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A B S T R A C T

We report a randomized study comparing fludarabine in combination with busulfan (FB) or thiotepa (FT), as con- ditioning regimen for hematopoietic stem cell transplantation (HSCT) in patients with myelofibrosis. The primary study endpoint was progression-free survival (PFS).

Sixty patients were enrolled with a median age of 56 years and an intermediate-2 or high-risk score in 65%, according to the Dynamic International Prognostic Staging System (DIPSS). Donors were HLA-identical sibling (n = 25), matched unrelated (n = 25) or single allele mismatched unrelated (n = 10). With a median follow-up of 22 months (range, 1 to 68 months), outcomes at 2 years after HSCT in the FB arm versus the FT arm were as follows: PFS, 43% versus 55% (P = .28); overall survival (OS), 54% versus 70% (P = .17); relapse/progression, 36% versus 24% (P = .24); nonrelapse mortality (NRM), 21% in both arms (P = .99); and graft failure, 14% versus 10% (P = .96). A bet- ter PFS was observed in patients with intermediate-1 DIPSS score (P = .03). Both neutrophil engraftment and platelet engraftment were significantly influenced by previous splenectomy (hazard ratio [HR], 2.28; 95% confi- dence interval [CI], 1.16 to 4.51; P = .02) and splenomegaly at transplantation (HR, 0.51; 95% CI, 0.27 to 0.94; P = .03). In conclusion, the clinical outcome after HSCT was comparable when using either a busulfan or thiotepa based conditioning regimen.

INTRODUCTION

Myelofibrosis (MF), including primary and post essential thrombocythemia and polycythemia vera, is a clonal myelo- proliferative disorder with a heterogeneous clinical course, associated with several clinical and biological prognostic fac- tors used to predict survival in several scoring systems [1-3]. These factors can identify patients with a favorable prognosis (median survival of 8 to 10 years) from those with a shorter life expectancy (median survival of 1 to 4 years).

Allogeneic hematopoietic stem cell transplantation (HSCT) is the sole curative treatment for MF and is currently recommended for fit patients with intermediate–2 or high–risk MF up to 70 years of age [4]. The indication to proceed to HSCT did not change for most patients after the discovery of the role of the V617F mutation of the *JAK 2* gene in the pathogenesis of the disease and the positive effects of JAK1/2 inhibitor ruxolitinib [5-7] for alleviating MF symptoms and reducing splenomegaly. However, the toxicity of HSCT remains a major reason for concern, and conventional myeloablative conditioning (MAC) regimens can be proposed for only a small subgroup of young patients. For this reason, they have been replaced by reduced–intensity regimens (RIC) [8-11], which can expand transplantation eligibility to older patients [12,13]. Moreover, the optimal intensity of the conditioning regimen remains to be defined [4]. Randomized clinical trials to address this point have not been carried out because of the lack of a formally recognized standard conditioning regimen. In addition, owing to the rarity of MF as an indication for HSCT, conducting large Phase III studies in a reasonable time period is challenging.

Nonetheless, based on the results obtained in a prospective, multicenter study by the European Group for Blood and Marrow (EBMT), a RIC regimen based on fludarabine and busulfan can currently be considered a reasonable comparator for any prospective study [14]. Thiotepa is an alkylating agent that has been introduced in the conditioning regimen for allogeneic transplantation for its double effect on stem cell depletion and immune suppression [15] even in the context of RIC programs applied to myeloid malignancies, including MF [16-18]. For these reasons, we decided to compare thiotepa + fludarabine with busulfan + fludarabine in a Phase II randomized study, with the aim of collecting prospective controlled data that could provide a basis for future phase III studies.

METHODS

Patient Population

This study was a multicenter, randomized, Phase II trial carried out in 21 hospital transplantation programs in Italy (Appendix 1), coordinated by the Gruppo Italiano per il Trapianto di Midollo Osseo e Terapia Cellulare (GITMO) network. Eligible patients were age 18 to 70 years, had a diagnosis of primary or secondary MF, and had at least 1 of the following unfavorable prognostic factors: hemoglobin <10 g/dL or leukocytes >25 × 10^9/L or >1% circulating blasts or constitutional symptoms, moreover, they had a Karnofsky Performance Status score ≥60, and a Hematopoietic Cell Transplantation-Specific Comorbidity Index (HCT-CI) score <5. Donor inclusion criteria were age 18 to 65 years and an HLA-identical sibling donor or HLA-matched unrelated donor by high–resolution DNA-based HLA-A, –B, C, -DRB1 typing or 1 allele–mismatched (class I) unrelated donor (for recipients up to age 60 years).

We conducted the study in accordance with the International Conference on Harmonization for Good Clinical Practice and the appropriate regulatory requirements. The study was approved by the ethic committees of the participating centers. The trial protocol was in accordance with the Declaration of Helsinki and is registered at www.ClinicalTrials.gov (NCT01814475).

Randomization

Patients were assigned at random (1:1) using a stratified biased coin algorithm with a variable block size strategy to receive the thiotepa + fludarabine or busulfan + fludarabine conditioning regimen. Randomization was centralized at the Fondazione Mario Negri Sud (Santa Maria Imbaro, Chieti, Italy) and was done via a dedicated web-based system with remote data entry. Patients were stratified by donor type (related versus unrelated donor).

Study Procedures

Patients could be treated before transplantation according to the local policy, including the administration of JAK1/2 inhibitors. Splenectomy before transplantation was considered in patients with massive splenomegaly (≥22 cm) [19] in the major diameter at ultrasound scan unresponsive to medical treatment.

