95% CI: 0.28–1.04). TREO had a similar relapse risk to BU-HD and BU-RIC (TREO vs BU-HD, HR: 0.71, 95% CI: 0.42–1.20; TREO vs BU-RIC, HR: 0.63, 95% CI: 0.36–1.11). Other parameters variably influencing the outcomes were the disease status at time of transplantation and *CALR* mutation status (Table 3).

DISCUSSION

This retrospective EBMT registry-based study is the largest to date comparing outcomes following either treosulfan or busulfan-based regimens in MF HSCT. We established three groups which were compared: treosulfan group, busulfan RIC, and busulfan non-RIC based on participating centers. The consideration of BU doses is based on last EBMT recommendations stipulating that patients who received less than 6.4 mg/kg received a low dose while

Table 3. Multiple variables Cox models with multiple imputations.

	Overall survival	*P* value	PFS	*P* value	NRM	*P* value	Relapse	*P* value
BU-RIC vs BU-HD	0.92 (0.68–1.26)	0.619	0.95 (0.72–1.26)	0.722	0.80 (0.54–1.20)	0.286	1.11 (0.76–1.64)	0.580
Treosulfan vs BU-HD	0.61 (0.39–0.93)	0.023	0.57 (0.38–0.84)	0.005	0.44 (0.24–0.80)	0.007	0.71 (0.42–1.20)	0.194
Treosulfan vs BU-RIC	0.66 (0.41–1.05)	0.076	0.60 (0.39–0.91)	0.016	0.54 (0.28–1.04)	0.067	0.63 (0.36–1.11)	0.111
Age	1.02 (1.00–1.03)	0.075	1.01 (0.99–1.02)	0.469	1.01 (0.99–1.03)	0.238	1.00 (0.98–1.02)	0.865
Disease stage								
Partial response	1		1		1		1	
Stable	1.32 (0.79–2.18)	0.283	1.16 (0.76–1.77)	0.481	1.11 (0.61–2.01)	0.722	1.23 (0.68–2.24)	0.488
Progressive	1.82 (1.07–3.08)	0.027	1.53 (0.99–2.37)	0.058	1.67 (0.90–3.11)	0.102	1.43 (0.76–2.72)	0.268
CALR								
Wild type	1		1		1		1	
Mutated	0.66 (0.41–1.06)	0.085	0.59 (0.39–0.90)	0.013	0.58 (0.32–1.05)	0.069	0.60 (0.33–1.09)	0.094
Karyotype								
Normal	1		1		1		1	
Abnormal	1.17 (0.86–1.61)	0.316	1.29 (0.98–1.69)	0.073	0.87 (0.57–1.31)	0.490	1.83 (1.25–2.68)	0.002
DIPSS								
Low-int1	1		1		1		1	
Int-2	1.32 (0.89–1.96)	0.170	1.40 (0.98–2.00)	0.068	1.51 (0.90–2.54)	0.118	1.25 (0.77–2.04)	0.361
High	1.36 (0.93–1.99)	0.116	1.36 (0.96–1.91)	0.082	1.35 (0.82–2.22)	0.236	1.38 (0.86–2.21)	0.178

