CLINICAL TRIALS AND OBSERVATIONS

Pretransplant administration of imatinib for allo-HSCT in patients with *BCR-ABL*–positive acute lymphoblastic leukemia

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Key Points

- Pretransplant imatinib improved both relapse and nonrelapse mortality in patients with *BCR-ABL*–positive acute lymphoblastic leukemia.

We aimed to evaluate the impact of pretransplant imatinib administration on the outcome of allogeneic hematopoietic stem cell transplantation (allo-HSCT) in adults with Philadelphia chromosome-positive (Ph^+) acute lymphoblastic leukemia (ALL). We retrospectively analyzed 738 patients with Ph^+ ALL that underwent allo-HSCT between 1990 and 2010 using data from the Transplant Registry Unified Management Program of the Japan Society of Hematopoietic Cell Transplantation. We compared the allo-HSCT outcomes between 542 patients who received imatinib before allo-HSCT during the initial complete remission period (imatinib cohort) and 196 patients who did not receive imatinib (non-imatinib cohort). The 5-year overall survival after allo-HSCT was significantly higher in the imatinib cohort than in the non-imatinib cohort (59% vs 38%; 95% confidence interval [CI], 31-45%; $P < .001$). Multivariate analysis indicated that pretransplant imatinib administration had beneficial effects on overall survival (hazard ratio [HR], 0.57; 95% CI, 0.42-0.77; $P < .001$), relapse (HR, 0.66; 95% CI, 0.43-0.99; $P = .048$), and nonrelapse mortality (HR, 0.55; 95% CI, 0.37-0.83; $P = .005$). In conclusion, our study showed that imatinib administration before allo-HSCT had advantageous effects on the clinical outcomes of allo-HSCT in patients with Ph^+ ALL. (*Blood.* 2014;123(15):2325-2332)

Introduction

The treatment of Philadelphia chromosome–positive (Ph^+) acute lymphoblastic leukemia (ALL) has changed dramatically since the introduction of imatinib. Most imatinib-treated patients achieve complete remission (CR), and hematopoietic stem cell transplantation (HSCT) can be performed in a substantial proportion of patients who have achieved major or complete molecular remission.[1-4] Several studies have shown improvements in overall survival (OS) since the incorporation of imatinib-based therapy.[5-9] However, the possible benefits of imatinib administration before HSCT have not been extensively examined. In Japan, imatinib was initially used to treat Ph^+ ALL in the Japan Adult Leukemia Study Group (JALSG) ALL202 study, which began in February 2002, and has been widely used since 2005.[4] A comparison of the clinical outcomes of the 60 patients enrolled in the JALSG ALL202 study with those of patients from the pre-imatinib era strongly suggested that Ph^+ ALL patients who received imatinib before allogeneic HSCT (allo-HSCT) during the initial CR period had significantly improved OS compared with those who did not receive imatinib.[10] In the present study, we used data from the Transplant Registry Unified Management Program of the Japan Society of Hematopoietic Cell Transplantation (JSHCT) to perform a large retrospective analysis of the clinical impact of imatinib administration before allo-HSCT.[11,12]

Methods

Data source and patient selection criteria

For this retrospective observational study, patient data were provided by the JSHCT, the Japan Marrow Donor Program, and the Japan Cord Blood Bank Network.[11] In the Transplant Registry Unified Management Program, patient survival, disease status, and long-term complications, including chronic

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Table 1. Characteristic of 738 patients with Ph$^+$ ALL who received allo-SCT

Characteristic	Non-imatinib cohort (n = 196)	Imatinib cohort (n = 542)	P
Age at SCT, years (%)			
≤29	48 (24)	99 (18)	
30-54	140 (71)	365 (67)	
≥55	8 (4)	78 (14)	
Median	39	42	<.001
Gender (male/female)	109/87	293/249	.708
Donor status (%)			
Related	121 (62)	178 (33)	
Unrelated	75 (38)	364 (67)	<.001
HLA disparity (%)			
Matched	139 (71)	330 (61)	
Mismatched	56 (29)	211 (39)	
Unknown	1 (0)	1 (0)	.010
Stem cell source (%)			
Bone marrow	151 (77)	345 (64)	
Peripheral blood	36 (18)	86 (16)	
Cord blood	9 (5)	111 (20)	<.001
PS at SCT (%)			
0	70 (36)	311 (57)	
1-4	45 (23)	218 (40)	
Unknown	81 (41)	13 (2)	.632
Days from diagnosis to SCT (%)			
<180	94 (48)	286 (53)	
≤180	98 (50)	255 (47)	
Unknown	4 (2)	1 (0)	.352
BCR-ABL subtype (%)			
Major	24 (12)	70 (13)	
Minor	69 (35)	352 (65)	
Major and minor	1 (0)	18 (3)	
Unknown	102 (52)	102 (19)	.039
Donor recipient gender match (%)			
Male-male	43 (22)	180 (33)	
Male-female	38 (19)	129 (24)	
Female-male	35 (18)	97 (18)	
Female-female	33 (17)	111 (20)	
Unknown	47 (24)	25 (5)	.463
Conditioning regimen (%)			
Reduced intensity	1 (1)	44 (8)	
Myeloabrative	121 (62)	479 (88)	
Unknown	74 (38)	19 (4)	<.001
WBC at diagnosis (%)			
<30 000/μL	109 (56)	288 (53)	
≤30 000/μL	74 (38)	247 (46)	
Unknown	13 (7)	7 (1)	.178
GVHD prophylaxis (%)			
CyA/methotrexate	133 (68)	228 (42)	
Tacrorimus/methotrexate	46 (23)	266 (49)	
Other/unknown	17 (9)	48 (9)	<.001
Cytogenetics (%)			
t(9;22) only	180 (92)	461 (85)	
Other abnormality	16 (8)	81 (15)	.016
ABO blood type disparity (%)			
Match	65 (33)	266 (49)	
Minor	24 (12)	119 (22)	
Major	37 (19)	152 (28)	
Unknown	70 (36)	5 (1)	.747
Transplant year (%)			
1990-2005	183 (93)	139 (26)	
2006-2010	13 (7)	403 (74)	<.001
MRD status at SCT			
Positive	44 (22)	144 (27)	
Negative	23 (12)	256 (47)	<.001

CyA, cyclospoline.

graft-versus-host disease (GVHD) and secondary malignancies, are reviewed annually using follow-up forms.[12] Ph$^+$ ALL was diagnosed by the presence of the *Ph* chromosome using cytogenetics and/or fluorescence in situ hybridization analysis and the determination of *BCR-ABL* fusion transcript positivity via real-time quantitative polymerase chain reaction (PCR) analysis. Grafts from unrelated donors were exclusively bone marrow derived because peripheral blood stem cell donation from unrelated donors was not approved in Japan during the study period. The timing and procedure of allo-HSCT, including the conditioning regimens, GVHD prophylaxis, and *BCR-ABL* transcript level assessments, were determined at each institution. *BCR-ABL* transcript levels were not compensated with a correction factor. In most laboratories, *BCR-ABL* mRNA copy numbers were normalized relative to glyceraldehyde-3-phosphate dehydrogenase mRNA copy numbers and expressed as copies per microgram of RNA. The quantification threshold was 50 copies/μg RNA, which corresponded to a minimal sensitivity of 10^{-5}; nondetection of *BCR-ABL* or samples below this threshold was designated as "not detected" or "<50 copies/μg" (presented herein as PCR negative). Minimum residual disease (MRD) was evaluated using real-time quantitative PCR within a 30-day period before transplantation. Therapeutic decisions regarding tyrosine kinase inhibitor (TKI) administration after allo-HSCT were made at each institution. This study was approved by the data management committees of the JSHCT, the Japan Marrow Donor Program, and the Japan Cord Blood Bank Network and by the Institutional Review Board of the Fujita Health University. This study was conducted in accordance with the Declaration of Helsinki.

Patient selection

To attain an adequate level of comparability in terms of the allo-HSCT regimens, the following inclusion criteria were used: (1) presence of de novo Ph$^+$ ALL; (2) age of 16 to 59 years; (3) allo-HSCT during the first CR; and (4) initial HSCT between 1990 and 2010. Additional data on pretransplant imatinib administration and MRD at the time of allo-HSCT were also collected for this study. Of the 865 patients who fulfilled these criteria, information on pretransplant imatinib administration was available for 739 patients. One patient was excluded because of missing information on the date of relapse. Finally, 738 patients with Ph$^+$ ALL who underwent allo-HSCT during the initial CR were analyzed.

Statistical considerations

The primary end point of our study was OS after allo-HSCT. Secondary end points included the incidence of nonrelapse mortality (NRM) and relapse. The observation periods for OS were calculated from the date of transplantation until the date of the event or the last known date of follow-up. The OS probabilities were estimated according to the Kaplan-Meier product limit method. The cumulative relapse and NRM incidences were estimated while considering the competing risk, as described elsewhere.[13] For each estimate of the cumulative event incidence, death without an event was defined as a competing risk. Risk factors were evaluated using a combination of univariate and multivariate analyses. The following variables were evaluated: imatinib use before HSCT (yes vs no), age group in years (40-54 and 55-59 vs <40), donor and stem cell source (bone marrow from unrelated donor, peripheral blood from related donor or cord blood vs bone marrow from related donor), human leukocyte antigen (HLA) disparity (matched [HLA identical siblings or 6/6 allele-matched unrelated] vs mismatched), performance status (PS) at allo-HSCT (0 vs 1-4), time from diagnosis to allo-HSCT (<180 vs ≥180 days), BCR-ABL subtype (major vs minor vs major and minor), donor-recipient gender match (male-male vs male-female vs female-male vs female-female), conditioning regimen (decreased intensity vs myeloablative), white blood cell (WBC) count at diagnosis (<30 000/μL vs ≥30 000/μL), GVHD prophylaxis (CyA/methotrexate vs tacrolimus/methotrexate), cytogenetics [t(9;22) only vs more/other abnormalities], and ABO blood type compatibility (match, minor mismatch, or major mismatch). Continuous CR was defined as the absence of any hematological recurrence. We defined the following dosages as decreased-intensity regimens: busulfan, <9 mg/kg; melphalan, ≤140 mg/m^2; and total body irradiation, <500 cGy (single or fractionated) or 500 to 800 cGy (fractionated).[14] Donor and recipient pairs were considered matched when the

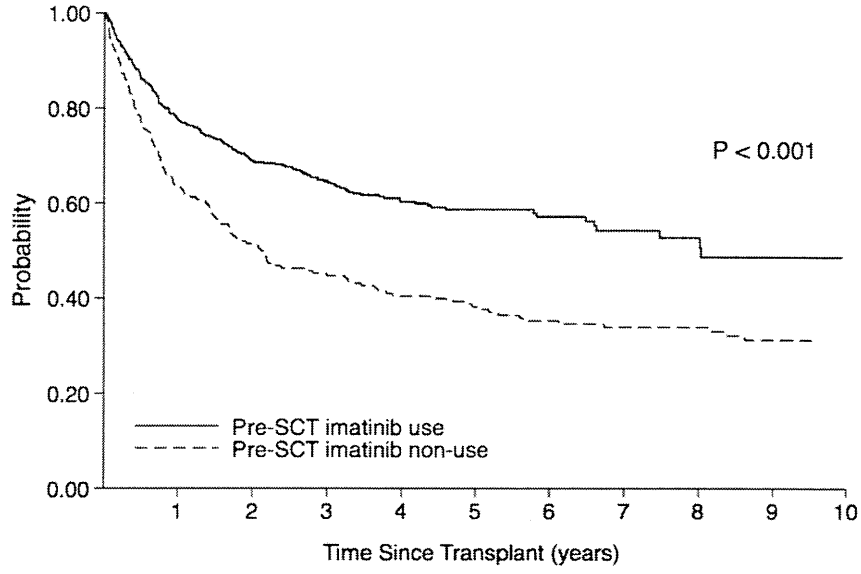

Figure 1. Effects of imatinib administration before stem cell transplantation on the overall survival of patients with Ph$^+$ ALL who underwent allo-HSCT during the initial CR period.

