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Impact of the Direction of HLA Mismatch on Transplantation Outcomes in Single Unrelated Cord Blood Transplantation

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A B S T R A C T

The impact of the direction of HLA mismatch (MM) on outcome in unrelated cord blood (UCB) transplantation has not yet been clarified. We conducted a retrospective study using national registry data on 2977 patients who underwent transplantation using a single UCB for leukemia or myelodysplastic syndrome. HLA matching was assessed by serologic data for HLA-A, -B, and -DR loci. The median age of the recipients at transplantation was 41 years (range, 0-82 years), and 2300 recipients (77%) were age ≥16 years. The 2-year overall survival rate was 0.46. The presence of MM only in the graft-versus-host direction or only in the host-versus-graft direction was not associated with overall mortality (hazard ratio [HR], 0.88; $P = .317$ and HR, 0.95; $P = .670$, respectively) compared with 1 bidirectional MM. This finding was consistent in both the child and adult cohorts. The presence of MM only in the graft-versus-host direction was associated with a lower incidence of nonrelapse mortality (HR, 0.65; $P = .040$), significant only in the child cohort. No MM category was associated with relapse. Our findings suggest that the direction of HLA MM does not have a significant impact on overall survival after UCB transplantation.

INTRODUCTION

Unrelated cord blood (UCB) has emerged as a promising alternative source of hematopoietic stem cells for adult and pediatric allogeneic hematopoietic cell transplantation [1-4], and the use of UCB transplantation (UCBT) has been rapidly increasing, particularly in the United States, Europe, and Japan. One advantage of using UCB as a hematopoietic stem cell source is that UCBT requires less stringent HLA matching compared with bone marrow or peripheral blood stem cell transplantation, making it easier to find candidate UCB units in UCB banks. One or 2 antigen/allele mismatches (MMs) in the HLA-A, -B, and -DR loci between a UCB unit and recipient are acceptable without ex vivo T cell depletion methods, and the clinical outcome of transplantation using a 0-2 antigen/allele-mismatched UCB unit was almost comparable to that from an HLA allele-matched unrelated donor [1-3].

Although the number of HLA MMs between a UCB unit and a recipient is usually counted without considering the MM direction, the effect of the immune reaction caused by HLA MM differs according to whether the MM is in the graft-versus-host (GVH) or host-versus-graft (HVG) direction. A mismatched antigen in the GVH direction can be a major target for donor T cells and can cause graft-versus-host disease (GVHD), whereas a mismatched antigen in the HVG direction can be a major target for the remaining recipient T cells and can lead to graft rejection. In related transplantation, the presence of HLA MMs in the GVH direction is associated with a higher incidence of GVHD, whereas the

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presence of HLA MMs in the HVG direction is associated with a higher incidence of rejection [5-7]. Therefore, from a biological perspective, the impact of HLA MM should be discussed separately according to the direction of MM. However, because most patients have an equal number of MMs in the GVH and HVG directions (bidirectional MM), studying an adequate number of patients to evaluate an MM imbalance in the GVH and HVG directions has proven difficult.

The few studies that have evaluated the impact of the HLA MM direction on UCBT outcome have reported inconsistent results [8-10]. Matsuno et al. [8] reported that an HLA MM in the GVH direction was associated with lower incidence of neutrophil engraftment. In contrast, Stevens et al. [9] showed that UCBT with an MM only in the GVH direction was associated with a lower incidence of nonrelapse mortality (NRM) and overall mortality compared with UCBT with an 1 bidirectional MM, whereas UCBT with an MM only in the HVG direction was associated with a lower incidence of neutrophil engraftment and a higher incidence of relapse.

To clarify the significance of the direction of HLA MM on transplantation outcomes, we conducted a retrospective study using national registry data in 2977 patients who underwent a single UCBT.

METHODS

Data Collection

Data for 2987 patients with acute myelogenous leukemia (AML), acute lymphoblastic leukemia (ALL), myelodysplastic syndrome (MDS), and chronic myelogenous leukemia (CML) who underwent a first transplantation using a single UCB unit between January 1, 1998, and December 31, 2009, were obtained from the Transplant Registry Unified Management Program (TRUMP) [11], in which all UCBTs are registered through the Japan Cord Blood Bank Network (JCBBN), a national network of all 11 cord blood banks in Japan. Ten patients lacking data on survival status or survival date were excluded. A total of 2977 patients met the criteria for study inclusion. The study design was approved by the TRUMP Data Management Committee and the Institutional Review Board of Saitama Medical Center, Jichi Medical University, where this study was organized.

Histocompatibility

Histocompatibility data for the HLA-A, -B, and -DR loci were obtained from reports collected from the institution at which the transplantation was performed or cord blood banks. HLA typing methods have been described previously [12]. To reflect current practice in Japan, HLA matching was assessed by serologic data for HLA-A, -B, and -DR loci. A secondary analysis using antigen level data for HLA-A, -B and available allele level data for HLA-DRB1 was also performed to compare our data with previously published data from the United States and Europe. HLA-DRB1 allele information was available in 84% of patients (2498 of 2977). Among these patients, 62% had the same number of MMs at HLA-DRB1 loci at either the antigen or allele level. An HLA MM in the GVH direction was defined as when the recipient's antigens or alleles were not shared by the donor, and an MM in the HVG direction was defined as when the donor's antigens or alleles were not shared by the recipient.

Endpoints

The primary study endpoint was overall survival (OS). Other endpoints assessed were relapse, NRM, neutrophil and platelet engraftment, grade II-IV or III-IV acute GVHD, and chronic GVHD. Neutrophil recovery was defined as an absolute neutrophil count exceeding 0.5×10^9/L for 3 consecutive days after UCBT. Platelet recovery was defined as an absolute platelet count exceeding 50×10^9/L without platelet transfusion. The physicians who performed transplantation at each center diagnosed and graded acute and chronic GVHD according to traditional criteria [13,14]. The incidence of acute GVHD was evaluated in patients who engrafted, and that of chronic GVHD was evaluated in patients who engrafted and survived for more than 100 days.

Statistical Analysis

The probability of OS was estimated according to the Kaplan–Meier method and the groups were compared using the log-rank test. The probabilities of relapse, NRM, neutrophil and platelet engraftment, and acute and chronic GVHD were estimated based on cumulative incidence curves [15]. Competing events were death without relapse for relapse, relapse for NRM, death without engraftment for neutrophil and platelet engraftment, and death or relapse without GVHD for acute and chronic GVHD. The groups were compared using Gray's test [16]. The Cox proportional hazards model was used to evaluate the effect of confounding variables on OS, and the Fine and Gray proportional hazards model was used for the other endpoints [17]. Based on the report by the Center for International Blood and Marrow Transplant Research, we classified the conditioning regimens as myeloablative if total body irradiation >8 Gy, oral busulfan ≥9 mg/kg, i.v. busulfan ≥7.2 mg/kg, or melphalan >140 mg/m^2 was used in the conditioning regimen; otherwise, the conditioning regimen was classified as reduced intensity [18]. For patients with insufficient data regarding dosages of the agents used in the conditioning regimen, we used the information on conditioning intensity (myeloablative or reduced intensity) reported by the treating clinicians. We defined AML and ALL in first or second remission, CML in first or second chronic phase or accelerated phase, and MDS with refractory anemia or refractory anemia with ringed sideroblasts as standard risk, and all other conditions as high risk.

