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ORIGINAL ARTICLE

Unrelated cord blood transplantation vs related transplantation with HLA 1-antigen mismatch in the graft-versus-host direction

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Little information is available regarding whether an unrelated cord blood (UCB) unit or a related donor with a 1-antigen mismatch at the HLA-A, HLA-B or HLA-DR locus in the graft-versus-host direction (RD/1AG-MM-GVH) should be selected as an alternative donor for patients without an HLA-matched related/unrelated donor. Therefore, we conducted a retrospective study using national registry data on patients with leukemia or myelodysplastic syndrome who received transplantation using a single UCB ($n = 2288$) unit or an RD/1AG-MM-GVH ($n = 525$). We found that the survival rate in the UCB group was comparable to that in the RD/1AG-MM-GVH group, although the RD/1AG-MM-GVH group with an HLA-B mismatch showed significantly higher overall and non-relapse mortality. Neutrophil and platelet engraftment were significantly faster, whereas the incidence of acute or chronic graft-versus-host disease (GVHD) was significantly higher in the RD/1AG-MM-GVH group. The incidence of acute or chronic GVHD in the RD/1AG-MM-GVH group with *in vivo* T-cell depletion was comparable to that in the UCB group, which translated into a trend toward better overall survival, regardless of the presence of an HLA-B mismatch. In conclusion, UCB and RD/1AG-MM-GVH are comparable for use as an alternative donor, except for RD/1AG-MM-GVH involving an HLA-B mismatch.

Leukemia (2013) 27, 286–294; doi:10.1038/leu.2012.203

Keywords: cord blood transplantation; related transplantation; HLA mismatch; alternative donor

INTRODUCTION

For patients who lack an HLA-identical sibling, an HLA-matched unrelated donor (MUD) is considered to be the preferred alternative donor in allogeneic hematopoietic cell transplantation (HCT).^{1–5} However, it is difficult to find an MUD for patients with rare HLA haplotypes. Furthermore, it takes at least a few months from the start of an unrelated donor search to actually receive a graft. Therefore, there is a large demand for an alternative source to an HLA-identical sibling or MUD, particularly for patients who have a rare haplotype or who need immediate transplantation.

Unrelated cord blood (UCB) has emerged as a promising alternative source for pediatric and adult patients.^{6–17} In UCB transplantation, up to two antigen/allele mismatches between a recipient and cord blood unit are acceptable without an increased risk of acute graft-versus-host disease (GVHD). The clinical outcome in UCB transplantation is improving, and is almost comparable to that in HLA 8/8 allele MUD transplantation, although a high risk of graft failure and early treatment-related complications are still major issues.^{15–17}

Another alternative source is an HLA-mismatched related donor, particularly when a related donor with a 1-antigen mismatch at the HLA-A, HLA-B, or HLA-DR locus in the graft-versus-host (GVH)

direction (RD/1AG-MM-GVH) is available. HCT from an RD/1AG-MM-GVH results in a higher but acceptable incidence of acute GVHD.^{18–20} In previous studies, HLA mismatches in the host-versus-graft (HVG) direction were associated with a higher incidence of graft failure and lower overall survival (OS).^{18,19,21} However, the risk of graft failure might have been improved by the use of conditioning regimens that strongly suppress the recipient's immune system.²² Therefore, in current clinical practice in Japan, stem cell transplantation from an RD/1AG-MM-GVH is being performed while accepting multiple antigen mismatches in the HVG direction without specific *ex vivo* stem cell manipulation.^{18,19,23} We have recently reported that OS in transplantation from an RD/1AG-MM-GVH involving an HLA-B antigen mismatch was inferior, whereas that from an RD/1AG-MM-GVH involving an HLA-A or -DR antigen mismatch was comparable to that from an 8/8-MUD in standard-risk diseases.²³

Unlike transplantation from an MUD, transplantation using a UCB unit or an RD/1AG-MM-GVH can be performed immediately when necessary. However, little information is available regarding the priority in selecting these alternative donors. Therefore, we conducted a retrospective study using national registry data on 2813 patients with leukemia or myelodysplastic syndrome (MDS)

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Received 15 June 2012; revised 5 July 2012; accepted 11 July 2012; accepted article preview online 18 July 2012; advance online publication, 10 August 2012

who received transplantation using a single UCB or an RD/1AG-MM-GVH.

