# Dual-alkylator Conditioning Regimen with Busulfan and Melphalan for Bone Marrow Allogeneic Hematopoietic Stem Cell Transplantation in Patients with Myelofibrosis : The Results of a Retrospective Study at a Single Institution in Japan

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Myelofibrosis (MF) is a myeloproliferative neoplasm associated with significant morbidity and mortality, and allogeneic hematopoietic stem cell transplantation (allo-HSCT) remains the only curative approach. While the optimal conditioning regimen before allo-HSCT for MF patients remains to be determined, recent studies have suggested that a thiotepa-busulfan-containing dual-alkylator regimen, FBT regimen, may be associated with favorable outcomes. In Japan, however, thiotepa is not indicated for MF. Here we describe the results of 6 cases of MF treated with melphalan-busulfan containing dual-alkylator regimen, FBM regimen, followed by their first allo-HSCT at a single institution. Neutrophil and platelet engraftment was achieved in all patients. And a full donor chimerism was confirmed in all patients at +30 days after allo-HSCT. The relatively small size and short observation period of our study make it difficult to draw a definitive conclusion ; however, our results suggest that a dual-alkylator regimen of FBM may be a candidate for an conditioning for allo-HSCT for MF, which should be verified with a large cohort of patients. *Shinshu Med J 71 : 393—402, 2023*

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**Key words** : myelofibrosis, allogeneic stem cell transplantation, conditioning regimen, dual alkylator

## I Introduction

Myelofibrosis (MF) is a Philadelphia chromosome-negative myeloproliferative neoplasm (MPN)[1]. It can present as a primary MF or a secondary MF to polycythemia vera (PV) or essential thrombocythemia (ET), and characterized by a progressive risk of blood transfusion dependency due to cytopenia, leukemic changes with clonal evolution, and multiple organ damage with extramedullary hematopoiesis[2)-5)]. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) remains the only curative procedure for patients with MF, even in the era of Janus kinase (JAK) inhibitors[2)6)7)].

Although allo-HSCT may provide a cure for a patient with MF, however, the rarity of the disease also precludes large-scale prospective control trials to establish a standard approach regarding indication, timing, and platform of allo-HSCT, and a tailor-made approach is mandatory to help guide the patients with MF[6)8)]. Clinical guides often recommend that patients with intermediate- or high-risk MF are eligible for allo-HSCT, if they are medically fit[6)9)]. Hence, transplant eligibility varies between institutions, but there has been a steady increase in the number of patients

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world-wide undergoing allo-HSCT as a potentially curative treatment over the past 15 years[4)10)11)].

However, some patients may be discouraged to proceed to allo-HSCT because of the challenges that are characteristic to MF : high risk of engraftment failure and/or poor graft function, largely due to fibrotic changes in the bone marrow and a splenomegaly associated extramedullary hematopoiesis of MF[4)12)]. The sequestration of transplanted stem cells in the enlarged spleen may negatively affect the transplantation outcome in MF patients[6)13)-15)]. Several strategies have been in practice to mitigate a risk of allo-HSCT due to splenomegaly in MF patients, including surgical removal of the spleen, pre-transplantation splenic eradiation, and administration of JAK inhibitor, ruxolitinib[6)]. However, an increased risk of development of acute leukemia after splenectomy, a risk of prolonged pancytopenia and infection after radiation therapy, and a limited durability of JAK inhibitor, remain to be concerns for each strategy[6)]. On the other hand, studies suggest that some conditioning regimens may be associated with better engraftment and overall transplant outcomes than those with other regimens, regardless of interventions for splenomegaly[6)12)]. Recently, Bacigalupo and others described that a dual-alkylator regimen, containing thiotepa and busulfan (FBT regimen), may improve the transplantation outcome in MF[16)17)].

In Japan, however, FBT regimen is not indicated for MF, and another set of dual alkylators has been frequently used in allo-HSCT for myeloid malignancies : a regimen containing melphalan and busulfan (FBM regimen)[18)-21)]. A favorable engraftment was also described in MF patients treated FBM regimen followed by cord blood transplantation[22)]. Hence, we herein report a result of a retrospective analysis of bone marrow allo-HSCT with FBM regimen in patients with MF at our institution over the past ten years.

