MPD-RC 101 Prospective Study of Reduced Intensity Allogeneic Stem Cell Transplantation in Patients with Myelofibrosis

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Key Points:

- High survival rate in primary or secondary MF patients transplanted from matched related donors using FluMel regimen

- FluMelATG in HSCT from unrelated donors for MF patients is associated with an increased risk of graft failure

ABSTRACT

From 2007 to 2011, 66 patients with primary myelofibrosis (PMF) or MF preceded by essential thrombocythemia or polycythemia vera were enrolled into a prospective phase II clinical trial of reduced intensity allogeneic hematopoietic stem cell transplantation (AHSCT), Myeloproliferative Disorder Research Consortium -101 trial. The study included patients with sibling donors (n=32) receiving fludarabine/melphalan (FluMel) as a preparative regimen and patients with unrelated donors (n=34) receiving conditioning with FluMel+anti-thymocyte globulin (rATG). Patient characteristics in the two cohorts were similar. Engraftment occurred in 97% of siblings and 76% unrelated transplants, while secondary graft failure occurred in 3% and 12%, respectively. With a median follow-up of 25 months for patients alive, the overall survival was 75% in the sibling (median not reached) and 32% in the unrelated group (median OS: 6 months, 95% CI:3,25) (HR 3.9, 95% CI: 1.8,8.9) ($p<0.001$). Non-relapse-mortality was 22% in siblings and 59% in unrelated AHSCT. Survival correlated with type of donor, but not with the degree of histocompatibility match, age or *JAK2*^{V617F}-status. In patients with myelofibrosis with sibling donors AHSCT is an effective therapeutic option while AHSCT from unrelated donors with FluMel+ATG conditioning led to high rate of graft failure and limited survival. (clinicaltrials.gov Identifier 00572897)

INTRODUCTION

Primary myelofibrosis (PMF) is a chronic myeloproliferative neoplasm that occurs more frequently in patients above 60 years of age. The *JAK2*^{V617F} mutation, is present in approximately 50% of primary myelofibrosis(PMF) patients and post-essential thrombocythemia (ET) MF and virtually all patients with post-polycythemia vera (PV) MF[1,2]. Among PMF and post-ET MF patients with wild-type *JAK2*, mutations of Calreticulin (CALR) have been identified in approximately 90% of the cases[3]. Survival in patients with MF depends on the time to transformation to acute myeloid leukemia or complications due to progressive cytopenias and or splenomegaly. In the past, PMF, post-ET or PV MF patients enrolled in clinical trials were stratified by prognosis according to the Lille prognostic scoring system[5] that divided patients in low, intermediate or high risk prognostic categories based on the presence of anemia, leukopenia or leukocytosis. More recently, additional independent variables affecting the survival of PMF patients have been identified , including , constitutional symptoms, presence of circulating blasts, thrombocytopenia, transfusion requirements and chromosomal abnormalities which have served as the basis for the Dynamic International Prognostic Score System (DIPSS) [6] and the DIPSS-Plus[7] . At this time, a therapeutic agent which is capable of curing MF patients is not available. Clinical trials with small molecule JAK1/2 inhibitors have proven beneficial in reducing the degree of splenomegaly and suppressing constitutional symptoms in a large fraction of patients with MF, but treatment with these agents do not extensively affect the degree of marrow fibrosis or eliminate molecular or cytogenetic abnormalities[8,9].

The only therapeutic option that can reverse the marrow fibrosis in MF patients [10,11] is an AHSCT. Initially AHSCT with myeloablative conditioning regimens was shown to be curative, especially in younger patients, thanks to a graft-versus-tumor effect from donor lymphocytes[12-15]. Nevertheless, because of a very high transplant-related mortality in patients 45 years of age and older[16], this approach was not routinely offered to the majority of MF patients. Studies from the MPD-RC and others[17-19] then demonstrated that reduced intensity conditioning regimens allow older patients to undergo AHSCT with limited treatment related mortality (TRM) and with a significant chance for long term survival. However, only a single multi-center large prospective study[20] of RIC AHSCT has been reported to date. In this study, patients were transplanted from related or unrelated donors after receiving a conditioning regimen including fludarabine, busulfan and rabbit anti-thymocyte globulin (ATG). The adverse prognostic factors that were identified[20,21] in patients who received this type of RIC HSCT included: age ≥57 years, a mismatched unrelated donor, an intermediate or high Lille score, *JAK2* wild type, the presence of constitutional symptoms and more recently the absence of CALR mutations[22].

Based on the initial transplant experience using a standard RIC regimen with fludarabine and melphalan, the MPD-RC launched in 2007 a prospective study of RIC- AHSCT using this regimen and investigated the efficacy of FluMel based RIC regimens in two parallel cohorts for matched sibling or matched unrelated donors transplantation, where ATG was utilized only in the latter group

PATIENTS AND METHODS

Patients

The MPD-RC 101 is a multicenter Phase 2 prospective study of reduced intensity AHSCT that was performed at 11 centers affiliated with the MPD-RC (6 from the US, 4 from Europe and 1 from Canada). Patients enrolled in the study had documented PMF or post-ET- or post-PV MF (blasts < 20%), age 18-65, no significant co-morbidities, intermediate or high risk Lille score, or low risk Lille score with a platelet count < 100 x 10^9/L, a sibling or unrelated available stem cell donor, and a signed consent form according to the MPD-RC 101 protocol approved by each MPD-RC institution's Institutional Review Board (IRB) or equivalent Ethical Committee (for European centers). The study included two parallel protocols: one for patients who received an AHSCT from a sibling donor and one who received grafts from unrelated donors.