MATERIALS AND METHODS

Data collection

Data for patients (age: ≥ 16 years) with acute myeloid leukemia, acute lymphoblastic leukemia, MDS and chronic myelogenous leukemia who received a first HCT using a single HLA 0–2 antigen-mismatched UCB unit or an RD/1AG-MM-GVH between 1 January 1998 and 31 December 2009 were obtained from the Transplant Registry Unified Management Program (TRUMP),²⁴ which includes data from the Japan Cord Blood Bank Network (JCBBN) and the Japan Society for Hematopoietic Cell Transplantation (JSHCT). Our analysis included 2306 patients who received a single UCB graft (UCB group) and 541 patients who received a graft from an RD/1AG-MM-GVH (RD/1AG-MM-GVH group). As of January 2012, double UCB grafts for HCT are not available in Japan. The following patients were excluded: 26 patients who lacked data on survival status, survival date, sex of recipient, or GVHD prophylaxis and 8 patients who received stem cells that had been manipulated by *ex vivo* T-cell depletion or CD34 selection. Overall, 2288 patients who received a UCB unit and 525 who received a graft from an RD/1AG-MM-GVH fulfilled the criteria. The study was approved by the data management committees of TRUMP and by the institutional review boards of Japanese Red Cross Nagoya First Hospital and Saitama Medical Center, Jichi Medical University, where this study was organized.

Histocompatibility

Histocompatibility data for the HLA-A, HLA-B and HLA-DR loci were obtained from reports from the institution where the transplantation was performed or from cord blood banks. To reflect current practice in Japan, HLA matching in UCB or RD/1AG-MM-GVH transplantation was assessed by serological data for HLA-A, HLA-B, and HLA-DR loci. An HLA mismatch in the GVH direction was defined as when the recipient's antigens or alleles were not shared by the donor, whereas a mismatch in the HVG direction was defined as when the donor's antigens or alleles were not shared by the recipient.

End points

The primary end point of the study was to compare OS rates between the UCB and RD/1AG-MM-GVH groups. Other end points were the cumulative incidences of neutrophil and platelet engraftment, acute and chronic GVHD, relapse, and non-relapse mortality (NRM). Neutrophil recovery was considered to have occurred when the absolute neutrophil count exceeded $0.5 \times 10^9/l$ for 3 consecutive days following transplantation. Platelet recovery was considered to have occurred when the absolute platelet count exceeded $50 \times 10^9/l$ without platelet transfusion. The physicians who performed transplantation at each center diagnosed and graded acute and chronic GVHD according to the traditional criteria.^{25,26} The incidence of chronic GVHD was evaluated in patients who survived for at least 100 days.

Statistical analysis

Descriptive statistics were used to summarize variables related to the patient characteristics. Comparisons between groups were performed with the χ^2 -test or extended Fisher's exact test as appropriate for categorical variables and the Mann–Whitney *U*-test for continuous variables. The probability of OS was estimated according to the Kaplan–Meier method, and the groups were compared with the log-rank test. The adjusted probability of OS was estimated according to the Cox proportional-hazards model, with other significant variables considered in the final multivariate model. The probabilities of neutrophil and platelet engraftment, acute and chronic GVHD, NRM, and relapse were estimated on the basis of cumulative incidence methods, and the groups were compared with the Gray test,^{27,28} competing events were death without engraftment for neutrophil and platelet engraftment, death or relapse without GVHD for acute and chronic GVHD, death without relapse for relapse, and relapse for NRM. The Cox proportional-hazards model was used to evaluate variables that may affect OS, whereas the Fine and Gray proportional-hazards model was used to evaluate variables that may affect engraftment, GVHD, NRM and relapse.²⁹ We classified the conditioning regimen as myeloablative if either total body irradiation > 8 Gy, oral busulfan ≥ 9 mg/kg,

