Allogeneic hematopoietic stem cell transplantation for primary and secondary myelofibrosis: a retrospective, multicenter study of the Kanto Study Group for Cell Therapy (KSGCT)


Introduction

Myelofibrosis (MF) is a clonal stem cell-derived myeloproliferative neoplasm (MPN) that results in bone marrow fibrosis, extramedullary hematopoiesis, splenomegaly, and insufficient hematopoiesis. Primary myelofibrosis (PMF) is the least common among chronic MPNs; one study reported an estimated incidence of 1.5 cases per 100,000 subjects per year. The median age at diagnosis is 50–67 years. In a Japanese cohort of 298 PMF patients, median survival was reported to be 10.0 years. The incidence of secondary myelofibrosis (sMF), which arises after essential thrombocytosis (ET) and polycythemia vera (PV), is 10–20% after 15–20 years of follow-up.

The optimal candidates and timing for allogeneic stem cell transplantation (allo-SCT) in patients with primary myelofibrosis (PMF) and secondary myelofibrosis (sMF) are unknown. We retrospectively examined the outcomes of PMF (n = 13) and sMF (n = 8) patients who underwent allo-SCT between 1997 and 2008. The median age at transplantation was 50 years (range, 21–60). Thirteen subjects (61.9%) received myeloablative conditioning. The source of hematopoietic cells was HLA-matched related (52.3%), -matched unrelated (33.3%), or -mismatched unrelated (9.5%) donors. All patients achieved engraftment, and the median time to neutrophil and platelet recovery was 19 (range, 13–36) and 75 (range, 15–411) days, respectively. With a median follow-up of 16.7 (range, 1–134) months, overall survival (OS) at 60 months was 55.6% (95% CI, 34.0–77.0%). No significant differences in OS were observed between PMF and sMF patients and between myeloablative and reduced-intensity conditioning. HLA-mismatched donors, lower platelet count (<10 x 10^4/μl), and previous blastic transformation were associated with a significantly worse prognosis. These data suggest that allo-SCT with myeloablative or reduced-intensity conditioning is potentially effective for PMF and sMF patients aged <60 years. However, a platelet count of <10 x 10^4/μl is a strong adverse factor. (Journal of Hematopoietic Cell Transplantation Vol. 1 No. 1; 15–23, 2012)
and sMF in the late stage.\cite{8} TEL-Lyn, TEL-JAK2, and long- 
term administration of thrombopoietin, which also activate 
STAT5, have been shown to induce MF in vivo.\cite{9-12} These 
results indicate that the JAK2–STAT5 signaling cascade is very 
important in MF development. Specific inhibitors of JAK2 
kine

Allogeneic hematopoietic stem cell transplantation (allo-
SCT) is the only curative treatment for MF. Recent studies 
revealed that reduced-intensity conditioning could be applied 
to MF patients.\cite{15-20} However, considering the small number of MF patients, relatively slow disease progression, and high-
er age of patients at MF diagnosis, the optimal timing and 
candidates for allo-SCT remain controversial. To answer 
these questions, we retrospectively examined the outcomes of 
21 PMF and sMF patients who underwent allo-SCT between 

Patients and methods

Patients

Twenty-one PMF (n = 13) and sMF (n = 8) patients who un-
derwent allo-SCT from 1997 to 2008 in 9 facilities of the 
Kanto Study Group for Cell Therapy (KSGCT; Kanto, Japan) 
were retrospectively analyzed in this study. sMF includes MF 
 arising after ET and PV. Myelodysplastic syndrome with 
bone marrow fibrosis was not included in this study. Trans-
formation into acute myeloid leukemia (AML) was defined 
as >20% blasts in blood or bone marrow. Pre-transplant pa-
tient characteristics are summarized in Table 1. The median 
age at transplantation was 50 years (range, 21–60), and the median time from diagnosis to transplantation was 30.9 
months (range, 6.6–242). No significant differences were ob-
erved between PMF and sMF patients with respect to pre-
transplant characteristics. Only 1 patient was assessed for the 
JAK2 mutational status; he was negative for the mutation but 
positive for the novel TEL-Lyn fusion gene.\cite{9} Ten patients re-
ceived chemotherapy prior to allo-SCT. Most patients had 
splenomegaly (n = 19, 90.5%), but only 2 patients received 
treatment for splenomegaly: 1 patient underwent splenectomy 
and the other received irradiation to the spleen.