Donors

HLA matching was determined by low resolution molecular typing for sibling donors and by high-resolution molecular typing for both class I (A, B and C) and class II (HLA-DRB1 and HLA-DQB1) antigens for unrelated donors. Donor graft consisted of either unmanipulated bone marrow or peripheral blood hematopoietic stem cells (PBSC). The PBSC donors were mobilized with recombinant human granulocyte colony-stimulating factor (rhG-CSF) 10 µg/kg s.c / day x 5 days and underwent PBSC collection by leukapheresis.

Treatment Plan

Patients were conditioned with the fludarabine 30 mg/m2/day intravenous (i.v.) for 5 days (day -6 to day -2) and melphalan 70 mg/m2/day i.v. for 2 days (day -2 to day -1). Graft versus-host disease (GVHD) prophylaxis consisted of tacrolimus 0.03 mg/kg i.v. from d-2, methotrexate 10 mg/m^2 iv d+1 and 8 mg/m^2 d+3 and d+6. Thymoglobulin (rabbit antithymocyte globulin, Genzyme Corp. Cambridge, MA) at 4.5 mg/kg total dose was used as additional GVHD prophylaxis only in patients receiving a graft from an unrelated donor.

Criteria for engraftment, response and GVHD

Hematopoietic engraftment was defined as time to absolute neutrophil count (ANC) ≥ 0.5 x 10^9/L and time to platelet count ≥ 50 x10^9/L for 3 consecutive days. Post-transplantation donor-recipient chimerism was assessed by means of DNA microsatellite analysis of blood mononuclear cells. Clinical responses were categorized according to criteria developed by the International Working Group for Myelofibrosis Research and Treatment (IWG-MRT) [24] and included complete response (CR), partial response (PR), clinical improvement (CI), stable disease (SD) or progressive disease (PD). Acute and chronic GVHD were graded according to standard criteria[25,26]

Study Design

The trial was designed to estimate progression free survival and overall survival of patients with MF undergoing an AHSCT prepared with a reduced intensity conditioning regimen in each of two cohorts separately (sibling and unrelated donor). With 32 patients in each stratum, an improvement in progression-free survival at 2 years without any evidence of disease from 50% of patients surviving to 75% is detectable with 2-sided

alpha of 0.05 and power of 80%. Within each stratum, if 22 or more patients survive progression-free at 2 years, we would conclude that the transplant regimen had a progression-free survival rate of 75% or greater; if there are 10 or fewer patients surviving progression-free at 2 years, we would conclude that the transplant regimen has a progression-free survival rate of 25% or lower.

A group sequential stopping rule was used to test whether there was evidence that the transplant-related mortality rate (i.e., mortality within 6 months of transplant) exceeded 50%. This stopping rule was applied separately to each stratum. The trial would have been terminated for a stratum if the stopping rule demonstrated that transplant-related mortality exceeded 50%.

Statistical analysis

Baseline patient characteristics, disease history, and treatment related variables were summarized separately for each cohort defined by donor type using descriptive summary statistics and graphical approaches. Summary test statistics were used to provide additional descriptive information regarding the differences between the sibling and unrelated groups of patients. Overall survival was calculated from the date of transplant to the date of death or last follow up date. Event-free survival was calculated from date of transplant to the date of the first occurrence of failure to engraftment (primary graft failure), loss of donor graft (secondary graft failure), or death, or last follow up date. Survival curves were estimated using Kaplan-Meier methods. The association of overall survival and current diagnosis (PMF or MF secondary to ET / PV), Lille Score (0 / 1 or 2), gender, age adjusted DIPSS (Low / Intermediate-1 Risk or Intermediate-2 / High Risk), donor HLA compatibility (Matched or Mismatched) and baseline *JAK* ^{V617F}

status (Positive or Negative) was examined using the log rank test. The association of overall survival and age at transplant, time from diagnosis to transplant, baseline white blood cell count, platelet count, percentage of blasts, and the dose of CD34+ cells within the graft was examined using the Cox proportional hazards model.

RESULTS

Patient Characteristics

The characteristics of patients in the sibling (n=32) and unrelated (n=34) groups are shown in Table 1. Although the study was not designed to compare these two groups, the clinical characteristics of patients were similar. Of 66 patients, 63 were had intermediate or high risk MF according to the Lille score system, whereas 3 patients in the sibling group had low risk disease with thrombocytopenia. The median age at the time of transplant was 55 years in the sibling and 56 in the unrelated group, respectively. Bone marrow biopsies of 51/66 were reviewed by a central group of pathologists to confirm the histological diagnosis and to grade the fibrosis according to the European consensus scale (0-3)[23]. A *Jak2*^{V617F} mutational analysis was available for 63 of 66 patients and was positive in approximately 50% of the patients. Of the 66 patients, 52 had splenomegaly at the time of transplant while 10 had been previously splenectomized (5 patients in each group). Karyotypic analyses just prior to transplant were available in 49 patients. 44% patients in the sibling group and 41% in the unrelated group had a normal karyotype. The median time from diagnosis to transplant in the two groups was 16 months (range: 1-247) in recipients with sibling and 20 months (range: 2-341) with unrelated donors, respectively.

Donors

Fifty seven patients (26/32 in the sibling and 31/34 in the unrelated group) received peripheral blood hematopoietic stem cells (PBSC) and 9 patients (6/32 in sibling and 3/34 in the unrelated group) received marrow grafts. The median dose of CD34+ cells

infused in recipients of PBSC was comparable in the sibling or unrelated group (Table 1). In recipients of bone marrow grafts, the median dose of total nucleated cells (TNC) infused in 6 sibling transplants (3.78 x 10^8/kg range: 2.65, 7.87) was slightly higher than in 3 unrelated transplants (2.37 x 10^8/kg range: 1.9, 2.7). Donors were HLA matched (10/10 antigens) in 30/32 sibling and 25/34 unrelated transplants (p=0.02). The remaining donors were mismatched for either 1 HLA antigen, or 1 HLA antigen + 1 allele, or only 1 or 2 alleles.

