Hematopoietic recovery

A total of 113 patients achieved primary engraftment with a median time to reach a neutrophil count of 0.5×10^9/L or higher and a platelet count of 2.0×10^9/L or higher of 14 d (range, 10–40 d) and 22 d (range, 8–105 d), respectively. The median times to reach these neutrophil and platelet counts were earlier in the RIC group than the MAC group (neutrophil: 14 vs. 19 d, $P < 0.001$; platelet: 21 vs. 29 d, $P = 0.005$), as shown in Table 2. None of the patients experienced primary graft failure. All but two patients, who died before day 30 after allo-HCT without evidence of engraftment, were assessed for hematopoietic recovery, and 6 (5%) experienced secondary graft failure.

Graft-versus-host disease

The 113 patients who achieved engraftment was evaluated for aGVHD. The incidence of grade II–IV aGVHD was 42% and that of grade III–IV aGVHD was 14%, as shown in Table 2. There was no significant difference between the RIC and MAC groups in the incidence of aGVHD. Among the 107 patients who survived more than 100 d after allo-HCT, 10 (9%) developed limited cGVHD and 48 (45%) developed extensive cGVHD. There was no significant difference between the RIC and MAC groups with regard to the incidence of cGVHD.

Non-relapse mortality

The 4-yr incidence of NRM was 29% in the MAC group and 33% in the RIC group ($P = 0.89$) (Fig. 1A). In a univariate analysis, covariates associated with a higher incidence of NRM were recipient sex [female, hazard ratio (HR) 2.9, 95% CI 1.1–7.5, $P = 0.03$], IPSS risk at diagnosis (Int-2/High, HR 2.2, 95% CI 1.1–4.7, $P = 0.04$), the FAB stage at peak (RAEB/CMMoL, HR 2.8, 95% CI 1.0–7.7, $P = 0.05$), cytogenetic risk at diagnosis (poor, HR 2.0, 95% CI 1.1–4.0, $P = 0.03$), BM blasts at HCT (20% or higher, HR 4.1, 95% CI 1.7–10.2, $P = 0.002$), and the presence of aGVHD (grade III–IV, HR 4.4, 95% CI 2.2–9.0, $P < 0.001$), as shown in Table S1. In a multivariate analysis (Table 3), the covariates associated with a higher incidence of NRM were the presence of aGVHD (grade III–IV, HR 6.9, 95% CI 2.7–17.4, $P < 0.001$) and BM blasts at HCT (20% or higher, HR 3.6, 95% CI 1.3–9.9, $P = 0.01$). cGVHD in this model was not an independent factor for NRM when substituted for grade III–IV aGVHD (data not shown).

Relapse

The 4-yr incidence of relapse was 26% in the MAC group and 25% in the RIC group ($P = 0.97$) (Fig. 1B). In a univariate analysis, the only covariate associated with a higher relapse rate was prior chemotherapy (HR 2.5, 95% CI 1.1–5.8, $P = 0.04$), as shown in Table S1. In a multivariate analysis (Table 3), covariates associated with a higher relapse rate were prior chemotherapy (HR 4.3, 95% CI 1.2–15.9, $P = 0.03$), BM blasts at HCT (5–19%, HR 4.3, 95% CI 1.5–12.8, $P = 0.008$) and the absence of cGVHD (HR 12.7, 95% CI 3.1–52.6, $P < 0.001$). Grade II–IV or III–IV aGVHD in this model was not an independent factor for relapse when substituted for cGVHD (data not shown).

Table 2 Transplantation outcome

No. of patients	All $N = 115$	MAC $N = 34$	RIC $N = 81$
Graft failure (%)			
Primary	0 (0)	0 (0)	0 (0)
Secondary	6 (5)	1 (3)	5 (6)
Engraftment			
Neutrophils $\geq 0.5 \times 10^9$/L Median days (range)	14 (10–40)	19 (10–40)	14 (10–27)
Platelets $\geq 20 \times 10^9$/L Median days (range)	22 (8–105)	29 (13–90)	21 (8–105)
Acute GVHD (%)			
II–IV	48 (42)	12 (35)	36 (44)
III–IV	16 (14)	4 (11)	12 (15)
Onset, median days (range)	30 (5–98)	34 (9–66)	31 (9–68)
Chronic GVHD (%)			
Limited	10 (10)	4 (14)	6 (8)
Extensive	48 (47)	11 (39)	37 (50)
Onset, median days (range)	138 (100–1090)	124 (100–245)	134 (100–1090)

MAC, myeloablative conditioning; RIC, reduced intensity conditioning; GVHD, graft-versus-host disease.

Overall survival

In the overall population, the 4-yr OS was 44%. Although patients in the RIC group were older and had a worse cytogenetic risk, no difference in OS was seen between the two groups (47% in the MAC group vs. 42% in the RIC group, $P = 0.84$) (Fig. 1C). Fifty two patients (45%) were alive and 63 (55%) had died. Disease relapse or progression (40%) was the most common cause of death, followed by non-relapse causes complicated by organ failure (23%), infection (19%), GVHD (6%), and others (12%) (Table 4). In a univariate analysis, covariates associated with a worse OS were older age (60 yrs or older, HR 1.7, 95% CI 1.0–2.9, $P = 0.04$), the FAB stage at diagnosis (RAEB/CMMoL, HR 1.8, 95% CI 1.0–3.2, $P = 0.04$), IPSS risk at diagnosis (Int-2/High, HR 2.4, 95% CI 1.3–4.4, $P < 0.001$), the FAB stage at peak (RAEB/CMMoL, HR 2.3, 95% CI 1.0–5.2, $P = 0.04$ RAEB-T/AML-MLD, HR 2.6, 95% CI 1.2–5.7,

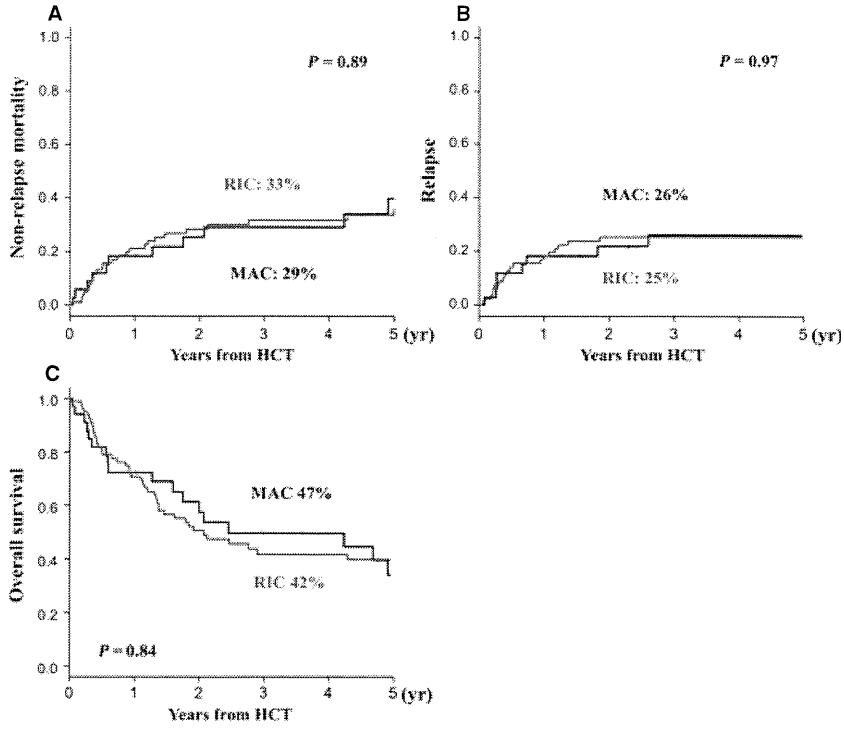

Figure 1 Outcomes stratified according to the intensity of the conditioning regimens. non-relapse mortality (A), Relapse (B) and overall survival (C) of patients with myelodysplastic syndrome receiving allo-hematopoietic cell transplantation after myeloablative conditioning or reduced-intensity conditioning regimens.

$P = 0.01$), IPSS risk at peak (Int-2/High, HR 2.3, 95% CI 1.1–5.0, $P = 0.02$), cytogenetic risk at diagnosis (poor, HR 2.2, 95% CI 1.3–3.7, $P < 0.001$), BM blasts at HCT (20% or higher, HR 3.4, 95% CI 1.6–7.2, $P < 0.001$), and the presence of aGVHD (Grade III–IV, HR 2.8, 95% CI 1.5–5.4, $P = 0.001$), as shown in Table S1. In a multivariate analysis (Table 3), covariates associated with a worse OS were the FAB stage at peak (RAEB-T/AML-MLD, HR 3.3, 95% CI 1.2–8.6, $P = 0.02$), cytogenetic risk at diagnosis (poor, HR 2.1, 95% CI 1.1–6.9, $P = 0.01$), BM blasts at HCT (20% or higher, HR 3.0, 95% CI 1.3–6.9, $P = 0.01$) and the absence of cGVHD (HR 2.0, 95% CI 1.1–4.0, $P = 0.04$). The presence of grade III–IV aGVHD was significantly associated with a worse OS (HR 5.4, 95% CI 2.5–11.4, $P < 0.001$) when this was substituted for cGVHD in this model.

In semi-landmark analyses for the entire population, the OS of patients with cGVHD tended to be better than that of patients without cGVHD ($P = 0.11$) (Fig. 2A). When the analysis was limited to the RIC group, the OS of patients with cGVHD was significantly better than that of patients without cGVHD ($P = 0.005$) (Fig. 2B). We also found that, in patients with poor cytogenetic risk, the OS of patients with cGVHD was significantly better than that of patients without cGVHD ($P = 0.003$) (Fig. 2C), whereas in patients with good/intermediate cytogenetic risk, there was no significant difference in OS between the two groups ($P = 0.76$) (Fig. 2D). In patients with BM blasts 5% or higher at HCT, the OS of patients with cGVHD was significantly better than that of patients without cGVHD ($P = 0.02$) (Fig. S1A), whereas in patients with BM blasts <5% at HCT, there was no significant difference in OS between the two groups ($P = 0.59$) (Fig. S1B).