Among all 21 patients, only 2 patients with PMF under-
went “upfront” allo-SCT. Other patients underwent allo-SCT 
if the disease progressed. The results of 2 patients included 
in the present study have been reported previously.\cite{9,21}

The source of hematopoietic cells was HLA-matched re-
lated (n = 11, 52.3%), -matched unrelated (n = 7, 33.3%), 
or-mismatched unrelated (n = 3, 9.5%) donors. Most patients 
(n = 18, 85.7%) underwent bone marrow transplantation. 
Only 3 patients received peripheral blood stem cells (PBSCs) 
(Table 1). All unrelated donors were recruited via the Japan 
Marrow Donor Program (JMDP).

Among the 21 patients, 13 patients underwent convention-
al myeloablative stem cell transplantation (CST) and 8 pa-
tients underwent reduced-intensity stem cell transplantation 
(RIST) (Table 1).

Criteria for engraftment and GVHD

The day of neutrophil engraftment was defined as the first 
day of 3 consecutive days with neutrophil counts of >500/\textmu l. 
The day of platelet engraftment was defined as the first day 
of 7 consecutive days with platelet counts of >20>10^3/\textmu l 
without transfusion. Acute and chronic GVHD were graded 
according to standard criteria.\cite{17}

Statistical analysis

All data were analyzed according to the intention-to-treat 
principle. For the baseline variables, summary statistics were 
constructed employing frequencies and proportions for cate-
gorical data, and medians and ranges for continuous varia-
bles. We compared patient characteristics using Fisher’s 
exact test for categorical outcomes and Mann–Whitney U test 
for continuous variables, as appropriate.

For time-to-event outcomes, the lengths of time to a first 
event were compared using the log-rank test, while the Ka-
plan–Meier method was used to estimate the absolute risk of 
each event for each group, and hazard ratios and 95% confi-
dence intervals (CIs) were estimated by the Cox propor

lational hazards model. Overall survival (OS) was defined as time 
from transplantation to death due to any cause, and patients 
alive at the time of last follow-up were censored at that date. 
Time to progression (TTP) was defined as “relapse or pro-
gression” with censoring of death unrelated to MF or patients 
who were lost to follow-up, and the corresponding time inter-
val was from transplantation to event. Non-relapse mortality 
(NRM) was defined as death due to all causes not related to MF. 
Univariate analyses of risk factors for OS was per-
fomed by Cox regression analyses, whereas risk factors for 
TTP and NRM were examined in a competing risks regres-

To identify baseline and clinical factors associated with OS, multivariate analysis was performed using the Cox proportional hazard model with a step-wise selection procedure. The stepwise procedure was set to a threshold of 0.15 for inclusion and 0.15 for exclusion.

All comparisons were planned and the tests were two-sid-
A p-value of less than 0.05 was considered to be statistically significant. All statistical analyses were performed by using the SAS software program, version 9.2 (SAS Institute Inc., Cary, NC).

Results

Engraftment
All patients achieved neutrophil and platelet engraftment at a median time of 19 (range, 13–36) and 75 (range, 15–411) days, respectively. No significant differences in median time to neutrophil engraftment were observed between patients who underwent CST and RIST (19 days vs. 22 days, p = 0.66) and between patients with PMF and sMF (19 days vs. 25.5 days, p = 0.71). Median time to platelet engraftment was not significant between patients who underwent CST and RIST (75 days vs. 75 days, p = 1.00) and between patients with PMF and sMF (26 days vs. 90 days, p = 0.30). Cumulative incidences of neutrophil and platelet engraftment are shown in Figure 1.

GVHD
The incidence of acute GVHD of grades II–IV and III–IV was 33.3% and 14.3%, respectively. No significant difference was observed in the incidence of acute GVHD between sibling and unrelated donors. Among 18 evaluable patients, the overall incidence of chronic GVHD was 50%, with limited disease in 28% and extensive disease in 22%.

Mortality and survival
Ten patients died during the follow-up period. The reasons for death were as follows: disease relapse or progression (n = 6); pulmonary complications (bronchiolitis obliterans, idiopathic pneumonia syndrome, and pneumonia; n = 3); and sinusoidal occlusive disease accompanied by acute GVHD (n = 1).