Engraftment

Donor cell engraftment was analyzed in each of the two groups separately (Table 2). In the sibling group, neutrophil and platelet engraftment was achieved in 31/32 (97%) and 28/32 (88%) of patients, respectively. The median time to engraftment for neutrophils was 22 days (range: 0-62) and for platelets was 28 days (range:0-62). In the Unrel group, neutrophil and platelet engraftment occurred in 26/34 (76%) and 20/34 (59%). Two patients in this group died prior to day 30 without hematopoietic cell engraftment. The median time to engraftment for neutrophils was 18 days (range: 11-43) and for platelets was 28 days (range: 9-365).

An analysis of donor cell chimerism of blood mononuclear cells was available in 56 patients. By day 30 post-transplant, 23/28 patients in the sibling and 21/28 in the unrelated cohort had ≥98% donor cell chimerism in the peripheral blood. Primary graft failure was observed in 1/32 (3%) sibling transplants and in 8/34 (24%) unrelated transplants. Secondary graft failure was observed in 1 sibling and 4 unrelated transplants, after a median of 32 and 48 days, respectively. Therefore, the overall graft failure rate was 6% in the sibling and 36% in the unrelated transplant groups,

respectively. Within the 12 patients with graft failure in the unrelated group, 8 had received an HLA matched and 4 an HLA mismatched graft.

GVHD

Overall, 61% of the patients participating in the study did not experience greater than grade I acute GVHD. In the sibling group, 12/32 patients (38%) experienced aGVHD grade II-IV with 4 cases (12%) at grade III-IV. In the unrelated group, 14/34 patients (41%) had aGVHD grade II-IV, with 7 cases (21%) at grade III-IV (Table 2). The degree of chronic GVHD could be assessed in 43 patients (28 sibling and 15 unrelated group, respectively). Of patients in the sibling cohort, 36% experienced cGVHD (extensive in 25%), while in the unrelated cohort 33% of the patients had cGVHD and in 20% it was extensive.

Mortality

The overall median follow-up for patients alive at last follow-up was 25 months (range: 10-73). In the sibling group 24/32 patients (75%) were alive. Progression of disease caused the death of 1 patient in this group while causes of non-relapse-mortality (NRM) in the remaining 7 patients (22%) included: secondary malignancy (n=1); aGVHD (n=3); hemorrhage (n=1); respiratory failure; (n=1); heart failure (n=1). In the unrelated group 11/34 patients (32%) are alive. Among the causes of death, 3 were related to progression of disease (in 2 cases after a second transplant) (9%), while 20 (59%) were due to transplant-related complications: aGVHD (n=5); hemorrhage (n=3); renal failure (n=2); pneumonia/respiratory failure (n= 2); venous occlusive disease (n=1); viral infection (n=1); other events secondary to graft failure (n=6).

Clinical Response

Clinical responses were assessed according to the IWG-MRT 2006 criteria in 46 patients (29 sibling and 17 unrelated transplants, respectively) who survived at least 180 days (Table 2). In the sibling group, the overall response rate (ORR) was 93% with 7 patients (25%) achieving a clinical CR, 8 patients a PR (29%), and 11 patients (39%) a CI. Two patients had stable disease and 1 patient had an unknown response. In the unrelated group, the ORR was 69% with 6/17 (35%) CR, 1 PR (6%) and 5 CI (29%). One patient experienced progression of the disease 180 days post-transplant. These results show that the clinical result of AHSCT from sibling or unrelated donors may be comparable for patients who achieve a sustained stem cell engraftment.

Survival

The median overall survival (OS) for the sibling group has not been reached while for the unrelated group was 6 months (95% CI: 3,25). A significantly higher risk of death was observed for patients receiving a transplant from an unrelated as compared to a sibling donor (Hazard Ratio 3.9, 95% CI: 1.8,8.9) ($p<0.001$). (Figure 1-top). Median event-free survival (EFS) has not been reached in the sibling and was 6 months (95% CI: 2,25) in the unrelated group (Figure 1-bottom). Analysis by Cox proportional hazards model in sibling and unrelated transplants did not show any association between overall survival and age of patient at transplant, time from diagnosis to transplant, baseline white blood cell count, baseline platelet count, baseline percentage of circulating blasts, and CD34+ cell dose in the graft. Survival curves based on diagnosis (primary or secondary MF), degree of donor HLA match, presence of *JAK2*^{V617F} and age ≥ 57 years showed no

statistical difference within sibling or unrelated groups (Figure 2). Patients with or without the *JAK2*^{V617F} mutation had a similar survival in the sibling cohort. In the unrelated cohort, however, the presence of *JAK2*^{V617F} mutation was associated with a trend for worse survival but this relationship did not reach statistical significance (p=0.29). Therefore none of these variables was found to predict survival in these patients. . When patients in each group were stratified based on the age-adjusted DIPSS[27,28] (Figure 3), patients with Low/Int-1 and Int-2/High risk in the sibling cohort had comparable survival, whereas patients in the unrelated cohort at Int-2/High Risk had lower survival rates than those at Low/Int-1 risk (2-year survival: 42% vs 17%, p=0.1). Survival was examined in each of the two transplant groups in relation to diagnosis, age, gender, Lille score and DIPSS score at the time of transplant, donor HLA compatibility and presence of *Jak2*^{V617F} mutation. The results, shown in Table 3, demonstrate that none of these factors predicted survival individually in either group of patients.