The conventional regimen was i.v. fludarabine 30 mg/m^2 on day -8 to day -3; i.v. busulfan (Busilvex; Pierre Fabre Pharma, Toulouse, France) 0.8 mg/kg for 4 doses on days -5 and -4 and for 2 doses on day -3, (total dose, 8 mg/kg). The experimental arm received i.v. fludarabine 30 mg/m^2 on day -8 to day -3 and i.v. thiotepa (Tepadina; Adienne, Lugano, Switzerland) 6 mg/kg for 2 doses on days -4 and -3. Allogeneic stem cell transplantation consisted of the reinfusion of 5 to 10 × 10^6/kg $CD34^+$ stem cells from sibling or unrelated donors. Peripheral blood (PB) was the preferred source of stem cells. Graft versus host disease (GVHD) prophylaxis consisted of cyclosporine and methotrexate on days +1, +3, and +6. Antithymocyte immunoglobulin (ATG; Genzyme, Cambridge, MA) 3.5 mg/kg, on days -3 and -2, was administered to recipients of unrelated donor grafts. No ATG was administered to recipients of matched sibling donor grafts.

The main outcome data were collected and the main assessments of adverse events performed at day +30, day +100, day +180, 1 year, and 2 years after transplantation. At the same time points, the achievement of full donor chimerism (FDC) (defined as >95% of cells being of donor origin as evaluated by molecular analysis of short tandem repeats) was evaluated on bone marrow cells and PB mononuclear cells. Acute GVHD was assessed weekly for the first 3 months after transplantation and graded according to the Glucksberg scale [20]. Chronic GVHD was assessed at each follow-up visit and classified as mild, moderate, or severe according to the National Institutes of Health criteria [21].

First-line treatment of grade II-IV acute GVHD was based on standard 2 mg/kg 6-methylprednisolone for 5 days. In responsive patients, the dose was reduced by 25% every 5 to 7 days, whereas unresponsive patients received second-line treatment in accordance with the protocol at each center.

Outcomes

The primary study endpoint was progression–free survival (PFS), assessed at 1 year after transplantation. PFS was defined as the time from the date of randomization to the date of the first documented disease progression or relapse (according to the criteria of International Working Group for Myelofibrosis Research and Treatment [IWG-MRT] [22]) or death due to any cause. Secondary endpoints for efficacy and safety were cumulative incidence of nonrelapse mortality (NRM), overall survival (OS), rate of clinical hematologic and histologic responses (according to IWG-MRT criteria), rate of molecular remissions in patients with a molecular marker, cumulative incidences of neutrophil and platelet engraftment, and cumulative incidences of acute and chronic GVHD. NRM was defined as death due to any other cause than progression of malignancy after HSCT.

Neutrophil engraftment was defined as the number of days after transplantation taken to achieve an absolute neutrophil count of at least ≥0.5 × 10^9 cells/L and platelet engraftment was defined as the number of days to maintain an untransfused platelet count of at least 20 × 10^9 cells/L. Primary graft failure was defined as the absence of donor cells in the bone marrow by day +30 after transplantation. Secondary graft failure or rejection was defined as the absence of donor cells in the bone marrow by day +60 after transplantation, following an initial hematopoietic chimerism. Adverse events were graded based on the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0.

Statistical Analysis

Because the number of patients with MF referred for HSCT is very limited, we chose our sample size on the basis of feasibility. The GITMO registry allowed a predicting accrual of 20 patients per year, so we calculated a sample size of 60 patients over a 3-year enrollment period.

Baseline categorical characteristics were compared between the FT and FB arms using the chi-square or Fisher exact test. Continuous variables were compared with Mann-Whitney *U* test. PFS and OS were estimated using the Kaplan-Meier method, and the log-rank test was applied to test differences between arms. NRM and cumulative incidence of relapse were estimated using cumulative incidence function, considering relapse and death as a competing event, respectively, and the Fine and Gray nonparametric test was used to assess between-arm differences. The univariate analyses were performed by fitting Cox models, and hazard ratios (HRs) with 95% confidence intervals (CIs) were reported. Acute and chronic GVHD were considered time-dependent variables, and their unadjusted effects on survival outcomes were tested with the Mantel-Byar test. All analyses were done according to the intention-to-treat principle. All reported *P* values were 2–sided, and the conventional 5% significance level was fixed. The Data Safety Monitoring Board for unexpected trends closely monitored the number of treatment failures and serious adverse events. All analyses were done with SAS version 9.4 (SAS Institute, Cary, NC).

RESULTS

Patient Characteristics

A total of 62 patients were enrolled between July 2011 and November 2015. Two patients had a leukemia transformation before conditioning and were excluded from the analysis. Thirty patients were randomized to the standard FB arm and 30 were randomized to the experimental FT arm, constituting the intention-to treat population. Three patients did not

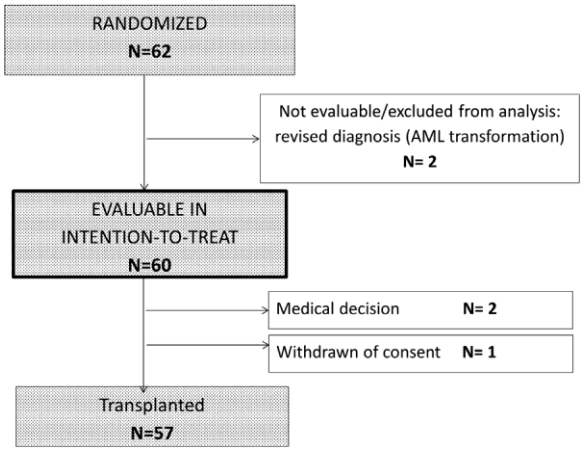

Figure 1. Patients disposition chart.

receive the allocated treatment because of medical decision (2 patients) or withdrawn of consent (1 patient), so the per-protocol population comprised 57 patients (Figure 1). Patient characteristics are summarized in Table 1. The main clinical features were balanced between the FT and FB arms. The only difference is that more patients with an HCT-CI score of ≥3 were allocated to the FT arm than to the FB arm ($P = .07$).