After a median follow-up of 16.7 months (range, 1–134), OS was 55.6% at 60 months (95% confidence interval (CI), 34.0–77.0%; Figure 2A). No significant differences in OS at 60 months were observed between patients aged ≤ 55 years and those aged > 55 years (54.7% vs. 60%, p = 0.77), patients with PMF and sMF (53.8% vs. 62.5%, p = 0.96), and patients who underwent CST and RIST (59.3% vs. 50.0%, p = 0.86; Figure 2B, 2C, 2D). NRM at day 100 and 12 months was 9.5% (95% CI, 0–22.1%) and 22.6% (95% CI, 2.6–42.6%), respectively.

In univariate analysis, significant factors for OS were lower platelet count (<10 × 10^4/μl) at transplantation (p = 0.004) and previous transformation into AML (p = 0.024) (Table 2). These two variables showed relatively strong correlation (p = 0.534); therefore, the latter variable was excluded from multivariate analysis. In multivariate analysis, HLA-mismatched transplantation (hazard ratio (HR) = 36.20; 95% CI, 1.02–1278.51; p = 0.038) and lower platelet count (<10 × 10^4/μl) at transplantation (HR = 32.85; 95% CI, 2.07–520.43; p = 0.0132) were significant prognostic factors for OS (Table 2).

In univariate analysis for NRM, acute GVHD (HR = 7.52; 95% CI, 0.78–72.6; p = 0.081) showed a relatively low p value, but none of the factors included in the present study was significant (Table 3). In univariate analysis for TTP, only lower platelet count at transplantation was a significant factor (HR = 14.5; 95% CI, 1.64–128; p = 0.016) (Table 3).

Figure 3 shows the Kaplan–Meier survival curve showing OS stratified by the platelet count at transplantation. Patients with a lower platelet count showed significantly worse survival in all disease categories.

Figure 1. Cumulative incidences of neutrophil (A) and platelet (B) engraftment after transplantation.
Discussion

In the present study, we showed that all patients successfully achieved marrow engraftment after CST and RIST, and no significant differences in OS were observed between PMF and sMF patients, which are compatible with the results of other studies.\textsuperscript{20,23} PV, ET, and PMF share a common background to some extent; therefore, it may be difficult to distinguish PMF from sMF if a patient is referred to hospital with advanced disease. In this context, whether we should distinguish between PMF and sMF when we consider the indications for allo-SCT is open to discussion.

Extensive marrow fibrosis associated with PMF was initially considered a contraindication for allo-SCT. However, despite earlier concerns that marrow fibrosis may hinder hematopoietic recovery after allo-SCT, multiple reports have shown that engraftment is obtained consistently and that extensive fibrosis is completely reversible with successful allo-SCT.\textsuperscript{24}

The median age at diagnosis in MF patients is ranging between 50–67 years,\textsuperscript{2,3} which means that most of them are not eligible for conventional myeloablative conditioning. RIST would be a promising alternative for older patients, but it may not be a sufficiently secure engraftment under severe marrow fibrosis. Nevertheless, reports have shown that allo-SCT can be successfully performed with standard myeloablative and reduced-intensity regimens.\textsuperscript{15,20,23,25,26} These results are consistent with those of the present study, which revealed prompt neutrophil recovery in all patients regardless of their conditioning intensity. No significant difference was observed in OS and NRM between patients with standard myeloablative and reduced-intensity regimens, which is also compatible with the results of previous reports.\textsuperscript{23,25} Although we could not find statistical significance in TTP between standard myeloablative and reduced-intensity regimens, Stewart et al. reported that the relapse rate of patients receiving reduced-in-
Intensity regimens (46%) is increased compared with that of those prepared by myeloablative conditioning (15%), approaching significance (P \( \leq 0.06 \)). Therefore, allo-SCT with reduced-intensity conditioning could be the potential treatment of choice for MF patients who are not eligible for myeloablative conditioning and should be carefully chosen for younger MF patients, considering its association with a higher risk of relapse compared with myeloablative conditioning.

Presentation and evolution of PMF is heterogeneous. The estimated median survival of 6 years is significantly affected by the presence or absence of readily available parameters such as the hemoglobin level, platelet count, leukocyte count, constitutional symptoms, and cytogenetic findings. Unfortunately, controlled studies that would facilitate patient selection for allo-SCT in MF are lacking. Reports suggested that patients with a higher risk of disease may benefit most by transplantation. However, these same retrospective studies also suggested that young patients with a low risk of disease could be expected to survive longer. Therefore, the optimal timing and candidates for allo-SCT remain controversial.