DISCUSSION

We report here the results of a large prospective phase 2 multicenter study of RIC AHSCT in patients with PMF, post-ET or post-PV related MF that included 66 patients transplanted in 11 centers within the MPD-Research Consortium.

That HSCT is the only curative intervention for patients with myelofibrosis has been known for over fifteen years. However, the parameters that should guide transplant physicians in defining treatment plans or assessment of risk associated with transplant remain still to be validated. In fact, most of the data related to factors that determine the outcome of transplant in MF are derived from retrospective studies[29-37] of cohorts of patients who have been transplanted at single centers or larger numbers of patients in national or international registries. These studies often include data related to transplants performed over decades, thus adding additional variables related to significant changes in supportive therapy in blood and marrow transplantation over the years. The only large prospective transplant study in primary or secondary myelofibrosis, was the European Society for Blood and Marrow Transplant (EBMT) trial[20] that utilized a RIC regimen with fludarabine, busulfan and rabbit ATG, in 33 transplants from sibling and 70 from unrelated donors. The results of this study differed from others especially because of the high survival rate (approximately 70%) and low non relapse-mortality (NRM) in transplants from matched unrelated donors. However, since in that study patients >55 years of age had a 48% survival as opposed to 82% for younger patients, it is conceivable that a large portion of patients who received a matched unrelated transplant may have been younger than those participating in the present study. Survival correlated also with HLA mismatched donors and intermediate and high risk Lille score at the time of transplant. Relapse after transplant was influenced by Lille score and

splenectomy before transplant. Based on this trial, the authors then reported a prognostic scoring system[21] that included age ≥ 57, absence of *JAK2*^{V617F} (*JAK2* wild type) and presence of constitutional symptoms at the time of transplant as independent adverse indicators. In our study we prospectively transplanted 66 patients in two groups based on the type of donor: 32 patients with a sibling donor (94% were HLA matched) and 34 with an unrelated donor (74% HLA matched). As compared with the EBMT study, the present study included a lower number of patients with low risk Lille score (4.5 vs 16.5%). Moreover, low risk patients in our study had more advanced disease according to a modified Lille score[37] based on each of them being thrombocytopenic. Our conditioning regimen included melphalan instead of busulfan and ATG was administered only to recipients of the group receiving unrelated grafts. The present results in the sibling group showed 75% OS and are consistent with the EBMT study, as well with our prior retrospective study[17] of transplants from matched siblings. However, in transplants from unrelated donors the OS in the present study was 32%, significantly inferior to the sibling group (HR 3.9, 95% CI: 1.8,8.9) ($p<0.001$). As opposed to the EBMT trial, in our study we did not detect a difference between HLA matched and mismatched unrelated transplants. Since the relapse-related mortality was only 6% in the study (3% in the sibling and 9% in the unrelated group), the high rate of NRM in the unrelated group was frequently secondary to graft failure. Previous studies have reported controversial conclusions concerning the risk associated with utilizing an unrelated donor. Similar to the EBMT prospective trial, a retrospective study from Seattle[37] did not find a significant difference of NRM with matched sibling or unrelated donors. However, a recent retrospective analysis of the EBMT registry in 250 patients with post-ET-MF or post-PV-MF who received a AHSCT from 1994 to 2010 showed that NRM in unrelated

transplants was 34% and that using an unrelated donor was an independent adverse prognostic factor[39]. Given that in our study we did not observe any difference in the outcome of PMF and post-ET- or post-PV-MF, our findings seem consistent with the EBMT retrospective report. Prior retrospective data from the Italian (GITMO)[36] and French (SFGM-TC)[35] registries also showed significantly higher rates of NRM in transplants from non-sibling donors. An intial analysis[40] from the Center for International Blood and Marrow Transplant Registry (CIBMTR) including MF patients receiving mostly a myeloablative regimen found a 55% and 70% overall mortality in sibling and unrelated transplants, respectively. A more recent retrospective study[41] from the CIBMTR of 233 patients with MF who received a RIC transplant showed a better outcome for those who received a transplant from a matched sibling as opposed to a matched unrelated donor . In another study of RIC HSCT, Bacigalupo et al[42] reported that the three independent adverse prognostic factors for outcome of AHSCT transplant for myelofibrosis were: any type of donor other than a matched sibling, a large spleen, and an excessive number of red blood cell transfusions prior to transplant. This scoring system suggests that other factors related to the disease can affect the transplant outcome and will be validated in future prospective trials. However, consistent with this study we found that transplant from an Unrel donor carried a higher risk of death.

None of the studies of transplant in MF have evaluated the possible role of HLA antibodies, especially in the Unrel setting, as a possible factor correlating with graft failure. Unfortunately these data were not measured in our study and we cannot rule out that this may have influenced the excessive rate of rejection in our cohort of unrelated transplants. Based on risk factors identified in prior studies, we analyzed whether the limited survival in unrelated transplants were related to HLA mismatch, age>57 or

absence of *JAK2*^{V617F}. None of these factors correlated with survival and only the type of donor (sibling vs unrelated) predicted the outcome. As opposed to the EBMT results, *JAK*^{V617F} mutation actually showed a trend toward a worse outcome in unrelated transplants. This, however, could be related to a smaller number of patients in the unrelated cohort in our study. Finally, we found that in transplants from unrelated but not sibling donors, a DIPSS Intermediate-2/High risk status for the recipient correlated with an inferior survival. A correlation between advanced DIPSS scores and NRM was reported in the retrospective analysis by Scott et al in 170 patients transplanted in Seattle from sibling or unrelated donors between 1990 and 2009[27]. However, this study included a variety of conditioning regimens ranging from non-myeloablative, to reduced intensity or fully myeloablative.