The following possible confounding variables were considered: recipient age group (0-5 years, 6-15 years, 16-49 years, or ≥50 years at transplantation), matching of ABO blood type between the recipient and UCB (match or major, minor, or bidirectional MM), recipient sex, sex MM between recipient and UCB (match, male donor–female recipient, or female donor–male recipient), disease (AML, ALL, CML, or MDS), disease status before transplantation (standard or high risk), type of conditioning regimen (myeloablative or reduced intensity), type of GVHD prophylaxis (calcineurin inhibitor plus methotrexate, calcineurin inhibitor only, others), and year of transplantation (1998-2004 or 2005-2009). Factors other than HLA MM and total nucleated cell (TNC) dose category were selected in a stepwise manner from the model with a variable retention criterion of $P < .05$. HLA MM and TNC dose category (≥10.0, 5.0-9.9, 2.5-4.9, 2.0-2.4, and <2.0 $\times 10^7$/kg) were then added to the final model. All tests were 2-sided, and a P value <.05 was considered statistically significant. All statistical analyses were performed with Stata version 12 (StataCorp, College Station, TX) and EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R 2.13.0 (R Foundation for Statistical Computing, Vienna, Austria) [19]. More precisely, EZR is a modified version of R commander (version 1.6-3) designed to add statistical functions used frequently used in biostatistics.

RESULTS

Patient Characteristics

Table 1 summarizes patient and transplant characteristics. The median age of the recipients at transplantation was 41 years (range, 0-82 years), and 2300 patients (77%) were age ≥16 years. Diagnoses for transplantation were AML in 1606 patients, ALL in 893, CML in 135, and MDS in 343. Half of the patients had standard-risk disease. UCBT was performed between 1998 and 2004 in 1153 patients (39%) and between 2005 and 2009 in 1824 patients (61%). The combination of a calcineurin inhibitor (tacrolimus or cyclosporine) and methotrexate was used in 62% of patients, whereas a calcineurin inhibitor alone was used in 22% of patients.

Some 40% of patients received a UCB unit containing <2.5 $\times 10^7$/kg TNCs, and 45% received a UCB unit containing 2.5-4.9 $\times 10^7$/kg TNCs. Roughly 12% of patients received ≥5.0 $\times 10^7$/kg TNCs, but 93% of these patients were age <16 years. Median body weight was 17 kg (range, 4-68 kg) for the children and 55 kg (range, 24-165 kg) for the adults. HLA MM was categorized as follows: HLA match in both the GVH and HVG directions (GVH 0/HVG 0 MM group; n = 273 [9%]), 1-2 antigen MMs in the GVH direction but 0 MMs in the HVG direction (GVH 1-2/HVG 0 MM group; n = 150 [5%]), 1-2 antigen MMs in the HVG direction but 0 MM in the GVH direction (GVH 0/HVG 1-2 MM group; n = 136 [5%]), 1 antigen MM in both the GVH and HVG directions at the same locus (GVH 1/HVG 1 MM group; n = 716 [24%]), 2 antigen MMs in both the GVH and HVG directions (GVH 2/HVG 2 MM group; n = 1170 [39%]), 2 antigen MMs in the GVH direction and 1 antigen MM in the HVG direction (GVH 2/HVG 1 MM group; n = 231 [8%]), 1 antigen MM in the GVH direction and

Table 1
Patient Characteristics

Characteristic	Total	Children (0-15 Years)	Adults (16+ Years)
Recipient age at UCBT, years, median (range)	41 (0-82)	5 (0-15)	49 (16-82)
Recipient age at UCBT, years, n (%)			
0-9	511 (17)	511 (75)	0 (0)
10-19	272 (9)	166 (25)	106 (5)
20-29	287 (10)	0 (0)	287 (12)
30-39	371 (12)	0 (0)	371 (16)
40-49	422 (14)	0 (0)	422 (18)
50-59	625 (21)	0 (0)	625 (27)
≥60	489 (16)	0 (0)	489 (21)
ABO matching, n (%)			
Match	994 (33)	248 (37)	746 (32)
Minor	815 (27)	174 (26)	641 (28)
Major	704 (24)	149 (22)	555 (24)
Bidirectional	458 (15)	104 (15)	354 (15)
Missing	6 (0)	2 (0)	4 (0)
Recipient sex, n (%)			
Female	1316 (44)	305 (45)	1011 (44)
Male	1661 (56)	372 (55)	1289 (56)
Donor–recipient sex match, n (%)			
Match	1157 (39)	290 (43)	867 (38)
Male donor and female recipient	635 (21)	153 (23)	482 (21)
Female donor and male recipient	768 (26)	172 (25)	596 (26)
Missing	417 (14)	62 (9)	355 (15)
Diagnosis, n (%)			
AML	1606 (54)	234 (35)	1372 (60)
ALL	893 (30)	391 (58)	502 (22)
CML	135 (5)	11 (2)	124 (5)
MDS	343 (12)	41 (6)	302 (13)
Disease risk at UCBT, n (%)			
Standard risk	1385 (47)	423 (62)	962 (42)
High risk	1450 (49)	226 (33)	1224 (53)
Missing	142 (5)	28 (4)	114 (5)
Conditioning regimen, n (%)			
Myeloablative	1980 (67)	585 (86)	1395 (61)
Reduced intensity	986 (33)	86 (13)	900 (39)
Missing	11 (0)	6 (1)	5 (0)
In vivo T cell depletion (ATG or alemtuzumab), n (%)			
No	2935 (99)	665 (98)	2270 (99)
Yes	42 (1)	12 (2)	30 (1)
GVHD prophylaxis, n (%)			
CSA only	250 (8)	59 (9)	191 (8)
TAC only	407 (14)	27 (4)	380 (17)
CSA + MTX	1105 (37)	209 (31)	896 (39)
TAC + MTX	755 (25)	241 (36)	514 (22)
CSA + MMF	104 (3)	0 (0)	104 (5)
TAC + MMF	148 (5)	6 (1)	142 (6)
CSA + corticosteroid	87 (3)	67 (10)	20 (1)
TAC + corticosteroid	34 (1)	26 (4)	8 (0)
Other	66 (2)	33 (5)	33 (1)
Missing	21 (1)	9 (1)	12 (1)
Year of UCBT, n (%)			
1998-2004	1153 (39)	389 (57)	764 (33)
2005-2009	1824 (61)	288 (43)	1536 (67)
TNC dose when frozen, n (%)			
$\geq 10.0 \times 10^7$/kg	99 (3)	99 (15)	0 (0)
$5.0\text{-}9.9 \times 10^7$/kg	259 (9)	234 (35)	25 (1)
$2.5\text{-}4.9 \times 10^7$/kg	1344 (45)	268 (40)	1076 (47)
$2.0\text{-}2.4 \times 10^7$/kg	924 (31)	44 (6)	880 (38)
$<2.0 \times 10^7$/kg	275 (9)	21 (3)	254 (11)
Missing	76 (3)	11 (2)	65 (3)
Weight, kg, median (range)	52 (4-165)	17 (4-68)	55 (24-165)
HLA MM			
0 MM	273 (9)	144 (21)	129 (6)
1-2 MM/GVH only	150 (5)	45 (7)	105 (5)
1-2 MM/rejection only	136 (5)	39 (6)	97 (4)
1 bidirectional MM	716 (24)	314 (46)	402 (17)
2 bidirectional MM	1170 (39)	98 (14)	1072 (47)
2 MM: bidirectional + GVHD	231 (8)	16 (2)	215 (9)
2 MM: bidirectional + rejection	264 (9)	19 (3)	245 (11)
2 MM: GVHD + rejection	37 (1)	2 (0)	35 (2)