patients received 9.6 mg/kg received an intermediate dose and those received 12.8 mg/kg a high dose. However, there is a gradation of the intensity of conditioning regimen between RIC and MAC. Indeed, patients with MF often received 2 days and half busulfan doses (8 mg/kg) on the basis of the German prospective trial [35] which is considered dose reduced as compared to full doses. We were not able to analyze the impact of all different doses of busulfan due to the myriad of doses used and small groups which had to be compared. In addition, outcome of patients received BU RIC or BU higher dose were very close, confirming that the intensity of BU may not have a major impact on general outcome. The TREO group had significantly better OS than BU-HD and a trend towards a better OS over BU-RIC. PFS was significantly better with TREO conditioning than with either BU-HD or BU-RIC. Relapse rates were similar between the TREO, BU-HD, and BU-RIC cohorts, suggesting that the differences are not linked to relapse risk but more to NRM. However, these conclusions may be taken with caution regarding the role of TREO on disease control. Indeed, more TREO patients had progressive disease at time of transplant which did not translate in a higher risk of relapse in this study. We could also show that the NRM rates were significantly lower in the TREO cohort compared to those undergoing BU-HD and there was a trend towards a lower NRM as compared to BU-RIC (p value = 0.067). These findings are in line with the recent randomized trial demonstrating that patients with myeloid disease undergoing HSCT had lower mortality using TREO-based conditioning compared with BU-RIC protocols [28, 36]. This randomized trial included patients with MDS or AML with a median age of 60 years and findings are in contrast to a recent registry study which did not show any benefit of treosulfan in this population [37, 38]. A specific study comparing TREO- with BU-based conditioning in patients with MF has yet to be performed. In this rare disease, it is challenging to conduct a randomized trial able to detect a significant difference among 2 arms, therefore, the best conditioning regimen remains largely unknown but some phase 2 prospective studies have been published. The Gruppo Italiano per il Trapianto di Midollo Osseo conducted a phase 2 randomized trial including 62 patients within 2 years and across 21 centers. Patients were randomized to receive either thiotepa and fludarabine or busulfan and fludarabine. At 2 years, OS and PFS were non-significantly better in the thiotepa arm (OS, 54% versus 70% ($P = 0.17$); PFS, 43% versus 55% ($P = 0.28$)) [17]. The phase 2 nature and limited number of patients in this study may have prevented demonstration of any statistical significance. Retrospective studies have generally shown that engraftment and donor chimerism are related to the intensity of the conditioning regimen in HSCT in patients with MF [13]. Unfortunately, we were unable to properly analyze chimerism data in this registry-based study. Conditioning regimen intensity, initially based on definitive myeloablation or reversible myeloablation, has now been challenged by new drugs. While treosulfan was introduced much more recently than for instance cyclophosphamide, busulfan, and melphalan into the conditioning arena, the definition of treosulfan intensity remains somewhat uncertain despite its reduced toxicity profile. Of note, doses of TREO were variable in this series, but high TREO doses (≥42 g/m^2) had not a significant impact on the outcome in univariate analysis (data not shown). These results should be interpreted with caution, due to the small number of subgroup categories precluding any adjusted analysis. Especially, it does not confirm a previous study of escalating dose showing higher toxicity of 42 g/m^2 as compared to 30 g/m^2 [25].

While more intensive conditioning regimens can reduce graft failure, rejection, and relapse rates in MF HSCT, unfortunately, it also increases the risk of NRM, without a benefit on PFS. This is the case when comparing fludarabine-melphalan to fludarabine-busulfan RIC, where relapse/progression is decreased with melphalan but with a detrimental rate of NRM [14, 39]. Similar

findings are reported when comparing 2 days of busulfan to 3 days of busulfan, the relapse rate is decreased by increased dose of busulfan but with an increased NRM without a resultant difference in OS [16]. The absence of a clear benefit of BU-HD over BU-RIC in HSCT in MF was reflected in our study, i.e., there was no difference in outcome between BU-RIC and BU-HD. It is striking that NRM or relapse is not different between BU-RIC and BU-HD in this study. Another potential limitation of our study is the center effect related to specific protocols as well as a potential bias related to participating units which may not reflect all centers. For instance, in this specific cohort, CIBMTR score was not prognostic so it did not reflect a previous EBMT cohort where CIBMTR score had been validated [34]. In addition, our study does not include sufficient haplo-identical donor transplants which currently represents a growing indication, including for MF patients.

To conclude, treosulfan-based conditioning regimens for HSCT in patients with MF offers encouraging results, in particular a better PFS, and compare favorably to conditioning regimens containing busulfan. More homogeneous data are required to confirm these results, including further comparison to various conditioning regimens commonly used.

DATA AVAILABILITY

Data are available on request to the corresponding author.

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AUTHOR CONTRIBUTIONS

MR initiated and proposed the study to the CMWP, MR, TC, NP, JDS, KR, JCHD, and DML were involved in the study design, SI made the statistics, LK made data management, MR, JP, AD, KW, US, PD, PVDB, SR, TCl, SB, JAPS recruited patients and checked the data, all co-authors approved the paper and contributed to the paper.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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