It is possible that differences between the only two prospective cooperative studies, ours and the EBMT one, may be due primarily to the different conditioning regimen. However, other possible differences could include a lower number of low risk patients in our study or the different type of ATG utilized. Since FluMel regimen was associated with low rates of graft failure, relapse and NRM in sibling transplants, we assume that the different results obtained in the unrelated group may be due, in part, to a strong in-vivo immunomodulatory effect/ in vivo T cell depletion of the graft due to the combination of FluMel/ATG as a conditioning regimen that could have favored a host-anti-donor immune response. The immune effect of the conditioning may play an important role in the success that will be encountered with new protocols that are being designed to include ruxolitinib or other JAK 1/2 inhibitors (MPD-RC114)[43-46]. In fact, these agents are being investigated in AHSCT because they can rapidly reduce constitutional symptoms and the spleen size thanks to a marked suppression of proinflammatory cytokines[9], but

also for a possible immunosuppressive activity that may limit GVHD [47]. Because of these considerations, and the high rate of graft failure in unrelated transplants prepared with FluMelATG observed in this study, the MPD-RC has recently launched a new prospective study combining ruxolitinib with a FluBuATG RIC regimen.

Based on the results of two large prospective studies of RIC AHSCT in MF, regimens including melphalan or busulfan are both very effective in transplants from sibling donors. However, a busulfan-based regimen seems preferable in case of transplant from an unrelated donor since comparable results in sibling or unrelated transplants were observed in the EBMT study using a reduced intensity regimen and previously in a large retrospective study using a myeloablative regimen [37]. These studies also suggest that an initial search for matched donors should be performed for all the MF patients at ≥ Int-1 risk. In patients at Int-2 or High risk patients AHSCT should be offered immediately. In patients at Int-1, especially if they have only a matched unrelated donor, AHSCT should be offered as soon as the disease shows any sign of overall progression, such as worsening of anemia, or symptoms, or increase of spleen size, even before they meet the criteria for Int-2 risk category.

Our prospective study exploited for the first time the use of a standard RIC regimen such as FluMel in AHSCT for myelofibrosis. More large prospective studies testing new strategies to reduce NRM in high risk AHSCT, such as from non sibling donor, or HLA mismatched, or in patients with more advanced disease are warranted.

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AUTHORSHIP CONTRIBUTION

DR, JDG, LRS and RH contributed to the study design; DR, JDG, LSP and RH contributed to the writing of the manuscript; MS, CM, GP, RM collected and assembled the data; VN, AO and RSW collected and analyzed patients' samples; JDG and LSP performed statistical analysis; DR, LI, TBS, MB, AB, AR, RBK, VG, BA, JM, MW, AMV, JTP, GB, and RH enrolled patients; and all authors assisted in the critical review of the manuscript and approved the final version of the manuscript for submission.

CONFLICT OF INTEREST

None of the authors has any conflict to disclose with this study.

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Table 1. Characteristics of 66 patients with myelofibrosis in the MPD-RC 101 study. Patients received a stem cell transplant from sibling (n=32) or unrelated (n=34) donors were enrolled. Patients at Low Risk were enrolled only if they were thrombocytopenic.

		Sibling Donor N=32	**Unrelated Donor N=34**
Diagnosis	Primary Myelofibrosis	14 (44%)	25 (74%)
	PV - Myelofibrosis	3 (9%)	5 (15%)
	ET - Myelofibrosis	15 (47%)	4 (12%)
Lille Score	0	3 (9%)	0 (0%)
	1	20 (63%)	23 (68%)
	2	9 (28%)	11 (32%)
Age at Transplant	Median (Range)	55 (40-65)	56 (30-65)
Gender	Female	13 (41%)	15 (44%)
	Male	19 (59%)	19 (56%)
Time from Diagnosis	Median [months] (range)	16.1 (1-247)	20 (2-341)
Patient:Donor Gender	F: F	6 (19%)	7 (21%)
	F: M	7 (22%)	8 (24%)
	M: F	10 (31%)	6 (18%)
	M: M	9 (28%)	13 (38%)
Age-adjusted DIPSS*	Low	5 (16%)	5 (15%)
	Int-1	13 (41%)	14 (41%)
	Int-2	8 (29%)	10 (29%)
	High	3 (9%)	2 (6%)
	Unknown	3 (9%)	3 (9%)
WBC** x 10^9/L	Median (Range)	5.1 (2-43)	6.8 (1.3-70)
Circulating Blasts %	Median (Range)	1.0 (0-10)	0.12 (0-16)
Platelets x 10^9/L	Median (Range)	104 (28-927)	120 (19-1662)
JAK-2 V617F	Positive	12 (38%)	18 (53%)
	Negative	17 (53%)	16 (47%)
	Unknown	3 (9%)	0 (0%)
Bone Marrow Fibrosis	Grade 1	0	2 (6%)
	Grade 2	2 (6%)	6 (18%)
	Grade 3	18 (56%)	23 (68%)
	Unknown	12 (38%)	3 (9%)
Splenomegaly	Yes	24 (75%)	28 (82%)
	No	3 (9%)	1 (3%)
	Splenectomy	5 (16%)	5 (15%)
Karyotype	Normal	14 (44%)	14 (41%)
	One Abnormality	7 (22%)	9 (26%)
	Complex Abnormality	5 (16%)	0 (0%)
	Unknown	6 (19%)	11 (32%)
Stem Cell Source	Peripheral Blood	26 (81%)	31 (91%)
	Bone Marrow	6 (19%)	3 (9%)
CD34 cells (x10^6/kg)	PBSC Median (range)	5.9 (3.2-14)	6.5 (2.9-11)
TNC*** (x10^8/kg)	BM Median (range)	3.7 (2.6-7.8)	2.3 (1.9, 2.7)
Full HLA Matched		30 (94%)	25 (74%)
HLA 1 Ag mismatched	No allele mismatched	2 (6%)	4 (12%)
HLA Ag matched +	1 or 2 Allelle Mismatched	0 (0%)	5 (15%)

* = Dynamic International Prognostic Score System; ** = white blood cell; *** = total nucleated cells

Table 2. Outcomes in patients with myelofibrosis in MPD-RC 101. Engraftment, GVHD and survival data are shown. Primary graft failure is defined as lack of engraftment of donor neutrophils and secondary graft failure as loss of the graft. Clinical response was assessed according to the IWG Criteria in patients with at least 180 days follow-up.