ATG indicates antithymocyte globulin; CSA, cyclosporine; MMF, mycophenolate mofetil; MTX, methotrexate; TAC, tacrolimus; 0 MM, HLA match in both the GVH and HVG directions; 1-2 MM/GVH only, antigen MMs in the GVH direction and 0 MMs in the HVG direction; 1-2 MM/rejection only, 1 or 2 antigen MMs in the HVG direction and no MMs in the GVH direction; 1 bidirectional MM, 1 antigen MM in both the GVH and HVG directions at the same locus; 2 bidirectional MM, 2 antigen MMs in both the GVH and HVG directions; 2 MM: bidirectional + GVHD, 2 antigen MMs in the GVH direction and 1 antigen MM in the HVG direction; 2 MM: bidirectional + rejection, 1 antigen MM in the GVH direction and 2 antigen MMs in the HVG direction; 2 MM: GVHD + rejection, 1 antigen MM in the GVH direction at one locus and 1 antigen MM in the HVG direction at another locus.

2 antigen MMs in the HVG direction (GVH 1/HVG 2 MM group; n = 264 [9%]), and 1 antigen MM in the GVH direction at 1 locus and 1 antigen MM in the HVG direction at another locus (GVH 1/HVG 1 2-antigen MM group; n = 37 [1%]).

OS, Relapse, and NRM

The median follow-up period in survivors was 2.2 years (range, 0.0-11.1 years). The 2-year OS rate was 0.46 (95% confidence interval [CI], 0.44-0.48) (Figure 1). To clarify the impact of HLA MM in each vector, the GVH 1/HVG 1 MM group was considered the reference group in the multivariate analyses, in accordance with the approach of Stevens et al. [9], and the following hazard ratios (HRs) were adjusted for the other significant variables, including TNC dose category. The GVH 1-2/HVG 0 MM (HR, 0.88; 95% CI, 0.69-1.13; $P = .317$), the GVH 0/HVG 1-2 MM (HR, 0.95; 95% CI, 0.74-1.22; $P = .670$), and other groups were not associated with overall mortality compared with the GVH 1/HVG 1 MM group (Table 2 and Figure 1). The GVH 0/HVG 0 MM group

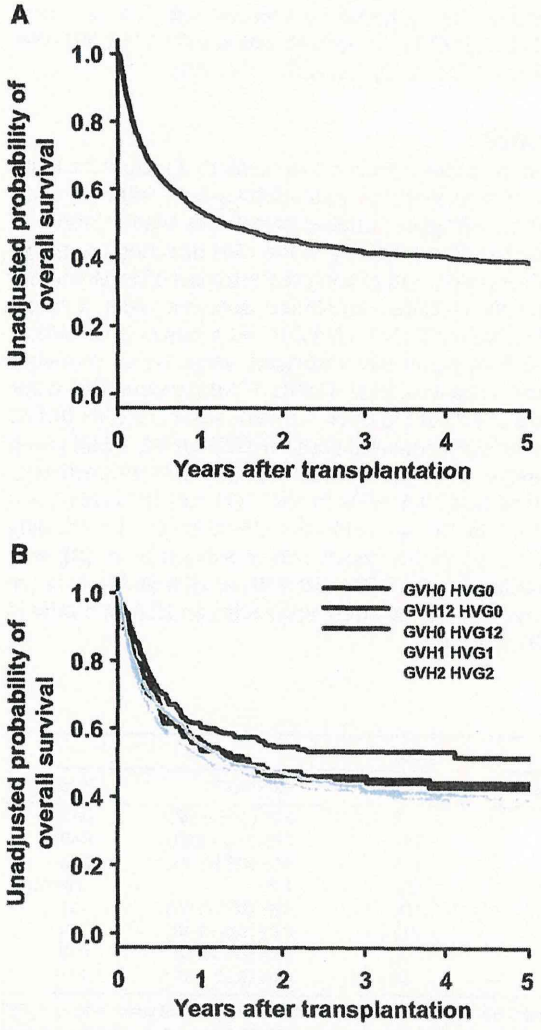

Figure 1. OS of total patients (A) and patients grouped according to HLA MM category (B). GVH0 HVG0, HLA match in both the GVH and HVG directions; GVH12 HVG0, antigen MMs in the GVH direction and 0 MMs in the HVG direction; GVH0 HVG12, 1 or 2 antigen MMs in the HVG direction and 0 MM in the GVH direction; GVH1 HVG1, 1 antigen MM in both the GVH and HVG directions at the same locus; GVH2 HVG2, 2 antigen MMs in both the GVH and HVG directions.

Table 2
Overall Mortality

HLA MM Category	Total*			Child			Adult		
	Number	HR 95% CI	*P* Value	Number	HR 95% CI	*P* Value	Number	HR 95% CI	*P* Value
0 MM	273	0.79 (0.64-0.97)	.025	144	0.74 (0.53-1.04)	.079	129	0.82 (0.63-1.06)	.134
1-2 MM/GVH only	150	0.88 (0.69-1.13)	.317	45	0.73 (0.44-1.20)	.208	105	0.92 (0.69-1.22)	.560
1-2 MM/rejection only	136	0.95 (0.74-1.22)	.670	39	0.85 (0.49-1.46)	.557	97	0.96 (0.72-1.29)	.796
1 bidirectional MM	716	1.00	Reference	314	1.00	Reference	402	1.00	Reference
2 bidirectional MM	1170	0.90 (0.79-1.03)	.122	98	0.88 (0.61-1.26)	.480	1072	0.89 (0.76-1.03)	.118
2 MM: bidirectional + GVHD	231	0.97 (0.79-1.18)	.737	16	1.10 (0.55-2.20)	.785	215	0.94 (0.76-1.17)	.594
2 MM: bidirectional + rejection	264	0.93 (0.77-1.13)	.481	19	2.08 (1.19-3.63)	.011	245	0.87 (0.71-1.08)	.208
2 MM: GVHD + rejection	37	0.55 (0.35-0.87)	.012	2			35	0.54 (0.33-0.87)	.012