Donors and Stem Cell Grafts

Donors were HLA-matched (10/10 antigens) in all 25 sibling donor transplants and in 25 of 35 unrelated donor transplants. The remaining 10 unrelated donors were mismatched for 1 single allele in HLA class I. The data presented in Table 2 indicate no significant differences in the distribution of the main characteristics of the donors between the 2 treatment arms. Of the 57 patients who proceeded to HSCT, 51 (89%) received a peripheral blood stem cell graft (median dose, 6 × 10^6/kg $CD34^+$ cells), and only 6 patients received a bone marrow graft (median dose, 4.7 × 10^6/kg $CD34^+$ cells).

Engraftment

There was no difference in the cumulative incidence of neutrophil engraftment at 30 days between the FB and FT arms (93% [95% CI, 80% to 99%] versus 89% [95% CI, 75% to 97%]; $P = .93$). However, the cumulative incidence of platelet engraftment at 30 days was significantly higher in the FB arm (82% [95% CI, 75% to 97%] versus 71% [95% CI, 54% to 86%]; $P = .04$] (Figure 2A and B). Donor type, stem cell source, and dose of $CD34^+$ cell dose infused did not have a significant impact on neutrophil and platelet engraftment. However, hematopoietic recovery was significantly influenced by spleen size at transplantation; in fact, patients with splenomegaly before HSCT had significantly slower neutrophil and platelet engraftment (HR, 0.51 [95% CI, 0.27 to 0.94], $P = .03$ versus HR, 0.41 [95% CI, 0.22 to 0.77], $P = .005$), whereas the patients who had undergone previous splenectomy had significantly faster neutrophil and platelet recovery (HR, 2.28 [95% CI, 1.16 to 4.51], $P = .02$ versus HR, 2.49 [95% CI, 1.26 to 4.92], $P = .009$) (Table 3). In patients with splenomegaly, the median times to neutrophil and platelet engraftment were 19 and 20 days, respectively—significantly longer than those in patients who were splenectomized or without spleen enlargement before HSCT (16 and 14 days, respectively; $P < .0001$).

Graft failure was observed in 4 out of 28 (14%) transplantations in the FB arm and in 3 out of 29 (10%) in the FT arm ($P = .96$). Primary graft failure occurred in 5 transplantations (3 after FB conditioning and 2 after FT conditioning), and secondary graft failure was observed in 2 transplantations (1 in each arm). Out of the 7 patients with overall graft failure, 5 had received a transplant from an unrelated donor (3 HLA-matched and 2 HLA mismatched donors), and 6 had received a PB stem cell graft.

GVHD

The cumulative incidence of grade II-IV acute GVHD was 20% (95% CI, 11% to 31%) and of grade III-IV GVHD was 8% (95% CI, 3% to 17%). Skin was the most commonly involved organ (8 out of 17 patients; 47%). The cumulative incidence of overall chronic GVHD at 18 months was 15% (95% CI, 7% to 26%), and that of moderate chronic GVHD was 8% (95% CI, 3% to 17%). No patient developed severe chronic GVHD. Conditioning type, patient age, disease risk, donor type, and stem cell source had no significant impact on the incidence of acute or chronic GVHD incidence.

NRM and Adverse Effects

In the intention-to-treat analysis, the cumulative incidence of NRM was 21% in both the FB and FT arms at 2 years post-HSCT (HR, 0.88; 95% CI, 0.28 to 2.73) (Figure 3A). Six out of 30 patients (20%) in the FB arm and 6 out 30 (20%) in the FT arm died from transplantation-related causes. Causes of death included infections (n = 6), GVHD (n = 2), encephalopathy (n = 2), severe renal insufficiency (n = 1), and poor marrow function (n = 1) and were distributed equally between the 2 arms.

The most common conditioning regimen-related toxic effects assessed within 30 days after transplantation are listed in Table 4. Fewer grade 3-4 adverse events were reported in the FB arm compared with the FT arm (6 versus 11). The most frequently reported grade 3-4 adverse events were infections in the FB arm (4 events; 66%) and gastrointestinal toxicities in the FT arm [5 events (46%)].

Outcomes

At 1 year after HSCT, responses according to the IWT-MRT criteria were evaluable in 17 patients in the FB arm and in 21 patients in the FT arm. The overall response rate was 12 (75%) in the FB arm and 17 (81%) in the FT arm ($P = .70$). Specifically, rates of complete response, partial response, clinical improvement, and stable disease were 41% (n = 10) versus 48% (n = 10), 18% (n = 3) versus 5% (n = 1), 12% (n = 2) versus 14% (n = 3), and 0 versus 14% (n = 3), respectively. Relapse or progressive disease occurred in 5 patients (29%) in the FB arm and in 4 patients (19%) in the FT arm. V617F JAK2 evaluation was available for 55 of the 60 patients (92%), including 37 with mutation (Table 1). Of these patients, 16 could be analyzed for measurable residual disease at 1 year after transplantation; 5 of 6 patients (83%) in the FB arm and 6 of 10 patients (60%) in the FT arm reached a molecular negativity ($P = .59$). With a median follow-up of 22 months (range, 0 to 68 months), by an intention-to-treat analysis, the PFS at 2 years was 43% (95% CI, 25% to 60%) in the FB arm versus 55% (95% CI, 35%-71%) in the FT arm ($P = .28$) (Figure 3B). The respective OS at 2 years in the 2 arms was 54% (95% CI, 34% to 70%) versus 70% (95% CI, 50% to 83%) ($P = .17$) (Figure 3C). At 2 years after randomization, the cumulative incidence of relapse and progression was 36% (95% CI, 18% to 53%) in the FB arm and 24% (95% CI, 10% to 41%) in the FT arm (HR, 0.55; 95% CI, 0.21 to 0.46; $P = .24$). The per-protocol analysis (Appendix 2) was in keeping with that described by the intention-to-treat analysis reported above.