## Ⅱ Patients and Methods

### A Patients

A list of patients with MF was extracted from the dataset of the recipients to whom allo-HSCT was performed at the Department of Hematology, Shinshu University School of Medicine between 2013 and 2022. The diagnosis of MF was confirmed in accordance with the WHO Classification Tumours of Haematopoietic and Lymphoid Tissues, 4th edition 2017[1)]. Patients with secondary myelofibrosis transformed from other MPNs, i.e. post-PV MF and post-ET MF, were included in the cohort. Patients with bone marrow fibrosis with other underlying non-MPN conditions (e.g., myelodysplastic syndrome with bone marrow fibrosis) were excluded. If a patient underwent multiple transplants, only the data obtained during the first allo-HSCT procedure were included in the analysis. Relevant clinical data were retrieved from the patients' medical records. Disease-based risk scores were calculated using three scoring systems : the Dynamic International Prognostic Scoring System (DIPSS)[23)], the DIPSS-plus[24)], and the Myelofibrosis Secondary to PV and ET (MYSEC-PM)[25)]. The relevant data within a month before the first day of a conditioning regimen were applied to each scoring system. A size of the spleen before allo-HSCT was measured with CT scans and a Kucybala's index, a product of the maximal length (Lmax) and the vertical hight (Hvert) of the spleen, was calculated for each patient to represent a volume of the spleen. A splenomegaly was determined when a Kucybala's index was higher than 115, according to the original literature[26)].

### B Chimerism analysis

DNAs extracted from the bone marrow, whole peripheral blood and a CD3-positive fraction of the peripheral blood, all of which were obtained at +1 month after transplantation, were subjected to a chimerism analysis, as previously described[27)]. Briefly, we semi-quantitatively compared the short tandem repeats between the donor-derived DNA and the patient-derived DNA, which had been identified in advance to distinguish them prior to transplantation, using a polymerase chain reaction-based method. Mixed chimerism was defined as the presence of ≥ 5 % recipient-derived DNA in the sample[28)].

### C Statistical analysis

The primary endpoint was neutrophil engraftment.

The day of neutrophil engraftment was defined as the first day of three consecutive days with an absolute neutrophil count $\geq 0.5 \times 10^3/\mu L$ by +28 days after transplantation. Delayed engraftment was defined as neutrophil engraftment between +28 days and +42 days after transplantation. Primary graft failure was defined as failure to exceed the threshold absolute donor-derived neutrophil count by day +43 after transplantation.

Secondary endpoints included platelet engraftment. The day of platelet engraftment was defined as the first day with an absolute platelet count of $\geq 2 \times 10^4/\mu L$, maintained for 3 days, without blood transfusion support in the previous 7 days after transplantation. Donor chimerism at 1 month after the transplantation, clinical status including GVHD and residual bone marrow fibrosis after allo-HSCT, platelet recovery of $\geq 5 \times 10^4/\mu L$ without transfusion support, 1-year overall survival (OS), and 1-year event-free survival (EFS) were also evaluated. OS was defined as the time from the date of allo-HSCT to the date of death from any cause, and EFS was defined as the time from the date of allo-HSCT to the date of events, including graft failure, disease progression with leukemic transformation, a veno-occlusive disease/sinusoidal obstruction syndrome (VOD/SOS), and death from any cause.

All statistical analyses were performed with EZR version 1.61 (available at Saitama Medical Center, Jichi Medical University, Saitama, Japan [https://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmedEN.html]), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). More precisely, it is a modified version of R commander designed to add statistical functions frequently used in biostatistics[29].

### D Ethical considerations

This study was conducted with approval by the institutional review board of Shinshu University School of Medicine (approval number #5749, approval date February 1st, 2023). Patients who did not agree to be included in the present study were excluded from the list using an opt-out method.

## Ⅲ Results

### A Patients

A total of 9 recipients of allo-HSCT for MF was identified during the study period. One of the patients underwent her second allo-HSCT and two patients used only single alkylator as their conditioning regimen; thus, the data from six transplants were included in this analysis. The characteristics of the enrolled patients are summarized in **Table 1**.