* Other significant variables were recipient age group, 0-5 years (reference, 1.00), 6-15 years (HR, 0.93; 95% CI, 0.69-1.24; $P = .603$), 16-49 years (HR, 1.32; 95% CI, 1.00-1.75; $P = .053$), ≥50 years (HR, 1.96; 95% CI, 1.48-2.60; $P < .001$); recipient sex, female (reference, 1.00), male (HR, 1.16; 95% CI, 1.05-1.28; $P = .005$); disease risk, standard risk (reference, 1.00), high risk (HR, 2.42; 95% CI, 2.16-2.71; $P < .001$); GVHD prophylaxis, CSA/TAC + MTX (reference, 1.00), CSA/TAC only (HR, 1.30; 95% CI, 1.15-1.46; $P < .001$), others (HR, 1.13; 95% CI, 0.98-1.31; $P = .091$), year of transplantation, 1998-2004 (reference, 1.00), 2005-2009 (HR, 0.83; 95% CI, 0.75-0.93; $P = .001$).

was associated with lower overall mortality compared with the GVH 1/HVG 1 MM group (HR, 0.79; 95% CI, 0.64-0.97; $P = .025$); however, in both the child and adult cohorts, the association was not significant, owing in part to a lack of statistical power. The GVH 1/HVG 1 2-antigen MM group, which was represented mostly in the adult cohort, was associated with lower overall mortality compared with the GVH 1/HVG 1 MM group (HR, 0.55; 95% CI, 0.35-0.87; $P = .012$).

We performed an additional analysis according to the HLA matching criteria used in the United States and Europe (HLA-A and -B for antigen level and -DRB1 for allele level) (Supplemental Table 1). Consistent with the result obtained using our criteria (HLA-A, -B, and -DR for antigen level), there were no differences in the impact of the MM direction (GVH or HVG) on OS. The difference in OS between the GVH 0/HVG 0 MM and GVH 1/HVG 1 MM groups was not significant in this analysis.

The cumulative incidence rates of relapse and NRM at 2 years were 0.34 (95% CI, 0.32-0.36) and 0.26 (95% CI, 0.24-0.27), respectively. There was no difference in the incidence of relapse between the GVH 1/HVG 1 MM and any other MM group (Table 3 and Figure 2). The GVH 1-2/HVG 0 MM group was significantly associated with lower NRM compared with the GVH 1/HVG 1 MM group (HR, 0.65; 95% CI, 0.44-0.98; $P = .040$) (Table 3 and Figure 2), but only in the child cohort (child, $P = .048$; adult, $P = .215$).

Because our cohorts were mainly adults, and most adults received a TNC dose of 2.0-4.9 $\times 10^7$/kg, we performed an additional analysis in the subset of adults who received a TNC dose of 2.0-2.4 $\times 10^7$/kg or 2.5-4.9 $\times 10^7$/kg (Supplemental Table 2). In the subset of adults who received a TNC dose of 2.0-2.4 $\times 10^7$/kg, compared with the GVH 1/HVG 1 MM group, the GVH 0/HVG 0 MM group was associated with lower overall mortality ($P = .027$) and NRM ($P = .007$) and a higher incidence of relapse ($P = .028$), and the GVH 2/HVG 2 MM group was associated with lower overall mortality ($P = .001$) and NRM ($P = .008$). The GVH 1-2/HVG 0 MM group was significantly associated with lower NRM compared with the GVH 1/HVG 1 MM group ($P = .033$). In the subset of adults who received a TNC dose of 2.5-4.9 $\times 10^7$/kg, no HLA MM group was associated with overall mortality, relapse, or NRM, except for lower overall mortality in the GVH 1/HVG 1 2-antigen MM group compared with the GVH 1/HVG 1 MM group ($P = .046$).

Neutrophil and Platelet Engraftment

The cumulative incidence rates of neutrophil and platelet engraftment in our study cohort were 0.76 (95% CI, 0.74-0.77) and 0.57 (95% CI, 0.55-0.59), respectively. The GVH 1-2/HVG 0 MM group was marginally associated with better neutrophil and platelet engraftment kinetics compared with the GVH 1/HVG 1 MM group (neutrophil engraftment: HR, 1.18; 95% CI, 0.98-1.42; $P = .081$; platelet engraftment: HR, 1.23; 95% CI, 1.00-1.51; $P = .053$) (Table 4 and Figure 3). The impact on neutrophil engraftment was significant only in the adult cohort (child, $P = .496$; adult, $P = .045$).

Acute and Chronic GVHD

In all engrafted patients, the cumulative incidence rates of grade II-IV and III-IV acute GVHD were 0.45 (95% CI, 0.43-0.47) and 0.15 (95% CI, 0.14-0.17), respectively. The GVH 0/HVG 0 MM group was significantly associated with a lower incidence of grade II-IV acute GVHD compared with the GVH 1/HVG 1 MM group (HR, 0.70; 95% CI, 0.54-0.90; $P = .006$) (Supplemental Table 3 and Figure 4), but only in the child cohort (child, $P = .002$; adult, $P = .506$). The GVH 0/HVG 0 MM group was marginally associated with a lower incidence of chronic GVHD compared with the GVH 1/HVG 1 MM group (HR, 0.72; 95% CI, 0.51-1.00; $P = .050$).

DISCUSSION

This nationwide retrospective study that included a large number of both pediatric and adult patients allowed us to consider an adequate number of patients who underwent UCBT with an HLA MM only in the GVH direction or only in the HVG direction, and to analyze the impact of an MM in the GVH or HVG direction on clinical outcomes after a single UCBT. Neither the GVH 1-2/HVG 0 MM group nor the GVH 0/HVG 1-2 MM group was associated with overall mortality compared with the GVH 1/HVG 1 MM group. The point estimates of HRs of the GVH 1-2/HVG 0 MM and GVH 0/HVG 1-2 MM groups compared with the GVH 1/HVG 1 MM group were similar and both <1 (HR, 0.88 and 0.95, respectively), suggesting that HLA MMs in the GVH and HVG directions post-UCBT do not have different effects on OS. This finding does not support the conclusion of Stevens et al. [9], who recommended using UCB units with an HLA MM only in the GVH direction and avoiding units with an HLA MM only in the HVG direction.