intravenous busulfan ≥ 7.2 mg/kg, or melphalan > 140 mg/m² was used in the conditioning regimen, and otherwise classified it as reduced intensity, based on the report by the Center for International Blood and Marrow Transplant Research.³⁰ For patients for whom the doses of agents used in the conditioning regimen were not available, we used the information on conditioning intensity (myeloablative or reduced intensity) reported by the treating clinicians. Acute leukemia in the first or second remission, chronic myelogenous leukemia in the first or second chronic phase or accelerated phase, and MDS with refractory anemia or refractory anemia with ringed sideroblasts were defined as standard-risk diseases, and other conditions were defined as high-risk diseases. The following variables were considered when comparing the UCB and RD/1AG-MM-GVH groups: the recipient's age group (≤ 50 years or > 50 years at transplantation), sex of recipient, disease (acute myeloid leukemia, acute lymphoblastic leukemia, chronic myelogenous leukemia or MDS), disease status before transplantation (standard- or high-risk), type of conditioning regimen (myeloablative or reduced intensity), type of GVHD prophylaxis (calcineurin inhibitor and methotrexate, calcineurin inhibitor only, or other), year of transplantation (1998–2004, 2005–2009), and the time from diagnosis to transplantation (< 6 months or ≥ 6 months). In the analysis within the RD/1AG-MM-GVH group, the use of *in vivo* T cell depletion (no vs yes), stem cell source (peripheral blood (PB) stem cells vs bone marrow (BM)), and the number of HLA mismatches in the HVG direction (0–1 vs 2–3) were also considered. Factors without a variable of main interest were selected in a stepwise manner from the model with a variable retention criterion of $P < 0.05$. We then added a variable of main interest to the final model. All tests were two-sided, and $P < 0.05$ was considered to indicate statistical significance. All statistical analyses were performed with Stata version 12 (Stata Corp., College Station, TX, USA) and EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan).³¹ EZR is a graphical user interface for R (The R Foundation for Statistical Computing, version 2.13.0, Vienna, Austria). More precisely, it is a modified version of R commander (version 1.6–3) that was designed to add statistical functions that are frequently used in biostatistics.

RESULTS

Characteristics of patients and transplants

Table 1 shows the patient and transplant characteristics. Recipients of an RD/1AG-MM-GVH were younger than recipients of a UCB unit. Approximately half of the recipients in the RD/1AG-MM-GVH group received PB. The number of HLA mismatches in the GVH direction between a UCB unit and recipient was 0 in 10%, 1 in 33% and 2 in 57%. In the RD/1AG-MM-GVH group, the number of antigen mismatches in the HVG direction was 0 in 12%, 1 in 68%, 2 in 18% and 3 in 3%. Most of the recipients of an RD/1AG-MM-GVH received a calcineurin inhibitor with methotrexate for GVHD prophylaxis, whereas 25% of UCB recipients received only calcineurin inhibitor. *In vivo* T-cell depletion including antithymocyte globulin (ATG) or alemtuzumab was used in 10% of the RD/1AG-MM-GVH group, but in only 1% of the UCB group. Alemtuzumab was used in only one patient, who received transplantation from an RD/1AG-MM-GVH. Information regarding the dose and type of ATG was missing in two-third of the patients who received ATG. Available data showed that the median dose of thymoglobulin was 2.5 (range 2.5–9.0, $n = 9$) and 2.5 (range 1.25–5.0, $n = 10$) mg/kg and the median dose of ATG-Fresenius was 8.0 (range 5.0–10.0, $n = 3$) and 8.0 (range 5.0–10.0, $n = 7$) mg/kg, in the UCB and RD/1AG-MM-GVH groups, respectively. Two-third of UCB transplantations were performed between 2005 and 2009. The median duration of follow-up for survivors was 2 and 4 years in the UCB and RD/1AG-MM-GVH groups, respectively.

Neutrophil and platelet engraftment

The incidence of neutrophil engraftment at day 50 in the RD/1AG-MM-GVH group was higher than that in the UCB group (UCB group, 73%, 95% confidence interval (CI), 71–75%; RD/1AG-MM-GVH group, 93%, 95% CI, 91–95%; Gray test, $P < 0.001$; Figure 1a). The incidence of platelet engraftment at day 150 in the