HLAs were matched at the A, B, and DRB1 loci, as determined by low-resolution HLA typing in allo-SCT from a related donor or cord blood. For unrelated allo-HSCT, matching at the HLA-A, B, Cw, and DRB1 loci in HLA high-resolution molecular typing was considered matched cases. Mismatches were defined by the presence of ≥1 disparity among these loci. Univariate analysis was performed using Cox regression models or a log-rank analysis. Multivariate analysis was performed using the Cox proportional hazards regression model or the competing risk regression model,[15] as appropriate. Demographic differences among groups were evaluated using the χ^2 or Wilcoxon rank-sum tests as appropriate. All statistical analyses were performed with STATA 11 software (STATA Corp., College Station, TX).

Results

Patient characteristics

The 738 study patients included 402 men and 336 women with a median age of 41 years (range, 16-59 years). HLA matching information was not available for 2 patients. The donor sources included HLA-identical sibling donors (n = 280), unrelated donors (n = 439), and other related donors (n = 19). There were no significant differences between the imatinib and non-imatinib cohorts with respect to gender, PS at allo-HSCT, interval between diagnosis and allo-HSCT, donor-recipient gender match, WBC count at diagnosis, or donor-recipient ABO compatibility, whereas significant differences were observed with respect to the age distribution at allo-HSCT, donor status, HLA disparity, stem cell source, BCR-ABL subtype, conditioning regimen, GVHD prophylaxis, and cytogenetics (Table 1). Of the 196 patients in the non-imatinib cohort, 183 (93%) underwent allo-HSCT between 1990 and 2005. In contrast, 403 of the 542 (74%) patients in the imatinib cohort underwent allo-HSCT between 2006 and 2010.

Outcomes

Overall survival. The median follow-up duration of the allo-HSCT survivors was 1551 days (range, 66-6648 days), and the 3- and 5-year OS rates for all patients were 59% (95% confidence interval [CI], 55-63%) and 53% (95% CI, 49-56%), respectively. The 5-year OS in the imatinib cohort was 59% (95% CI, 54-63%), which was significantly higher than that in the non-imatinib cohort (38%; 95% CI, 31-45%; $P < .001$; Figure 1). Table 2 shows the OS risk factor analysis. Imatinib administration before allo-HSCT had a significantly favorable effect on OS, as revealed by univariate analysis (hazard ratio [HR], 0.56; 95% CI, 0.45-0.70; $P < .001$] and confirmed by multivariate analysis (HR, 0.57; 95% CI, 0.42-0.77; $P < .001$). In addition, age, interval between diagnosis and HSCT, and WBC count at diagnosis were significant prognostic factors for OS in the multivariate analysis.

Relapse. Relapse after allo-HSCT occurred in 116 (21%) and 66 (34%) patients in the imatinib and non-imatinib cohorts, respectively, after median periods of 232 (range, 19-2560 days) and 258 days (range, 42-2350 days), respectively. In the imatinib cohort, the estimated 3-year cumulative incidence of relapse was 23% (95% CI, 20-27%), which was significantly lower than that in the non-imatinib cohort (39%; 95% CI, 31-47%; $P < .001$; Figure 2). Table 3 shows the relapse risk factor analysis. Imatinib administration before allo-HSCT had a significantly favorable effect on relapse, as determined by univariate analysis (HR, 0.52; 95% CI, 0.39-0.71; $P < .001$) and confirmed by multivariate analysis (HR, 0.66; 95% CI, 0.43-0.99; $P = .048$). In addition, the following were significant prognostic factors for relapse: age, 30 to 54 years; HLA disparity; and female-male donor-recipient matching.

NRM. Overall, 207 (38%) patients in the imatinib cohort and 131 (67%) in the non-imatinib cohort died after allo-HSCT within median periods of 178 (range, 8-2935 days) and 177 days (range, 5-4549 days), respectively. Of these, 124 (23%) and 71 (36%) deaths in the former and latter cohorts, respectively, were not related to relapse after allo-HSCT. The major causes of all deaths and their respective frequencies in the imatinib and non-imatinib cohorts were as follows: relapse (40% vs 47%), infection (18% vs 9%), organ failure (12% vs 9%), interstitial pneumonia (6% vs 4%), GVHD (5% vs 9%), transplantation-associated thrombotic microangiopathy (2% vs 2%), bleeding (2% vs 5%), sinusoidal obstruction syndrome (1% vs 5%), and others (13% vs 11%). The estimated cumulative incidence of NRM at 3 years was significantly lower in the imatinib cohort (22%; 95% CI, 18-26%) than in the non-imatinib cohort (30%; 95% CI, 24-37%; $P = .002$; Figure 2). Table 4 shows the NRM risk factor

Table 2. Results of univariate and multivariate analysis of overall survival among 738 patients with Ph$^+$ ALL

	Univariate analysis		Multivariate analysis	
Variable	RR (95% CI)	*P*	RR (95% CI)	*P*
Imatinib use before SCT				
No	1 (Reference)		1 (Reference)	
Yes	0.56 (0.45-0.70)	<.001	0.57 (0.42-0.77)	<.001
Age at SCT (regression)	1.02 (1.01 −1.03)	.002	1.02 (1.01-1.03)	<.001
HLA disparity				
Matched	1 (Reference)		1 (Reference)	
Mismatched	0.89 (0.71-1.11)	.30	0.89 (0.66-1.20)	.430
Stem cell source				
Related bone marrow	1 (Reference)		1 (Reference)	
Unrelated bone marrow	0.81 (0.62-1.10)	.13	0.92 (0.63-1.33)	.640
Related peripheral blood	1.08 (0.79-1.48)	.64	1.27 (0.90-1.78)	.180
Cord blood	0.86 (0.61-1.22)	.39	1.25 (0.78-2.0)	.360
PS at SCT				
0	1 (Reference)	.24	1 (Reference)	
1-4	1.15 (0.91-1.47)		1.05 (0.82-1.36)	.690
Duration from diagnosis to SCT				
>180 days	1 (Reference)		1 (Reference)	
≤180 days	1.26 (1.02-1.57)	.03	1.31 (1.03-1.67)	.030
BCR-ABL subtype				
Major	1 (Reference)		1 (Reference)	
Minor	0.77 (0.36-1.66)	.51	NA	
Major and minor	0.90 (0.44-1.83)	.78		
Donor recipient gender match				
Male-male	1 (Reference)		1 (Reference)	
Male-female	0.83 (0.61-1.11)	.21	0.78 (0.57-1.06)	.110
Female-male	0.78 (0.56-1.08)	.13	0.77 (0.55-1.07)	.120
Female-female	0.71 (0.51-0.98)	.03	0.70 (0.50-0.98)	.040
Conditioning regimen				
Reduced intensity	1 (Reference)		1 (Reference)	
Myeloabrative	0.96 (0.60-1.53)	.87	1.04 (0.64-1.70)	.150
WBC at diagnosis				
<30 000/μL	1 (Reference)		1 (Reference)	
≤30 000/μL	1.29 (1.04-1.61)	.02	1.07 (0.99-1.14)	.053
GVHD prophylaxis				
CyA/MTX	1 (Reference)		1 (Reference)	
Tacrorimus/MTX	0.78 (0.62-0.98)	.03	0.98 (0.73-1.31)	.899
Cytogenetics				
t(9;22)only	1 (Reference)		1 (Reference)	
Other abnormality	0.97 (0.71-1.34)	.87	NA	
ABO blood type disparity				
Match	1 (Reference)		1 (Reference)	
Minor	1.12 (0.83-1.51)	.48	NA	
Major	1.21 (0.93-1.59)	.16	NA	

CyA, cyclospoline; NA, not applicable; RR, relative risk.

analysis. Imatinib administration before allo-HSCT had a significantly favorable effect on NRM, as determined by univariate (HR, 0.65; 95% CI, 0.49-0.88; $P < .001$) and multivariate analyses (HR, 0.55; 95% CI, 0.37-0.83; $P = .005$). Age was also found to be a significant prognostic factor in multivariate analysis.

MRD. Data regarding MRD status before allo-HSCT were available for 67 (34%) patients in the non-imatinib cohort and 400 (74%) patients in the imatinib cohort (Table 1). Among the 467 patients, the MRD negativity rate before allo-HSCT was significantly higher in the imatinib cohort than in the non-imatinib cohort (64%; 95% CI, 59-69% vs 34%; 23-47%; $P < .001$). The estimated cumulative incidence of relapse at 3 years was significantly lower in the MRD-negative patients than in the MRD-positive patients (20%; 95% CI, 15-25% vs 32%; 95% CI, 25-40%, respectively; $P = .0017$), and this tendency was significant in the imatinib cohort (19%; 95% CI, 15-25% vs 34%; 95% CI, 25-42%, respectively; $P = .0016$), but not in the non-imatinib cohort (27%; 95% CI, 10-48% vs 28%; 95% CI, 15-43%, respectively; $P = .566$). There was no significant difference in NRM between the MRD-negative and MRD-positive patients (19%; 95% CI, 14-24% vs 22%; 95% CI, 16-28% at 3 years, respectively; $P = .0642$).

Discussion

Although many studies have confirmed the beneficial effects of imatinib on the clinical outcomes of patients with Ph$^+$ ALL,[1-6] the potential benefits of pretransplant imatinib administration has not been investigated in a sufficient number of patients. In our study, which is the largest of its type to date, we analyzed the records of 738 patients during a long-term follow-up period to analyze the benefits of pretransplant imatinib administration in patients with Ph$^+$ ALL. We observed significant improvements in the relapse rate and NRM in patients who received imatinib before allo-HSCT compared with those who did not receive imatinib (23% vs 39%; $P < .001$ and 22% vs 30%; $P = .002$, respectively). In the MVA, pretransplant imatinib administration was shown to have a significant favorable effect on both relapse and NRM after allo-HSCT.

Some investigators have reported that MRD before HSCT can serve as a powerful predictor of a lower relapse rate. In an analysis of the outcomes of 95 patients with Ph$^+$ ALL who received pretransplant imatinib-based therapy, Lee et al showed that the strongest predictor of relapse was the patient's MRD status at the end of 2 courses of pretransplant imatinib-based chemotherapy.[16] In the present study, patients who were MRD negative before HSCT had a significantly lower relapse rate after HSCT compared with those who were initially MRD positive (20.0% vs 32%, $P = .0017$), and this tendency was remarkable in the imatinib cohort (19% vs 34%, $P = .0016$). Moreover, the MRD negativity rate for *BCR-ABL* patients before allo-HSCT was significantly higher in the imatinib cohort than in the non-imatinib cohort (62% vs 37%, $P < .001$). These data suggest that in the imatinib cohort, the powerful antileukemia activity associated with pretransplant imatinib administration extensively decreased the MRD before allo-HSCT and prevented subsequent relapse after allo-HSCT.