Table 3
Relapse and NRM

HLA MM category	Relapse*			NRM†		
	Number	HR 95% CI	*P* Value	Number	HR 95% CI	*P* Value
0 MM	258	1.07 (0.84-1.37)	.560	258	0.74 (0.53-1.02)	.063
1-2 MM/GVH only	147	1.20 (0.90-1.59)	.215	147	0.65 (0.44-0.98)	.040
1-2 MM/rejection only	131	1.18 (0.84-1.64)	.338	131	0.81 (0.55-1.19)	.292
1 bidirectional MM	667	1.00	Reference	667	1.00	Reference
2 bidirectional MM	1106	0.99 (0.83-1.19)	.930	1106	0.88 (0.72-1.07)	.191
2 MM: bidirectional + GVHD	217	1.00 (0.76-1.33)	.979	217	0.81 (0.60-1.10)	.184
2 MM: bidirectional + rejection	243	1.27 (0.99-1.63)	.060	243	0.66 (0.49-0.91)	.010
2 MM: GVHD + rejection	36	0.64 (0.32-1.24)	.184	36	0.61 (0.32-1.16)	.131

* Other significant variables were recipient age group, 0-5 years (reference, 1.00), 6-15 years (HR, 0.61; 95% CI, 0.44-0.84; $P = .002$), 16-49 years (HR, 0.71; 95% CI, 0.52-0.97; $P = .030$), ≥50 years (HR, 0.72; 95% CI, 0.52-0.98; $P = .040$); diagnosis, AML (reference, 1.00), ALL (HR, 1.11, 95% CI, 0.94-1.30, $P = .210$), CML (HR, 1.33, 95% CI, 0.99-1.79, $P = .059$), MDS (HR, 0.67, 95% CI, 0.51-0.87, $P = .003$); disease risk, standard risk (reference, 1.00), high risk (HR, 2.93; 95% CI, 2.54-3.39; $P < .001$); GVHD prophylaxis, CSA/TAC + MTX (reference, 1.00), CSA/TAC only (HR, 0.72; 95% CI, 0.61-0.86; $P < .001$), others (HR, 0.87; 95% CI, 0.71-1.05; $P = .145$).

† Other significant variables were recipient age group, 0-5 years (reference, 1.00), 6-15 years (HR, 1.44; 95% CI, 0.90-2.30; $P = .128$), 16-49 years (HR, 2.04; 95% CI, 1.29-3.22; $P = .002$), ≥50 years (HR, 3.52; 95% CI, 2.24-5.52; $P < .001$); GVHD prophylaxis, CSA/TAC + MTX (reference, 1.00), CSA/TAC only (HR, 1.90; 95% CI, 1.60-2.26; $P < .001$), others (HR, 1.42; 95% CI, 1.14-1.75; $P = .001$), year of transplantation, 1998–2004 (reference, 1.00), 2005-2009 (HR, 0.71; 95% CI, 0.61-0.83; $P < .001$).

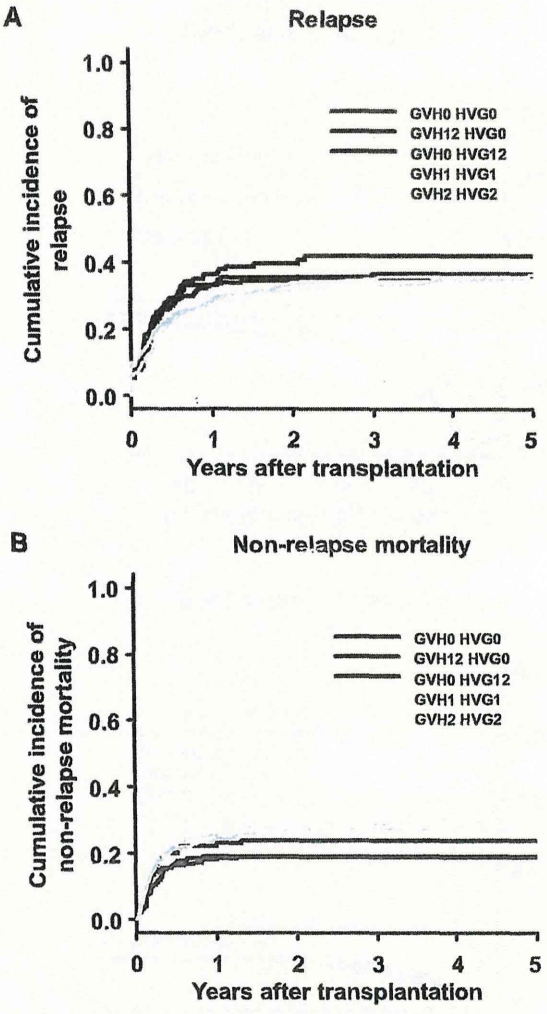

Figure 2. Relapse and NRM.

Several differences in patient background between the study of Stevens et al. [9] and the present study warrant clarification. The first difference is in the age distribution of patients. Stevens et al.'s series included 907 pediatric patients age <16 years and 295 adult patients; in contrast, our series included 677 pediatric patients and 2300 adult patients, which can provide useful information for both pediatric and adult transplant physicians. Since the cell doses of UCB units in child and adult cohorts are significantly different, which may affect the impact of HLA MM, we performed stratified analyses in the child and adult cohorts. Our results consistently showed that the direction of MM had no apparent impact on overall mortality in either cohort. Consistent with the results of Stevens et al. [9], the GVH 1-2/HVG 0 MM group was associated with lower NRM in the child cohort, but this advantage was offset by a higher incidence of relapse in this cohort. A second difference between the 2 studies is in conditioning regimens. A myeloablative regimen was used in 92% of the patients in the Stevens et al. study, compared with 67% in our study. Consequently, we performed a separate analysis in the patients who received a myeloablative regimen and confirmed that the direction of MM had no apparent impact on overall mortality (data not shown).

The third difference between the 2 studies relates to GVHD prophylaxis. Cyclosporine and steroids were used as GVHD prophylaxis in 62% of the patients in the Stevens et al. study, but in only 3% of the patients (10% of the child cohort) in our study, which might have affected outcomes. The fourth difference is in the number of patients with an HLA MM only in the GVH direction or only in the HVG direction. The Stevens et al. study included 35 patients with a GVH 1-2/HVG 0 MM and 22 patients with a GVH 0/HVG 1-2 MM in the overall mortality analysis, compared with 150 and 136 patients, respectively, in our study. Finally, the level of HLA typing used to determine the number of HLA MMs differed between the 2 studies. In the present study, MMs in HLA-DR loci were counted at the antigen level in accordance with current practice in Japan, whereas Stevens et al. counted HLA-DRB1 MMs at the allele level. Consequently, we performed an additional analysis using the same HLA matching criteria as in previous studies from the United States and Europe (HLA-A and -B for antigen level and -DRB1 for allele level), and reached a similar conclusion that an MM only in the GVH or only in the HVG direction had no impact on overall mortality.