Table 1. Patient characteristics

Variable	UCB (n = 2288)	RD/1AG-MM-GVH (n = 525)	P	
Age at transplant, median (range)	49 (16–82)	43 (16–74)	<0.001	
<i>Recipient sex</i>				
Female	1004 (44%)	239 (46%)	0.494	
Male	1284 (56%)	286 (54%)		
<i>Disease</i>				
Acute myelogenous leukemia	1365 (60%)	269 (51%)	0.003	
Acute lymphoblastic leukemia	498 (22%)	137 (26%)		
Chronic myelogenous leukemia	124 (5%)	42 (8%)		
Myelodysplastic syndrome	301 (13%)	77 (15%)		
<i>Duration from diagnosis to transplant</i>				
Median time (range), months	7.9 (0.2–768.5)	7.6 (0–251.7)	0.233	
<i>Disease risk</i>				
Standard	959 (42%)	249 (47%)	0.050	
High	1217 (53%)	257 (49%)		
Unknown	112 (5%)	19 (4%)		
<i>Source of stem cells</i>				
Bone marrow	—	251 (48%)	—	
Peripheral blood	—	274 (52%)		
Cord blood	2288 (100%)	—		
<i>HLA compatibility in the graft-versus-host direction</i>				
Matched	225 (10%)	—	<0.001	
One-antigen mismatch	753 (33%)	525 (100%)		
Two-antigen mismatch	1310 (57%)	—		
<i>HLA compatibility in the host-versus-graft direction</i>				
Matched	233 (10%)	62 (12%)	<0.001	
One-antigen mismatch	716 (31%)	355 (68%)		
Two-antigen mismatch	1339 (59%)	94 (18%)		
Three-antigen mismatch	—	14 (3%)		
<i>Conditioning regimen</i>				
Myeloablative	1390 (61%)	253 (48%)	<0.001	
CY + TBI ±	1062	164		
Other TBI regimen	130	20		
BU + CY ±	88	45		
Other non-TBI regimen	110	24		
Reduced intensity	894 (39%)	162 (31%)		
FLU ± TBI ±	840	138		
Other regimen	54	24		
Unclassifiable	4 (0.2%)	110 (21%)		
<i>GVHD prophylaxis</i>				
CSA/TAC + MTX	1410 (62%)	448 (85%)		<0.001
CSA/TAC + MMF	246 (11%)	12 (2%)		
CSA/TAC + Steroid	28 (1%)	13 (2%)		
CSA/TAC only	571 (25%)	45 (9%)		
Unknown	33 (1%)	7 (1%)		
<i>Use of in vivo T-cell depletion</i>				
No	2258 (99%)	472 (90%)	<0.001	
Yes	30 (1%)	53 (10%)		
<i>Year at transplant</i>				
1998–2004	760 (33%)	260 (50%)	<0.001	
2005–2009	1528 (67%)	265 (50%)		
<i>Follow-up of survivors</i>				
Median time (range), years	2.1 (0.0–10.0)	4.0 (0.1–12.2)	<0.001	

Abbreviations: BU, busulfan; CSA, cyclosporine; CY, cyclophosphamide; FLU, fludarabine; MMF, mycophenolate mofetil; MTX, methotrexate; TAC, tacrolimus; TBI, total body irradiation; UCB, unrelated cord blood.

RD/1AG-MM-GVH group was also higher than that in the UCB group (UCB group, 53%, 95% CI, 51–55%; RD/1AG-MM-GVH group, 70%, 95% CI, 66–74%; Gray test, $P < 0.001$; Figure 1b). The use of

RD/1AG-MM-GVH was significantly associated with a higher incidence of neutrophil and platelet engraftment in the multivariate analysis (neutrophil engraftment, hazard ratio (HR), 3.46,

95% CI, 3.00–3.98, $P < 0.001$; platelet engraftment, HR 2.20, 95% CI, 1.89–2.57, $P < 0.001$; Supplementary Table 1). As our previous study revealed that an HLA-B mismatch had an adverse effect on OS in transplantation from an RD/1AG-MM-GVH, patients in the RD/1AG-MM-GVH group with an HLA-A, -B, or -DR mismatch were

separately compared with the UCB group. We consistently observed superior neutrophil and platelet engraftment in each RD/1AG-MM-GVH group as compared with the UCB group (Supplementary Table 1).

Acute and chronic GVHD

The incidence of grade II–IV or grade III–IV acute GVHD in the RD/1AG-MM-GVH group was significantly higher than that in the UCB group (grade II–IV acute GVHD at day 100: UCB group, 34%, 95% CI, 32–36%; RD/1AG-MM-GVH group, 50%, 95% CI, 45–54%; Gray test, $P < 0.001$; grade III–IV acute GVHD at day 100: UCB group, 11%, 95% CI, 10–13%; RD/1AG-MM-GVH group, 21%, 95% CI, 17–24%; Gray test, $P < 0.001$; Figures 2a and b). The incidence of chronic GVHD or extensive type of chronic GVHD in the RD/1AG-MM-GVH group was also significantly higher than that in the UCB group (chronic GVHD at 3 years: UCB group, 25%, 95% CI, 23–27%; RD/1AG-MM-GVH group, 42%, 95% CI, 38–47%; Gray test, $P < 0.001$; extensive chronic GVHD at 3 years: UCB group, 11%, 95% CI, 10–13%; RD/1AG-MM-GVH group, 29%, 95% CI, 25–34%; Gray test, $P < 0.001$; Figures 2c and d). A multivariate analysis confirmed a higher risk of grade II–IV or grade III–IV acute GVHD, chronic or extensive chronic GVHD in the RD/1AG-MM-GVH group than in the UCB group (grade II–IV acute GVHD; HR 1.64, 95% CI, 1.43–1.90, grade III–IV acute GVHD; HR 2.28, 95% CI, 1.80–2.88, chronic GVHD; HR 1.47, 95% CI, 1.24–1.73, extensive chronic GVHD; HR 2.35, 95% CI, 1.90–2.91, Supplementary Table 2).