The Ph chromosome is an adverse prognostic factor in patients with ALL, and only allo-HSCT offers a curative option for patients with Ph ALL. However, the probability of NRM in patients who undergo transplantation during the initial CR is relatively high; therefore, methods to decrease NRM were investigated. Recently, the UKALLXII/ECOG2993 study confirmed the superiority of allogeneic transplantation over chemotherapy on the basis of prospective outcome data from 267 unselected adult patients and reported that high NRM remained a significant problem in the pre-imatinib era.[17] Patient age, donor status, and HLA disparity are well-known prognostic factors for NRM after allo-HSCT.[1,3,17-19] In the present study, the risk of NRM was significantly lower in the imatinib cohort than in the non-imatinib cohort ($P = .002$), despite the former comprising significantly larger proportions of older recipients and unrelated and/or HLA-mismatched donors ($P < .001$, $P < .001$, and $P = .01$, respectively). Imatinib-based therapy has increased the proportion of patients who achieve sustained remission, thus providing additional

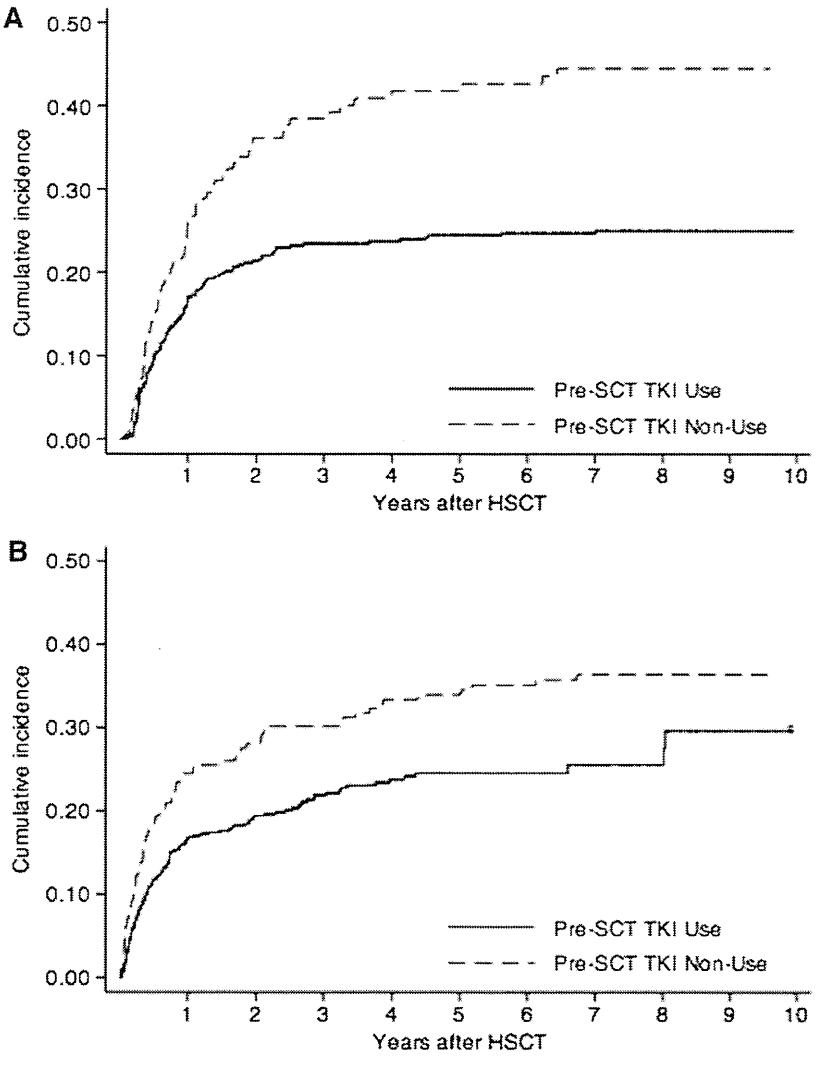

Figure 2. Cumulative incidence of relapse- or nonrelapse-related mortality of patients with Ph$^+$ ALL who underwent allo-HSCT during the initial CR period. (A) Relapse mortality. (B) NRM.

time for suitable donor selection and allo-HSCT and enabling individualized treatment approaches.[1-3] These secondary benefits may have contributed to the lower NRM in the imatinib cohort. Moreover, several recent studies have reported improved NRM following the incorporation or dose escalation of imatinib before allo-HSCT.[18-22] Given these findings, we believe that imatinib administration has allowed more patients with Ph$^+$ ALL to undergo allo-HSCT while in a better condition, resulting in the achievement of a lower NRM.

Over the last few decades, there have been many attempts to improve patient outcomes after allo-HSCT, including changes in the conditioning regimens and donor selection and the prophylaxis and treatment of organ complications, GVHD, and infectious diseases. In Japan, the period of 1990 to 2005 marked a pioneering era of cord blood transplantation, during which the relevance of cell doses and HLA matching had not yet been recognized. Laport et al reported their experiences with 79 patients with Ph$^+$ ALL who underwent allo-SCT with matched sibling donors; in these patients, the 5-year OS and NRM were examined according to the decade in which SCT was performed (1985-1995 vs 1996-2005), and no significant difference were observed between these 2 time periods.[23] In Japan, Kurosawa et al used a nationwide registry database of >6000 patients to retrospectively assess changes in the incidence and causes of NRM during 3 consecutive 4-year periods (1997-2000, 2001-2004, and 2005-2008).[24] The authors reported that the incidence of NRM after allo-HCT had significantly decreased during the entire 12-year period, which led to improvements in OS and decreases in NRM in subgroups comprising older patients (50-70 years of age) and/or those who received unrelated bone marrow transplants.[24] According to the present study, patients who underwent allo-HSCT with alternative donors and/or elderly patients would benefit from recent improvements in transplantation procedures, and this progress in transplantation may have partly contributed to the improved NRM in the imatinib cohort.

A strength of the present study was its large sample size; this permitted a more accurate estimation of the end points and added statistical power to the analyses. However, because this was a retrospective multicenter study, our results may be susceptible to the disadvantages of any retrospective study, such as heterogeneity in the treatment strategies selected by the physicians. With regard to patient selection bias, changes in patient selection and transplantation procedures throughout the study period (1990-2010) should also be considered. In Japan, the widespread use of alternative donors after 2000 facilitated the extension of allo-HSCT eligibility. Furthermore, cord blood cells were more frequently used in the imatinib cohort (20%) than in the non-imatinib

Table 3. Results of univariate and multivariate analysis of relapse among 738 patients with Ph$^+$ ALL

	Univariate analysis		Multivariate analysis	
Variable	RR (95% CI)	*P*	RR (95% CI)	*P*
Imatinib use before SCT				
No	1 (Reference)		1 (Reference)	
Yes	0.52 (0.39-0.71)	<.001	0.66 (0.43-0.99)	.048
Age at SCT (years)				
≤29	1 (Reference)		1 (Reference)	
30-54	0.58 (0.42-0.81)	.001	0.63 (0.45-0.89)	.009
≥55	0.60 (0.36-1.03)	.062	0.71 (0.40-1.30)	.250
HLA disparity				
Matched	1 (Reference)		1 (Reference)	
Mismatched	0.52 (0.3-0.74)	<.001	0.48 (0.29-0.80)	.005
Stem cell source				
Related bone marrow	1 (Reference)			
Unrelated bone marrow	0.50 (0.35-0.70)	<.001	0.76 (0.48-1.21)	.251
Related peripheral blood	0.76 (0.50-1.16)	.202	0.91 (0.58-1.42)	.670
Cord blood	0.51 (0.31-0.84)	.008	1.2 (0.58-2.5)	.618
PS at SCT				
0	1 (Reference)		1 (Reference)	
1-4	1.038 (0.75-1.44)	.821	NA	
Days from diagnosis to SCT				
>180 days	1 (Reference)		1 (Reference)	
≤180 days	0.91 (.68-1.22)	.538	1.16 (0.84-1.61)	.366
BCR-ABL subtype				
Major	1 (Reference)		1 (Reference)	
Minor	0.61 (0.20-1.91)	.400	NA	
Major and minor	1.22 (0.44-3.39)	.703	NA	
Donor recipient gender match				
Male-male	1 (Reference)		1 (Reference)	
Male-female	0.95 (0.65-1.40)	.790	1.02 (0.69-1.52)	.908
Female-male	0.59 (0.37-0.93)	.024	0.51 (0.32-0.81)	.004
Female-female	0.49 (0.30-0.80)	.004	0.53 (0.33-0.87)	.013
Conditioning regimen				
Reduced intensity	1 (Reference)		1 (Reference)	
Myeloabrative	1.15 (0.61-2.17)	.675	0.95 (0.51-1.79)	.864
WBC at diagnosis				
<30 000/μL	1 (Reference)		1 (Reference)	
≤30 000/μL	1.39 (1.03-1.87)	.029	1.08 (1.00-1.16)	.057
GVHD prophylaxis				
CyA/MTX	1 (Reference)		1 (Reference)	
Tacrorimus/MTX	0.56 (0.40-0.77)	<.001	0.74 (0.50-1.09)	.135
Cytogenetics				
t(9;22) only	1 (Reference)		1 (Reference)	
Other abnormality	1.01 (0.65-1.57)	.955	NA	
ABO blood type disparity				
Match	1 (Reference)		1 (Reference)	
Minor	0.75 (0.49-1.16)	.199	NA	
Major	0.86 (0.59-1.24)	.413	NA	

CyA, cyclospoline; NA, not applicable; RR, relative risk.

cohort (5%). These discrepancies resulted in different donor status, HLA disparity, and stem cell source frequencies in the present study.

An important difference in the pretransplant chemotherapy regimens should also be noted. Although detailed information about pretransplant chemotherapy was not available, the majority of the non-imatinib cohort was likely treated according to the JALSG ALL93[25] or JALSG ALL97 protocols,[26] whereas most of the imatinib cohort was likely to be treated according to the JALSG ALL202 protocols,[4] in which the chemotherapeutic regimen was similar to that used in the earlier protocols, except for the use of imatinib, because these were widely used regimens in Japan during the study period. Therefore, the influence of pretransplant chemotherapy appears to be limited.