Impact of extensive cGVHD in the RIC group

The median age in the RIC group was 57 (19–68) yrs. Among the 81 patients in the RIC group, 46 patients (58%) had cGVHD. The majority (86%) of patients with cGVHD developed extensive cGVHD. We also conducted a multivariate analysis limited to the patients pre-treated with RIC (Table S2) and found that the absence of extensive cGVHD was significantly associated with a worse OS (HR 2.4, 95% CI 1.2–5.5, $P = 0.001$) and a higher relapse rate (HR 13.1, 95% CI 4.0–43.9, $P < 0.001$). The presence of extensive cGVHD in this model was not an independent factor for NRM (HR 0.9, 95% CI 0.3–2.7, $P = 0.85$) when substituted for Grade III–IV aGVHD.

Discussion

We performed retrospective analyses of 115 patients with *de novo* MDS or AML-MLD who received their first allo-HCT at our center. By multivariate analyses, we found that the presence of cGVHD significantly reduced relapse and improved OS. To evaluate these results, we considered GVHD to be a time-dependent covariate and analyzed data from all patients to avoid bias from not considering patients

Table 3 Multivariate analysis for NRM, relapse, and OS

Variable	NRM		Relapse		OS	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age						
<60 yrs			1	0.72	1	0.33
≧60 yrs			1.2 (0.5–3.2)		1.4 (0.7–2.6)	
Prior chemotherapy						
No			1	0.03		
Yes			4.3 (1.2–15.9)			
Conditioning regimens						
MAC	1	0.33	1	0.77	1	0.63
RIC	0.7 (0.3–1.5)		0.9 (0.3–2.6)		1.2 (0.6–2.5)	
FAB stage at peak						
RA/RARS	1		1		1	
RAEB/CMMoL	1.2 (0.5–2.7)	0.68	0.6 (0.1–4.8)	0.57	1.9 (0.6–5.9)	0.28
RAEB-T/AML-MLD	2.3 (0.7–7.3)	0.14	0.7 (0.1–4.8)	0.73	3.3 (1.2–8.6)	0.02
Cytogenetic risk group						
Good/Intermediate	1	0.68	1	0.04	1	0.01
Poor	1.2 (0.5–2.7)		2.7 (1.1–6.9)		2.1 (1.1–6.9)	
BM blasts at HCT						
≦4%	1		1		1	
5–19%	1.2 (0.5–2.9)	0.75	4.3 (1.5–12.8)	0.008	1.6 (0.7–3.4)	0.28
≧20%	3.6 (1.3–9.9)	0.01	4.6 (0.9–23.4)	0.07	3.0 (1.3–6.9)	0.01
GVHD						
Grade III–IV aGVHD						
No	1	<0.001				
Yes	6.9 (2.7–17.4)					
cGVHD						
Yes			1	<0.001	1	0.04
No			12.7 (3.1–52.6)		2.0 (1.1–4.0)	

NRM, non-relapse mortality; OS, overall survival; HCT, allogeneic hematopoietic cell transplantation; HR, hazard ratio; CI, confidence interval; MAC, myeloablative conditioning; RIC, reduced intensity conditioning; FAB, French-American-British; RA, refractory anemia; RARS, refractory anemia with ringed sideroblasts; RAEB, refractory anemia with excess blasts; CMMoL, chronic myelomonocytic leukemia; RAEB-T, refractory anemia with excess blasts in transformation; AML-MLD, acute myeloid leukemia with multilineage dysplasia; BM, bone marrow; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease.

Covariates examined for NRM; Period of HCT, Patient sex, Conditioning regimens, FAB stage at peak, Cytogenetic risk group, BM blast at HCT, The presence of Grade III–IV aGVHD. Covariates examined for Relapse rate; Period of HCT, Age, Patient sex, Prior chemotherapy, Conditioning regimens, FAB stage at peak, Cytogenetic risk group, BM blast at HCT, The presence of cGVHD. Covariates examined for OS; Period of HCT, Conditioning regimens, FAB stage at peak, Cytogenetic risk group, BM blast at HCT, The presence of cGVHD.

who died or relapsed too early to develop acute or chronic GVHD. Some studies that used the same statistical method reported that cGVHD had beneficial effects on relapse in patients receiving allo-HCT after MAC (14, 15). In addition, others showed that the presence of cGVHD was an independent factor in reducing relapse and improving progression-free survival (PFS) in the setting of non-MAC regimens (12) or RIC regimens (16). Similar to our study, Valcárcel *et al.* (16) demonstrated that the development of cGVHD was the strongest factor in reducing relapse and improving survival in patients with high-risk MDS and AML receiving allo-HCT after RIC.

There has been no previous study on the effect of cGVHD on OS according to the conditioning regimen and disease status at allo-HCT. To clarify these questions, we used semi-landmark analyses to evaluate the effect of cGVHD on OS in various subgroups. In the current study, the presence of cGVHD predominantly improved OS in the setting of RIC, but did not affect OS in the MAC group (data not shown). In addition, the presence of cGVHD was significantly associated with the improvement in OS in high-risk patients with BM blasts of 5% or higher at allo-HCT or poor cytogenetic risk, whereas it did not affect OS in low-risk patients. These findings suggest that the benefit of the GVL effect appeared to be more prominent in patients with high-risk MDS who did not receive intensive preparative regimens.

Our findings may suggest that extensive cGVHD is beneficial for patients pre-treated with RIC because of elderly age or less-fit conditions. Valcárcel *et al.* reported that cGVHD was significantly associated with reducing relapse and improving OS without increasing NRM in high-risk AML and MDS patients pre-treated with RIC. In their study,

Table 4 Cause of death

No. of patients	All $N = 115$	MAC $N = 34$	RIC $N = 81$
Cause of death			
All Causes (% of all patients)	63 (55)	18 (53)	45 (56)
Progression (% of all death)	25 (40)	7 (39)	18 (40)
Organ failure (%)	14 (23)	5 (28)	9 (20)
Multiple organ failure	3	1	2
Veno-occlusive disease	3	1	0
Renal failure	1	0	1
Cardiac failure	1	1	0
Diffuse alveolar hemorrhage	7	2	5
Infection (%)	12 (19)	3 (17)	9 (20)
Bacterium	7	2	5
Fungus	3	0	3
Virus	2	1	1
Bleeding (%)	2 (3)	0 (0)	2 (4)
Secondary cancer (%)	4 (6)	0 (0)	4 (10)
GVHD (%)	4 (6)	2 (11)	2 (4)
Unknown (%)	2 (3)	1 (5)	1 (2)

MAC, myeloablative conditioning; RIC, reduced intensity conditioning; GVHD, graft-versus-host disease.

the cumulative incidence of cGVHD was 53% and extensive cGVHD accounted for the majority (94%) of that (16). Baron *et al.* (12) showed a comparable incidence of extensive cGVHD and reported the same results in AML and MDS patients with extensive cGVHD pre-treated with non-MAC regimens.

It is difficult to induce cGVHD 'moderately' on purpose, and the induction of cGVHD may lead to an increased risk of NRM. When we wish for the presence of cGVHD without a devastating outcome, there are two possible choices. First, G-CSF-mobilized peripheral blood mononuclear cells (G-PBMC) may be a preferable stem cell source when compared with BM. Some studies have shown that the use of G-PBMC as a stem cell source increased the frequency of cGVHD with comparable survival as compared with BM (17–19). Second, GVHD prophylaxis without ATG may be another beneficial option, as ATG has been shown to significantly decrease the incidence of cGVHD (20–22).

As the major causes of treatment failure were disease relapse and progression, treatment strategies before or after allo-HCT to reduce the risk of relapse remain a significant consideration for patients with high-risk MDS. The use of some additional treatment might be effective, especially for patients with high-risk MDS without cGVHD. Azacitidine is a DNA hypomethylating agent to show a significantly prolonged OS compared with conventional care regimens in patients with intermediate-2 and high-risk MDS (23, 24). The use of low-dose azacitidine as pre-emptive and maintenance treatment may prolong survival in patients with higher-risk MDS or AML after allo-HCT (25–27). Azacitidine also appears to induce leukemic cell differentiation and increase the expression of human leukemic antigen DR-1 (HLA-DR) and several tumor-associated antigens that could potentially enhance the GVL effect (28–30). We were not

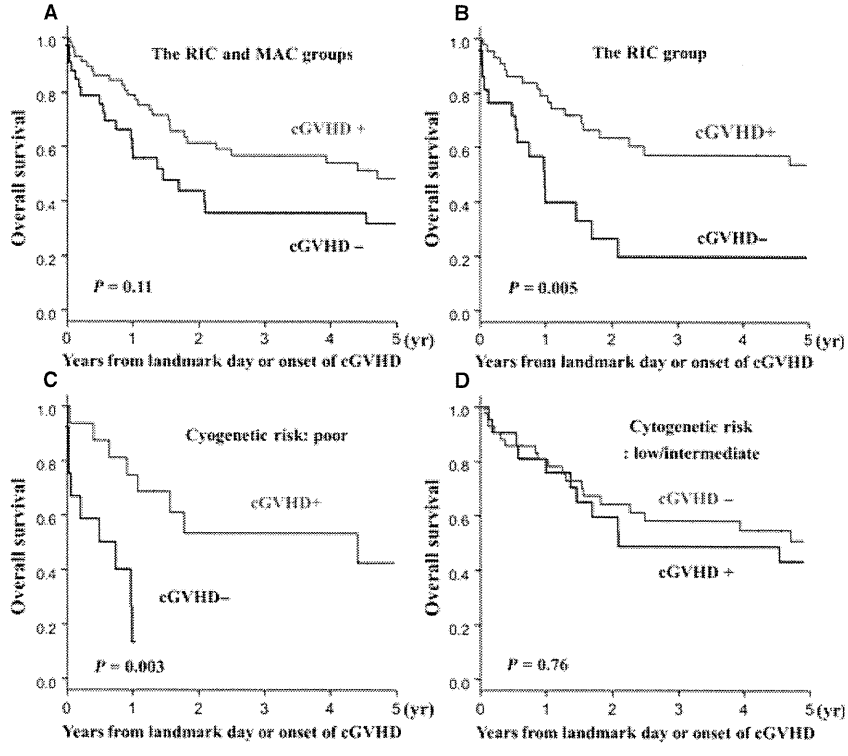

Figure 2 Semilandmark plots illustrating the impact of chronic graft-versus-host disease (GVHD) on overall survival (OS) of patients with myelodysplastic syndrome receiving allo-hematopoietic cell transplantation. OS curves of patients with or without chronic GVHD are shown for the entire population (A), the reduced-intensity conditioning group (B), patients with poor cytogenetic risk (C), and patients with low/intermediate cytogenetic risk (D).

able to assess the effect of Azacitidine before or after allo-HCT in patients with MDS, because patients who received Azacitidine were not included in our study. These issues need to be addressed in a prospective study.