OS

The 3-year unadjusted OS rates in the UCB and RD/1AG-MM-GVH groups were 38% (36–41%) and 39% (34–43%), respectively ($P = 0.115$). The use of either UCB or RD/1AG-MM-GVH was not associated with OS rates in the multivariate analysis (UCB vs RD/1AG-MM-GVH, HR, 0.99, 95% CI, 0.87–1.12, $P = 0.833$) in all-risk patients, or either standard-risk ($P = 0.588$) or high-risk patients ($P = 0.639$; Table 2), after adjusting for the following significant risk factors: age > 50 years, male recipient, acute myeloid leukemia vs MDS, high-risk disease, GVHD prophylaxis using only calcineurin inhibitor vs calcineurin inhibitor + methotrexate, and earlier year

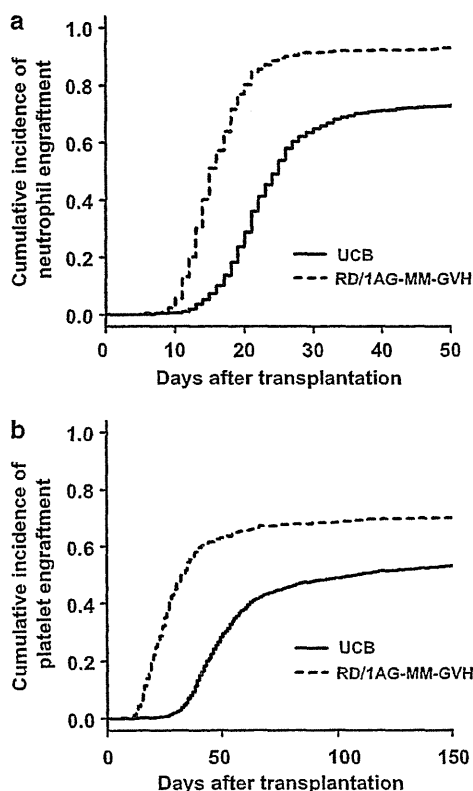


Figure 1. Neutrophil (a) and platelet engraftment (b).

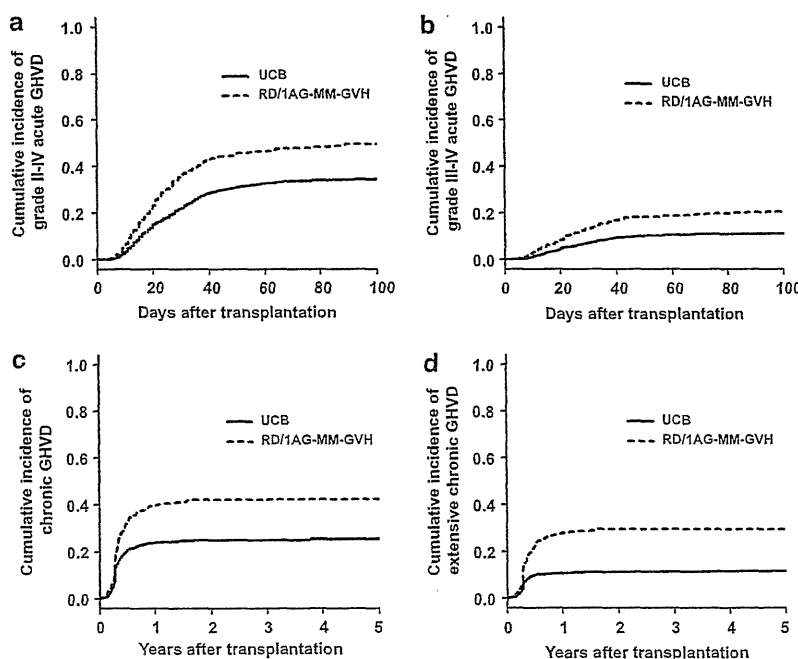


Figure 2. Acute and chronic GVHD. Cumulative incidences of grade II–IV (a) and grade III–IV acute GVHD (b) and chronic (c) and extensive chronic GVHD (d) are shown.