There were 2 males and 4 females with a median age (range) of 63 (31-69) years at the time of allo-HSCT. Their diagnoses were primary MF (n=1), post-PV MF (n=1) and post-ET MF (n=4). Driver mutations were detected in all the patients: JAK2 V617F (n=5) or CALR 52bp del (n=1). Chromosomal abnormalities were evaluated in all patients and an abnormal karyotype was found in 3 patients. Two of the abnormalities included a trisomy 9, and one of the patients had a complex karyotype, defined as harboring 3 or more abnormal karyotypes[30]. The disease risks were also summarized in **Table 1**; the DIPSS were either int-1 risk (n=2) or int-2 risk (n=3); the DIPSS-plus were either int-1 risk (n=1), int-2 risk (n=4), or high risk (n=1); and MYSEC-PM were either low risk (n=2), int-1 risk (n=2), or int-2 risk (n=1). Five of the six patients had splenomegaly, four of which were palpable below the left subcostal margin, at the time of allo-HSCT. One of the five patients had splenomegaly of 11cm below the subcostal margin despite of ruxolitinib treatment. The other patient (UPN#3) had received a splenectomy 6 months before allo-HSCT because he had an intrasplenic nodule, 7cm in diameter with a high uptake on $^{18}$F-FDG positron emission tomography, which might have been contraindicative to an organ transplantation if it were a metastatic lesion of another malignancy. It was, however, pathologically determined to be an extramedullary hematopoietic area of MF, and he was eventually considered to be indicated to allo-HSCT. Two patients with an int-1 risk with the DIPSS had increased blasts (>1 %) in their peripheral blood in repeated manual measurements (2-5 % in UPN #2 and 1-7 % in UPN #5). The per-

Table 1　Patients' demography

| UPN# | age at allo-HSCT | sex | At diagnosis | | | | Before allo-HSCT | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | diagnosis | BM fibrosis | driver mutation (allele burden %) | chromosomal abnormality | DIPSS# | DIPSS-plus# | MYSEC-PM# | subcostal splenomegaly palpable before HSCT | PS | Rux | Kucybala's index (Lmax) x (H vert) |
| 1 | 64 | M | primary MF | MF-2 | JAK2 (NA) | 46,XY | int-2 | int-2 | int-1 | 11cm | 0 | + | 2693 |
| 2 | 61 | F | post-ET MF | MF-3 | JAK2 (70.9) | 47,XX,add(6)(p21),+9, add(16)(p11.2) / 46,XX | int-2 | high | int-1 | 11cm | 0 | − | 4769 |
| 3 | 69 | M | post-ET MF | MF-1 | JAK2 (64.3) | 46,XY | int-1 | int-1 | int-2 | splenectomized** | 1 | − | 0 |
| 4 | 31 | F | post-ET MF | MF-2 | CALR (NA) | 46,XX | int-2 | int-2 | low | 4 cm | 0 | − | 2656 |
| 5 | 69 | F | post-PV MF | MF-2 | JAK2 (91.3) | 46,XX,del(20)(q11.2q13.3) / 46,XX | int-2 | int-2 | int-2 | not palpable | 0 | − | 1210 |
| 6 | 57 | F | post-ET MF | MF-3 | JAK2 (68.5) | 48,XX,+9,+21 / 46,XX | int-1 | int-2 | low | 6.5 cm | 0 | − | 2314 |

**The spleen, palpable for 2.5cm subcostal margin, was resected 6 months before allo-HSCT. # Risk scores were calculated within a month before allo-HSCT.

Table 2　Allo-HSCT with dual alkylators in patients with MF

| UPN# | Stem Cell Source | relationship of donor | CMV status (donor/recipient) | HLA disparity (HGV direction) | HLA disparity (GVH direction) | Conditioning regimen | intensity | ATG | ABO mismatch |
|---|---|---|---|---|---|---|---|---|---|
| 1 | BM | unrelated | +/+ | serum HLA-DR | serum HLA-DR | Flu-Bu-Mel | RIC | + | minor |
| 2 | BM | unrelated | −/+ | allele HLA-B | allele HLA-B | Flu-Bu-Mel | MAC | + | minor |
| 3 | BM | unrelated | +/+ | serum HLA-DR | matched | Flu-Bu-Mel | MAC | − | minor |
| 4 | BM | unrelated | −/+ | matched | matched | Flu-Bu-Mel | MAC | − | major |
| 5 | BM | unrelated | −/+ | matched | matched | Flu-Bu-Mel | RIC | − | major |
| 6 | BM | unrelated | +/+ | allele HLA-DR | allele HLA-DR | Flu-Bu-Mel | MAC | + | major |