Table 4
Neutrophil and Platelet Engraftment

HLA MM Category	Neutrophil Engraftment*			Platelet Engraftment†		
	Number	HR 95% CI	*P* Value	Number	HR 95% CI	*P* Value
0 MM	272	1.03 (0.88-1.20)	.718	272	1.06 (0.88-1.27)	.559
1-2 MM/GVH only	149	1.18 (0.98-1.42)	.081	149	1.23 (1.00-1.51)	.053
1-2 MM/rejection only	136	1.01 (0.82-1.26)	.899	136	0.84 (0.66-1.07)	.164
1 bidirectional MM	716	1.00	Reference	714	1.00	Reference
2 bidirectional MM	1167	0.98 (0.87-1.09)	.672	1166	0.96 (0.85-1.10)	.590
2 MM: bidirectional + GVHD	231	0.91 (0.76-1.08)	.278	230	0.91 (0.74-1.13)	.406
2 MM: bidirectional + rejection	264	0.86 (0.72-1.02)	.089	264	0.98 (0.80-1.19)	.816
2 MM: GVHD + rejection	37	1.40 (1.03-1.89)	.030	37	2.21 (1.46-3.33)	<.001

* Other significant variables were TNC category, 2.5-4.9 × 10^7/kg (reference, 1.00), ≥10.0 × 10^7/kg (HR, 1.76; 95% CI, 1.33-2.33; $P < .001$), 5.0-9.9 × 10^7/kg (HR, 1.26; 95% CI, 1.05-1.52; $P = .015$), 2.0-2.4 × 10^7/kg (HR, 0.87; 95% CI, 0.79-0.95; $P = .003$), <2.0 × 10^7/kg (HR, 0.82; 95% CI, 0.71-0.95; $P = .007$); diagnosis, AML (reference, 1.00), ALL (HR, 1.11, 95% CI, 1.00-1.22, $P = .040$), CML (HR, 0.87, 95% CI, 0.73-1.04, $P = .124$), MDS (HR, 0.88, 95% CI, 0.75-1.04, $P = .129$); disease risk, standard risk (reference, 1.00), high risk (HR, 0.74; 95% CI, 0.67-0.80; $P < .001$); GVHD prophylaxis, CSA/TAC + MTX (reference, 1.00), CSA/TAC only (HR, 1.16; 95% CI, 1.04-1.30; $P = .010$), others (HR, 1.09; 95% CI, 0.96-1.23; $P = .169$), year of transplantation, 1998-2004 (reference, 1.00), 2005-2009 (HR, 1.21; 95% CI, 1.11-1.33; $P < .001$).

† Other significant variables were TNC category, 2.5-4.9 × 10^7/kg (reference, 1.00), ≥10.0 × 10^7/kg (HR, 1.49; 95% CI, 1.09-2.03; $P = .013$), 5.0-9.9 × 10^7/kg (HR, 1.26; 95% CI, 1.01-1.57; $P = .040$), 2.0-2.4 × 10^7/kg (HR, 0.95; 95% CI, 0.84-1.06; $P = .365$), <2.0 × 10^7/kg (HR, 0.81; 95% CI, 0.67-0.97; $P = .022$); recipient age group, 0-5 years (reference, 1.00), 6-15 years (HR, 1.00; 95% CI, 0.79-1.25; $P = .971$), 16-49 years (HR, 0.99; 95% CI, 0.77-1.26; $P = .909$), ≥50 years (HR, 0.70; 95% CI, 0.55-0.90; $P = .006$); recipient sex, female (reference, 1.00), male (HR, 0.90; 95% CI, 0.82-0.99; $P = .034$); disease risk, standard risk (reference, 1.00), high risk (HR, 0.58; 95% CI, 0.53-0.64; $P < .001$); GVHD prophylaxis, CSA/TAC + MTX (reference, 1.00), CSA/TAC only (HR, 0.81; 95% CI, 0.71-0.91; $P = .001$), others (HR, 0.88; 95% CI, 0.76-1.01; $P = .074$), and year of transplantation, 1998-2004 (reference, 1.00), 2005-2009 (HR, 1.26; 95% CI, 1.14-1.40; $P < .001$).

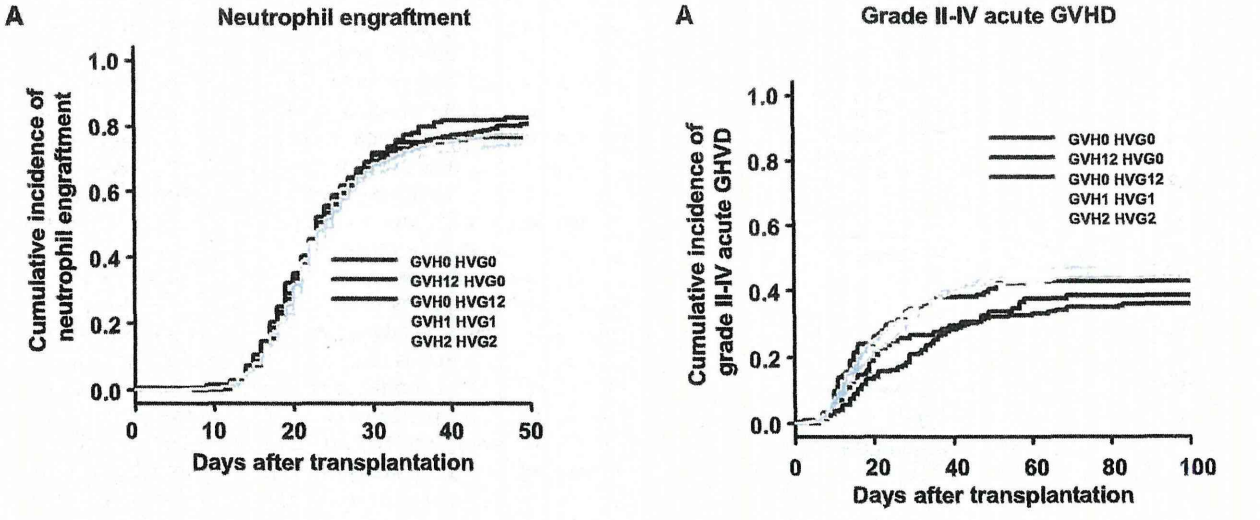

Figure 3. Neutrophil and platelet engraftment.