We also analyzed the impact of aGVHD on outcomes after allo-HCT. The presence of grade II–IV aGVHD did not significantly influence the outcome. On the other hand, the presence of grade III–IV aGVHD was significantly associated with a worse OS and a higher incidence of NRM. Several studies have analyzed the effect of aGVHD on the prognosis after allo-HCT, but only a few have shown that aGVHD has a positive impact (12, 15, 16, 31). Kanda *et al.* (31) reported that grade I aGVHD had a beneficial effect on PFS in high-risk patients. However, we were not able to evaluate the effect of grade I aGVHD because of the small number of patients.

In the present study, OS, relapse and NRM did not differ significantly between the MAC and RIC groups, although the RIC group had significantly higher proportions of elderly patients and those with poor cytogenetic risk. Several previous studies have analyzed MDS and AML patients who received allo-HCT after MAC or RIC regimens (2, 6, 32, 33). In some studies, OS and PFS tended to be similar between the MAC and RIC groups, with a decreased incidence of NRM offset by an increased incidence of relapse in the RIC group. In other studies, there were no differences in relapse or NRM between the MAC and RIC groups, with a comparable OS (34, 35), and our results were consistent with the latter results.

The other major covariates that influenced OS in the present study were poor cytogenetic risk at diagnosis and the disease status at allo-HCT. Poor cytogenetic risk was also a significant factor for the increased risk of relapse, which was consistent with previous reports (32, 33, 36, 37). Although some studies have reported that a low pre-transplant tumor burden was essential for the success of allo-HCT in patients with MDS (35, 38, 39), it remains to be determined whether induction chemotherapy should be given to reduce the tumor burden before allo-HCT. Previous studies have shown that chemotherapy prior to allo-HCT did not improve OS because of the possibility of an increased incidence of NRM (38–40). In the present study, prior chemotherapy was significantly associated with an increased risk of relapse, but did not affect OS or NRM. This result may be explained by the fact that patients who need chemotherapy prior to HCT are probably those with high-risk disease.

Our study has several limitations, and thus the results must be interpreted with caution. These limitations include the retrospective nature of the study including the fact that therapeutic strategies were chosen at the discretion of physicians, the small number of patients analyzed, the heterogeneity of the groups of patients, and a short follow-up period. Nevertheless, the present data from more than 100 patients treated in a single center allowed us to identify factors that were associated with the prognosis in patients with MDS after allo-HCT.

In summary, the presence of cGVHD significantly reduced the risk of relapse and improved OS without increasing the incidence of NRM in patients with MDS. We also found that the presence of cGVHD significantly improved OS in high-risk patients or the RIC group, which suggests that the GVL effect may be beneficial in high-risk patients who do not receive intensive preparative regimens. For elderly or unfit patients with MDS, allo-HCT with RIC regimens was a potentially curative therapeutic option comparable with MAC regimens. As the major causes of treatment failure were disease relapse and progression, the treatment strategies to reduce the risk of relapse before and after allo-HCT are still a significant consideration for patients with high-risk MDS.

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Conflicts of interest

The authors declare no conflicts of interest.

Author contributions

N.H. designed the study, prepared the data file, performed the analysis, interpreted data, and wrote the manuscript; S.K was primarily responsible for the study design, data analysis, and interpretation of the data; K.O., T.K., Y.K., A.S., Y.I., R.U. and T.T. provided the patients' data; S-W.K., Y.T., and Y.H. interpreted data and reviewed the manuscript; K.T. supported the statistical analysis; T.F. provided the patients' data, interpreted data, and helped to write the manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Semilandmark plots illustrating impact of chronic GVHD on OS of patients with MDS receiving allo-HCT.

Table S1. Univariate analysis for NRM, relapse, and OS.

Table S2. Multivariate analysis for NRM, relapse and OS in the RIC group (patients pretreated with RIC).

Analysis of outcomes following autologous stem cell transplantation in adult patients with Philadelphia chromosome-negative acute lymphoblastic leukemia during first complete remission

The optimal treatment for adult Philadelphia chromosome-negative acute lymphoblastic leukemia [Ph(–)ALL] during first complete remission (CR1) remains a matter of debate. One treatment option for Ph(–)ALL is autologous hematopoietic stem cell transplantation (auto-SCT).[1,2] Previous studies have reported that the successful eradication of residual disease either before or after auto-SCT led to favorable clinical outcomes in patients with adult acute lymphoblastic leukemia (ALL) and yielded disease-free survival rates ranging from 57% to 77%.[3,4] Furthermore, auto-SCT was associated with a similarly increased overall survival (OS) duration to that associated with allogeneic hematopoietic stem cell transplantation (allo-SCT) in patients with lymphoblastic lymphoma,[5] a disease entity similar to ALL. However, a recent meta-analysis[1] demonstrated that the 5-year OS among adult ALL patients was significantly better in patients who underwent allo-SCT or chemotherapy alone compared to those who underwent auto-SCT. To evaluate the clinical relevance of auto-SCT for Ph(–)ALL, we conducted a retrospective study of a Japanese nationwide multicenter database to analyze the outcomes of auto-SCT for Ph(–)ALL during CR1.

A total of 155 Ph(–)ALL patients who underwent auto-SCT between 1983 and 2009 were analyzed (Table 1). Median follow-up duration was ten years (range 0.02-24 years), and the 10-year OS rate was 41% [95% confidence interval (CI): 33-49%] (Figure 1). The cumulative 10-year incidence rates of relapse and non-relapse mortality (NRM) were 47% (95%CI: 39-55%) and 10% (95%CI: 6-16%), respectively. The minimal residual disease (MRD) data could not be obtained for this study. Among patients under 45 years of age, the survival rate of adolescent/young adult (AYA; those aged ≤24 years) patients was similar to that of patients aged 25-44 years (P=0.94). A multivariate analysis revealed that age under 45 years [hazard ratio (HR): 0.60 (95%CI: 0.36-0.96); P=0.03] and the use of a total body irradiation (TBI) conditioning regimen [HR: 0.54 (95%CI: 0.30-0.98); P=0.04] were associated with increases in OS and decreases in the relapse rate, respectively (*Online Supplementary Table S1*). No significant factors were associ-

Table 1. Patients' characteristics.

Characteristic	Autologous (n=155) N.	%	Allogeneic (n=919) N.	%	P
Sex (Male)	86	55.5	515	56.0	0.90
Age at transplant, years					0.07
Median	25		30		
Range	16-74		16-66		
Age ≥ 45 years at transplant	33	21.3	129	14.0	0.02
Immunophenotypes					0.83
B lineage	80	51.6	588	64.0	
T lineage	21	13.6	146	15.9	
Unspecified or missing	54	34.8	185	20.1	
WBC at diagnosis, x10⁹/L					0.25
<30x10⁹/L	80	51.6	560	60.9	
≥30x10⁹/L	26	16.8	239	26.0	
Missing	49	31.6	120	13.1	
Cytogenetics					
Normal karyotypes	69	44.5	486	52.9	0.26
t(4;11) or complex	3	1.9	49	5.3	
Others or missing	83	53.6	384	41.8	
Year of transplant, year					<0.01
≤2000	142	91.6	378	41.1	
>2000	13	8.4	541	58.9	
Conditioning regimens					<0.01
TBI regimens	42	27.1	803	87.4	
Non-TBI regimens	111	71.6	114	12.4	
Missing	2	1.3	2	0.2	
Donor source					–
Autologous	155	100.0		–	–
Related allogeneic	–	–	670	72.9	
Unrelated allogeneic	–	–	249	27.1	
HLA matching					–
Matched	–	–	630	68.6	
Class I locus-mismatched	–	–	47	5.1	
Class II locus-mismatched	–	–	61	6.6	
Class I+II locus-mismatched	–	–	13	1.4	
Missing	–	–	168	18.3	

WBC: white blood cell; TBI: total body irradiation; BM: bone marrow; PB: peripheral blood; HLA: human leukocyte antigen.

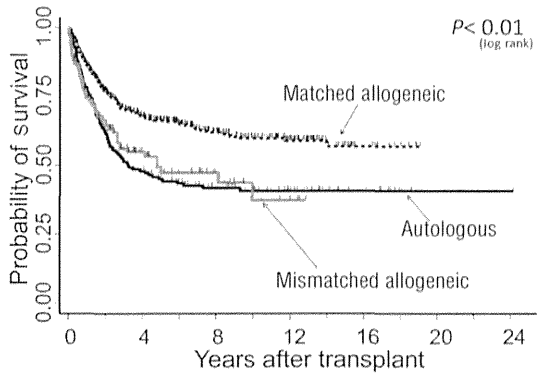

Figure 1. Overall survival according to the donor source.

ated with NRM.

Patients who had undergone myeloablative preparative regimens[6] followed by allo-SCT were selected for comparison (Table 1). With a median follow up of 4.9 years, allo-SCT yielded a better OS rate than auto-SCT (63% *vs.* 48% at 4 years; $P<0.01$). The cumulative incidence of relapse at four years was higher among patients who underwent auto-SCT than among those who underwent allo-SCT [46% (95%CI: 37-54%) *vs.* 23% (95%CI: 20-26%); $P<0.01$]. The NRM rates at four years after auto-SCT and allo-SCT were 9% (95%CI: 5-14) and 16% (95%CI: 14-19), respectively ($P=0.04$). With respect to the donor source, matched allo-SCT yielded a better OS than did auto-SCT, whereas auto-SCT and mismatched allo-SCT showed similar outcomes (Figure 1). In a multivariate analysis, autologous graft use was identified as a risk factor for relapse; however, this factor was not a significant risk factor for OS.