In univariate analysis, we observed that patients with intermediate-2 or higher DIPSS risk at transplantation had a

Table 1
Patient Clinical and Laboratory Characteristics at Transplantation

Characteristic		All (N = 60)	FB Arm (N = 30)	FT Arm (N = 30)	*P* Value
Age at randomization, yr, median (range)		56 (36-66)	55.5 (41-65)	57 (36-66)	.31
Sex, n (%)					
	Female	16 (27)	10 (33)	6 (20)	.24
	Male	44 (73)	20 (67)	24 (80)	
Myelofibrosis, n (%)					
	Primary	33 (55)	17 (57)	16 (53)	.79
	Secondary to PV/ET	27 (45)	13 (43)	14 (47)	
Time between diagnosis and HSCT, d, median (range)		645 (36-6389)	393 (126-6389)	1047 (36-3536)	.17
DIPSS, n (%)					
	Low	0	0	0	.84
	Intermediate-1	21 (35)	10 (33)	11 (37)	
	Intermediate-2	36 (60)	19 (62)	17 (57)	
	High	3 (5)	1 (3)	2 (7)	
Platelets, median (range)		82.5 (4-2388)	56 (4-806)	163.5 (14-2388)	.04
Platelets <100 £ 10^9/L, n (%)		32 (53)	19 (63)	13 (43)	.12
CD34⁺ cells/㎛L in PB, median (range)		2.8 (0-2628)	3.5 (0-419)	2.1 (0-2628)	.94
Splenomegaly, n (%)		45 (75)	23 (77)	22 (73)	.76
Spleen >22 cm, n (%)		7 (16)	5 (21)	2 (9)	.41
Blasts in marrow aspirate, n (%) (N = 44)					
	0	18 (41)	9 (41)	9 (41)	.77
	1-5	20 (45)	9 (41)	11 (50)	
	5-20	6 (14)	4 (18)	2 (9)	
Fibrosis in bone marrow biopsy, n (%) (N = 58)					
	0	3 (5)	1 (3)	2 (7)	.80
	1	9 (15)	6 (21)	3 (10)	
	2	18 (31)	9 (31)	9 (31)	
	3	21 (36)	9 (31)	12 (41)	
	4	7 (12)	4 (14)	3 (10)	
Karyotype					
	Normal	23 (38)	8 (27)	15 (50)	.32
	Unfavorable alterations	5 (8)	3 (10)	2 (7)	
	Other alterations	12 (20)	7 (23)	5 (17)	
	Unknown	20 (33)	12 (40)	8 (27)	
Jak mutation					
	Wild type	18 (30)	8 (27)	10 (33)	.63
	Mutated	37 (62)	19 (63)	18 (60)	
	not done	5 (8)	3 (10)	2 (7)	
Splenectomy, n (%)		12 (20)	5 (17)	7 (23)	.52
Previous erythroid transfusions, n (%)		39 (65)	19 (63)	20 (67)	.79
>20 erythroid transfusions, n (%)		1 (2)	1 (3)	0	1.00
HCT-CI, median (range)		1 (0-6)	0 (0-5)	1 (0-6)	.15
HCT-CI c3, n (%)		14 (23)	4 (13)	10 (33)	.07
Previous therapy, n (%)					
	Hydroxyurea	22 (37)	14 (47)	8 (27)	.56
	Other chemotherapy	3 (5)	3 (10)	0	
	Steroids	9 (15)	6 (20)	3 (10)	
	Ruxolinitib	7 (12)	2 (7)	5 (17)	
	Erythroid-stimulating agents	6 (10)	2 (7)	4 (13)	
	Immune-modulating agents	3 (5)	2 (7)	1	(3)

PV indicates polycythemia vera; ET, essential thrombocythemia.
*DIPSS Plus score was calculated in 20 patients without considering karyotype, which was unavailable.

significantly lower PFS at 2 years compared with patients with intermediate-1 DIPSS risk (38% versus 68%; P = .03), owing to an elevated risk of relapse (38% versus 10%; P = .02). We did not find any other significant associations among PFS, OS, and NRM and the main patient and transplantation characteristics. Specifically, the 2-year NRM, PFS, and OS were 17%, 57%, and 71%, respectively, when HSCT was performed with a HLA-identical sibling donor graft, compared with 24%, 42%, and 56% when HSCT was performed with an unrelated donor graft (P = .48, .23, and .18, respectively). Moreover, no significant difference in transplantation outcomes between matched unrelated donors and mismatched unrelated donors was identified, and the development of acute and chronic GVHD had no significant influence on outcomes (Appendix 3).

Seven patients (2 patients in the FB arm and 5 in the FT arm) received donor lymphocyte infusion at a median of 155 days (range, 32 to 376 days) after HSCT because of mixed chimerism (in 6 patients) or relapse (in 1 patient). Two patients underwent a second HSCT, at 119 days and 458 days after the first transplantation, for graft failure in 1 patient and hematologic relapse in the other.