In conclusion, our study involving a large number of patients observed over a long-term follow-up period clearly demonstrates that imatinib administration before allo-HSCT had advantageous effects on the clinical outcomes of patients with Ph$^+$ ALL. This finding encourages us to consider allo-HSCT for patients with Ph$^+$ ALL even during the imatinib era; however, we should continue to investigate

Table 4. Results of univariate and multivariate analysis of NRM among 738 patients with Ph$^+$ ALL

	Univariate analysis		Multivariate analysis	
Variable	RR (95% CI)	*P*	RR (95% CI)	*P*
Imatinib use before SCT				
No	1 (Reference)		1 (Reference)	
Yes	0.65 (0.49-0.88)	<.001	0.55 (0.37-0.83)	.005
Age at SCT (years)				
≤29	1 (Reference)		1.03 (1.02-1.05)	<.001
30-54	1.77 (1.16-2.70)	.008	(Regression)	
≥55	2.54 (1.51-4.30)	<.001		
HLA disparity				
Matched	1 (Reference)		1 (Reference)	
Mismatched	1.15 (0.86-1.54)	.330	1.27 (0.87-1.87)	.219
Stem cell source				
Related bone marrow	1 (Reference)		1 (Reference)	
Unrelated bone marrow	1.47 (1.01-2.13)	.044	1.32 (0.76-2.32)	.327
Related peripheral blood	1.37 (0.87-2.14)	.174	1.55 (0.95-2.53)	.081
Cord blood	1.49 (0.93-2.38)	.097	1.59 (0.81-3.11)	.181
PS at SCT				
0	1 (Reference)		1 (Reference)	
1-4	1.04 (0.76-1.42)	.810	0.90 (0.64-1.26)	.542
Days from diagnosis to SCT				
>180 days	1 (Reference)		1 (Reference)	
≤180 days	1.60 (1.20-2.13)	.001	1.35 (0.97-1.88)	.075
BCR-ABL subtype				
Major	1 (Reference)		1 (Reference)	
Minor	1.20 (0.40-3.62)	.750	NA	
Major and minor	0.96 (0.33-2.76)	.940	NA	
Donor recipient gender match				
Male-male	1 (Reference)		1 (Reference)	
Male-female	0.83 (0.55-1.25)	.380	0.73 (0.48-1.12)	.150
Female-male	1.01 (0.67-1.51)	.970	1.07 (0.71-1.62)	.737
Female-female	0.95 (0.63-1.42)	.790	0.85 (0.55-1.30)	.446
Conditioning regimen				
Reduced intensity	1 (Reference)		1 (Reference)	
Myeloabrative	0.76 (0.44-1.31)	.328	0.93 (0.52-1.67)	.819
WBC at diagnosis				
<30 000/μL	1 (Reference)		1 (Reference)	
≤30 000/μL	1.04 (0.78-1.38)	.810	1.03 (0.94-1.14)	.468
GVHD profiraxis				
CyA/MTX	1 (Reference)		1 (Reference)	
Tacrorimus/MTX	1.19 (0.89-1.60)	.250	1.23 (0.84-1.81)	.287
Cytogenetics				
t(9;22) only	1 (Reference)		1 (Reference)	
Other abnormality	1.03 (0.68-1.54)	.900	NA	
ABO blood type disparity				
Match	1 (Reference)		1 (Reference)	
Minor	1.31 (0.89-1.92)	.170	NA	
Major	1.28 (0.91-1.82)	.160	NA	

CyA, cyclospoline; NA, not applicable; RR, relative risk.

alternative treatment options for patients who are not eligible for allo-HSCT because of older age and/or comorbidity. For example, in recent years, MRD monitoring has been increasingly used as an independent prognostic factor in response to a number of studies that have demonstrated its importance. Ravandi et al analyzed the clinical outcomes of patients with Ph^+ ALL treated with TKI combined chemotherapy without allogeneic SCT and demonstrated that the achievement of a major molecular response status at 3 months (and beyond) after treatment initiation was associated with a decreased likelihood of relapse and a longer OS.[27] Bachanova et al used data from the Center for International Bone Marrow Transplant Research to analyze 197 patients with Ph^+ ALL and reported that the achievement of a MRD-negative status may lead to a low relapse rate and prolonged survival in response to either myeloablative conditioning or decreased-intensity conditioning HSCT. They also reported that MRD status may be more helpful than a predefined age cutoff in guiding decisions regarding the conditioning intensity before allo-HSCT.[28] In the TKI era, the potential of MRD monitoring via PCR was demonstrated; this technique allows us to identify patients who would benefit from treatment intensification and to select continued therapy without transplantation in older patients with poorer conditions. In addition, recent studies have shown that imatinib therapy before autologous HSCT is also beneficial.[7,29] The clinical relevance of autologous HSCT in patients with Ph^+ ALL should also be investigated as an alternative stem cell source in the TKI era.

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Authorship

Contribution: S.M., S.N., K.I., and J.T. designed the study and wrote the manuscript; S.M., Y.A., and K. Matsuo performed the statistical analysis and interpreted the data; H.K., K.O., T.F., Y.O., K. Miyamura, S.T., and M.O. provided the patient data; and Y.A., R.S., Y.M., K.K., and H.S. collected the patient data.

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Graft-Versus-Host Disease and Survival after Cord Blood Transplantation for Acute Leukemia: A Comparison of Japanese versus White Populations

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A B S T R A C T

An earlier report identified higher risks of acute and chronic graft-versus-host disease (GVHD) in White children compared with the Japanese after HLA-matched sibling transplantations. The current analysis explored whether racial differences are associated with GVHD risks after unrelated umbilical cord blood transplantation. Included are patients of Japanese descent (n = 257) and Whites (n = 260; 168 of 260 received antithymocyte globulin [ATG]). Transplants were performed in the United States or Japan between 2000 and 2009; patients were aged 16 years or younger, had acute leukemia, were in complete remission, and received a myeloablative conditioning regimen. The median ages of the Japanese and Whites who received ATG were younger at 5 years compared with 8 years for Whites who did not receive ATG. In all groups most transplants were mismatched at 1 or 2 HLA loci. Multivariate analysis found no differences in risks of acute GVHD between the Japanese and Whites. However, chronic GVHD was higher in Whites who did not receive ATG compared with the Japanese (hazard ratio, 2.16; $P < .001$), and treatment-related mortality was higher in Whites who received ATG compared with the Japanese (relative risk, 1.81; $P = .01$). Nevertheless, there were no significant differences in overall survival between the 3 groups.

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INTRODUCTION

Graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation is associated with significant morbidity and mortality [1]. Acute GVHD risks are higher after HLA-mismatched compared with HLA-matched transplantations and primarily attributed to mismatching at major histocompatibility antigens. On the other hand, donor–recipient mismatching for minor histocompatibility antigens may explain GVHD after HLA-matched transplantations. In an earlier report, Oh et al. [2] compared GVHD and overall survival between different ethnic populations after HLA-matched related bone marrow transplantation for hematologic malignancies. That report, which included children and adults, showed higher acute but not chronic GVHD risks for adult U.S. Whites compared with adults of Japanese descent. However, among children, both acute and chronic GVHD risks were higher in U.S. Whites compared with the Japanese. In that report, the observed differences between the Japanese and Whites were attributed to the relative homogeneity of minor histocompatibility antigens in persons of Japanese descent [3].

Umbilical cord blood (UCB) is less immunogenic, and consequently HLA mismatches that are prohibitive between unrelated adult donors and recipients are considered acceptable up to 2 mismatches when selecting UCB units. The practice of transplanting UCB units (UCBT) that are HLA mismatched to recipients is common in both children and adults worldwide [4-8]. To our knowledge, no published reports explore whether racial differences exist in acute and

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chronic GVHD risks after UCBT. Almost all UCB units used in Japan are from cord blood banks in Japan, allowing for a homogeneous cohort of donors. On the other hand, the U.S. population is genetically diverse, and UCB units may have been obtained through either a U.S. or international cord blood bank. Additionally, differences in transplantation strategies exist. In Japan, UCB units generally contain fewer total nucleated cells (TNCs) than in the United States and consider lower resolution HLA match (antigen level) at HLA-A, -B, and -DR. In the United States, HLA matching at the DR locus considers allele-level match. Additionally, antithymocyte globulin (ATG) was routinely included in the transplant preparatory regimen before 2007 in the United States [8,9]. Another key difference between the 2 countries is the co-infusion of 2 UCB units, routine in the United States, for adults. Consequently, the current analysis is limited to younger patients such that the comparison is between appropriately aged patients who were transplanted with a single UCB unit. In this report we compare acute and chronic GVHD and mortality risks after UCBT between Japanese and White children with acute leukemia to test whether the genetic diversity of donors and recipients influenced the likelihood of GVHD.

METHODS

Data Source

Data were obtained from the Center for International Blood and Marrow Transplant Research [10] for U.S. transplants and from the Japan Society for Hematopoietic Cell Transplantation (JSHCT) and the Japan Cord Blood Bank Network [11] for Japanese transplants. All U.S. patients provided consent for research participation. Patient consent is not required for registration of the JSHCT because registry data consist of anonymized clinical information. The institutional review board of the National Marrow Donor Program, Medical College of Wisconsin, Nagoya University Graduate School of Medicine, and the Data Management Committees of the JSHCT and the Japan Cord Blood Bank Network approved this study.

Inclusion Criteria

Patients were 16 years old and younger with acute myeloid leukemia or acute lymphoblastic leukemia and in first, second, or third complete remission (CR). All patients received a myeloablative transplant conditioning regimen and were transplanted in Japan or the United States between 2000 and 2009. Excluded were non-Whites transplanted in the United States, transplantations in relapse, prior allogeneic transplantation, infusion of 2 UCB units, or reduced-intensity transplant conditioning regimens. Five hundred seventeen patients were eligible: 257 transplants from Japan, 168 transplants for Whites that included ATG in their transplant regimen, and 92 transplants for Whites without ATG. Units were matched to patients at the antigen level at HLA-A and -B and at the allele level at HLA-DRB1; for the Japanese cohort, this occurred retrospectively.

Outcomes

Acute and chronic GVHD were defined as time to occurrence of GVHD, using standard criteria [12,13]. Treatment-related mortality (TRM) was defined as death without leukemia relapse. Relapse was defined as hematological/morphological recurrence of leukemia. Overall mortality was defined as death from any cause.

Statistical Analysis

To compare the outcomes of interest, Cox proportional hazards models were used to adjust for potential imbalance in baseline characteristics between the 3 treatment groups [14,15]. The main effect term, Japanese versus Whites who received ATG versus Whites who did not receive ATG, was held in all steps of model building regardless of level of significance. Other variables considered were age at transplantation (≤5 versus 6 to 16 years), gender, recipient cytomegalovirus (CMV) serostatus, disease, disease status, TNCs (≤3 versus >3 × 10^7/kg), HLA match (6/6 versus 5/6 versus 4/6), transplant preparative regimen (containing total body irradiation [TBI] versus not), GVHD prophylaxis (containing cyclosporine versus tacrolimus), and period (2000 to 2006 versus 2007 to 2009). All variables met the proportionality assumption, and there were no first-order interactions between variables in the final model and the main effect term. The effects of acute and chronic GVHD were also tested for their effect on overall mortality as time-dependent covariates. Results are expressed as hazard ratios (HRs) together with 95% confidence intervals (CIs).

Adjusted cumulative incidences of acute GVHD, chronic GVHD, relapse, and TRM [16] and adjusted probabilities of survival [17] were calculated using the multivariate models, stratified on the 3 treatment groups, and weighted by the pooled sample proportion value for each prognostic factor. SAS version 9.3 (Cary, NC) was used in the analyses.

RESULTS

Table 1 shows characteristics of patients, their diseases, and transplant regimens by the 3 treatment groups: Japanese, Whites who received ATG, and Whites who did not receive ATG. None of the Japanese patients received ATG. The median ages of the Japanese and Whites who received ATG was 5 years, whereas Whites who did not receive ATG were slightly older at 8 years.