This study demonstrated that auto-SCT during CR1 could produce favorable outcomes in a proportion of Ph(–)ALL patients who exhibited long-term survival plateaus. The multivariate analysis revealed that the donor source (autograft *vs.* allograft) was not a prognostic factor for OS. These findings appear to be encouraging. However, the current strategy has uncovered a strong trend toward omitting auto-SCT. With advances in allo-SCT methods and the improved transplant success rate, many physicians have placed the highest priority on allo-SCT as consolidation when a suitable donor is available during CR1. Besides, given the near 100% health insurance system coverage, the improved co-ordination of the Japan Marrow Donor programs,[7] and improved outcomes from the use of pediatric-based chemotherapy regimens in adult ALL, the number of patients undergoing auto-SCT decreased rapidly in the 2000s. Approximately half of the cases in our study population were patients aged 24 years or under. The prognosis of younger patients, especially AYA patients, could be improved by the current intensive pediatric protocols.[8] Further studies are needed to compare the consolidative role of auto-SCT to that of chemotherapy alone.

A high relapse rate is among the main factors leading to the poorer clinical outcomes of ALL patients.[1] One important factor that has been associated with subsequent relapse is the conditioning regimen selected. TBI has been widely used as a component in the conditioning regimens of ALL patients undergoing allo-SCT.[9] In the present study, we identified TBI as a potential prognostic factor associated with reduced relapse rates in Ph(–)ALL patients who underwent auto-SCT, a finding that was consistent with those reported in earlier studies.[10] TBI might be a powerful tool for disease control along with both allo-SCT and auto-SCT. However, among mature lymphoid malignancies, the Dana-Farber group documented secondary malignancy rates of 16% at ten years and 38% at 15 years in patients who underwent auto-SCT with TBI-based conditioning during CR1.[11] Physicians should be careful when applying TBI regimens, especially to younger patients.

Ph(–)ALL adults who benefit from allo-SCT are primarily those who present with post-induction positive MRD, whereas patients with negative MRD fare equally well with conventional chemotherapy.[12] Whether auto-SCT would be beneficial compared to chemotherapy for patients with high post-induction MRD and no suitable donor is a matter of debate. A recent meta-analysis[1] showed a lack of benefit from auto-SCT compared to treatment with chemotherapy alone. Nevertheless, no prospective studies have compared auto-SCT with chemotherapy alone in adult Ph(–)ALL patients while stratifying according to MRD status. Recent advances in MRD detection technologies might lead to a more precise selection of transplant candidates; moreover, the use of novel agents could reduce MRD at transplantation[13-15] which might help to expand the indications for auto-SCT. Auto-SCT might reduce the treatment duration and, in addition, would provide relatively easily available grafts. As the optimal post-remission therapy timing is sometimes critical for adult Ph(–)ALL patients, auto-SCT during CR1 might represent a rational treatment option for some adult ALL patients. However, high relapse rates remain a well-described and significant problem among ALL patients who have undergone auto-SCT, and the prognosis of relapsed ALL is usually extremely poor. To re-define the role of auto-SCT, further investigations that compare the results of auto-SCT with those of intensive chemotherapy without stem cell transplantation and that take into account MRD status will be needed.

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Key words: outcome, Philadelphia-negative acute lymphoblastic leukemia, autologous stem cell transplantation, first complete remission.

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ORIGINAL ARTICLE

Effect of graft sources on allogeneic hematopoietic stem cell transplantation outcome in adults with chronic myeloid leukemia in the era of tyrosine kinase inhibitors: a Japanese Society of Hematopoietic Cell Transplantation retrospective analysis

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Abstract We retrospectively compared transplant outcomes for related bone marrow transplantation (rBMT), related peripheral blood stem cell transplantation (rPBSCT), unrelated bone marrow transplantation (uBMT), and unrelated cord blood transplantation (CBT) in 1,062 patients with chronic myeloid leukemia (CML) aged 20 years or over between January 1, 2000 and December 31, 2009 in Japan. The disease status was as follows: chronic phase 1 (CP1, $n = 531$), CP 2 or later including accelerated phase (CP2-AP, $n = 342$) and blastic crisis (BC, $n = 189$). Graft sources (GS) were rBMT ($n = 205$), uBMT ($n = 507$), rPBSCT ($n = 226$) or CBT ($n = 124$). In multivariate analysis in CP1, lower overall survival (OS) (relative risk [RR]: 6.01, 95 % confidence interval [CI]: 1.20–29.97, $P = 0.029$) and leukemia-free survival (LFS) (RR: 4.26, 95 % CI: 1.24–14.62, $P = 0.021$) were observed in uBMT compared with those in rBMT. For patients in the advanced phase of CML beyond CP1, GS had no significant impact on OS or LFS. Our results support the use of rBMT for adults with CML in CP1, but in contrast to previous reports, the superiority of rPBSCT in advanced stage of CML was not confirmed in our cohorts.

Keywords Chronic myeloid leukemia · Allogeneic hematopoietic stem cell transplantation · Graft sources

On behalf of Choric Myeloid Leukemia Working Group of the Japan Society for Hematopoietic Cell Transplantation.

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Introduction

According to the Japan Society for Hematopoietic Cell Transplantation (JSHCT), the number of transplants reported annually for the treatment of CML was 306 in 2000, but drastically dropped to 46 transplants in the year 2009. Unsurprisingly, the drop in transplant activity was observed in Japan after imatinib (IM) became available as an experimental drug in 2000 and subsequently as a frontline treatment for CML in 2001. Thus, the excellent outcomes demonstrated by tyrosine kinase inhibitors (TKIs) argue against the use of allogeneic hematopoietic stem cell transplantation (allo-HSCT) as an upfront therapy for CML in CP1; allo-HSCT is currently recommended for patients with a T315I mutation, or who failed TKIs and progress to advanced phase disease [1–6]. Moreover, the newly launched third generation TKI, ponatinib, having a unique binding mechanism allowing inhibition of BCR-ABL kinases, including those with the T315I mutation may further narrow the range of transplant indication [7, 8]. Therefore, those CML patients who undergo allo-HSCT represent a selection of high-risk patients due to more advanced disease with high rates of accelerated or blast phase. To improve transplant outcomes, comprehensive approaches in transplant strategies including timing, choice of conditioning and GS, maintenance therapy might be needed for those CML patients being selected nowadays for allo-HSCT. The main purpose of this study was to analyze the impact of GS on transplant outcome for patients with CML in the era of TKIs, particularly the role of GS in each disease status. We also clarified the prognostic factors for transplant outcomes in each disease status. We herein report our analysis of 1,062 patients, whose complete registry-based clinical data which were provided by the JSHCT.

Patients and methods

Patients

Data on a total of 1,143 patients of at least 20 years of age who had undergone allogeneic bone marrow, peripheral blood, or cord blood transplantation for CML between January 1, 2000 and December 31, 2009 were initially collected through the Transplant Registry Unified Management Program (TRUMP). Eighty-one patients were excluded from the analysis, because one or two critical data such as alive, relapse, and engraftment status with or without date of onset were missing. Other missing data were dealt as missing data in the study and the analysis numbers in each variable were described, respectively. This included data from the Japan Cord Blood Bank Network (JCBBN), the Japan Marrow Donor Program (JMDP), and JSHCT. These are the 3 largest allo-HSCT registries in Japan, and their roles have been described previously [9]. The study was approved by the data management committees of JSHCT, as well as by the ethical committee of Tokyo Metropolitan Cancer and Infectious Disease Center, Komagome Hospital (Tokyo, Japan), where this study was organized.

Statistical analysis

The outcome endpoints were neutrophil recovery, platelet recovery, acute and chronic GVHD, relapse, transplantation-related mortality (TRM), overall survival (OS), and leukemia-free survival (LFS). The definitions of the statistical models used were in accordance with the statistical guidelines of the European Group for Blood and Marrow Transplantation (EBMT) (http://www.ebmt.org/1Whatis EBMT/whatisebmt2.html). Neutrophil recovery was defined by an absolute neutrophil count (ANC) of at least 0.5×10^9/L for 3 consecutive days, with the first day considered as the recovery day. Platelet recovery was defined by a non-transfused platelet count of at least 20×10^9/L for 3 consecutive days. Deaths occurring before day 90 or day 180 were considered as competing risks for neutrophil or platelet recovery, respectively. The graft failure rate for neutrophils was calculated for patients living without relapse for more than 30 days. Acute and chronic GVHD were diagnosed and graded at each center according to the standard criteria [10–12]. Relapse was defined on the basis of the reappearance of the blast or Philadelphia chromosome (Ph) or *BCR-ABL1* transgene by cytogenetic and/or molecular analysis, including polymerase chain reaction and fluorescence in situ hybridization. TRM was considered a sole cause of non-leukemic deaths occurring after transplantation; OS was defined as the time between transplantation and death due to any cause; LFS was defined as the time interval from allo-HSCT to a first event, either relapse or death, in patients achieving complete remission. HLA antigen disparities were categorised as either GVHD or rejection direction. Low-resolution antigens of HLA-A and HLA-B were identified for all patients by serologic typing or low-resolution molecular typing methods. While, HLA-DRB1 alleles were

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determined by high-resolution molecular typing using the sequence-based HLA typing method. In rBMT, HLA-DRB1 alleles were counted as identical, if the low-resolution antigens of HLA-A, B, and DR were identical. Data on HLA-DRBI allele were not fully available; there were 2 lacking data in CP1, 4 lacking data on CP2-AP and 2 lacking data in BC. Detail of HLA disparity toward either rejection or GVHD are noted in Table 1 and Supplementary Table 1.

Adjusted probabilities of OS and LFS were analyzed using Cox proportional-hazards regression model. The variables used were patients' age at HSCT, patients' sex, body weight at HSCT, time from diagnosis to HSCT, ABO mismatch, conditioning regimen, imatinib administration, kind of GVHD prophylaxis, and year of HSCT. Variables with more than two categories were dichotomized for the final multivariate analyses. Variables were dichotomized as the followings: patient's age at HSCT younger or older than median; patient's body weight at HSCT lighter or heavier than median; time from diagnosis to HSCT <1 year or >1 year. ABO major mismatch or others; myeloablative conditioning regimen or others; cyclosporine-based GVHD prophylaxis regimen or tacrolimus-based; year of HSCT before or after 2004. The endpoints of neutrophil and platelet recovery, acute GVHD and chronic GVHD, relapse and TRM were analyzed using cumulative incidence curves that estimated incidence according to the Fine and Gray models, in which we first used univariate models that contained each of the variables one at a time. Then all variables with a $P < 0.05$ by the likelihood-ratio test were included in a multivariate model.