Abbreviations ; allo-HSCT, allogeneic stem cell transplantation ; MF, myelofibrosis ; HVG direction, host-versus-graft direction ; GVH direction, graft-versus-host direction ; CB, cord blood ; BM, bone marrow ; ATG, anti-thymocyte immunoglobulin, Flu, fludarabine ; Mel, melphalan ; Bu, busulfan ; TBI, total body irradiation ;

formance status at the time of allo-HSCT was 0 or 1 in all patients.

**B Transplantation characteristics (Table 2)**

All transplantation procedures were performed at the Department of Hematology, Shinshu University Hospital. A conditioning was uniformly an FBM regimen ; intravenous busulfan (Bu ; 3.2 mg/kg) on day −7 to −4, melphalan (40 mg/m$^2$) on day −3 and −2, and fludarabine (Flu ; 30 mg/m$^2$) on day −7 to −2. Busulfan was reduced to a half-dose when a reduced intensity regimen was preferred to a myeloablative one for patients who were older than 65 years old and less fit.

The donors were unrelated bone marrow in all cases. The donor-recipient pairs of HLA typing were either one-locus serum mismatched (n = 2), one-locus allele mismatched (n = 2), or 8/8 matched (n = 2) in the HVG direction. In the GVH direction, the donor-recipient pairs were either one-locus serum mismatched (n = 1), one-locus allele mismatched (n = 2), of fully matched (n = 3). The ABO blood types were mismatched in a major direction (n = 3) and in a minor direction (n = 3).

A GVHD prophylaxis was a combination of a calcineurin inhibitor and short-term methotrexate. Rabbit anti-thymocyte immunoglobulin (ATG) 2 mg/m$^2$ was administered on day −1 if severe GVHD was anticipated because of HLA mismatch between the donor and recipient.

Prophylaxis for infections included levofloxacin (500 mg, daily) from day −7 until neutrophil engraftment, acyclovir (200 mg, twice daily) and fluconazole (200 mg, daily) from day −7 until a month after the cessation of GVHD prophylaxis or treatment. Fluconazole was replaced by voriconazole (200 mg, twice daily) or posaconazole (300 mg, twice daily) when a renovative construction was started at different wards in the same hospital building in August 2021. Since letermovir was approved in 2018, it was administered at a dose of 480 mg, once daily, for three months from the initiation of the conditioning regimen. Sulfamethoxazole (400 mg, daily) and trimethoprim (80mg, daily) were prescribed soon after the day of neutrophil engraftment until one month after the cessation of GVHD prophylaxis or treatment. Anti-epileptic agents were administered prophylactically along with busulfan.

**C Outcomes of transplantation**

Neutrophil engraftment was obtained in 6 of the 6 patients (100 %) with the median (range) time to neutrophil engraftment was +20 (19-31) days **(Table 3)**. One of them showed a delayed neutrophil engraftment (UPN#2). Platelet recovery of $\geq 2 \times 10^4/\mu$L was also observed in all the patients ; the patient with a delayed neutrophil engraftment required about 10 months to achieve the platelet engraftment. The median (range) days to platelet engraftment was +40 (29-292). All patients achieved platelet recovery of $\geq 5 \times 10^4/\mu$L but required even longer period of time ; the median (range) days was 51 (34-565). Chimerism analyses at +30 days after transplantation showed that full donor chimerism was observed in all the patients who received a dual-alkylator conditioning regimen. Acute GVHD, grade II, was observed in one patient. SOS/VOD event was not found in this cohort. Splenomegaly was significantly ameliorated in all the cases with enlarged spleen at the time of transplantation **(Table 3, Fig. 1)**. The bone marrow biopsy was performed in five of the six patients between 3 and 12 months after allo-HSCT ; pathological improvement was observed in all the subjects, including disappearance of fibrotic changes in three of them **(Table 3, Fig. 2)**. The median (range) period during which blood transfusion was required was 169 (37-278) days for red blood cell product and 36 (27-278) days for platelet product. All the patients did not require blood transfusion a year after allo-HSCT. All the patients are alive with the median (range) period of observation of 25.5 (11-54) months from allo-HSCT. The estimated 1-year OS was 100 %.