There were other differences: Japanese patients were more likely to be CMV seropositive, to be transplanted in first CR, and more likely to receive a UCB unit with TNCs < 3 × 10^7/kg compared with Whites. Median TNC doses were 5.1 × 10^7/kg (range, 1.3 to 48), 7.4 × 10^7/kg (range, 1.6 to 50), and 5.7 × 10^7/kg (range, 1.6 to 21) for Japanese, Whites who received ATG, and Whites who did not receive ATG,

Table 1
Characteristics of Study Patients

	Japanese (n = 257)	White, with ATG (n = 168)	White, no ATG (n = 92)	*P*
Age at transplant, yr				<.0001
≤5	135 (53%)	85 (51%)	25 (27%)	
6-16	122 (47%)	83 (49%)	67 (73%)	
Gender				.73
Female	116 (45%)	81 (48%)	40 (43%)	
Male	141 (55%)	87 (52%)	52 (57%)	
Recipient CMV status				<.0001
Negative	78 (30%)	88 (52%)	43 (47%)	
Positive	115 (45%)	80 (48%)	48 (52%)	
Unknown	64 (25%)	—	1 (1%)	
Disease				<.0001
AML	70 (27%)	88 (52%)	23 (25%)	
ALL	187 (73%)	80 (48%)	69 (75%)	
Disease risk				<.0001
CR1	156 (61%)	61 (36%)	32 (35%)	
CR2/CR3	101 (39%)	107 (64%)	60 (65%)	
Year of transplant				<.0001
2000-2006	174 (68%)	105 (63%)	27 (29%)	
2007-2009	83 (32%)	63 (38%)	65 (71%)	
No. of cryopreserved TNCs ×10^7/kg				<.0001
<3	48 (19%)	4 (2%)	9 (10%)	
≥3	201 (78%)	164 (98%)	83 (90%)	
Unknown	8 (3%)	—	—	
HLA match status (A B; intermediate resolution, DRB1; allele level)				.51
6/6	47 (18%)	42 (25%)	17 (18%)	
5/6	127 (49%)	75 (45%)	47 (51%)	
4/6	83 (32%)	51 (30%)	28 (30%)	
Conditioning regimen				<.0001
Non-TBI	75 (29%)	74 (44%)	8 (9%)	
TBI	182 (71%)	94 (56%)	84 (91%)	
GVHD prophylaxis				<.0001
Cyclosporine containing	122 (47%)	138 (82%)	54 (59%)	
Tacrolimus containing	135 (53%)	30 (18%)	38 (41%)	
Median follow-up of survivors, mo (range)				<.0001
	61 (10-138)	52 (12-123)	36 (13-85)	

CMV indicates cytomegalovirus; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia.

respectively. Acute lymphoblastic leukemia was the predominant indication for the Japanese and Whites after 2006.

Differences were found in GVHD prophylaxis. About half of the Japanese patients received tacrolimus-containing GVHD prophylaxis, whereas most Whites used cyclosporine-containing GVHD prophylaxis. There were no significant differences in degree of donor–recipient HLA match by race; about half of all transplants were mismatched at a single HLA locus and about a third were mismatched at 2 HLA loci. The median follow-up for Whites who did not receive ATG was 3 years compared with 4 years Whites who received ATG and 5 years for the Japanese.

Acute and Chronic GVHD

There were no significant differences in the risks of grades II to IV and grades III to IV acute GVHD among the 3 groups (Table 2). No other factors were associated with grades II to IV acute GVHD, but grades III to IV acute GVHD risks were higher for patients aged 6 to 16 years compared with younger patients (HR, 1.93; 95% CI, 1.19 to 3.14; $P = .008$) and boys (HR, 1.71; 95% CI, 1.07 to 2.75; $P = .03$). The 100-day adjusted cumulative incidences of grades II to IV acute GVHD were 38% (95% CI, 32% to 43%), 37% (95% CI, 30% to 44%), and 45% (95% CI, 35% to 55%) for the Japanese, Whites who received ATG, and Whites who did not receive ATG, respectively (Figure 1A). The corresponding probabilities for grades III to IV acute GVHD were 14% (95% CI, 10% to 19%), 18% (95% CI, 13% to 24%), and 12% (95% CI, 7% to 19%, Figure 1B).

Chronic GVHD risks differed by transplant strategy. Compared with the Japanese, risks were significantly higher in Whites who did not receive ATG (HR, 2.16; 95% CI, 1.40 to 3.32; $P < .001$) than for Whites who received ATG (Table 2). There were no differences in the severity of chronic GVHD; among those with chronic GVHD, the proportion of patients with extensive chronic GVHD was 33% for the Japanese, 48% for Whites receiving ATG, and 44% for Whites who did not receive ATG ($P = .28$). The 3-year cumulative incidences of chronic GVHD were 21% (95% CI, 16% to 26%) for the Japanese, 27% (95% CI, 20% to 34%) for Whites who received ATG, and 40% (95% CI, 30% to 50%) for Whites who did not receive ATG (Figure 1C).

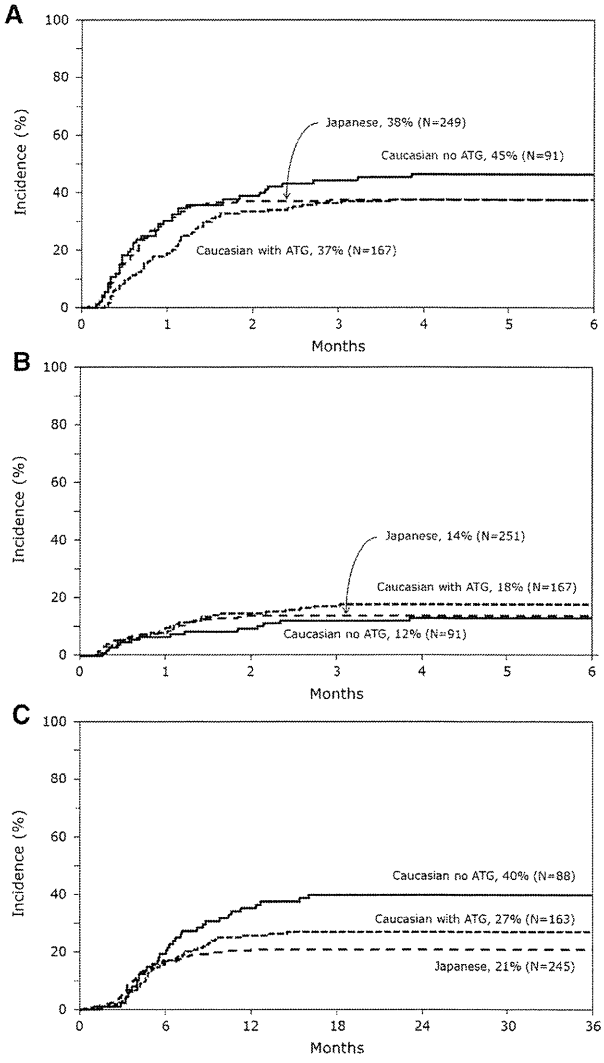

Figure 1. Cumulative incidence of GVHD after single-unit UCBT. (A) Cumulative incidences of grades II to IV acute GVHD. (B) Cumulative incidences of grades III to IV acute GVHD. (C) Cumulative incidences of chronic GVHD.

Table 2
Multivariate Analysis of Acute and Chronic GVHD, TRM, Relapse, and Overall Mortality

	Number	HR (95% CI)	*P*
Grades II-IV acute GVHD			
Japanese	249	1.00	Reference
Whites, received ATG	167	.93 (.68-1.28)	.64
Whites, did not receive ATG	91	1.27 (.88-1.82)	.20
Grades III-IV acute GVHD			
Japanese	251	1.00	Reference
White, with ATG	167	1.34 (.82-2.18)	.25
White, no ATG	91	.93 (.50-1.75)	.83
Chronic GVHD			
Japanese	245	1.00	Reference
White, with ATG	163	1.43 (.96-2.14)	.08
White, no ATG	88	2.16 (1.40-3.32)	<.001
TRM			
Japanese	242	1.00	Reference
White, with ATG	167	1.81 (1.16-2.83)	.01
White, no ATG	91	1.28 (.74-2.22)	.39
Relapse			
Japanese	242	1.00	Reference
White, with ATG	167	.92 (.62-1.35)	.66
White, no ATG	91	.77 (.47-1.28)	.32
Overall mortality*			
Japanese	253	1.00	Reference
White, with ATG	167	1.40 (1.00-1.94)	.05
White, no ATG	91	1.17 (.78-1.76)	.45

* Two degrees of freedom overall test for overall mortality showed no statistical significance ($P = .14$).

TRM and Relapse

Compared with the Japanese, risks of TRM were significantly higher for Whites who received ATG but not for Whites who did not receive ATG (Table 2). Independent of geographical region and use of ATG, transplantations mismatched at 2 HLA loci (HR, 2.17; 95% CI, 1.14 to 4.12; $P = .02$) were associated with higher TRM compared with HLA-matched transplantations. TBI-containing regimens were also associated with higher TRM compared with non-TBI regimens (HR, 2.26; 95% CI, 1.32 to 3.89; $P = .003$). The 3-year cumulative incidence of TRM was 15% (95% CI, 11% to 20%) for the Japanese, 26% (95% CI, 19% to 33%) for Whites who received ATG, and 19% (95% CI, 12% to 28%) for Whites who did not receive ATG (Figure 2A). Relapse risks were not different among the 3 groups (Table 2). Relapse was associated with disease status at transplantation; transplants in second or third CR were associated with higher risks compared with first CR (HR, 1.61; 95% CI, 1.12 to 2.30; $P = .01$).

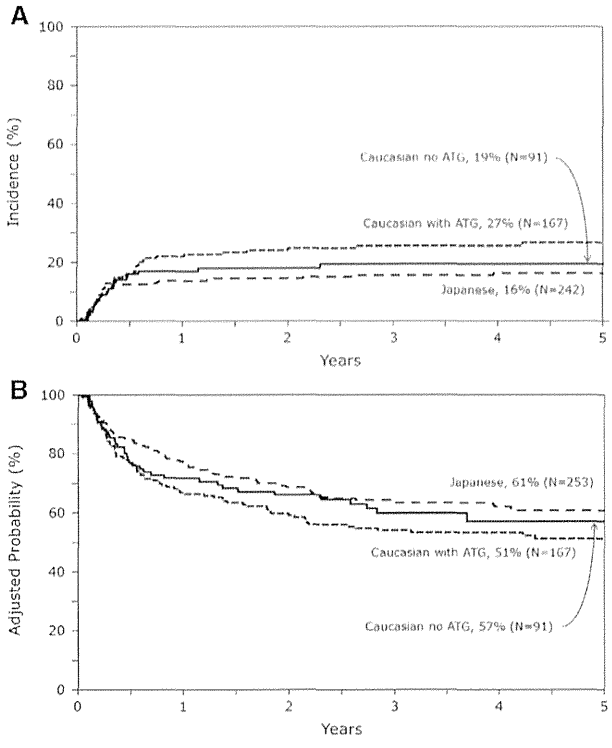

Figure 2. Cumulative incidence of TRM and overall survival curves. (A) Cumulative incidences of TRM. (B) Probability of overall survival after single-unit UCBT adjusted for CMV-seropositive status and disease risk at transplantation.

The 3-year cumulative incidence of relapse was 28% (95% CI, 23% to 34%) for the Japanese, 26% (95% CI, 20% to 33%) for Whites who received ATG, and 22% (95% CI, 14% to 31%) for Whites who did not receive ATG.

Overall Mortality

After adjusting for CMV serostatus and disease status at transplantation, factors associated with overall mortality, there were no differences in mortality risks between the Japanese and Whites who did not receive ATG (Table 2). However, the observed marginal increase in overall mortality risk for Whites who received ATG compared with the Japanese must be interpreted with caution. The level of significance for the overall mortality model did not reach the level of significance set for this analysis ($P = .14$; 2 degrees of freedom test). Consequently, any observed differences between exposure categories within the model are suspect and call for confirmation in an independent data set. Independent of region, mortality risks were higher for patients who were CMV seropositive (HR, 1.57; 95% CI, 1.16 to 2.11; $P = .003$) and for transplantations in second or third CR (HR, 1.54; 95% CI, 1.15 to 2.06; $P = .003$).