Cause-specific hazard ratios were estimated with 95 % confidence intervals (CIs). Statistical analysis was performed with the R Foundation statistical computing package, version 2.12.2 (http://www.r-project.org/).

Table 1 Characteristics of patients with CML in CP1, CP2-AP, and BP

	CP1 ($n = 531$)	CP2-AP ($n = 342$)	BP ($n = 189$)
Graft source rBMT/uBMT/rPBSCT/CBT	138/258/125/10	43/176/59/64	24/73/42/50
Gender	338/193 ($P < 0.001$)	215/127 ($P < 0.001$)	123/66 ($P < 0.001$)
Male/female			
Median age at transplantation (range)	40 (20–67)	43 (21–69)	43 (20–74)
GVHD prophylaxis CyA + MTX/CyA based/FK + MTX/FK based/ others	331/27/144/12/14[a]	148/17/145/19/9[a]	88/22/58/17/2[a]
Pre-transplant IM	133/249[b]	187/108[b]	94/95 ($P = 0.94$)
Yes/no	($P < 0.001$)	($P < 0.001$)	
Duration from diagnosis to transplantation, months median (range)	12.5 (0.8–169.0)	18.2 (1.6–255.3)	15.5 (2.4–322.7)
Duration from diagnosis to transplantation ≤1 year/> 1 year	248/258[c] ($P = 0.65$)	135/195[c]	80/100[c] ($P = 0.14$)
		($P < 0.001$)	
Patient's body weight, kg Median (range)	61 (40–104)	60 (34–104)	58.5 (34–96)
Conditioning regimen Myeloablative/reduced intensity	475/53[d] ($P < 0.001$)	289/53 ($P < 0.001$)	161/28 ($P < 0.001$)
Years at transplantation 2000–2004/2005–2009	447/84 ($P < 0.001$)	211/131 ($P < 0.001$)	116/73 ($P < 0.01$)
ABO mismatch No/yes	189/161[e] ($P = 0.13$)	132/156[e] ($P = 0.16$)	64/91[e] ($P = 0.03$)
HLA disparities (rejection direction)[g] 0–1/> 2	510/19[f] ($P < 0.001$)	281/57[f] ($P < 0.001$)	145/42[f]
			($P < 0.001$)
HLA disparities (GVHD direction)[g] 0–1/> 2	507/22[f] ($P < 0.001$)	285/53[f] ($P < 0.001$)	140/47[f]
			($P < 0.001$)

CP chronic phase, *AP* accelerated phase, *BP* blastic phase, *rBMT* related bone marrow transplantation, *rPBSCT* related peripheral blood stem cell transplantation, *uBMT* unrelated bone marrow transplantation, *CBT* unrelated cord blood transplantation, *GVHD* graft-versus-host disease, *CyA* cyclosporine, *MTX* methotrexate, *FK* tacrolimus, *IM* imatinib mesylate, *HLA* human leukocyte antigen

[a] Data on GVHD prophylaxis were not fully available; there were 3 missing data in CP data, 4 missing data on CP2-AP and 2 missing data in BC

[b] Data on pre-transplant imatinib administration were not fully available; 149 data and 47 data were not retrieved in CP1 and in CP2-AP, respectively

[c] Loss of data on duration from diagnosis to transplantation (≤ 1 year/> 1 year) was noted; 25 data in CP, 12 data in CP2-AP, and 9 data in BP were not retrieved

[d] Three data regarding conditioning regimen in CP were not retrieved

[e] Loss of data on ABO mismatch was noted; 181 data in CP, 54 data in CP2-AP, and 34 data in BP were not retrieved

[f] Data on HLA-DRBI allele were not fully available; there were 2 lacking data in CP, 4 lacking data on CP2-AP and 2 lacking data in BC

[g] More detail of HLA disparity toward either rejection or GVHD is noted in supplementary Table 1

Results

Patient characteristics

Of 1,062 patients (676 men, 386 women; median age, 41 years; range, 20–74), 414 patients (39 %) had a clear history of pre-transplant IM use. Disease status was as follows: CP1 ($n = 531$), CP2-AP ($n = 342$) and BC ($n = 189$). GS were related rBMT ($n = 205$), uBMT ($n = 507$), rPBSCT ($n = 226$) and CBT ($n = 124$). The unrelated PBSCT has not been allowed in Japan until 2012 and, therefore, our data included only unrelated BMT, not PBSCT. In addition, during the study period, there were no related CBTs at all. The other variables, including GVHD prophylaxis, pre-transplant IM, body weight at allo-HSCT, duration from diagnosis to transplant, conditioning intensity, years at transplantation (2000–2004 vs. 2005–2009), ABO mismatch, HLA mismatch in either GVHD or rejection direction, are shown in Table 1.

Overall survival and leukemia-free survival

The median follow-up period was 914 days after transplantation (range 2–3,902) and 1,914 days after diagnosis (range 29–9,120). Three-year OS was 70.6 % (95 % CI, 66.8-74.7 %) for patients in CP1 at the time of transplantation, 58.9 % (95 % CI, 53.7–64.7 %) for those with CP2-AP, and 26.9 % (95 % CI, 20.9–34.6 %) for those in BC. The probability of 3-year LFS for patients in CP1, CP2-AP and BC was 64.6 % (95 % CI, 60.4–68.6 %), 46.1 % (95 % CI, 40.9–51.9 %) and 19.2 % (95 % CI, 14.1–26.1 %), respectively (data not shown).

OS and LFS according to GS in CP1, CP2-AP, and BC are shown in Fig. 1a–c, and d–f, respectively. In view of OS and LFS according to GS, 3-year OS after rBMT, rPBSCT, uBMT, and CBT in CP1 was 84.4, 70.0, 64.4, and 48.0 %, respectively (Fig. 1a). Three-year LFS after rBMT, rPBSCT, uBMT, and CBT in CP1 was 76.3, 64.3, 59.3, and 30 %, respectively (Fig. 2d). Multivariate analysis for OS identified the following factors as adverse prognostic factors for

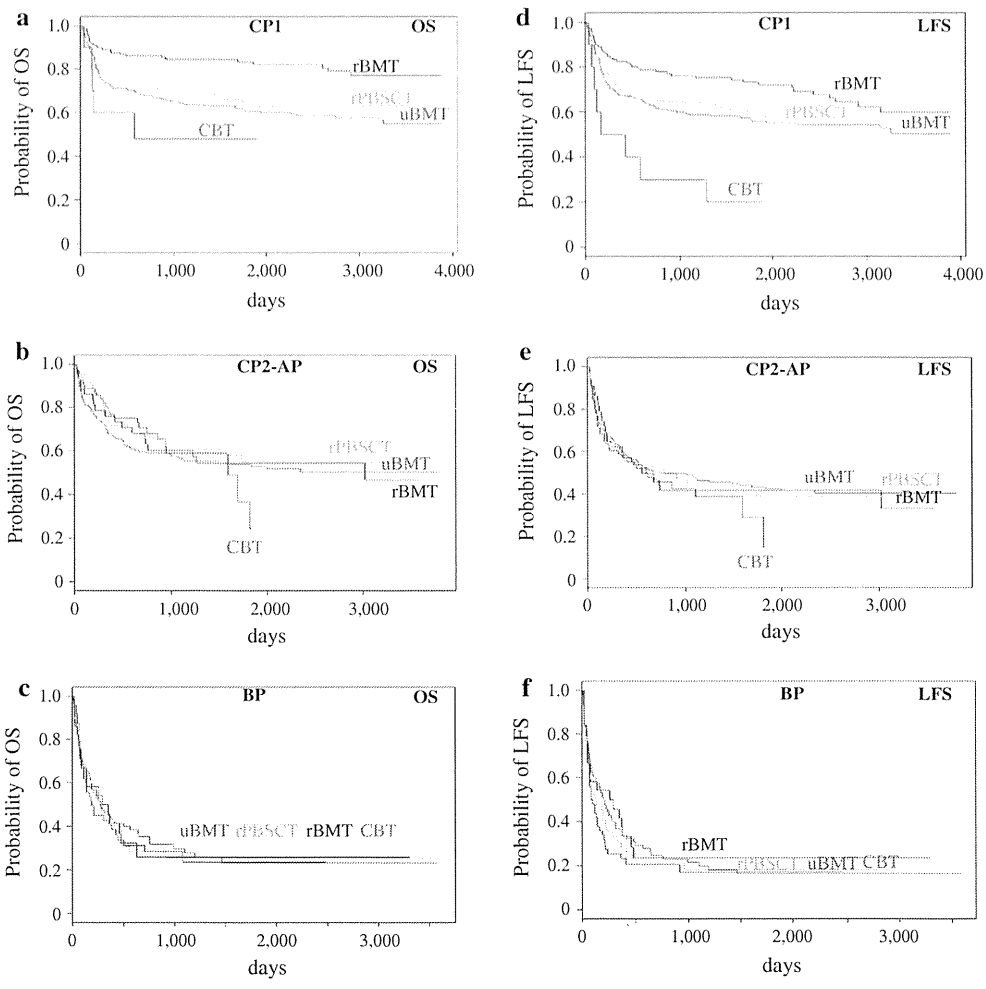

Fig. 1 Kaplan–Meier estimate of overall survival (OS) for patients in CP1 (**a**), CP2-AP (**b**) and BC (**c**); and leukemia-free survival (LFS) for patients in CP1 (**d**), CP2-AP (**e**) and BC (**f**)