Table 2. Multivariate analysis of overall mortality

Variable	Total ^a		Standard risk ^b		High risk ^c	
	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value
(A)						
UCB	1.00	reference	1.00	reference	1.00	reference
RD/1AG-MM-GVH	0.99 (0.87–1.12)	0.833	1.06 (0.86–1.31)	0.588	0.96 (0.81–1.13)	0.639
(B)						
UCB	1.00	reference	1.00	reference	1.00	reference
RD/HLA-A-MM-GVH	0.92 (0.72–1.18)	0.519	0.99 (0.66–1.48)	0.959	0.90 (0.64–1.26)	0.551
RD/HLA-B-MM-GVH	1.20 (1.01–1.44)	0.043	1.44 (1.05–1.96)	0.023	1.12 (0.89–1.41)	0.326
RD/HLA-DR-MM-GVH	0.85 (0.70–1.02)	0.084	0.88 (0.66–1.19)	0.411	0.84 (0.65–1.08)	0.170

Abbreviations: AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; CI, confidence interval; CML, chronic myelogenous leukemia; CSA, cyclosporine; HR, hazard ratio; MDS, myelodysplastic syndrome; MMF, mycophenolate mofetil; MTX, methotrexate; TAC, tacrolimus. ^aOther significant variables in model A were; patient age, 16–49 (reference, 1.00), 50–(HR, 1.50, 95% CI, 1.35–1.66, $P < 0.001$); sex of recipient, female (reference, 1.00), male (HR, 1.12; 95% CI, 1.02–1.24; $P = 0.023$); diagnosis, AML (reference, 1.00), ALL (HR, 1.11, 95% CI, 0.98–1.26, $P = 0.112$), CML (HR, 0.90, 95% CI, 0.72–1.13, $P = 0.374$), MDS (HR, 0.81, 95% CI, 0.68–0.95, $P = 0.001$); disease risk, standard risk (reference, 1.00), high risk (HR, 2.24; 95% CI, 2.00–2.50; $P < 0.001$), status not known, (HR, 1.59; 95% CI, 1.21–2.09; $P = 0.001$); GVHD prophylaxis, CSA/TAC + MTX (reference, 1.00), CSA/TAC only (HR, 1.23; 95% CI, 1.09–1.39; $P = 0.001$), CSA/TAC + steroid/MMF (HR, 1.02; 95% CI, 0.86–1.21; $P = 0.820$), other/missing (HR, 1.21; 95% CI, 0.82–1.78; $P = 0.342$); year of transplantation, 1998–2004 (reference, 1.00), 2005–2009 (HR, 0.89; 95% CI, 0.80–0.99; $P = 0.038$). ^bOther significant variables in model A were; patient age, 16–49 (reference, 1.00), 50–(HR, 1.72, 95% CI, 1.42–2.07, $P < 0.001$); GVHD prophylaxis, CSA/TAC + MTX (reference, 1.00), CSA/TAC only (HR, 1.43; 95% CI, 1.14–1.78; $P = 0.002$), CSA/TAC + steroid/MMF (HR, 1.00; 95% CI, 0.73–1.37; $P = 0.995$), other/missing (HR, 1.51; 95% CI, 0.67–3.39; $P = 0.319$). ^cOther significant variables were; patient age, 16–49 (reference, 1.00), 50–(HR, 1.41, 95% CI, 1.23–1.61, $P < 0.001$); diagnosis, AML (reference, 1.00), ALL (HR, 1.13, 95% CI, 0.95–1.34, $P = 0.183$), CML (HR, 0.94, 95% CI, 0.70–1.27, $P = 0.704$), MDS (HR, 0.73, 95% CI, 0.60–0.89, $P = 0.002$).

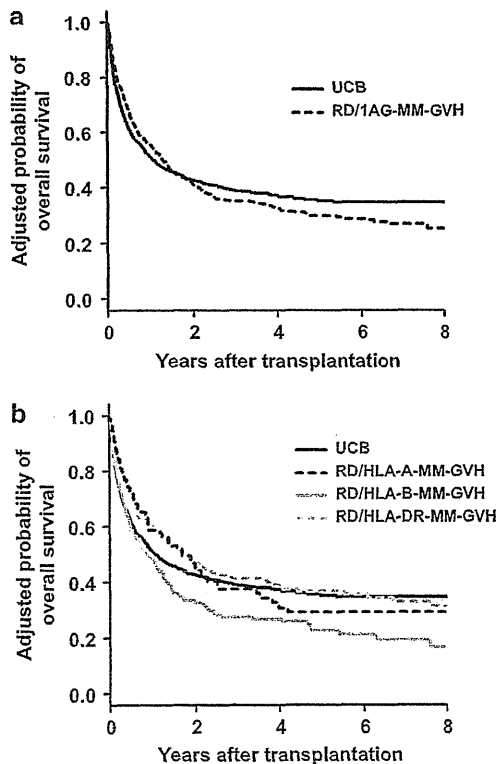


Figure 3. Overall survival. Overall survival rates in the transplantation using an unrelated cord blood vs a related donor with a 1-antigen mismatch at the HLA-A, HLA-B or HLA-DR locus in the GVH direction (a) or with an HLA-A, -B, or -DR antigen mismatch in the GVH direction (b) are shown.