Table 2
Donor Characteristics

Characteristic	All (N = 60)	FB Arm (N = 30)	FT Arm (N = 30)	*P* Value
HLA matching				
Sibling	25 (42)	13 (43)	12 (40)	.37
Unrelated matched	25 (42)	14 (47)	11 (37)	
Unrelated mismatched	10 (17)	3 (10)	7 (23)	
Age, yr, median (range)	35 (20-66)	37 (20-60)	34 (20-66)	.82
Sex matching, n (%)				
Male donor/male recipient	32 (53)	14 (47)	18 (60)	.54
Female donor/female recipient	5 (8)	4 (13)	1 (3)	
Male donor/female recipient	11 (18)	6 (20)	5 (17)	
Female donor/male recipient	12 (20)	6 (20)	6 (20)	
ABO matching				
Compatibility	34 (57)	18 (60)	16 (53)	.94
Major incompatibility	14 (23)	6 (17)	8 (23)	
Minor incompatibility	12 (20)	6 (20)	6 (20)	
CMV matching*				
CMV^- donor/CMV^- recipient	2 (3)	0	2 (7)	.06
CMV^- donor/CMV^+ recipient	23 (40)	8 (28)	15 (52)	
CMV^+ donor/CMV^- recipient	2 (3)	1 (3)	1 (3)	
CMV^+ donor/CMV^+ recipient	31 (52)	20 (69)	11 (38)	

CMV indicates cytomegalovirus.
* Data are missing for 2 donor-recipient pairs.

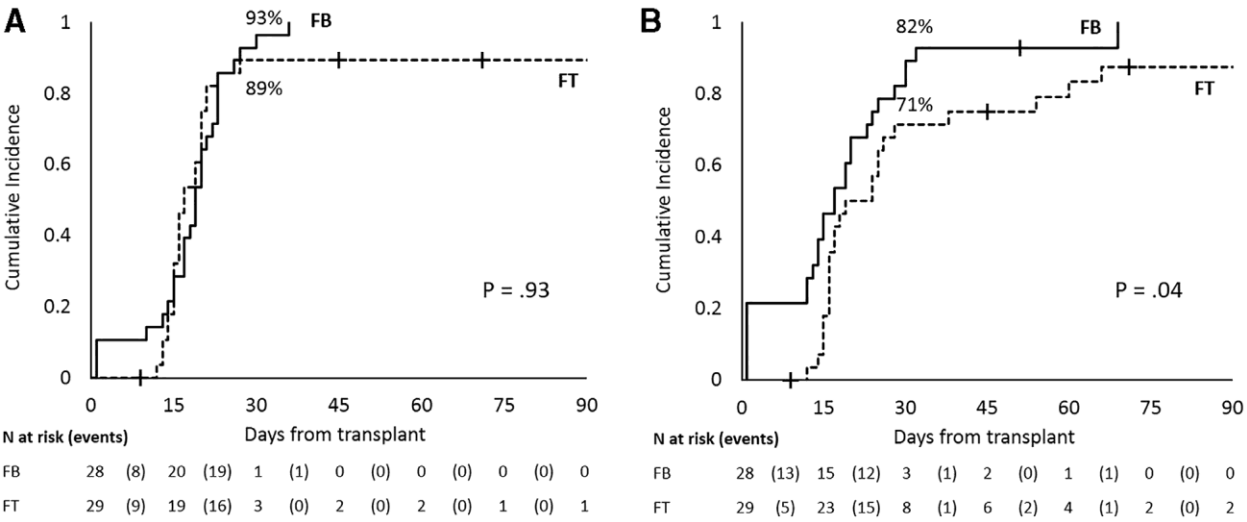

Figure 2. Neutrophil (A) and platelet (B) engraftment.

Chimerism Analysis

There was no significant difference in the rate of FDC in PB at day +30 after HSCT between the 2 treatment arms (62% [13 of 21] in the FB arm versus 86% [19 of 22] in the FT arm; $P = .14$). However, rate of FDC was significantly lower in the FB arm compared with the FT arm at day +100 (24% [5 of 21] in the FB arm versus 68% [13 of 19] in the FT arm; $P = .02$), but the differences were cancelled at subsequent time points (Figure 4). Rates of FDC in PB were similar after HSCT with sibling donor and unrelated donor grafts (sibling transplants: 64% [9 of 14] at day +30 and 50% [9 of 18] at day +100; unrelated transplants: 79% [23 of 29] at day +30 and 41% [9 of 22] at day +100; $P = .49$ and .70, respectively].

DISCUSSION

Only a few prospective trials and several retrospective studies have been conducted in patients with MF undergoing HSCT (Appendix 4). The first prospective study conducted within the EBMT showed a 1-year NRM of 16%, a 5-year disease-free-survival (DFS) of 51%, and a 5-year OS of 67% after receipt of a fludarabine + busulfan (8 mg/kg i.v.) conditioning regimen [14]. The second Phase II prospective trial, supported by the International Myeloproliferative Disorder Research Consortium (MDPC), used a fludarabine + melphalan preparative regimen and reported quite encouraging clinical results after transplants from HLA-matched sibling donors (2-year NRM, 22%; 2-year OS, 75%), but an unexpectedly high NRM of 59%, linked in part to graft failure, affected the outcome after transplantations from unrelated donors (2-year OS, 36%) [23]. As detailed in Appendix 4, more intensive fludarabine-based preparative regimens (including melphalan or 2 alkylating agents or 1 alkylating agent plus TBI) generally reported a higher rate of NRM and lower risk of relapse; on the other hand, less intensive conditioning regimens appeared to be safer, albeit associated with an increased risk of recurrence. Moreover, several retrospective comparisons of different RIC regimens failed to recognize the superiority of 1 preparative treatment over another in terms of OS and PFS [24-28]. In 2010, the GITMO designed the first prospective randomized study with the

Table 3
Univariate Analysis of Factors Influencing Neutrophil and Platelet Engraftment