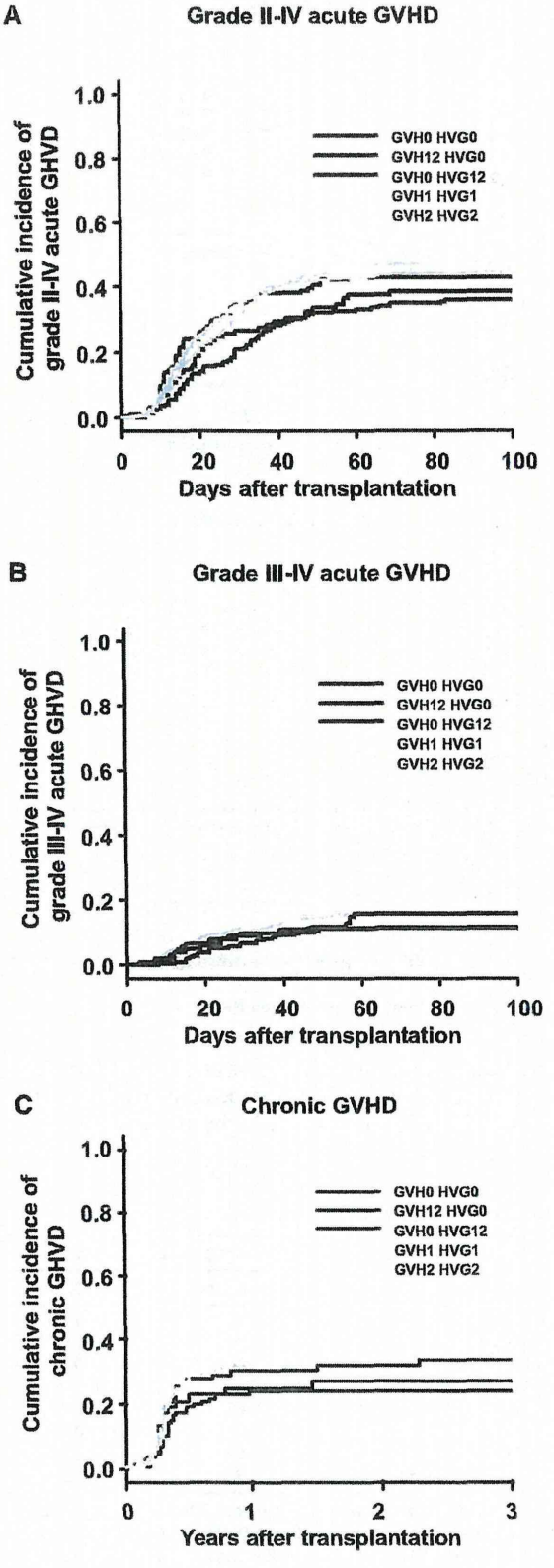

Figure 4. Acute and chronic GVHD.

Similar to Stevens et al. [9], we found a tendency for better neutrophil and platelet engraftment kinetics in the GVH 1-2/HVG 0 MM group. This finding suggests that an HLA MM in the GVH direction enhances engraftment by eradicating or suppressing the host residual immune cells responsible for the rejection or inhibition of donor cell engraftment. In contrast to our findings, Matsuno et al. [8] analyzed the impact of GVH/HVG MM on 152 patients who underwent a single UCBT in a single center, and found that the presence of a 2-antigen MM in the GVH direction was associated with slower and lower neutrophil engraftment compared with a 0- or 1-antigen MM in the GVH direction. Because Matsuno et al. used only a calcineurin inhibitor for GVHD prophylaxis in all of the patients in their cohort, we recategorized the HLA MM group according to HLA category (GVH 0-1/HVG 0-1 MM, GVH 0–1/HVG 2 MM, GVH 2/HVG 0-1 MM, and GVH 2/HVG 2 MM) and performed additional analyses in which patients were stratified according to GVHD prophylaxis (calcineurin inhibitor plus methotrexate, calcineurin inhibitor only, or other). Similar to the findings of Matsuno et al., an MM in the GVH direction was significantly associated with a lower incidence of engraftment in patients who received only a calcineurin inhibitor (data not shown). In contrast, an MM in the GVH direction was associated with a higher incidence of engraftment in patients who received a calcineurin inhibitor plus methotrexate. These findings suggest that the impact of HLA MM differs according to GVHD prophylaxis. A possible explanation for the different

effects of GVHD prophylaxis on engraftment is the high incidence of hemophagocytic syndrome (HPS) and pre-engraftment immune reaction in patients who received only a calcineurin inhibitor as GVHD prophylaxis [20,21]. Takagi et al. [20] reported HPS in 20 of 119 patients who underwent UCBT with mostly tacrolimus alone as GVHD prophylaxis, resulting in a high incidence of graft failure. Less-intensive GVHD prophylaxis may enhance the immune reaction caused by donor T cells that recognize the HLA MM antigen in the GVH direction in the early phase after transplantation, which could lead to HPS or similar conditions and decrease the rate of neutrophil engraftment. These findings demonstrate the need for a prospective study using uniform GVHD prophylaxis to further evaluate the impact of HLA MM on neutrophil engraftment.

This study has several limitations. First, the patients' heterogeneous backgrounds might have produced statistical bias, although we attempted to reduce this bias by adjusting the impact in the multivariate analyses. Second, the number of subjects in each HLA MM group category was limited. Nevertheless, the number of subjects in the GVH 1-2/HVG 0 and GVH 0/HVG 1-2 MM groups was much greater than that in previous studies [8,9]. Third, we might have underestimated the degree of HLA MM, given our incomplete allelic and HLA-C antigen information; for example, the group that had only an HLA MM in the GVH direction might have included an allelic MM in the HVG direction. A potential HLA-C antigen MM or KIR ligand MM also might have affected outcomes, but we did not evaluate HLA-C in the present study. The foregoing issues might have weakened the power of this study to detect differences.

In conclusion, our findings do not support a strategy for selecting UCB donors based on the direction of the HLA MM, although GVH 1-2/HVG 0 MMs may be associated with better neutrophil engraftment, particularly when a calcineurin inhibitor plus other immunosuppressive agents, such as methotrexate, are used for GVHD prophylaxis. The impact of HLA MMs in only the GVH direction remains to be clarified further under a uniform GVHD prophylaxis regimen.

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Authorship statement: J.K. and Y.K. designed the research, organized the project, and wrote the manuscript. J.K., Y.A., and Y.K. performed the statistical analysis and analyzed the data; K.K. and T.N.-I. collected data from JCBBN; and all of the authors interpreted the data, and reviewed and approved the final manuscript.

SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.bbmt.2012.09.017.

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Bone Marrow Transplantation (2013) 48, 529–536

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ORIGINAL ARTICLE

Changes in incidence and causes of non-relapse mortality after allogeneic hematopoietic cell transplantation in patients with acute leukemia/myelodysplastic syndrome: an analysis of the Japan Transplant Outcome Registry

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The outcomes for allogeneic hematopoietic cell transplantation (allo-HCT) are heavily influenced by non-relapse mortality (NRM). We retrospectively assessed the changes in the incidence and causes of NRM after allo-HCT over the past 12 years. NRM, relapse rate and OS were analyzed using the Japan transplant outcome database of 6501 adult patients with acute leukemia or myelodysplastic syndrome who received their first allo-HCT in remission from 1997 through 2008. In multivariate analysis in patients aged 16–49 years, the adjusted hazard ratios (HRs) for NRM for 2001–2004 and 2005–2008 were 0.78 (95% confidence interval, 0.65–0.93) and 0.64 (0.54–0.78), respectively, compared with 1997–2000. The HR for overall mortality in 2005–2008 was 0.81 (0.70–0.93) compared with 1997–2000. In patients aged 50–70 years, the HRs for NRM and overall mortality in 2005–2008 were 0.56 (0.46–0.68) and 0.66 (0.47–0.93), respectively, compared with those in 2001–2004. We found that causes of death that contributed to the changes in NRM varied among subgroups. In conclusion, our study indicated that the incidence of NRM after allo-HCT has significantly decreased over the past 12 years, which has led to an improvement of OS, and also showed reductions in NRM in subgroups consisting of older patients and those who received unrelated cord blood transplantation.

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Keywords: leukemia; allogeneic hematopoietic cell transplantation; non-relapse mortality; GVHD; cord blood transplantation

INTRODUCTION

Allogeneic hematopoietic cell transplantation (allo-HCT) has been recognized as a potent strategy for curing hematological malignancies. However, there have always been concerns about the risk of non-relapse mortality (NRM). As the risk of relapse is known to be significantly reduced after allo-HCT, the outcome of and indications for allo-HCT are heavily influenced by the risk for NRM.