## Ⅳ Discussion

The present study provides the result of a retrospective analysis of the consecutive patients who received a dual alkylator conditioning regimen followed by bone marrow allo-HSCT for MF at a single institution over the past 10 years. The relatively small size of cohort and short observation period of

Table 3 The Outcome of Allo-HSCT in the 6 consecutive patients with MF

| UPN# | Days from HSCT to Engraftment | | acute GVHD | chronic GVHD | SOS/VOD | period from allo-HSCT (months) | Clinical Status at Last Observation | | | BM fibrosis after HSCT |
|---|---|---|---|---|---|---|---|---|---|---|
| | neutrophil | platelet | | | | | survival | PS | subcostal splenomegaly | |
| 1 | 20 | 51 | – | – | negative | 54 | alive | 1 | not palpable | MF-1 |
| 2 | 31 | 292 | – | – | negative | 43 | alive | 1 | not palpable | no fibrosis |
| 3 | 21 | 36 | Grade II | – | negative | 30 | alive | 1 | not applicable | no fibrosis |
| 4 | 20 | 29 | – | – | negative | 21 | alive | 1 | 2.5cm palpable | biopsy not available |
| 5 | 19 | 35 | – | – | negative | 18 | alive | 1 | not palpable | no fibrosis |
| 6 | 20 | 44 | – | – | negative | 11 | alive | 1 | 4cm palpable | MF-2 |

Abbreviations ; allo-HSCT, allogeneic stem cell transplantation ; MF, myelofibrosis ; GVHD, graft versus host disease ; SOS/VOD, sinusoidal obstruction syndrome/veno-occlusive disease ; PS, performance statuts ; NA, not available ; *An increase of neutrophil was observed, but a chimerism analysis conducted a week later suggested that the engraftment was recepient origin.

our study make it difficult to draw definitive conclusions ; however, our results may suggest several points that are worth mentioning.

Splenomegaly was often attributed to delayed hematologic recovery after allo-HSCT in MF in previous literatures[6]. A sequestration of transplanted stem cells and progeny platelet in the residual enlarged spleen has been a suggested mechanism. Indeed, a platelet engraftment was apparently delayed in one of our patients, UPN#2, who had presented the largest spleen in size among the cohort. However, neutrophil and platelet engraftment were eventually achieved in all the patients, while 83 % of the patients had significant splenomegaly at the time of transplantation **(Table 1)**. Spearman rank-order correlations showed that there were only weak associations between the spleen volume at the time of transplantation and the days required for engraftment of neutrophil ($r = 0.334$, $p = 0.518$) and platelet ($r = 0.6$, $p = 0.242$) **(Fig. 3)**. A favorable result in terms of engraftment in this cohort was also supported by a full donor chimerism at +30 days after allo-HSCT attained in all the subjects. Although a comparative analysis between different conditioning regimens is beyond a scope of the present study, we could speculate that FBM regimen may have a potential to overcome the negative effect of a splenomegaly and to guarantee an engraftment of the transplanted donor's hematopoietic precursors in MF patients.

The rate of VOD/SOS occurrence after allo-HSCT was generally described as about 5 to 10 % of allo-HSCT recipients, and a busulfan-containing conditioning regimen is known to impart a high risk of VOD/SOS, especially in a high dose regimen or in a combination with another alkylator[31)32)]. The rate of VOD/SOS was described as 3-9 % of patients with MF after another dual alkylator regimen, FBT[17)33)]. Our cohort, which a dual alkylator FBM regimen was universally adopted to, did not have a case of VOD/SOS, possibly because our cohort was too small for a clinician to encounter an event when the incidence rate was up to 10 %.