of transplantation (1998–2004). Figure 3a shows the adjusted survival curves of the two groups. Next, the HLA-A, HLA-B and HLA-DR mismatched groups in transplantation from an RD/1AG-MM-GVH were compared with the UCB group. The OS rate of

patients who received transplantation from an RD/1AG-MM-GVH involving an HLA-B mismatch was significantly lower than that in the UCB group ($P = 0.043$; Figure 3b and Table 2), and a subgroup analysis revealed that the adverse effect of an HLA-B mismatch was significant only in standard-risk patients (standard-risk, $P = 0.023$; high-risk, $P = 0.326$; Table 2).

Relapse and NRM

The 3-year relapse rates in the UCB and RD/1AG-MM-GVH groups were 35% (95%CI, 33–37%) and 32% (95% CI, 28–36%), respectively (Gray test; $P = 0.041$; Figure 4a), and a significant decrease in the incidence of relapse was found in the RD/1AG-MM-GVH group in the multivariate analysis (RD/1AG-MM-GVH vs UCB, HR, 0.78, 95%CI, 0.64–0.95, $P = 0.012$; Table 3). The impact of reducing the incidence of relapse did not differ according to the HLA mismatch antigen in the RD/1AG-MM-GVH group (Table 3 and Figure 4b). The 3-year NRM rates in the UCB and RD/1AG-MM-GVH groups were 30% (95% CI, 28–32%) and 32% (95% CI, 28–36%), respectively (Gray test; $P = 0.474$; Figure 4c), and a significant increase in the NRM rate was observed in the RD/1AG-MM-GVH group in the multivariate analysis (RD/1AG-MM-GVH vs UCB, HR, 1.24, 95% CI, 1.04–1.47, $P = 0.016$; Table 3). In particular, the NRM rate of patients who received transplantation from an RD/1AG-MM-GVH with an HLA-B mismatch was significantly higher than that in the UCB group (RD/1AG-MM-GVH vs UCB, HR, 1.50, 95% CI, 1.17–1.92, $P = 0.001$; Figure 4d and Table 3).

The causes of death in patients who died without relapse are shown in Supplementary Table 3. The rates of GVHD and organ failure in the RD/1AG-MM-GVH group were higher than those in the UCB group (GVHD, 18 vs 10%, organ failure, 28 vs 19%), whereas the rates of graft failure and infection were lower in the RD/1AG-MM-GVH group (graft failure, 1 vs 5%; infection, 26 vs 38%).

The impact of the use of *in vivo* T-cell depletion in the RD/1AG-MM-GVH group

Based on the fact that the leading causes of death in the RD/1AG-MM-GVH group were GVHD and organ failure, we analyzed the risk factors for the development of acute GVHD in this group.