Variable		Neutrophil Engraftment, HR (95% CI)	*P* Value	Platelet Engraftment, HR (95% CI)	*P* Value
Treatment					
	FB	1.00		1.00	
	FT	0.98 (0.57-1.68)	.93	0.57 (0.33-1.00)	.04
Splenomegaly		0.51 (0.27-0.94)	.03	0.41 (0.22-0.77)	.005
Splenectomy		2.28 (1.16-4.51)	.02	2.49 (1.26-4.92)	.009
Donor					
	Sibling	1.07 (0.49-2.32)	.87	1.63 (0.73-3.61)	.23
	URD, identical	0.92 (0.42-2.02)	.84	1.28 (0.58-2.84)	.54
	URD, mismatched	1.00		1.00	
Stem cell source					
	Bone marrow	1.15 (0.49-2.70)	.76	1.40 (0.60-3.31)	.44
	PB	1.00		1.00	
Infused $CD34^+$ cell dose, £ 10^6/kg					
	<2	0.75 (0.18-3.18)	.70	1.58 (0.38-6.64)	.53
	2-5	0.72 (0.37-1.37)	.32	0.58 (0.30-1.12)	.10
	>5	1.00		1.00	

URD indicates unrelated donor.

aim of identifying the most suitable RIC regimen for HSCT from sibling donors and unrelated donors. The conventional arm was represented by a fludarabine + busulfan regimen, based on the results of the prospective EBMT study [14]. We chose thiotepa because of its excellent antitumor and immune suppressive activity when included in conditioning regimens and its common use in MF within the GITMO study group [16,19]. This Phase II study did not have the statistical power to claim any superiority between the 2 arms; however, it could generate useful data for future Phase III trials. A "pick the winner" design that has been used for quite some time could be similarly good, if not better, and should be considered for future trials testing different conditioning regimens in MF [29]. The patients and the donors recruited had clinical features comparable with those in the previous prospective trials. Indeed, in this study, the proportion of unrelated donors was 58%, compared with 68% in the EBMT trial and 51% in the MDPC trial.

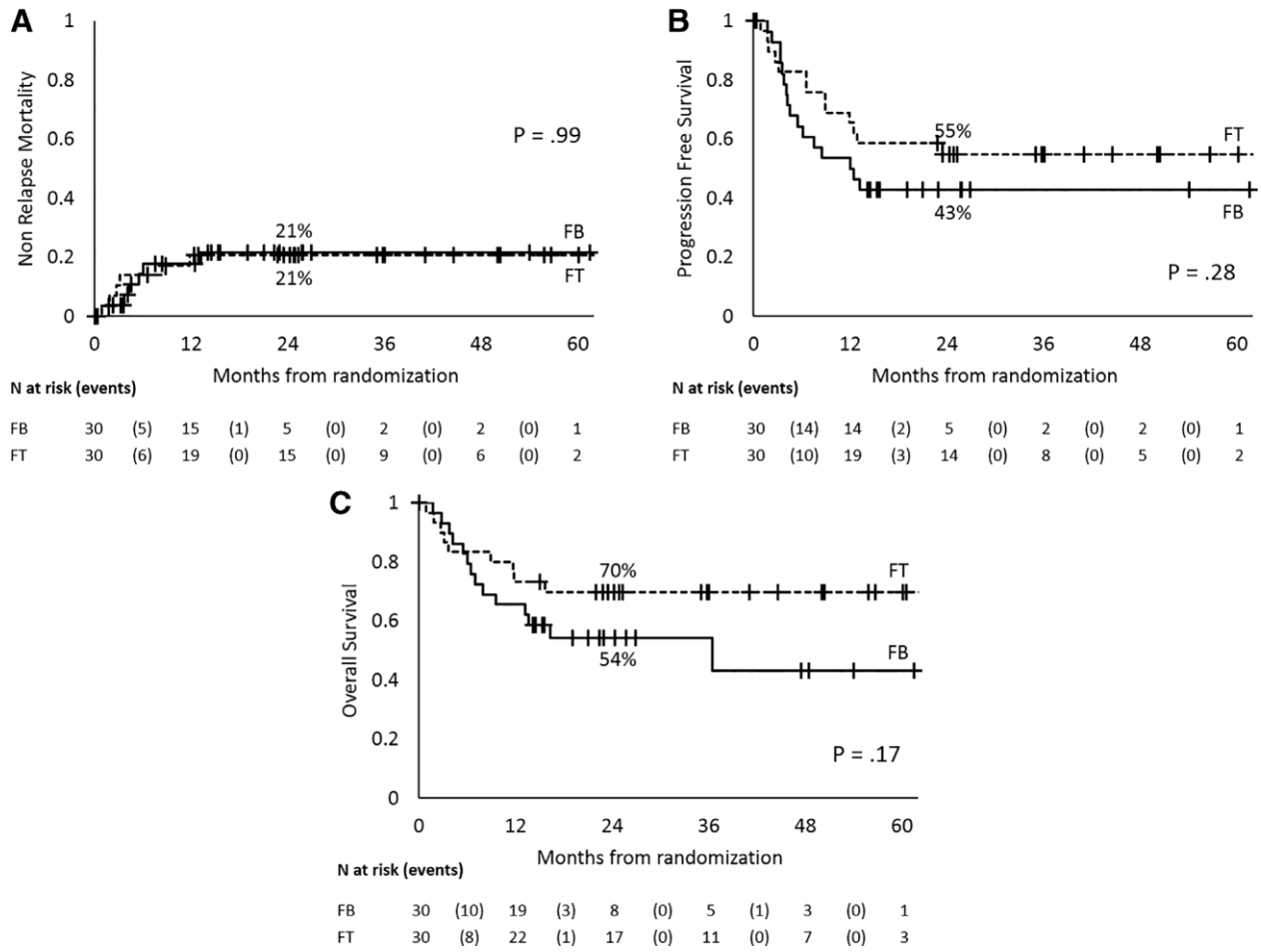

Figure 3. NRM by treatment (intention to treat) (A), PFS by treatment (intention to treat) (B), and OS by treatment (intention to treat) (C).