We also tested for the effect of acute (grades II to IV: HR, 1.19; 95% CI, .90 to 1.57; $P = .23$, grades III to IV: HR, 1.38; 95% CI, .96 to 1.98; $P = .08$) and chronic GVHD (HR, 1.14; 95% CI, .78 to 1.65; $P = .5$) on overall mortality and found none. The 3-year probabilities of overall survival adjusted for CMV serostatus and disease status at transplantation were 63% (95% CI, 57% to 69%) for the Japanese, 54% (95% CI, 46% to 62%) for Whites who received ATG, and 60% (95% CI, 49% to 70%) for Whites who did not receive ATG (Figure 2B). Table 3 shows the causes of death. Relapse was the most frequent cause of death in all groups and accounted for about half of all deaths in all groups. The most frequent causes of nonrelapse death for Japanese and Whites who received ATG were infection, GVHD, interstitial pneumonitis, and organ failure. On the other hand, for Whites who did not receive ATG, graft failure and infection were the most frequent causes of nonrelapse deaths.

Table 3
Causes of Death

	Japanese (n = 257)	White, with ATG (n = 168)	White, no ATG (n = 92)
Total death	93	82	37
Relapse	54 (58%)	41 (50%)	18 (49%)
Graft failure	3 (3%)	1 (1%)	5 (14%)
GVHD	9 (10%)	14 (17%)	2 (5%)
Infection	10 (11%)	11 (13%)	9 (24%)
Ipn/ARDS	8 (9%)	2 (2%)	2 (5%)
Organ failure	6 (6%)	9 (11%)	1 (3%)
Others*	2 (2%)	3 (3%)	—
Unknown	1 (1%)	1 (1%)	—

Ipn indicates interstitial pneumonitis; ARDS, acute respiratory distress syndrome.

* Other causes in Japanese include hemorrhage, n = 1, and thrombocytic microangiopathy, n = 1; in Whites with ATG include intracranial hemorrhage, n = 1; Epstein-Barr virus–associated lymphoproliferative disorder, n = 1; and anaplastic astrocytoma, n = 1.

The day-28 cumulative incidences of neutrophil recovery were higher for the Japanese (76%; 95% CI, 71% to 81%) compared with Whites who received ATG (66%; 95% CI, 59% to 73%; $P = .02$) and Whites who did not receive ATG (53%; 95% CI, 43% to 63%; $P < .001$). However, by day 42 cumulative incidences of neutrophil recovery were not different between the Japanese (88%; 95% CI, 84% to 92%) and Whites who received ATG (84%; 95% CI, 79% to 90%; $P = .31$) and those who did not receive ATG (82%; 95% CI, 74% to 90%; $P = .22$).

DISCUSSION

The current analyses, in children with acute leukemia, sought to identify differences in acute and chronic GVHD risks after UCBT in a relatively homogenous population, the Japanese, to a more genetically diverse population, Whites. Three major observations have not been reported previously. First, grades II to IV and grades III to IV acute GVHD risks were not different between the 2 populations. Second, chronic GVHD risks were higher for Whites who received regimens that did not include ATG. Third, significant differences were not found in survival between the populations. Our findings are different from a previous report that compared GVHD risks and survival after HLA-matched sibling bone marrow transplantation [2]. In that report, acute and chronic GVHD risks were higher for younger Whites compared with the Japanese. We hypothesize in the setting of UCBT that the use of units mismatched to recipients at the major histocompatibility antigens may have ameliorated any advantages associated with the sharing of minor histocompatibility antigens in the more homogenous Japanese population. There are also qualitative and quantitative differences in the composition of bone marrow and UCB grafts leading to differences in post-transplant immune reconstitution, which may explain the observed differences between the current analysis and the observations after matched sibling transplantation [18,19]. It is also plausible that transplant period may have influenced the observations in the current analysis, which studied transplantations in a more recent era. A recent report showed lower GVHD risks and improved transplant outcomes in recent years compared with the previous era [20], and it may be that current GVHD prophylaxis regimens

reduce the incidence of clinically significant GVHD in all patients, which further ameliorated the impact of genetic disparity for major and minor histocompatibility antigens.

Compared with the Japanese, who did not receive ATG, chronic GVHD was higher in Whites who did not receive ATG as part of their transplant conditioning but not for those who received ATG. We hypothesize the Japanese are inherently less likely to develop chronic GVHD. Despite the higher chronic GVHD in a subset of the Whites, we did not observe differences in survival between the treatment groups. Our findings differ from that of Narimatsu et al. [21] from the Japan Cord Blood Bank Network. In that report, chronic GVHD after UCBT was associated with improved survival. In the current analyses, despite the higher risks of chronic GVHD in 1 group, the severity of chronic GVHD was similar across the groups and may explain our inability to detect significant differences in survival between the groups. Generally, chronic GVHD adds to the burden of morbidity and mortality. Our study is limited by lack of data on health-related quality of life in patients with and without chronic GVHD. Another limitation is differential follow-up; non-ATG–containing preparative regimens for Whites is relatively recent, allowing for a median follow-up time of 3 years compared with 4 to 5 years for the other 2 groups. With extended follow-up it is possible that differences may exist between the non-ATG group and the other groups.

ATG is routinely included in the conditioning regimen package for UCBT in the United States and Europe to promote engraftment and lower GVHD risks [22]. However, ATG is seldom used for UCBT in Japan. Although we were unable to study immune recovery, delayed immune recovery and higher rate of infections with ATG is well documented [23] and has led to increasing use of fludarabine [24–26]. In the current analysis, we observed an absolute survival difference of 4% between the Japanese and Whites who did not receive ATG compared with an absolute difference of 10% between the Japanese and Whites who received ATG, suggesting that avoiding ATG-containing transplant conditioning regimens might be better for long-term survival.

Ethnic differences are reportedly contributors to related and unrelated bone marrow transplantation outcomes [2,27–29]. Ballen et al. [30] reported inferior survival in African Americans after UCBT, but the etiology for higher mortality was attributed to these patients having received units with greater HLA disparity and lower cell dose compared with Whites. In our study, despite the Japanese patients having received UCB units with lower cell dose compared with the U.S. Whites, survival rates were similar. We hypothesize that a minimum cell dose is needed for engraftment and selecting units with cell doses in excess of the minimum required does not lower mortality risks. It is possible differences may exists in the minimum cell dose for different populations. In the United States, reports from the Center for International Blood and Marrow Transplant have consistently shown the minimum required cell dose as 3.0×10^7/kg. Among the Japanese, the minimum cell dose is 2.0×10^7/kg. Others have shown better matching between UCB units and their recipients can lower some of the excess TRM associated with UCBT [9]. We were unable to test for the effect of matching at the HLA-C locus or matching at the allele level, because these data were not available for all patients in the current analyses. At least among Whites, most transplantations reported in the current analysis are likely to be mismatched at 3 or greater HLA loci when considering allele-level HLA-match [31]. Whether there exist differences in survival between the Japanese and Whites when units are better HLA matched remains to be tested.

In summary, unlike in the setting of HLA-matched sibling transplantation, we did not observe differences in acute GVHD or overall mortality after UCBT between the Japanese and U.S. Whites. The observed higher chronic GVHD in a subset of transplantations in the United States warrants a study that focuses on health-related quality of life and long-term survival to fully evaluate the effect of chronic GVHD in these patients. Our findings are limited to children with acute leukemia. Because most older patients in the United States receive 2 UCB units and those in Japan a single UCB unit, a comparative analyses as performed in children remains a challenge.

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Conflict of interest statement: There are no conflicts of interest to report.

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Changes in the Clinical Impact of High-Risk Human Leukocyte Antigen Allele Mismatch Combinations on the Outcome of Unrelated Bone Marrow Transplantation

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A B S T R A C T

Several high-risk HLA allele mismatch combinations (HR-MMs) for severe acute graft-versus-host disease (GVHD) have been identified by analyzing transplantation outcomes in Japanese unrelated hematopoietic stem cell transplant recipients. In this study, we analyzed the effects of HR-MMs in 3 transplantation time periods. We confirmed that the incidence of grade III to IV acute GVHD in the HR-MM group was significantly higher than that in the low-risk (LR) MM group (hazard ratio [HR], 2.74; $P < .0001$) in the early time period (1993 to 2001). However, the difference in the incidence of grade III to IV acute GVHD between the HR-MM and LR-MM groups was not statistically significant (HR, 1.06; $P = .85$ and HR, .40; $P = .21$, respectively) in the mid (2002 to 2007) and late (2008 to 2011) time periods. Similarly, survival in the HR-MM group was significantly inferior to that in the LR-MM group (HR, 1.46; $P = .019$) in the early time period, whereas the difference in survival between the 2 groups was not statistically significant in the mid and late time periods (HR, 1.06; $P = .75$ and HR, .82; $P = .58$, respectively). In conclusion, the adverse impact of HR-MM has become less significant over time. Unrelated transplantation with a single HR-MM could be a viable option in the absence of a matched unrelated donor or an unrelated donor with a single LR-MM.

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INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) from an unrelated donor has been established as an effective treatment option for patients with hematological diseases who lack a human leukocyte antigen (HLA)–matched related donor. However, an HLA mismatch at the genetic level (allele mismatch) may be observed even in HSCT from a serologically HLA-matched donor (antigen match), and the presence of an allele mismatch adversely affects the incidence of severe acute graft-versus-host disease (GVHD) and survival [1-4]. We recently showed that the presence of single HLA allele mismatches at the HLA-A, -B, -C, or -DRB1 loci equivalently affect the outcome of HSCT, although a previous study from Japan reported that an HLA-A or -B allele mismatch impairs overall survival more strongly than an HLA-C or -DRB1 allele mismatch [4,5]. These findings suggest that the

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Table 1
Patient Characteristics

Characteristic	Match n = 2504			Low-Risk Mismatch n = 1057			High-Risk Mismatch n = 157		
	Early	Mid	Late	Early	Mid	Late	Early	Mid	Late
	802	814	888	412	351	294	64	71	22
Age (recipient)									
Median	32	38	43	31	38	43	33	39	41
Age (donor)									
Median	34	34	36	33	34	37	35	36	37
Sex (recipient)									
Female	292	305	378	162	165	123	27	27	9
Male	510	509	510	250	186	171	37	44	13
Sex (donor)									
Female	286	262	266	164	158	107	20	28	5
Male	512	548	622	247	190	187	43	43	17
N.A.	4	4	0	1	3	0	1	0	0
Sex mismatch									
Match	507	537	512	238	209	166	35	40	14
Male to female	148	158	244	85	72	72	17	15	6
Female to male	143	115	132	88	67	56	11	16	2
N.A.	4	4	0	1	3	0	1	0	0
ABO blood type									
Match	454	462	500	167	151	121	33	31	9
Minor mismatch	154	162	175	112	84	81	15	18	3
Major mismatch	125	114	142	82	67	61	9	18	4
Bidirectional mismatch	58	70	71	45	46	31	7	4	6
N.A.	11	6	0	6	3	0	0	0	0
Disease									
AML	269	415	495	134	168	170	15	29	12
ALL	229	229	249	116	96	76	11	23	8
CML	237	84	29	125	42	14	30	3	0
MDS	67	86	115	37	45	34	8	16	2
Disease risk									
Low	552	533	607	265	219	181	40	38	12
High	230	239	280	135	116	113	21	28	10
Others	20	42	1	12	16	0	3	5	0
Cell dose (cells/kg)									
Median	3.0	2.7	2.7	3.0	2.6	2.6	3.1	2.8	2.6
GVHD prophylaxis									
CSA-based	545	306	185	267	114	47	45	21	2
TAC-based	240	499	689	135	227	240	19	50	20
N.A.	17	9	14	10	10	7	0	0	0
Conditioning regimen									
TBI regimen	760	639	560	394	272	194	59	53	15
Non-TBI regimen	30	114	328	17	52	100	3	11	7
N.A.	12	61	0	1	27	0	2	7	0

N.A. indicates not available; AML, acute myeloblastic leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome; GVHD, graft-versus-host disease; CSA, cyclosporine; TAC, tacrolimus; TBI, total body irradiation.

clinical impact of an HLA mismatch may have changed over time periods.