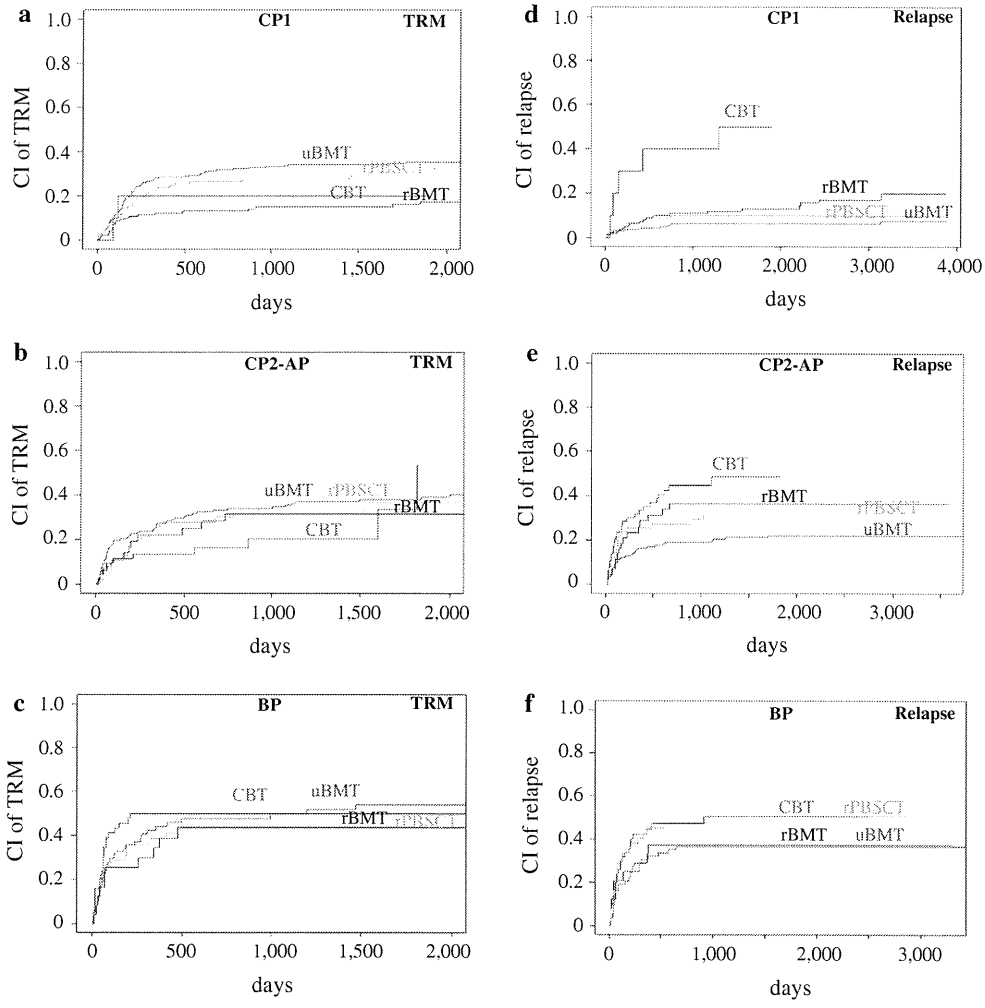

Fig. 2 The cumulative incidence of transplantation-related mortality (TRM) for patients in CP1 (**a**), CP2-AP (**b**) and BC (**c**); and relapse for patients in CP1 (**d**), CP2-AP (**e**) and BC (**f**)

patients in CP1: older age (>median age, 40 years: HR 1.67, 95 % CI, 1.15–2.41, $P = 0.007$), ABO mismatch (HR 1.44, 95 % CI, 1.003–2.06, $P = 0.048$) (Table 2), and uBMT (RR 6.01, 95 % CI, 1.20–29.97, $P = 0.029$) (Table 3). In CP2-AP, older age (> median age, 43 years: HR 1.74, 95 % CI, 1.25–2.43, $P < 0.001$) was the only factor an adverse prognostic factor (Table 2). In BC, pre-transplant IM (HR 0.61, 95 % CI, 0.49–0.89, $P = 0.011$) was the only factor for better OS (Table 2). Concerning LFS, multivariate analysis showed that uBMT (RR 4.26, 95 % CI, 1.24–14.62, $P = 0.021$) and older age (>median age, 40 years: HR 1.43, 95 % CI, 1.02–1.99, $P = 0.038$) were adverse risk factors in CP1 (Table 2, 3). For patients in CP2-AP and BC, no significant factor for OS or LFS was found. Thus, for patients in CP1, GS could have a significant impact on survival outcomes. While, for patients in the advanced phase of CML of beyond CP1, GS could have no significant impact on OS or LFS (Table 3).

TRM and relapse

The 1-year cumulative TRM rate by disease stage was 23.1 % (95 % CI, 19.5–26.7 %) in CP1, 24.2 % (95 % CI, 19.5–28.9 %) in CP2-AP, and 43.2 % (95 % CI, 35.9–50.5 %) in BC. TRM by GS is shown in Fig. 2a–c. The TRM rate appeared low in rBMT compared with uBMT or rPBSCT in CP1 (Fig. 2a). Multivariate analysis showed that uBMT (RR 2.49, 95 % CI 1.02–6.10, $P = 0.046$) and older age (>median age, 40 years: HR 1.69, 95 % CI, 1.19–2.39, $P = 0.003$) were factors associated with a significantly increased risk of TRM in CP1 (Table 2, 3).

The 3-year cumulative relapse rate by disease stage was 9.0 % (95 % CI, 3.9–7.9 %) in CP1, 28.2 % (95 % CI, 23.3–33.1 %) in CP2-AP, and 43.6 % (95 % CI, 36.3–50.9 %) in BC. Relapse rate by GS is demonstrated in Fig. 2d–f. For patients in CP1, the relapse rate after CBT appeared to be higher than that after other GS (Fig. 2d). In multivariate analysis by the effect of GS in CP1, CBT (RR

Table 2 Multivariate analysis of risk factors for the main outcomes after allo-HSCT for CML in CP1, CP2-AP, and BP

Main outcomes	Factors	CP1				CP2-AP				BP			
		Factors	HR	(95 % CI)	*P* value	Factors	HR	(95 % CI)	*P* value	Factors	HR	(95 % CI)	*P* value
OS	Age	≤40	1			≤43	1						
		>40	1.67	1.15–2.41	0.007	>43	1.74	1.25–2.43	< 0.001				
	ABO mismatch	No	1										
		Yes	1.44	1.003–2.06	0.048								
	Pre-transplant IM									No	1		
										Yes	0.61	0.41–0.89	0.011
LFS	Age	≤40	1										
		>40	1.43	1.02–1.99	0.038								
TRM	Age	≤40	1										
		>40	1.69	1.19–2.39	0.003								
Relapse	HLA mismatch (rejection)									0, 1	1		
										≥2	1.7	1.04–2.76	0.033
	HLA mismatch (GVHD)					0, 1	1						
						≥2	3.57	1.55–8.21	0.003				
Acute GVHD (all grades[a])	Pre-transplant IM	No	1										
		Yes	0.75	0.57–0.99	0.04								
	BW					≤60 kg	1						
						>60 kg	1.35	1.01–1.82	0.045				
Acute GVHD	BW					≤60 kg	1						
(≥grade 2)						> 60 kg	1.53	1.05–2.24	0.028				
Chronic GVHD (extensive[b])	BW					≤60 kg	1						
						>60 kg	1.75	1.06–2.73	0.028	0			

OS overall survival, *LFS* leukemia-free survival, *TRM* transplantation-related mortality, *ANC* absolute neutrophil count, *GVHD* graft-versus-host disease, *IM* imatinib, *HLA* human leukocyte antigen, *BW* body weight, *HR* hazard ratio, *CI* confidence interval, *CP* chronic phase, *AP* accelerated phase, *BP* blastic phase, *imatinib* imatinib mesylate

[a] Overall grade of acute GVHD assigned according to the Center for International Blood and Marrow Transplant Research (CIBMTR) severity index

[b] Chronic GVHD was graded as limited or extensive based on the Seattle criteria

Table 3 Impact of graft sources on main outcomes after allo-HSCT for CML in CP1, CP2-AP, and BP