In the present study, in most cases, allo-SCT was performed at disease progression, such as thrombocytopenia, leukocytopenia, excess of blasts, and anemia. In univariate analysis, lower platelet count (<10 \( \times \) \( 10^4 / \mu l \)) at transplantation and previous transformation into AML were associated with poor OS; these findings are consistent with those of a previous report. Furthermore, lower platelet count at transplantation was significantly associated with TTP, which suggested that patients with a lower platelet count at transplantation were more likely to suffer a relapse after allo-SCT.

Previous transformation into AML showed significant association with a lower platelet count at transplantation. This is reasonable because thrombocytopenia is a common manifestation of leukemic transformation in MF patients. Considering our finding, platelet and blast counts may be useful indicators to decide the optimal timing for transplantation.

### Table 2. Univariate and multivariate analyses of OS data using a Cox regression model

<table>
<thead>
<tr>
<th>Variables</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
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<tbody>
<tr>
<td>Sex</td>
<td></td>
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<tr>
<td>Male vs. female</td>
<td>HR (95% CI) 0.58 (0.15 – 2.33)</td>
<td>p 0.44</td>
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<td>Age at transplantation</td>
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<tr>
<td>≥50 vs. &lt;50</td>
<td>HR (95% CI) 1.21 (0.32 – 4.51)</td>
<td>p 0.78</td>
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<tr>
<td>Diagnosis</td>
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<tr>
<td>PMF vs. sMF</td>
<td>HR (95% CI) 1.028 (0.26 – 4.14)</td>
<td>p 0.97</td>
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<tr>
<td>Conditioning regimen</td>
<td></td>
<td></td>
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<tr>
<td>CST vs. RIST</td>
<td>HR (95% CI) 0.886 (0.24 – 3.30)</td>
<td>p 0.86</td>
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<tr>
<td>Stem cell source</td>
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<tr>
<td>BM vs. PBSC</td>
<td>HR (95% CI) 1.554 (0.19 – 12.45)</td>
<td>p 0.68</td>
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<tr>
<td>Previous transformation into AML</td>
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<tr>
<td>Yes vs. No</td>
<td>HR (95% CI) 4.366 (1.21 – 15.75)</td>
<td>p 0.024</td>
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<tr>
<td>PLT at transplantation</td>
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<tr>
<td>&lt;10 vs. ≥10 (( \times 10^4 / \mu l ))</td>
<td>HR (95% CI) 10.173 (2.06 – 50.31)</td>
<td>p 0.004</td>
</tr>
<tr>
<td>Hb at transplantation (g/dl)</td>
<td></td>
<td></td>
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<tr>
<td>≤10 vs. 10</td>
<td>HR (95% CI) 4.864 (0.61 – 39.07)</td>
<td>p 0.14</td>
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<tr>
<td>HLA matching</td>
<td></td>
<td></td>
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<tr>
<td>Full match vs. mismatch</td>
<td>HR (95% CI) 3.369 (0.64 – 17.70)</td>
<td>p 0.15</td>
</tr>
<tr>
<td>Grade II—IV acute GVHD</td>
<td></td>
<td></td>
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<tr>
<td>Yes vs. No</td>
<td>HR (95% CI) 3.255 (0.87 – 12.24)</td>
<td>p 0.081</td>
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<tr>
<td>Chronic GVHD</td>
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<td></td>
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<tr>
<td>Yes vs. No</td>
<td>HR (95% CI) 6.382 (0.74 – 54.91)</td>
<td>p 0.091</td>
</tr>
</tbody>
</table>
In multivariate analysis, HLA-mismatched SCT and lower platelet count were associated with a significantly worse prognosis (Table 2). We could not find statistical significance with HLA-mismatched SCT in univariate analysis, which was probably due to the small number of patients. Several reports have shown that HLA-mismatched SCT is associated with a poor prognosis mainly because of a higher incidence of NRM. Although we could not find a significant association between NRM and HLA-mismatched SCT (HR = 3.79; 95% CI, 0.33–43.5; p = 0.29), we consider that NRM affected the inferior OS in our cohort.