Some investigators have tried to identify specific donor-recipient allele combinations that may be associated with a higher risk of severe acute GVHD [6,7]. Kawase et al. found 16 high-risk HLA allele mismatch combinations (HR-MMs) for severe acute GVHD [7]. They also showed that the number of HR-MMs was associated with severe GVHD and poor survival, whereas the presence of mismatch combinations other than HR-MMs (low-risk mismatch combinations, LR-MMs) did not affect the outcome of HSCT. However, their study included a variety of benign and malignant hematological diseases. In addition, they included donor-recipient pairs with more than 1 HLA mismatch. The impact of each specific mismatch combination was evaluated after adjusting for the number of HLA mismatches in other loci in a multivariate model, but the possible presence of HR-MMs in other loci or the interaction between HLA mismatch combinations could not be appropriately treated in their model. At that time, the study design was inevitable, because the number of each HLA mismatch combination was limited. However, several years have passed and the amount of unrelated HSCT data in the Transplant Registry Unified Management Program (TRUMP) has increased to more than 13,500 donor-recipient pairs. Therefore, in this study, we reanalyzed the impact of HR-MMs, excluding HSCT with multiple HLA mismatches in patients with relatively homogeneous background diseases. In addition, we evaluated the impact of HLA mismatch on transplantation outcomes considering the period effect, because the impact of HR-MM mismatch might have changed over time periods, as we previously reported in an analysis of single HLA allele mismatches at the HLA-A, -B, -C, and -DRB1 loci [5].

METHODS

Patients

Patients aged at least 16 years with acute myeloblastic leukemia, acute lymphoblastic leukemia, myelodysplastic syndrome, or chronic myelogenous leukemia (CML) who underwent a first HSCT from a serologically HLA-A, -B, and -DR matched unrelated donors between 1993 and 2011, and who had full HLA-A, -B, -C, and -DRB1 allele data, were included in this study. Bone marrow was exclusively used as a stem cell source. Clinical data for

Table 2
Multivariate Analysis to Evaluate the Impact of Single HLA Allele Mismatches on the Incidence of Grade III to IV Acute GVHD Stratified according to the Transplantation Time Period

Year	Factor		Hazard Ratio	*P* Value
1993-2001				
	Donor age		1.02 (1.00-1.03)	.082
	Donor sex	Female	1.00	
		Male	1.65 (1.05-2.60)	.031
	Female to male transplantation	No	1.00	
		Yes	1.52 (.91-2.55)	.11
	Disease	AML	1.00	
		ALL	1.15 (.79-1.68)	.47
		CML	1.62 (1.11-2.36)	.012
		MDS	.65 (.32-1.35)	.25
	Disease risk	Low	1.00	
		High	1.30 (.93-1.83)	.13
		Others	.80 (.23-2.85)	.74
	GVHD prophylaxis	CSA-based	1.00	
		TAC-based	.83 (.61-1.14)	.25
	HLA	Low-risk mismatch	1.00	
		Match	.89 (.65-1.21)	.44
		High-risk mismatch	2.74 (1.73-4.32)	<.0001
2002-2007				
	Donor age		1.03 (1.01-1.05)	.0028
	Donor sex	Female	1.00	
		Male	1.50 (.96-2.33)	.076
	Female to male transplantation	No	1.00	
		Yes	1.53 (.89-2.64)	.13
	Disease	AML	1.00	
		ALL	1.36 (.95-1.96)	.094
		CML	1.27 (.74-2.20)	.38
		MDS	1.25 (.77-2.02)	.37
	Disease risk	Low	1.00	
		High	1.76 (1.25-2.48)	.0011
		Others	1.65 (.82-3.34)	.16
	GVHD prophylaxis	CSA-based	1.00	
		TAC-based	.86 (.63-1.19)	.37
	HLA	Low-risk mismatch	1.00	
		Match	.64 (.46-.89)	.008
		High-risk mismatch	1.06 (.58-1.93)	.85
2008-2011				
	Donor age		1.03 (1.01-1.06)	.0016
	Donor sex	Female	1.00	
		Male	1.28 (.78-2.12)	.33
	Female to male transplantation	No	1.00	
		Yes	.98 (.52-1.88)	.96
	Disease	AML	1.00	
		ALL	1.18 (.80-1.74)	.42
		CML	1.53 (.69-3.37)	.3
		MDS	.66 (.36-1.20)	.17
	Disease risk	Low	1.00	
		High	1.53 (1.08-2.17)	.018
		Others	NA (NA-NA)	NA
	GVHD prophylaxis	CSA-based	1.00	
		TAC-based	.82 (.55-1.24)	.34
	HLA	Low-risk mismatch	1.00	
		Match	.56 (.39-.80)	.0014
		High-risk mismatch	.40 (.10-1.64)	.21

AML indicates acute myeloblastic leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome; GVHD, graft-versus-host disease; CSA, cyclosporine; TAC, tacrolimus.

these patients were obtained from the TRUMP [8]. We excluded patients who lacked data on survival status, those with more than 1 allele or antigen mismatch, those who received a reduced-intensity conditioning regimen, and those who received ex vivo or in vivo T cell depletion, such as antithymocyte globulin or alemtuzumab. Finally, 3718 patients were included in the main part of this study. As a post hoc analysis, 415 patients with 2 LR-MMs and 66 patients with 2 allele mismatches including at least 1 HR-MM were added to compare the impact of 1 HR-MM and 2 LR-MMs and to analyze the statistical interaction between HR-MM and the presence of an additional allele mismatch. The study was approved by the data management committee of TRUMP and by the institutional review board of Saitama Medical Center, Jichi Medical University.

Histocompatibility

Histocompatibility data for serological and genetic typing for the HLA-A, HLA-B, HLA-C, and HLA-DR loci were obtained from the TRUMP database, which includes HLA allele data determined retrospectively by the Japan Marrow Donor Program using frozen samples [7,9]. In this study, the following donor-recipient HLA-mismatch combinations were regarded as HR-MMs: A*02:06-A*02:01, A*02:06-A*02:07, A*26:02-A*26:01, A*26:03-A*26:01, B*15:01-B*15:07, C*03:03-C*15:02, C*03:04-C*08:01, C*04:01-C*03:03, C*08:01-C*03:03, C*14:02-C*03:04, C*15:02-C*03:04, C*15:02-C*14:02, DR*04:05-DR*04:03, and DR*14:03-DR*-DR1401, as we did not have enough data on HLA-DP and -DQ [7]. In HR-MM pairs, the donor and the recipient must have the HLA allele as shown above, and at the same time, these donor and recipient HLA alleles should not be shared by the recipient and the donor, respectively. For example, if the donor has HLA-A*02:06/02:06 and the recipient has HLA-A*02:01/02:06, this pair was not regarded as HR-MM pair, as the donor's HLA-A*02:06 was shared by the recipient. Other HLA mismatch pairs were regarded as LR-MM pairs. Only the HLA-C mismatch group included HLA mismatch at a serological (antigen) level.

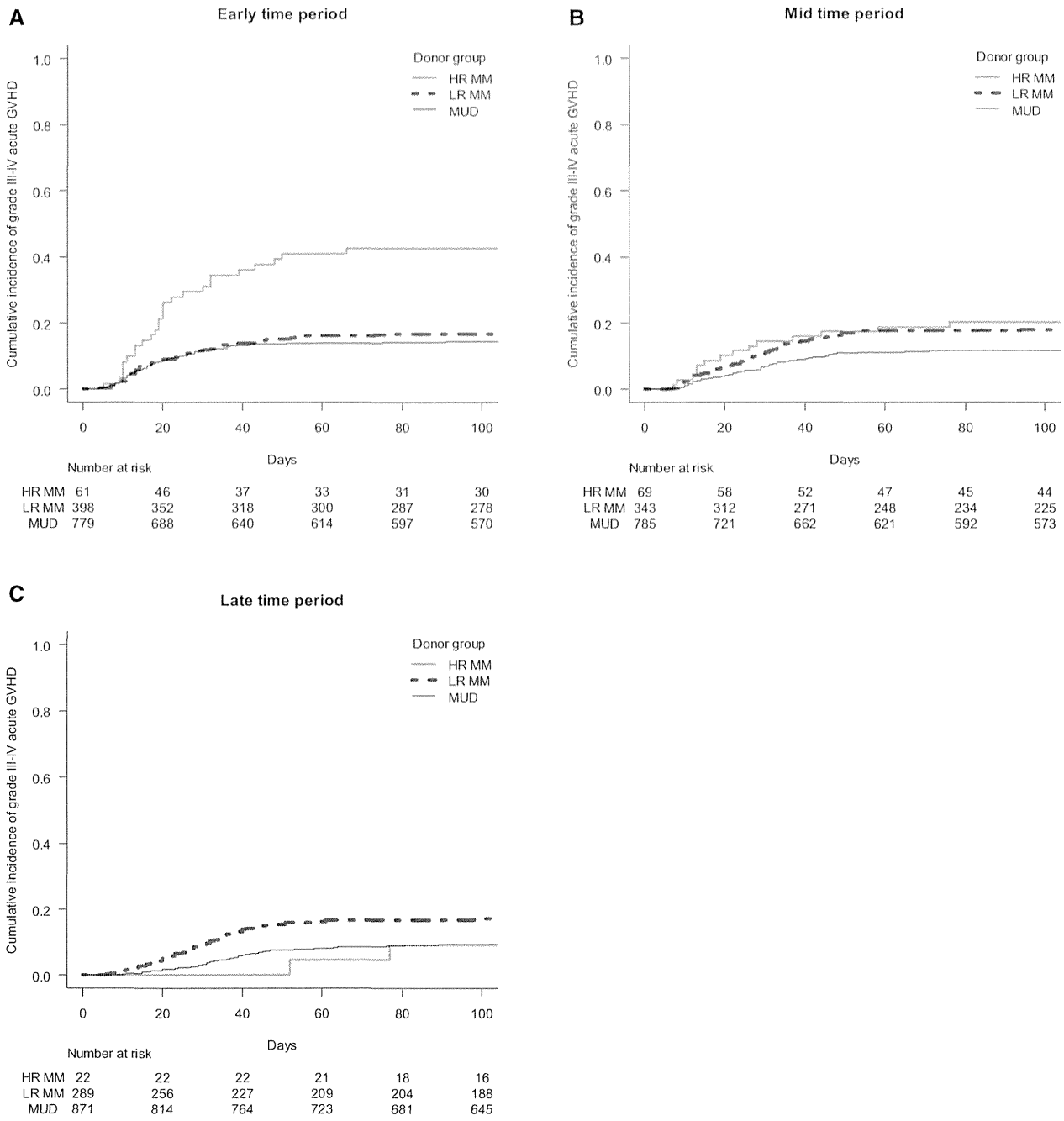

Figure 1. The cumulative incidence of grade III to IV acute GVHD grouped according to the HLA mismatch between the donor and recipient in the early (A), mid (B), and late time periods (C). HR-MM indicates high-risk mismatch; LR-MM, low-risk mismatch; MUD, matched unrelated donor.