		Sibling Donor n=32	**Unrelated n=34**
Primary graft failure	N (%)	1 (3%)	8 (24%)
Patients with ANC ≥ $0.5x10^9$/L	N	31	26
Days to ANC engraftment	Median (range)	22 (0-62)	18 (11-43)
Patients with PLT ≥ $20x10^9$/L	N	28	20
Days to PLT engraftment	Median (range)	28 (0-62)	29 (9-365)
Secondary graft failure	N (%)	1 (3%)	4 (12%)
Acute GVHD grade II-IV	N %	12 (38%)	14 (41%)
grade III-IV	N %	4 (13%)	7 (21%)
Chronic GVHD*	N %	10/30 (33%)	5/23 (22%)
Patients Alive	N (%)	24 (75%)	11 (32%)
Deaths < 6 months	N (%)	3 (9%)	17 (50%)
Clinical response	N (%)	29	17
Overall Response Rate (ORR)	N (%)	26/28 (93%)	11 (69%)
Clinical Complete Response	N (%)	7 (24%)	6 (35%)
Partial response	N (%)	8 (28%)	1 (6%)
Clinical Improvement	N (%)	11 (38%)	5 (29%)
Stable Disease	N (%)	2 (7%)	4 (24%)
Progressive Disease	N (%)	0	1 (6%)
Unknown Response	N (%)	1 (3%)	

Table 3: Univariate survival analysis in sibling and unrelated donor groups.

Sibling Donors (32 patients) Variable	N	2 Year Survival	Logrank P Value
Current Diagnosis			
PMF	14 (44%)	71% (48%, 95%)	0.67
ET / PV	18 (56%)	76% (56%, 97%)	
Lille Score			
0 / 1	23 (72%)	73% (54%, 91%)	0.81
2	9 (28%)	78% (51%, 100%)	
Gender			
Female	13 (41%)	69% (44%, 94%)	0.55
Male	19 (59%)	78% (58%, 97%)	
Age Adjusted DIPSS			
Low Risk / Int-1	18 (56%)	71% (49%, 92%)	0.67
Int-2 / High Risk	11 (34%)	82% (59%, 100%)	
Donor HLA			
Match	30 (94%)	72% (56%, 89%)	0.43
Mismatch	2 (6%)	100%	
Baseline JAK-2 V617F			
Positive	12 (38%)	76% (56%, 97%)	0.68
Negative	17 (53%)	64% (36%, 92%)	

Unrelated Donors (34 patients) Variable	N	2 Year Survival	Logrank P Value
Current Diagnosis			
PMF	25 (74%)	36% (17%,55%)	0.94
ET / PV	9 (26%)	33% (3%, 64%)	
Lille Score			
0 / 1	23 (68%)	35% (15%, 54%)	0.88
2	11 (32%)	36% (8%, 65%)	
Gender			
Female	15 (44%)	40% (15%, 65%)	0.81
Male	19 (56%)	32% (11%, 52%)	
Age Adjusted DIPSS			
Low Risk / Int-1	19 (56%)	42% (20%, 64%)	0.14
Int-2 / High Risk	12 (35%)	17% (0%, 38%)	
Donor HLA			
Match	25 (74%)	40% (21%, 59%)	0.33
Mismatch	9 (26%)	22% (0%, 49%)	
Baseline JAK-2 V617F			
Positive	18 (53%)	28% (7%, 48%)	0.29
Negative	16 (47%)	44% (19%, 68%)	

Figure legends:

Figure 1: Survival after AHSCT with FluMel conditioning regimen in MF patients. Cumulative overall survival (OS) **(A)** and event-free-survival (EFS) **(B)** in 32 MF patients who received a transplant from a sibling donor. Median survival has not been reached since 75% of patients were alive at last follow-up and 71% without disease progression. Cumulative overall survival (OS) **(C)** and event-free-survival (EFS) **(D)** in 34 MF patients who received a transplant from an unrelated donor. Median OS and EFS in unrelated transplants are shown.

Figure 2: Diagnosis, HLA matching, *JAK2*^{V617F}, and age do not correlate with survival after AHSCT with FluMel conditioning regimen in MF patients. OS in recipients with grafts from sibling (Sib) and unrelated (Unrel) donors based on: diagnosis (PMF, ET-MF or PV-EF) (A); HLA matched or mismatched donor (B) (*2/32 patients in HLA matched Sib group received a 1 Ag mismatched transplant from their sibling and none of them died); presence or *JAK2*^{V617F} mutation (Jak2 pos) (C); age < or ≥ 57 years (D).

Figure 3: OS in sibling (Sib) or unrelated (Unrel) transplants by age-adjusted DIPSS categories. Patients in each group were classified as low-Int-1 or Int-2/High risk.