Main outcomes	Graft sources	CP1			CP2-AP			BP		
		RR	(95 % CI)	*p* value	RR	(95 % CI)	*p* value	RR	(95 % CI)	*p* value
OS	rBMT	1.00			1.00			1.00		
	uBMT	6.01	(1.20–29.97)	0.029	1.12	(0.33–3.79)	0.851	>99	(0.00–99.99)	0.999
	rPBSCT	1.76	(0.77–4.04)	0.180	0.84	(0.21–3.43)	0.809	1.13	(0.56–2.30)	0.727
	CBT	1.00	(0.00–99.99)	1.000	NA	NA	NA	NA	NA	NA
LFS	rBMT	1.00			1.00			1.00		
	uBMT	4.26	(1.24–14.62)	0.021	1.61	(0.55–4.74)	0.383	0.00	(0–99.99)	0.999
	rPBSCT	1.72	(0.95–3.11)	0.073	0.42	(0.14–1.31)	0.135	0.67	(0.31–1.44)	0.299
	CBT	1.00	(0.00–99.99)	1.000	NA	NA	NA	NA	NA	NA
TRM	rBMT	1.00			1.00			1.00		
	uBMT	2.49	(1.02–6.10)	0.046	1.36	(0.60–3.09)	0.47	2.71	(0.74–9.96)	0.13
	rPBSCT	1.03	(0.52–2.07)	0.93	0.94	(0.52–1.70)	0.83	1.43	(0.64–3.22)	0.39
	CBT	0.33	(0.04–2.63)	0.29	0.98	(0.60–1.60)	0.94	1.26	(0.82–1.92)	0.29
Relapse	rBMT	1.00			1.00			1.00		
	uBMT	0.33	(0.12–0.95)	0.041	0.66	(0.29–1.55)	0.34	2.23	(0.28–17.61)	0.45
	rPBSCT	1.13	(0.62–2.07)	0.68	1.17	(0.64–2.14)	0.6	1.06	(0.44–2.54)	0.9
	CBT	25.16	(1.76–369.10)	0.018	1.15	(0.74–1.80)	0.53	0.77	(0.39–1.60)	0.49
ANC recovery	rBMT	1.00			1.00			1.00		
	uBMT	0.82	(0.55–1.23)	0.35	0.83	(0.53–1.31)	0.43	0.58	(0.27–1.26)	0.17
	rPBSCT	1.31	(1.02–1.69)	0.036	1.2	(0.90–1.59)	0.21	0.91	(0.33–2.52)	0.86
	CBT	2	(0.67-5.98)	0.22	0.53	(0.42–0.67)	<0.001	0.55	(0.37–0.82)	0.003
Platelet recovery	rBMT	1.00			1.00			1.00		
	uBMT	0.75	(0.46–1.21)	0.24	0.89	(0.51–1.56)	0.68	0.21	(0.07–0.61)	0.0039
	rPBSCT	0.93	(0.69–1.26)	0.65	0.91	(0.61–1.35)	0.63	0.67	(0.28–1.57)	0.35
	CBT	1.07	(0.35–3.28)	0.9	0.78	(0.62–0.99)	0.049	0.44	(0.26–0.74)	0.0018
Acute GVHD (all grades[a])	rBMT	1.00			1.00			1.00		
	uBMT	3.35	(1.50–6.22)	<0.001	1.67	(0.92–3.02)	0.09	1.22	(0.46–3.25)	0.69
	rPBSCT	1.49	(0.94–2.37)	0.091	0.86	(0.51–1.44)	0.56	0.94	(0.32–2.73)	0.91
	CBT	1.67	(0.68–4.11)	0.26	0.76	(0.58–1.01)	0.054	1.05	(0.56–1.96)	0.87
Acute GVHD (≥grade 2)	rBMT	1.00			1.00			1.00		
	uBMT	4.28	(1.92–9.53)	<0.001	2.14	(0.93–4.94)	0.075	1.34	(0.39–4.61)	0.65
	rPBSCT	1.5	(0.82–2.72)	0.19	1.53	(0.82–2.86)	0.18	2.23	(0.36–1.39)	0.39
	CBT	1.00	(0.00–99.99)	1.000	0.84	(0.58–1.22)	0.36	1.45	(0.55–3.81)	0.45
Chronic GVHD	rBMT	1.00			1.00			1.00		
	uBMT	0.95	(0.53–1.70)	0.86	1.1	(0.45–2.68)	0.84	0.27	(0.06–1.33)	0.11
	rPBSCT	1.37	(0.97–1.92)	0.075	1.24	(0.70–2.19)	0.47	0.84	(0.22–3.20)	0.8
	CBT	8.52	(0.64–11.43)	0.11	0.8	(0.52–1.25)	0.33	0.73	(0.32–1.66)	0.46
Chronic GVHD (extensive[b])	rBMT	1.00			1.00			1.00		
	uBMT	1	(0.49–2.04)	1	0.84	(0.33–2.15)	0.72	0.69	(0.14–3.46)	0.65
	rPBSCT	1.31	(0.87–1.96)	0.19	1.19	(0.60–2.34	0.62	1.08	(0.27–4.24)	0.92
	CBT	6.61	(0.22–200.8)	0.28	0.63	(0.36–1.09)	0.097	0.77	(0.31–1.88)	0.56

OS overall survival, *LFS* leukemia-free survival, *TRM* transplantation-related mortality, *ANC* absolute neutrophil count, *GVHD* graft-versus-host disease, *RR* relative risk, *CI* confidence interval, *CP* chronic phase, *AP* accelerated phase, *BP* blastic phase, *rBMT* related bone marrow transplantation, *rPBSCT* related peripheral blood stem cell transplantation, *uBMT* unrelated bone marrow transplantation, *CBT* unrelated cord blood transplantation, *NA* not available

[a] Overall grade of acute GVHD assigned according to the Center for International Blood and Marrow Transplant Research (CIBMTR) severity index

[b] Chronic GVHD was graded as limited or extensive based on the Seattle criteria

25.16, 95 % CI 1.76–369.10, $P = 0.018$) showed higher relapse, while uBMT (RR 0.33, 95 % CI 0.12–0.95, $P = 0.041$) was lower relapse compared with those in rBMT (Table 3).

Engraftment

The cumulative neutrophil recovery rate on day 90 was 97.5 % (95 % CI, 96.1–98.9 %) in CP1, 93.2 % (95 % CI, 90.5–95.9 %) in CP2-AP, and 82.3 % (95 % CI, 76.8–87.8 %) in BC. On day 180, the cumulative platelet recovery rate, as indicated by more than 2×10^{10}/L of platelets in blood, was 91.9 % (95 % CI, 89.5–94.3 %) in CP1, 85.1 % (95 % CI, 81.2–89.0 %) in CP2-AP, and 67.2 % (95 % CI, 60.3–74.1 %) in BC. Note that the neutrophil recovery and platelet recovery rates were lower after CBT, especially in patients in the advanced phase; i.e., neutrophil recovery in CBT: 90 % in CP1, 79.4 % in CP2-AP, and 64.0 % in BC; platelet recovery after CBT: 90.0 % in CP1, 72.5 % in CP2-AP, and 52.0 % in BC (Fig. 3a–f). Multivariate analysis showed that rPBSCT (RR 1.31, 95 % CI 1.02–1.69, $P = 0.0396$ was a significant factor for early neutrophil recovery in CP1. While, CBT (RR 0.53, 95 % CI 0.42–0.67, $P < 0.001$) was a significant factor for delayed neutrophil recovery in CP2-AP (Table 3). The factor statistically associated with delayed platelet recovery was CBT in CP2-AP (RR 0.78, 95 % CI 0.62–0.99, $P = 0.0049$) and in BC (RR 0.44, 95 % CI 0.26–0.74, $P = 0.0018$). Unrelated BMT (RR 0.21, 95 % CI 0.07–0.61, $P = 0.0039$) was also a significant factor for delayed platelet recovery in BC (Table 3).

Acute and chronic GVHD

The cumulative incidence of acute GVHD at all grades before day 100 was 62.8 % (95 % CI, 58.6–67.0 %) in CP1, 63.5 % (95 % CI, 58.2–58.8 %) in CP2-AP, and 68.6 % (95 % CI, 61.3–74.9 %) in BC. Patients who underwent uBMT showed a higher incidence of acute GVHD (all grades) in CP1 and CP2-AP (Fig. 4a, b). This association was confirmed by multivariate analysis; uBMT (RR 3.35, 95 % CI 1.50–6.22, $P < 0.001$) was a significant factor in CP1 (Table 3). Pre-transplant IM (HR 0.75, 95 % CI 0.57–0.99, $P = 0.04$) was a significant risk factor for acute GVHD (all grades) in CP1 (Table 2). Focusing exclusively on grade II or higher acute GVHD, uBMT (RR 4.28, 95 % CI 1.92–9.53, $P < 0.001$) (Table 3) was a significant risk factor in CP1 (Table 2). For patients in CP2-AP, body weight (>60 kg) was a factor significantly associated with increased risk of aGVHD (all grade; RR 1.35, 95 % CI, 1.01–1.82, $P = 0.045$, grade II or higher grade; RR 1.53, 95 % CI, 1.05–2.24, $P = 0.028$) (Table 2).

The cumulative incidence of chronic GVHD among evaluable patients who survived at least 100 days after allo-HSCT was 49.4 % (95 % CI, 44.7–54.1 %) in CP1, 42.2 % (95 % CI, 36.4–48.0 %) in CP2-AP, and 37.8 % (95 %CI, 30.0–45.6 %) in BC. For patients in CP1, rPBSCT showed a higher incidence of chronic GVHD (71.4 %), which was compared to other GS (Fig. 4d); however, this significant association was not confirmed in multivariate analysis (rPBSCT: RR 1.37 95 % CI 0.97–1.92, $P = 0.075$). For patients in CP2-AP and BC, chronic GVHD after CBT occurred at rates of 23.1 and 23.8 %, respectively, which were apparently lower than that of other GS (Fig. 4e, f), but these statistical associations were not also confirmed by multivariate analysis in CP2-AP or BC (Table 3). Concerning extensive chronic GVHD, multivariate analysis showed the significant association between body weight (>60 kg; RR 1.75, 95 % CI, 1.06–2.73, $P = 0.028$) and chronic GVHD in CP2-AP (Table 2).

Discussion

Our study reviewed 1,062 Japanese adult patients who underwent allo-HSCT during the past decade (2000–2009); thus, our cohort reflects the current use and results of allo-HSCT for CML in Japan. Moreover, the TRUMP database offers the advantage of a large number of patients with extensive data, which permits multivariate analysis. The 3-year OS was 70.6 % for patients in CP1, and the probability of 3-year LFS for patients in CP1 was 64.6 %. These survival data for patients in CP1 were comparable to those reported by others [12]. Based on the report from the EBMT, which included 13,416 CML patients and was apparently the largest CML transplant database including the 3 times cohorts (i.e., 1980–1990, 1991–1999, 2000–2003), the probability of OS at 2 years for patients transplanted in CP1 from an HLA-identical sibling was 74 %, with a cumulative incidence of TRM at 2 years of 22 % and of relapse of 18 % among the most recent cohort transplanted between 2000 and 2003 ($n = 3{,}018$) [13]. The Center for International Blood and Marrow Transplant Research (CIBMTR) recently reported the transplant outcomes of 449 patients with advanced phase CML; the disease-free survival rates remained as low as 35–40 % for CP2, 26–7 % for AP, and 8–11 % for BC [14]. Our series including 432 cases of CP2-AP and 189 cases of BC showed similar survival rates, as the probabilities of 3-year LFS in CP2-AP and BC were 46.1 and 19.2 %, respectively.