Splenomegaly is a common feature of MF patients because once bone marrow hematopoiesis is impaired by fibrosis, extramedullary hematopoiesis may recur in the spleen. Although splenectomy before allo-SCT is a treatment option for MF patients, whether splenectomy is associated with improved outcome remains uncertain. Li et al. analyzed the impact of pre-transplant splenectomy on post-transplant outcome in 26 patients. Post-transplant granulocyte recovery was faster among splenectomized patients, and the need for red blood cell and platelet transfusion was greater among patients who had intact spleens. However, in their series, no significant improvement was observed in prognosis after splenectomy. Recently, Bacigalupo et al. reported that splenectomy in patients with a spleen length >22 cm showed a lower incidence of relapse-related death in the setting of transplantation without peri-operative mortality in splenectomized patients. In the present study, only 2 patients received treatment for splenomegaly (splenectomy (n=1) and irradiation to the spleen (n=1)) before transplantation. Because of a smaller number of patients, we could not find differences between those who had their spleen treated and those with untreated spleens. In many reports, splenectomy is associated with mortality of 5–10%, and the role of splenectomy remains controversial. Hence, we consider that splenectomy before allo-SCT should be indicated only for patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>NRM HR (95% CI)</th>
<th>p</th>
<th>TTP HR (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male vs. female</td>
<td>0.39 (0.04–3.74)</td>
<td>0.41</td>
<td>0.74 (0.12–4.44)</td>
</tr>
<tr>
<td>Age at transplantation</td>
<td>≥50 vs. &lt;50</td>
<td>0.99 (0.14–7.04)</td>
<td>0.99</td>
<td>1.30 (0.22–7.83)</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>PMF vs. sMF</td>
<td>0.64 (0.07–6.24)</td>
<td>0.70</td>
<td>1.21 (0.20–7.38)</td>
</tr>
<tr>
<td>Conditioning regimen</td>
<td>CST vs. RIST</td>
<td>0.67 (0.09–4.72)</td>
<td>0.68</td>
<td>0.99 (0.17–5.98)</td>
</tr>
<tr>
<td>Previous transformation into AML</td>
<td>Yes vs. No</td>
<td>3.27 (0.34–31.7)</td>
<td>0.31</td>
<td>2.13 (0.39–11.6)</td>
</tr>
<tr>
<td>PLT at transplantation</td>
<td>&lt;10 vs. ≥10 (10⁴/µl)</td>
<td>5.33 (0.45–62.7)</td>
<td>0.18</td>
<td>14.5 (1.64–128)</td>
</tr>
<tr>
<td>Hb at transplantation (g/dl)</td>
<td>≤10 vs. &gt;10</td>
<td>NA</td>
<td>1.00</td>
<td>2.34 (0.26–21.0)</td>
</tr>
<tr>
<td>HLA matching</td>
<td>Full match vs. mismatch</td>
<td>3.79 (0.33–43.5)</td>
<td>0.29</td>
<td>2.44 (0.25–24.1)</td>
</tr>
<tr>
<td>Grade II–IV acute GVHD</td>
<td>Yes vs. No</td>
<td>7.52 (0.78–72.6)</td>
<td>0.08</td>
<td>1.51 (0.25–9.11)</td>
</tr>
<tr>
<td>Chronic GVHD</td>
<td>Yes vs. No</td>
<td>NA</td>
<td>1.00</td>
<td>3.19 (0.33–30.9)</td>
</tr>
</tbody>
</table>

NA, Not available
who are symptomatic from splenomegaly, who present refractory hemolytic anemia, or who exhibit complications of portal hypertension.24,30

In conclusion, allo-SCT with myeloablative and reduced-intensity conditioning are potentially effective treatment strategies for PMF and sMF patients aged <60 years with a high engraftment rate. A platelet count of $<10^4/\mu l$ is a strong risk factor for allo-SCT in MF patients. Thus, transplant-eligible MF patients with decreasing platelet count should be considered for allo-SCT.

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Authors’ contributions

M.T., C.N. and C.O. designed the study, analyzed data, and wrote the manuscript; K. O., K. K., T. M., Y. A., Y. K., S. T., A. Y., T. K., T. S., N. H., J. T., H. T., H. K., A. M., H. S. and S. O. analyzed the data and approved the manuscript; M. T., C. O. and Y. S. performed statistical analysis.

Conflict-of-interest disclosure

The authors declare no competing financial interests.

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