Statistical Analyses

We divided the patients into 3 groups according to the time period when HSCT was performed to evaluate whether the impact of HR-MM changed over time periods: the early, mid, and late groups included HSCT performed from 1993 through 2001, 2002 through 2007, and 2008 through 2011, respectively. The break points among groups were determined to make the number of patients in each group equivalent (n = 1278, 1236, and 1204, respectively). To avoid making misleading conclusions by arbitrary grouping, we confirmed that there was a statistically significant interaction between the presence of HR-MMs and transplantation year as a continuous variable, both for overall survival ($P = .0098$) and the incidence of grade III to IV acute GVHD ($P < .001$). The following analyses were performed separately in each group. However, in post hoc analyses to evaluate the impact of HR-MMs at each locus and to compare 1 HR-MM and 2 LR-MMs, the mid and late groups were combined to increase the statistical power, after confirming that similar results were obtained in the 2 groups.

The primary endpoint was the incidence of grade III to IV acute GVHD. Overall survival was evaluated as a secondary endpoint. The chi-square test or Fisher exact test was used to compare categorical variables and Student *t*-test or an analysis of variance test was used for continuous variables to evaluate the homogeneity of background characteristics of the HR-MM, LR-MM, and HLA-matched (MUD) groups. *P* values were adjusted using the Bonferroni's method and Tukey's method for multiple comparisons between each pair. Overall survival was estimated according to the Kaplan-Meier method, and compared among groups with the log-rank test. The incidence of acute GVHD was calculated treating death without GVHD as a competing event, and it was compared using Gray's test [10].

The impact of HR-MMs was evaluated using multivariate models: the Cox proportional hazards model was used for overall survival and Fine and Gray's proportional hazards model was used for acute GVHD [11]. The LR-MM group was regarded as the reference group. Potential confounding factors that were considered in these analyses included recipient/donor age, recipient/donor sex, sex mismatch, ABO major/minor mismatch, the use of

Table 3
Multivariate Analysis to Evaluate the Impact of Single High-Risk Allele Mismatches on Overall Survival Stratified According to the Transplantation Time Period

Year	Factor		Hazard Ratio	*P* Value
1993-2001				
	Age		1.02 (1.01-1.03)	<.0001
	Sex	Female	1.00	
		Male	1.06 (.90-1.23)	.51
	Disease	AML	1.00	
		ALL	1.20 (.99-1.45)	.065
		CML	.89 (.72-1.10)	.29
		MDS	.61 (.45-.83)	.0015
	Disease risk	Low	1.00	
		High	2.72 (2.30-3.23)	<.0001
		Others	2.03 (1.27-3.23)	.0029
	ABO major mismatch	Absent	1.00	
		Present	1.25 (1.06-1.47)	.0092
	GVHD prophylaxis	CSA-based	1.00	
		TAC-based	.85 (.72-1.00)	.049
	HLA	Low-risk mismatch	1.00	
		Match	.86 (.73-1.01)	.063
		High-risk mismatch	1.46 (1.06-2.01)	.019
2002-2007				
	Age		1.01 (1.00-1.02)	.0025
	Sex	Female	1.00	
		Male	1.20 (1.02-1.41)	.0027
	Disease	AML	1.00	
		ALL	1.16 (.96-1.39)	.13
		CML	.84 (.62-1.12)	.23
		MDS	.56 (.43-.73)	<.0001
	Disease risk	Low	1.00	
		High	2.87 (2.41-3.40)	<.0001
		Others	2.23 (1.58-3.15)	<.0001
	ABO major mismatch	Absent	1.00	
		Present	.97 (.81-1.16)	.77
	GVHD prophylaxis	CSA-based	1.00	
		TAC-based	.97 (.83-1.15)	.76
	HLA	Low-risk mismatch	1.00	
		Match	.83 (.69-.98)	.032
		High-risk mismatch	1.06 (.75-1.48)	.75
2008-2011				
	Age		1.02 (1.01-1.03)	<.0001
	Sex	Female	1.00	
		Male	1.08 (.89-1.31)	.42
	Disease	AML	1.00	
		ALL	.97 (.76-1.25)	.83
		CML	.97 (.57-1.64)	.9
		MDS	.65 (.48-.87)	.004
	Disease risk	Low	1.00	
		High	2.73 (2.23-3.35)	<.0001
		Others	NA (NA-NA)	NA
	ABO major mismatch	Absent	1.00	
		Present	1.14 (.92-1.41)	.22
	GVHD prophylaxis	CSA-based	1.00	
		TAC-based	.95 (.75-1.21)	.69
	HLA	Low-risk mismatch	1.00	
		Match	.86 (.69-1.06)	.15
		High-risk mismatch	.82 (.42-1.62)	.58

AML indicates acute myeloblastic leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome; GVHD, graft-versus-host disease; CSA, cyclosporine; TAC, tacrolimus.

total body irradiation in the conditioning regimen, cell dose in the bone marrow graft, the use of cyclosporine or tacrolimus as GVHD prophylaxis, background disease, and disease risk. Acute leukemia in first or second remission, CML in first or second chronic phase, CML in accelerated phase, and myelodysplastic syndrome of refractory anemia or refractory anemia with excess blasts were considered low-risk diseases, and other conditions were considered high-risk diseases. All of these potential confounding factors were included in the multivariate analyses and then deleted in a stepwise fashion from the model to exclude factors with a *P* value of .05 or higher. Finally, HLA mismatch was added to the model. Different multivariate models were compared using the likelihood ratio test. The quantity of interest was the deviance difference between the 2 models, under the null hypothesis that 2 models fit the data equally well and the deviance difference has an approximate chi-square distribution with degrees of freedom equal to the difference in the number of independent variables between the compared models.

All *P* values were 2 sided and *P* values of .05 or less were considered statistically significant. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University) [12], which is a graphical user interface for R (The R Foundation for Statistical Computing). More precisely, it is a modified version of R commander that was designed to add statistical functions frequently used in biostatistics.

RESULTS

Patients

The patient characteristics are summarized in Table 1. HR-MMs were observed in 64 of 1278, 71 of 1236, and 22 of 1204 donor-recipient pairs in the early, mid, and late time periods, respectively. On the other hand, 412, 351, and 294 pairs had LR-MMs, respectively. With regard to the

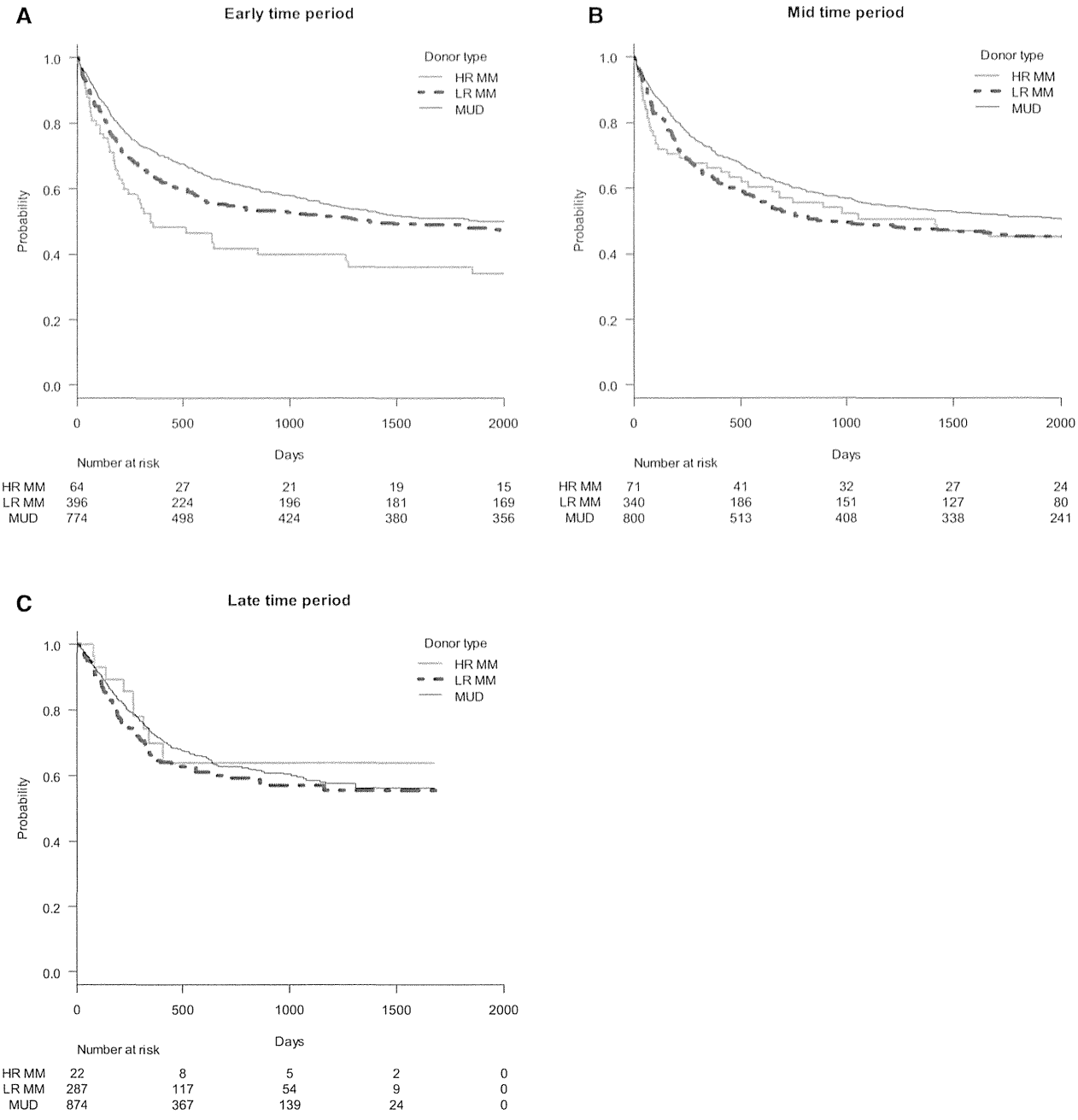

Figure 2. Overall survival grouped according to the HLA mismatch between the donor and recipient in the early (A), mid (B), and late time periods (C). The survival curves were adjusted for other significant factors by the mean of covariates method, in which average values of covariates are entered into the Cox proportional hazards model. HR-MM, high-risk mismatch; LR-MM, low-risk mismatch; MUD, matched unrelated donor.

differences among transplantation time periods, the numbers of LR-MMs and HR-MMs decreased in the late time periods, ie, after the introduction of routine typing for HLA-C and the publication of a paper about HR-MMs [7]. The proportion of HSCTs for CML also dramatically decreased over time periods (30.7%, 10.4%, and 3.6% in the early, mid, and late periods, respectively). With regard to the difference among HLA mismatch groups, the proportion of patients with high-risk underlying disease in the MUD group (29.9%) was significantly lower than those in the HR-MM (37.6%) and LR-MM groups (34.4%). In addition, the proportion of HSCTs for CML was significantly higher in the HR-MM group in the early time period (29.6%, 30.3%, and 46.9% in the MUD, LR-MM, and HR-MM groups, respectively).

Incidence of Grade III to IV Acute GVHD

To adjust the impact of HLA mismatch for possible confounding factors, we identified the following independently significant factors for the incidence of grade III to IV acute GVHD: donor age, donor sex, sex mismatch, disease, disease risk, and GVHD prophylaxis. After we adjusted for these factors, we confirmed that the incidence of grade III to IV acute GVHD in the HR-MM group was significantly higher than that in the LR-MM group (hazard ratio [HR], 2.74; 95% confidence interval [CI], 1.73 to 4.32; $P < .0001$) in the early time period, whereas the difference between the MUD and LR-MM groups was not significant (HR, .89; 95% CI, .65 to 1.21; $P = .44$) (Table 2, Figure 1). On the other hand, in the mid and late time periods, the difference in the incidence of