Table 4
Adverse Events in the First 30 Days Post-Transplantation

Adverse Event	FB Arm, n (%)		FT Arm, n (%)	
	Grade I-II	Grade III-IV	Grade I-II	Grade III-IV
All adverse events	24	6	19	11
Gastrointestinal toxicities	8 (33)	0	3 (16)	5 (46)
Fever	3 (13)		4 (21)	1 (9)
Infections	3 (13)	4 (66)	6 (32)	0
Cardiac toxicity	0	1 (17)	0	0
Hepatic toxicity	0	0	0	1 (9)*
Respiratory toxicity	2 (8)	1 (17)	0	1 (9)
Renal and urinary disorders	2 (8)	0	3 (16)	0
Nervous system disorders	1 (4)	0	0	1 (9)
Vascular disorders	0	0	1 (5)	2 (18)
Metabolism disorders	1 (4)	0	2 (10)	0
Eye disorders	1 (4)	0	0	0
Musculoskeletal disorders	2 (8)	0	0	0
Psychiatric disorders	1 (4)	0	0	0

* Venous occlusive disease of the liver.

Comparing the distribution of disease stages among recipients is more difficult, given the different MF scoring systems applied in these 3 studies. It is likely that a proportion of patients with low-stage disease enrolled in these trials would have not been considered suitable candidates for HSCT according to the present guidelines [4]. Moreover, the application of more recent and accurate scoring systems would change the disease stage distribution; in our study, the proportion of patients with intermediate-2 and high-risk disease increased from 63% according to the DIPSS to 85% according to the DIPSS Plus, taking into account transfusion dependence and platelet count in all patients and cytogenetic abnormalities in patients with available karyotype data [30]. Mutational status analysis could further improve disease stratification; however, all 3 prospective studies were designed before 2010 and limited their analysis to JAK2 V617F mutations [31] and MPL mutations [32]; thus, to date, the prognostic significance of driver and transforming mutations after HSCT have been evaluated only in retrospective studies [33,34].

HSCT recipients in the FT arm had slower platelet recovery and higher rates of severe extrahematologic toxicities, particularly in the gastrointestinal tract. However, the NRM rate was the same in the 2 arms. Our overall NRM of 21% at 2 years was similar to that reported in the EBMT study (16% at 1 year) and within the range of NRM rates reported in previous studies (Appendix 4); moreover, in the unrelated donor transplant recipients in our study, both conditioning regimens were apparently safer than the fludarabine + melphalan regimen of the MDPC trial. Nonetheless, in most studies, transplants from unrelated donors exhibited a higher risk of toxicity and mortality; in some of these, the increased risk was limited to mismatched donor transplants [14], whereas in others it included even transplants from matched unrelated donors [12,16,23-26,31,35,36], in which a high risk of graft failure and related-mortality are reported [26]. In the present study, GVHD did not seem to contribute to NRM, given the very low GVHD rates in both arms.

Our 15% cumulative incidence of overall chronic GVHD may be related to a still short patient follow-up and to the decreasing rates of FDC during post-HSCT follow-up. On the other hand, both graft failure and mixed chimerism were matter of concern in our study, even if our graft failure of 12% was in the range of that reported in previous trials [8,11-14,16,19,37] (Appendix 4). High rates of FDC were reported in the first studies using RIC regimens, particularly when chimerism was evaluated shortly after transplant, at the time of engraftment [38,39]. However, recent studies evaluating chimerism at different time points are concordant with our observation of an increased rate of mixed chimerism during follow-up, particularly after less-intensive conditioning regimens (Appendix 4) [28,40]. Donor lymphocyte