Over the past few decades, many changes have been made to improve the outcome after allo-HCT, including improvements in the conditioning regimen, donor selection, and prophylaxis and treatment for organ complications, GVHD and infectious diseases, which have led to a reduction in NRM.[1–4]

Although an improvement in NRM has been reported in relatively younger patients who have received allo-HCT from a BM or peripheral blood (PB) donor, NRM has not been fully examined in other settings, such as in elderly patients, or in cord blood (CB) transplantation.

To evaluate the effects of these advances, we retrospectively assessed the changes in the incidence and causes of NRM over the past 12 years, using a nationwide registry database of more than 6000 patients who received various types of allo-HCT.

PATIENTS AND METHODS

Data source

The clinical data were extracted from a nationwide transplant outcome registry database provided by the Japan Society for Hematopoietic Cell Transplantation (JSHCT), the Japan Marrow Donor Program (JMDP) and the Japan Cord Blood Bank Network (JCBBN). The JSHCT collect clinical data through the Transplant Registry Unified Management Program, as described previously.[5] This study was approved by the data management committees of JSHCT, JMDP and JCBBN, and by the Institutional Review Board at the National Cancer Center Hospital.

Patients and definitions

We evaluated the data on patients aged between 16 and 70 years who had AML, acute lymphocytic leukemia (ALL) or myelodysplastic syndrome (MDS), and who received their first allo-HCT between 1997 and 2008. We compared the incidence of NRM after allo-HCT in three consecutive 4-year

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periods (1997–2000, 2001–2004 and 2005–2008) for younger patients (16–49 years), and in the latter two periods for older patients (50–70 years). NRM was defined as death without recurrent disease after allo-HCT. Analyses were performed for patients with acute leukemia/MDS in remission or low-risk MDS (refractory anemia with or without ringed sideroblast: RA/RARS). Analyses were performed on the basis of patients' age (16–49 years and 50–70 years) and donor source (HLA-6/6-serum-matched or 1-Ag-mismatched related, unrelated BM and unrelated CB). In the era considered by this study, only BM from unrelated volunteer donors was used in Japan. In 2003, JMDP nationally recommended DNA typing of HLA-A and B, as well as HLA-DRB1. Since 2005, JMDP required all the candidates of unrelated allo-HCT to examine high-resolution typing of HLA-A, B and DRB1, and also recommended high-resolution typing of the C-locus. Conditioning regimens were classified as indicated by Giralt *et al.*[6] The incidences of mortality associated with GVHD, infection and organ failure were analyzed. In patients who had multiple causes among GVHD, infection and organ failure, information regarding the main cause of death was prioritized.

Statistical analysis

Data were retrospectively reviewed and analyzed as of June 2011. Among the three time periods, patient characteristics were compared using the χ^2 test. The primary end point of the study was NRM after allo-HCT. Probabilities of NRM and relapse were estimated with the use of cumulative incidence curves, with relapse viewed as a competing risk of NRM, and with NRM viewed as a competing risk of relapse. The Pepe and Mori test was used to evaluate the differences between groups. For the 151 patients (2%) who were known to have relapsed but whose date of relapse was unavailable, mid-point imputation was performed by substituting the midpoint from HCT to date of last contact as the date of relapse. The probability of OS was estimated using the Kaplan-Meier product limit method, and 95% confidence intervals (CIs) were calculated using the Greenwood formula. To compare the OS between groups, the log-rank test was used. Incidences of NRM, relapse and OS were estimated as probabilities at 3 years from allo-HCT. Multivariate analyses for NRM and relapse were performed using competing risk regression by the method of Fine and Gray, and for OS using a Cox proportional hazard regression model. The multivariate analyses were performed separately among patients aged 16–49 years and patients aged 50–70 years, where the year of allo-HCT (1997–2000 vs 2001–2004 or 2005–2008 among younger patients, 2001–2004 vs 2005–2008 among older patients or those who received unrelated CB transplantation (UCBT)), disease type (AML vs ALL or MDS), patient age (16–29 years vs 30–39 or 40–49 among younger patients, 50–59 vs 60–70 among older patients), patient gender (male vs female), donor source (HLA-6/6-Ag-matched sibling vs other family donors, HLA-6/6-Ag-matched unrelated BM, mismatched unrelated BM or unrelated CB) and conditioning regimens (myeloablative vs reduced-intensity) were considered as covariates. Multivariate analyses were also performed separately for those receiving related allo-HCT, where HLA-6/6-Ag-matched sibling vs other family donors were considered as covariates for the donor source, those receiving unrelated BM transplantation (UBMT), where HLA-6/6-Ag-matched unrelated BM vs mismatched BM were considered as covariates, and those who received UCBT, where the covariates above were examined other than the donor source. We considered two-sided *P*-values of <0.05 to be statistically significant. Statistical analyses were performed with SAS version 9.1.3 (SAS, Cary, NC, USA) and the SPSS software version 11.0.1 (SPSS, Chicago, IL, USA).

RESULTS

Patients

A total of 6501 patients registered from 266 institutions across the country[5] were analyzed, with a median age of 40 years and a median follow-up of 39 months. Characteristics of the patients and

Table 1. Patients' characteristics according to the time period of transplant

Characteristics	*1997–2000,* N *(%)*	*2001–2004,* N *(%)*	*2005–2008,* N *(%)*	P
Total number of patients	1354	2292	2855	
Age at transplant (years)				<0.001
16–34	740 (55)	892 (39)	862 (30)	
35–49	491 (36)	783 (34)	939 (33)	
50–59	116 (9)	489 (21)	743 (26)	
60–70	7 (1)	128 (6)	311 (11)	
Donor source				<0.001
Related BM	511 (38)	367 (16)	504 (18)	
Related peripheral blood	158 (12)	546 (24)	456 (16)	
Unrelated BM	588 (43)	998 (44)	1312 (46)	
Unrelated cord blood	14 (1)	321 (14)	534 (19)	
Others	83 (6)	60 (3)	49 (2)	
Disease type				0.991
AML	699 (52)	1226 (53)	1516 (53)	
ALL	505 (37)	744 (32)	949 (33)	
MDS	150 (11)	322 (14)	390 (14)	
Disease status				0.001
CR1	811 (60)	1288 (56)	1802 (63)	
CR2	311 (23)	552 (24)	654 (63)	
CR3 or beyond	76 (6)	96 (4)	77 (3)	
MDS RA/RARS	83 (6)	202 (9)	267 (9)	
Other remission state/no detailed data	73 (5)	154 (7)	55 (2)	
Conditioning				<0.001
Myeloablative	1131 (84)	1585 (69)	1788 (63)	
Reduced-intensity	21 (2)	394 (17)	689 (24)	
Not categorized	202 (15)	313 (14)	378 (13)	
GVHD prophylaxis				<0.001
CYA-based	1041 (77)	1367 (60)	1354 (47)	
Tacrolimus-based	270 (20)	825 (36)	1373 (48)	
No data available	43 (3)	100 (4)	128 (4)	

Abbreviations: MDS = myelodysplastic syndrome; RA/RARS = refractory anemia with or without ringed sideroblast.