Our primary object in this study was to assess the clinical impact of GS according to each disease status. Our study results revealed that the patients in CP1 who were

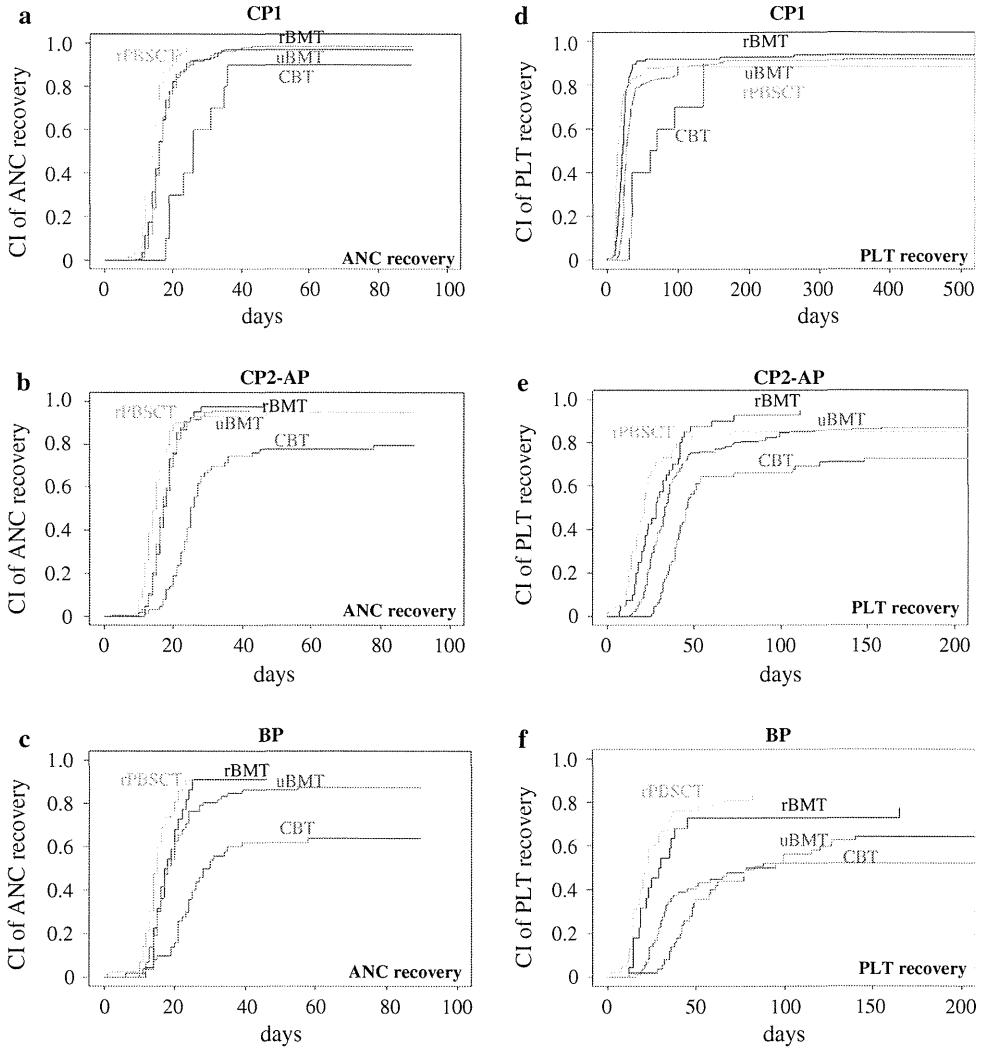

Fig. 3 The cumulative incidence of absolute neutrophil count (ANC) recovery for patients in CP1 (**a**), CP2-AP (**b**) and BC (**c**); and platelet (PLT) recovery for patients in CP1 (**d**), CP2-AP (**e**) and BC (**f**)

treated by rBMT showed a better 3-year OS (84.4 %) with a lower 1-year cumulative incidence of TRM, but the 3-year LFS and relapse rates were similar between patients receiving rBMT and patients receiving rPBSCT. These data were essentially in line with previous reports in which the CIBMTR reported the data of CML patients undergoing rPBSCT or rBMT in CP1; the 1-year LFS and relapse rates were similar for patients receiving rBMT or rPBSCT [14]. We also assessed the clinical impact of GS in CP2-AP; our results showed that there were no significant differences in OS or LFS between GS, despite lower probabilities of relapse after uBMT and lower probabilities of TRM after CBT. These results differ from the IBMTR reports in that for patients in CP2 or AP, rPBSCT was associated with a lower incidence of treatment failure and a higher probability of LFS at 1 year [15]. Regarding GVHD, a recent prospective randomized trial showed a trend toward a higher incidence of chronic GVHD after rPBSCT (59 % after rPBSCT vs. 40 % after rBMT, $P = 0.11$) for patients in CP1 [16]. Our results may confirm this report; although multivariate analysis in our study showed that rPBSCT (RR 1.37 95 % CI 0.97–1.92, $P = 0.075$) was not a significant risk factor for developing chronic GVHD (Table 3), rPBSCT showed a higher incidence of chronic GVHD (71.4 %), which was compared to other GS in CP1 (Fig. 4d).

Several investigators have addressed the clinical impact of pre-transplant IM on post-transplant outcomes for CML [14, 17–20]. The CIBMTR data demonstrated that pre-transplant IM was associated with better survival, but revealed no statistically significant differences in TRM, relapse, and LFS for patients in CP1 [17]. Among patients transplanted in the more advanced phases beyond CP1, pre-transplant IM was not associated with TRM, relapse, LFS, OS, or acute GVHD [17]. In contrast to these studies, our analysis showed that pre-transplant IM was significantly associated with better OS for patients in BC. In addition, multivariate analysis found pre-transplant IM was a

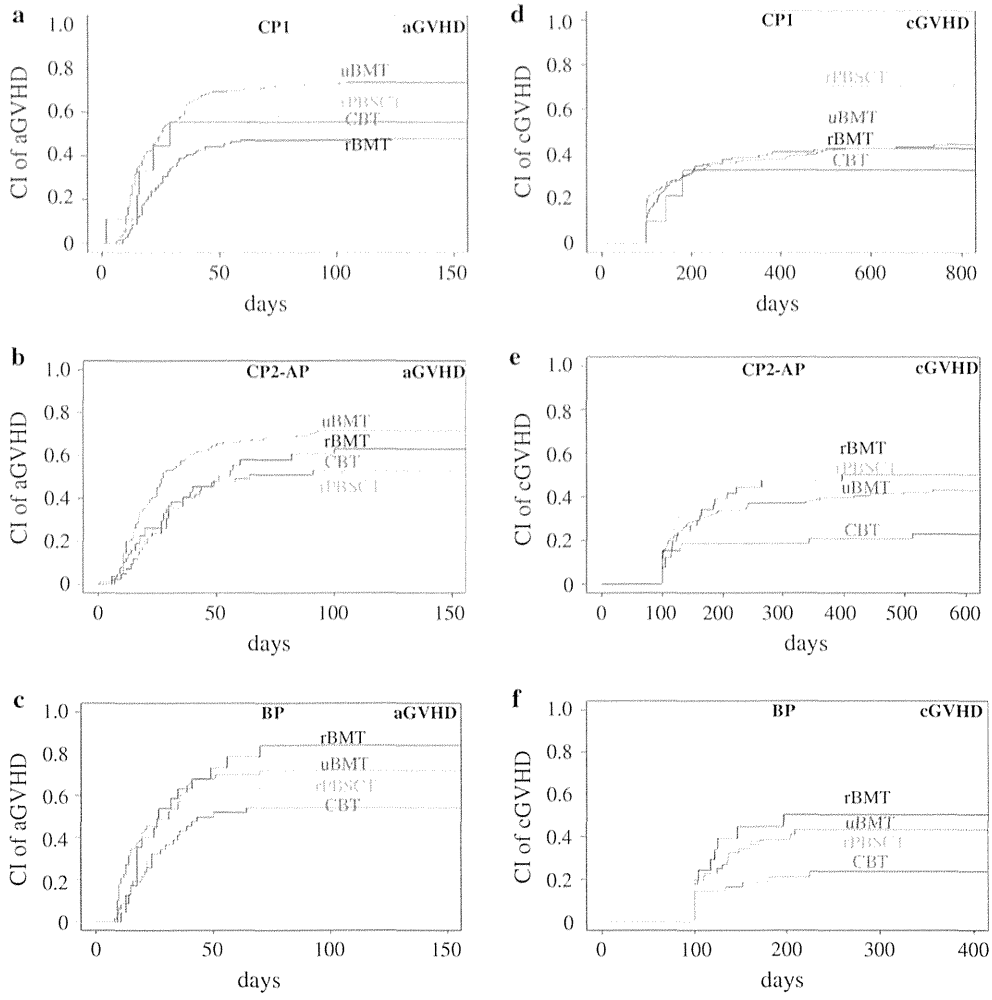

Fig. 4 The cumulative incidence of acute GVHD at all grades for patients in CP1 (**a**), CP2-AP (**b**) and BC (**c**); and chronic GVHD at all grades for patients in CP1 (**d**), CP2-AP (**e**) and BC (**f**)

significant factor associated with acute GVHD (>grade II) in CP1 (data not shown). Despite the study in the era of TKI, half of patients were in CP1, and 61 % of patients underwent allo-HSCT without use of pre-transplant TKI in this study. We should interpret these findings with utmost caution. We assume that most patients had already initiated the conventional treatment but could not reach a new, but expensive IM treatment before allo-HSCT, as a reason for these findings. Moreover, the findings that the number of patients in CP1 underwent allo-HSCT was 447 in the early period of IM from 2000 to 2004 and only 84 from 2005 to 2009 might support our assumption. Deininger et al. reported an effect of pre-transplant IM in their study that included 70 cases of CML and 21 cases of Ph (+) acute lymphoid leukemia. These investigators compared the outcomes with historical controls identified in the EBMT database [21], and observed a trend towards higher relapse mortality and significantly less chronic GVHD in patients with pre-transplant IM (OR = 0.44, $P = 0.027$). Thus, the clinical impact of pre-transplant IM is still a contentious issue; additional studies evaluating the long-term use of IM with a larger number of patients might permit a more refined analysis of the effect of pre-transplant IM.

Although data on clinical outcomes after CBT are conflicting, CBT has apparent advantages over uBMT, including no risk to the donor and ease of availability. Previous reports, mostly from pediatric studies, have shown that, despite higher HLA mismatch, CBT carries a lower risk of acute GVHD and chronic GVHD in comparison with uBMT [22–24]. A recent Japanese retrospective analysis assessing 86 patients, including pediatric patients, disclosed the transplant outcomes of CBT: 2-year OS was 53 %; for patients in CP, AP and BC, the OS rates were 71, 59 and 32 %, respectively [25]. Although our small population with only 10 cases of CBT in CP1 may prohibit drawing meaningful conclusions, a trend of higher relapse and lower TRM, OS and LFS in CP1 was similar to results obtained by previous study groups. Nevertheless, in CP2-AP and BC, transplant outcomes after CBT were comparable to those of other GS,