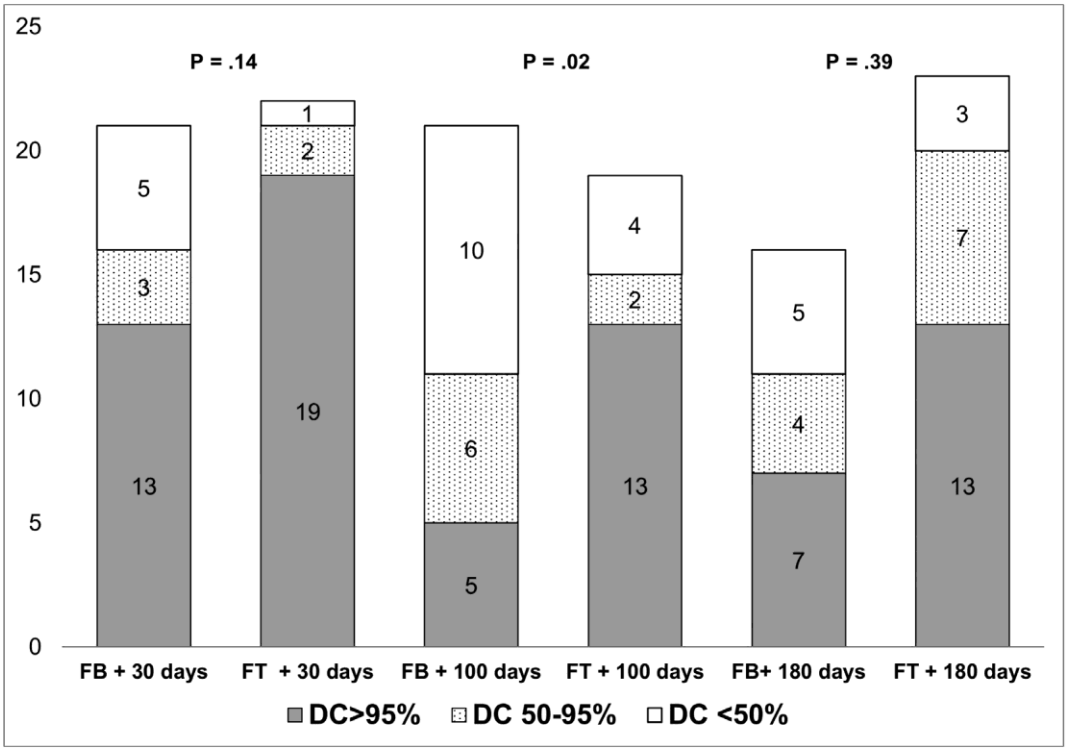

Figure 4. Chimerism evaluation in peripheral blood at days +30, +100, and +180, according to treatment arm. DC, donor chimerism.

infusion to maintain or achieve FDC has been reported in several studies [14,38,39]. We found that graft failure occurred independent of donor type, stem cell source, and conditioning regimen. Indeed, the burden of immune suppression in the conditioning regimen and the GVHD prophylaxis varied among the 3 prospective studies and could help explain the different clinical results. Either ATG or fludarabine was administered at lower doses in the MDPC study (120 mg/m^2 fludarabine and 4.5 mg/kg Thymoglobuline), which reported higher graft failure and NRM compared with the EBMT and GITMO studies (180 mg/m^2 fludarabine plus 60 mg/kg Fresenius or 7 mg/kg Thymoglobuline), respectively. For the future, alternative GVHD prophylaxis may be considered, as proposed by Bregante et al [37], who reported a possible beneficial effect on NRM associated with the substitution of ATG with post-transplantation cyclophosphamide.

In our study, the kinetics of hematopoietic engraftment was significantly influenced by the spleen size at the time of HSCT, as reported previously [33-36]. Only 15% of our patients had a spleen larger than 22 cm, but we observed that even a moderate splenic enlargement had a negative impact on hematopoietic engraftment, although it did not significantly influence overall outcome. At present, the management of splenomegaly before HSCT should be reconsidered after the introduction of JAK1/2 inhibitors. In the present study, we cannot draw any conclusions in this regard, because only 7 patients were pretreated with ruxolitinib. We also saw faster engraftment of splenectomized patients, and this may be an additional option for patients with splenomegaly.

Therefore, NRM remains a significant unmet need for patients with MF. Today, it is likely that the improvement in this setting will be achieved not by a different conditioning regimen, but rather via a global treatment strategy in which patients are not referred to HSCT too late. As expected, our multivariate analysis found a significantly higher PFS in patients with intermediate-1 MF compared with those with more advanced–stage MF, confirming a lower disease burden as a favorable prognostic factor, as was previously reported after MAC [9,10,12] and RIC [14] HSCT. Because a subgroup of DIPSS Plus intermediate-1 patients can be considered for HSCT if they have adverse features as per National Comprehensive Cancer Network recommendations [41,42], the 68% PFS at 2 years found in our study should be taken into consideration, even if the plateau of the curve needs to be confirmed with a longer follow-up, and comparisons with nontransplantation treatments are warranted [43,44].

Relapse, the other major determinant of long-term outcome, was comparable in our 2 treatment arms. Moreover, relapse rate is similar to that previously reported after busulfan based RIC regimens [14,25], but higher than that observed after melphalan [25]. As suggested by the transplant group in Genova, adding thiotepa to busulfan and fludarabine [32], with the aim of improving complete donor chimerism and reducing the risk of relapse, may represent an option that deserves further clinical investigation.

We must acknowledge some limits of the present study, which are linked predominantly to the rarity of MF as indication for HSCT and the long time required to recruit patients. First, the lack of analysis of driver and transforming mutations before transplantation did not allow a full evaluation of current prognostic factors [45]. Second, the limited number of patients enrolled did not allow us to score primary and secondary MF separately [46]. Finally, the study's phase II design, although randomized, does not allow us to draw firm conclusions, and the issue of the best conditioning regimen in MF remains open.

In conclusion, this prospective and comparative study shows that both fludarabine + busulfan and fludarabine + thiotepa are reasonably safe and moderately effective preparative treatments before HSCT from HLA-identical sibling and unrelated donors. The substitution of busulfan with thiotepa provided comparable disease control to that of the conventional RIC regimen. Spleen size significantly influenced hematopoietic engraftment, whereas disease stage was the sole independent predictor for PFS. Our study findings demonstrate the need to address some open problems, such as the prevention of graft failure and recurrence, in future studies.

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Conflict of interest statement: F.P. has had an advisory role at Janssen, Celgene, MSD Italy and has received travel, accommodations, and expenses from Celgene, Jazz, and Medac. A.R. has had a consulting and advisory role with Novartis, Roche, Genetech, Amgen, and Italfarmaco, and has received travel, accommodations, and expenses from Novartis, Celgene, Amgen, Sanofi, and Roche. The other authors have no conflicts of interest to report.

Authorship statement: F.P., F.B., R.F., A. Bacigalupo, A. Bosi, and A.R. designed the study; F.P., S.B., M.C.F., D.R., F.N., G.M., E. P.A., N.C., G.M., B.B., and F.B. enrolled patients; F.P., A.M., C.P., S. M., and B.B. collected, analyzed, and interpreted the clinical data; F.P. and A.R. wrote the report; and all the authors revised and approved the final manuscript. F.B. and A.R. are considered co-last authors.

SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at doi:10.1016/j.bbmt.2018.12.064.

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