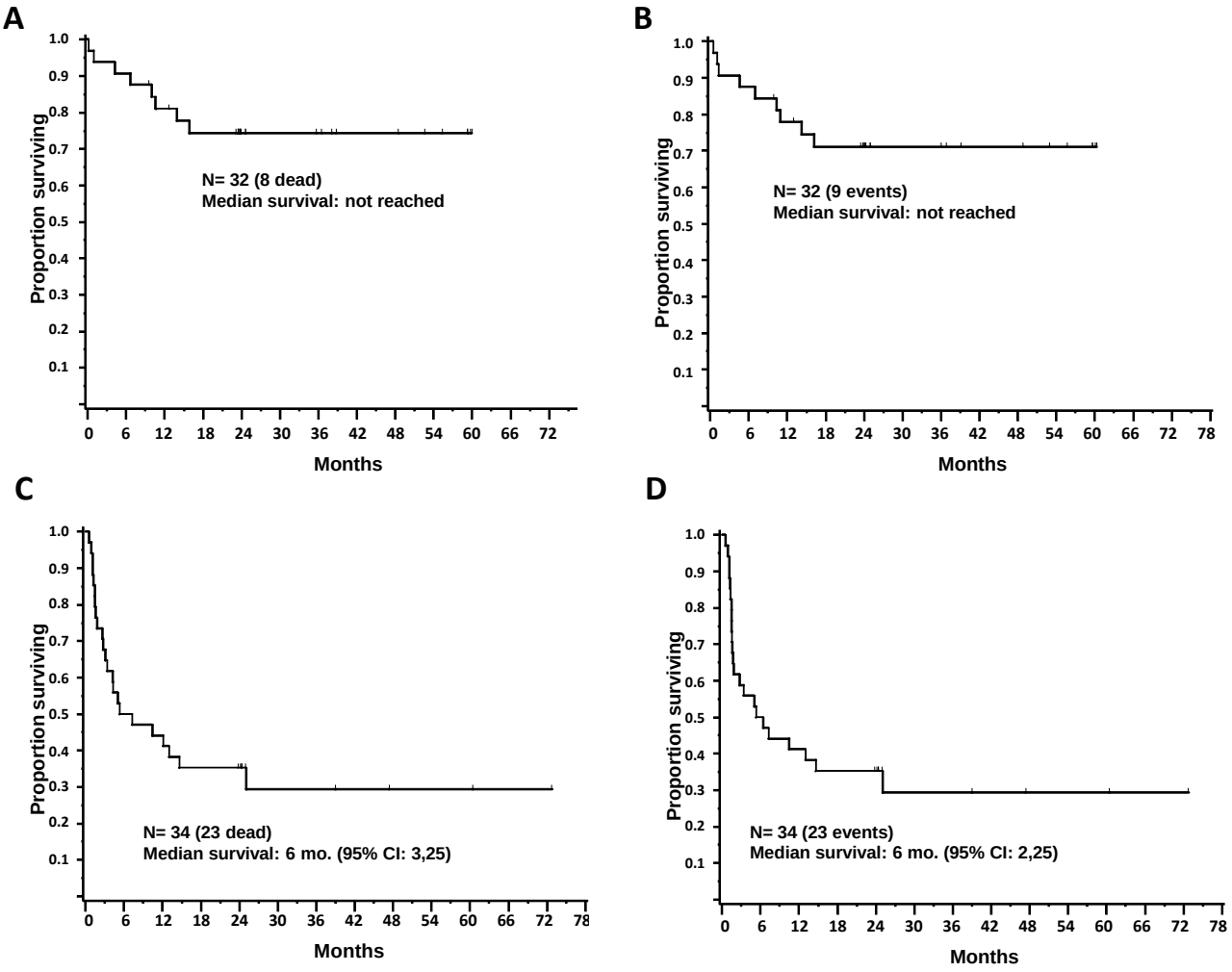
A
Proportion surviving
N= 32 (8 dead)
Median survival: not reached
Months
B
Proportion surviving
N= 32 (9 events)
Median survival: not reached
Months
C
Proportion surviving
N= 34 (23 dead)
Median survival: 6 mo. (95% CI: 3,25)
Months
D
Proportion surviving
N= 34 (23 events)
Median survival: 6 mo. (95% CI: 2,25)
Months