Several limitations associated with the present study warrant mention. We speculated that the favorable

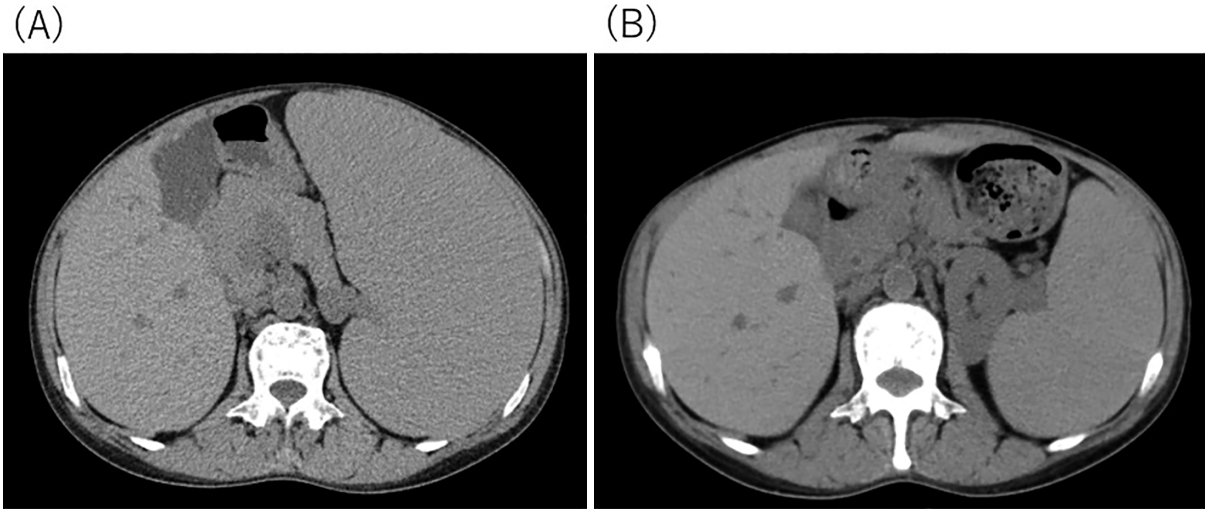


Fig. 1　Improvement of splenomegaly after allo-HSCT

The enlarged spleen was significantly ameliorated after allo-HSCT. Abdominal computed tomography scans before (A) and 3 months after allo-HSCT (B) in UPN#2, as a representative case of splenomegaly in MF patient. Kucybala's index was decreased from 4769 to 1932 in 3 months after allo-HSCT in this case.

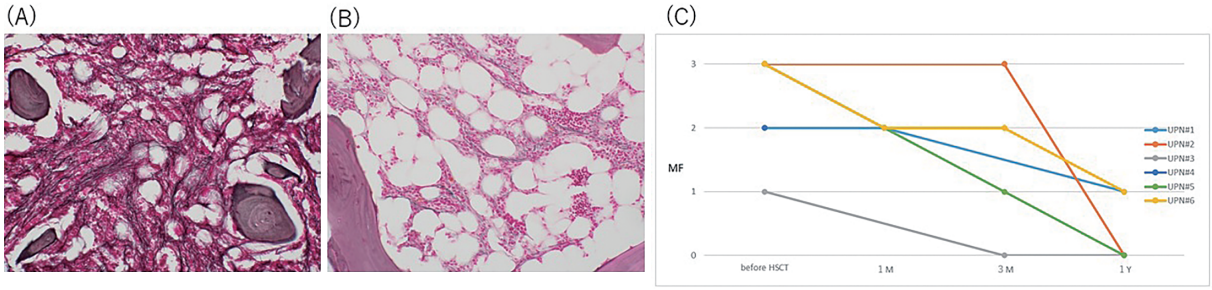


Fig. 2　Improvement of bone marrow fibrosis after allo-HSCT

The bone marrow biopsy samples of UPN#5, as a representative case of MF, showed pathological improvement of bone marrow fibrosis (original magnification, ×10; Silver stain); before (A) and 3 months after allo-HSCT (B). The fibrosis was improved from MF-2 to MF-1 in 3 months after allo-HSCT. Fig. 2(C) shows longitudinal changes of fibrosis; the vertical axis indicates the gradings of bone marrow fibrosis and each colored line represents a case of MF. Pathological improvement was observed in all the subjects, including disappearance of fibrotic changes in three cases.