Figure 1

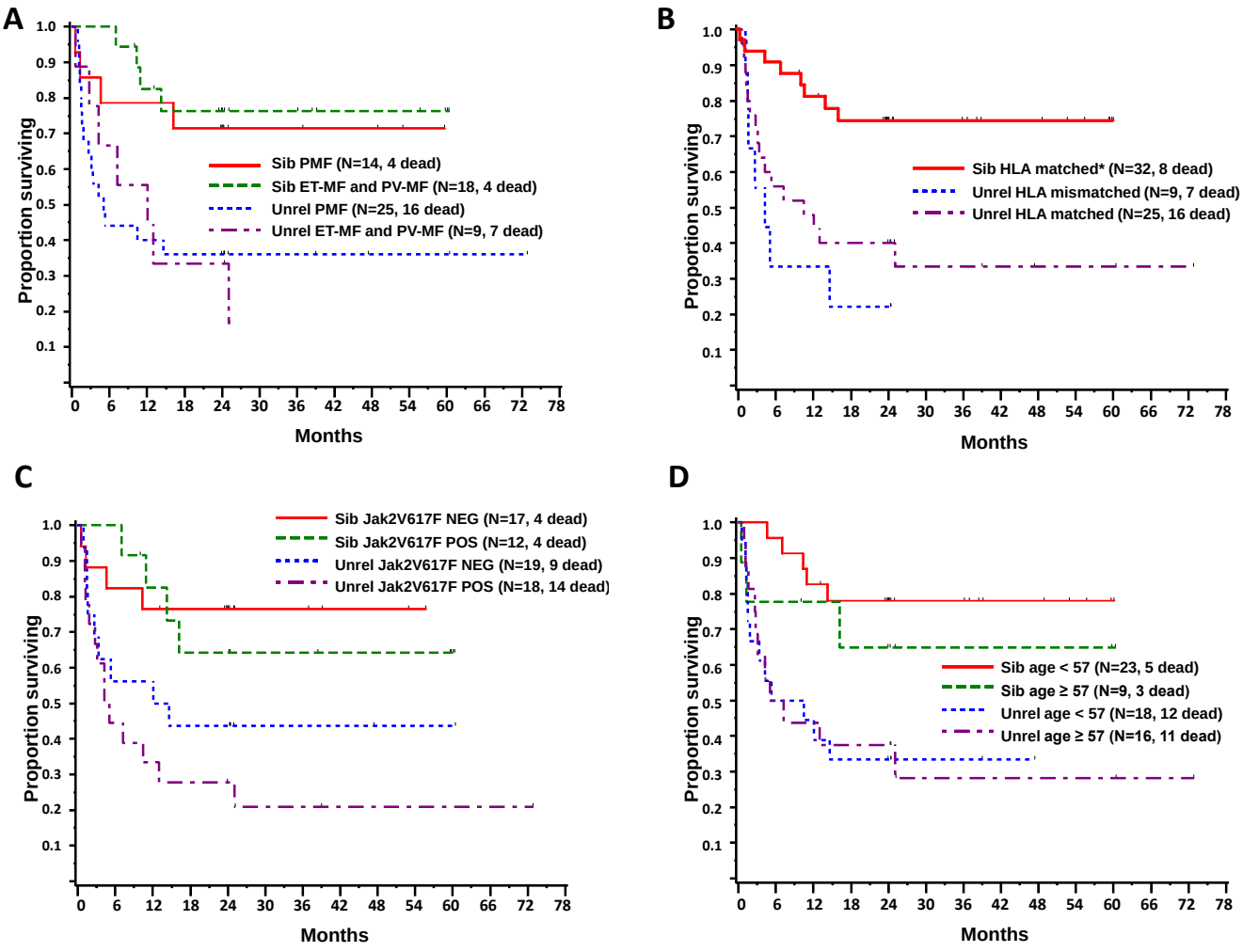
A
Proportion surviving
Months
Sib PMF (N=14, 4 dead)
Sib ET-MF and PV-MF (N=18, 4 dead)
Unrel PMF (N=25, 16 dead)
Unrel ET-MF and PV-MF (N=9, 7 dead)
B
Sib HLA matched* (N=32, 8 dead)
Unrel HLA mismatched (N=9, 7 dead)
Unrel HLA matched (N=25, 16 dead)
C
Sib Jak2V617F NEG (N=17, 4 dead)
Sib Jak2V617F POS (N=12, 4 dead)
Unrel Jak2V617F NEG (N=19, 9 dead)
Unrel Jak2V617F POS (N=18, 14 dead)
D
Sib age < 57 (N=23, 5 dead)
Sib age ≥ 57 (N=9, 3 dead)
Unrel age < 57 (N=18, 12 dead)
Unrel age ≥ 57 (N=16, 11 dead)

Figure 2

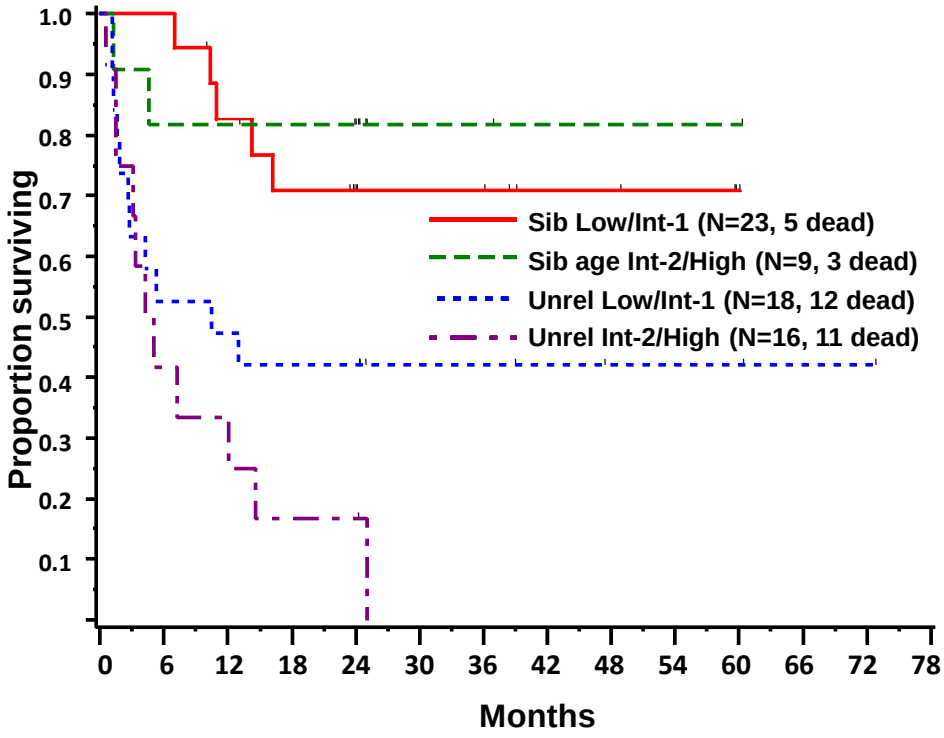
Proportion surviving
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
6
12
18
24
30
36
42
48
54
60
66
72
78
Months
Sib Low/Int-1 (N=23, 5 dead)
Sib age Int-2/High (N=9, 3 dead)
Unrel Low/Int-1 (N=18, 12 dead)
Unrel Int-2/High (N=16, 11 dead)

Figure 3