engraftment result of our cohort may possibly be associated with a dual alkylator regimen that we universally adopted. However, a comparative analysis with a large number of allo-HSCT recipients with MF may be necessary to draw a firm conclusion of superiority of one regimen to another. For example, more than 500 registry of MF patients may be required to show a superiority of one conditioning regimen to another in 1-year overall survival, if we perform a cox hazard ratio analysis with a presupposition of 10 % of event rate and five confounding factors included. However, an estimated incidence of MF is 0.3 per 100 thousand a year and majority of them are elderly and thus ineligible for transplant[8]. The largest cohort study of allo-HSCT for MF in Japan had 224 cases for 24 years[8]. The goal of the present study may thus be to summarize the characteristics and outcomes of the transplantation for MF at our institution in the previous ten years, which should eventually provide a dataset to be referred to in a clinical comparative study in the future to develop a better transplantation platform at this institution.

Besides a conditioning regimen, other factors of transplantation may also be confoundings to analyze

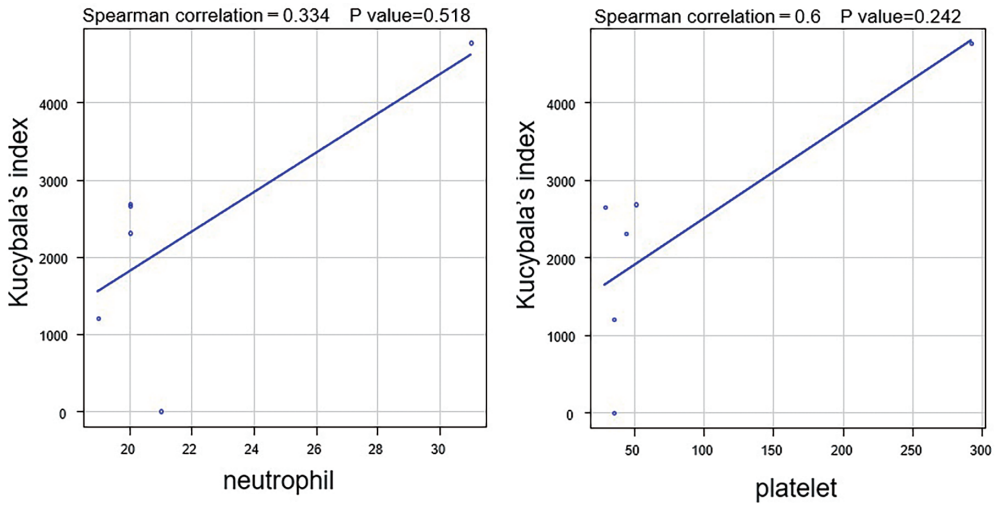


Fig. 3 Associations between the spleen volume before transplantation and days required for engraftment
A spleen volume was represented by a product of the maximal length (Lmax) and the vertical hight (Hvert) on the abdominal CT scans, a Kucybala's index. Spearman's rank-order correlation coefficient was calculated to analyze associations between the Kucybala's indices and the days required for the engraftment of neutrophil (A) and platelet (B). The correlations were weak, implying that neutrophil and platelet engraftment was attained regardless of the size of the spleen at the time of transplantation.

the outcomes in patients with MF, such as the intensity of regimens, HLA disparity, GVHD prophylaxis, degree of BM fibrosis and splenic size at the time of transplantation, and usage of ruxolitinib before transplantation. These factors should have been included in a propensity score matching analysis, for example, with a larger size of cohort to draw a more solid conclusion about a role of dual alkylator regimen as a conditioning for MF, although the small size of our cohort precluded such an analysis. Furthermore, new treatment strategies have been recently introduced to allo-HSCT for MF, such as alternative donors, a post-transplantation cyclophosphamide as a GVHD prophylaxis, and a GVHD treatment with ruxolitinib and ibrutinib. These novel strategies will also confer different outcomes of allo-HSCT for MF in near future.

## V Conclusion

We retrospectively summarized the transplantation outcomes of all the consecutive cases of MF receiving allo-HSCT after a dual alkylator regimen at a single institution. We speculated that a dual-alkylator regimen of FBM may be a candidate conditioning for bone marrow allo-HSCT in patients with MF, which needs to be verified in studies with a large sized cohort of patients.

## Conflict of Interest

The authors declare that they have no competing interests.

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