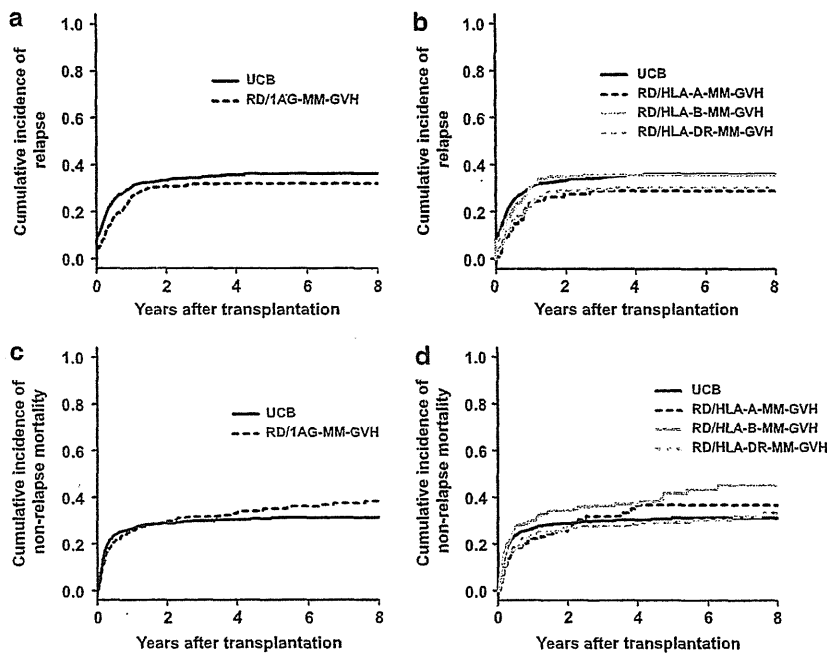


Figure 4. Relapse and non-relapse mortality. Cumulative incidence of relapse and non-relapse mortality after transplantation using an unrelated cord blood vs a related donor with a 1-antigen mismatch at the HLA-A, HLA-B or HLA-DR locus in the GVH direction (a, c) or with an HLA-A, -B, or -DR antigen mismatch in the GVH direction (b, d) are shown.

Variable	Relapse ^a		Non-relapse mortality ^b	
	HR (95% CI)	P value	HR (95% CI)	P value
(A)				
UCB	1.00	reference	1.00	reference
RD/1AG-MM-GVH	0.78 (0.64–0.95)	0.012	1.24 (1.04–1.47)	0.016
(B)				
UCB	1.00	reference	1.00	reference
RD/HLA-A-MM-GVH	0.70 (0.49–1.00)	0.050	1.28 (0.93–1.76)	0.130
RD/HLA-B-MM-GVH	0.81 (0.62–1.07)	0.134	1.50 (1.17–1.92)	0.001
RD/HLA-DR-MM-GVH	0.80 (0.61–1.04)	0.096	1.02 (0.78–1.32)	0.901

Abbreviations: AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; CI, confidence interval; CML, chronic myelogenous leukemia; CSA, cyclosporine; HR, hazard ratio; MDS, myelodysplastic syndrome; MMF, mycophenolate mofetil; MTX, methotrexate; TAC, tacrolimus. ^aOther significant variables in model A were: diagnosis, AML (reference, 1.00), ALL (HR, 1.09, 95% CI, 0.92–1.29, $P=0.336$), CML (HR, 1.39, 95% CI, 1.05–1.82, $P=0.019$), MDS (HR, 0.59, 95% CI, 0.46–0.76, $P<0.001$); time from diagnosis to transplantation, <6 months (reference, 1.00), ≥ 6 months (HR, 0.80; 95% CI, 0.70–0.92; $P=0.002$); disease risk, standard risk (reference, 1.00), high risk (HR, 2.81; 95% CI, 2.41–3.27; $P<0.001$), status not known, (HR, 2.17; 95% CI, 1.45–3.23; $P<0.001$); conditioning intensity, myeloablative (reference, 1.00), reduced intensity (HR, 1.22; 95% CI, 1.04–1.44; $P=0.014$); GVHD prophylaxis, CSA/TAC + MTX (reference, 1.00), CSA/TAC only (HR, 0.65; 95% CI, 0.53–0.78; $P<0.001$), CSA/TAC + steroid/MMF (HR, 0.75; 95% CI, 0.59–0.96; $P=0.024$), other/missing (HR, 0.94; 95% CI, 0.55–1.61; $P=0.825$). ^bOther significant variables in model A were: patient age, 16–49 (reference, 1.00), 50–(HR, 1.70, 95% CI, 1.47–1.98, $P<0.001$); GVHD prophylaxis, CSA/TAC + MTX (reference, 1.00), CSA/TAC only (HR, 1.70; 95% CI, 1.44–2.01; $P<0.001$), CSA/TAC + steroid/MMF (HR, 1.18; 95% CI, 0.94–1.49; $P=0.158$), other/missing (HR, 1.47; 95% CI, 0.86–2.51; $P=0.154$); year of transplantation, 1998–2004 (reference, 1.00), 2005–2009 (HR, 0.76; 95% CI, 0.66–0.88; $P<0.001$).

In multivariate analysis, two factors were found to be significantly associated with the risk of developing grade II–IV acute GVHD in the RD/1AG-MM-GVH group: the use of *in vivo* T-cell depletion and source of stem cells (use of *in vivo* T-cell depletion, yes vs no, HR 0.40, $P=0.002$, PB vs BM, HR 1.61, $P<0.001$).

Because the use of *in vivo* T-cell depletion significantly lowered the risk of acute GVHD, we re-compared the RD/1AG-MM-GVH group and the UCB group while focusing on the use of *in vivo* T-cell depletion in the RD/1AG-MM-GVH group. The incidence of grade II–IV or grade III–IV acute GVHD or chronic or extensive chronic GVHD in the RD/1AG-MM-GVH group using *in vivo* T-cell depletion was comparable to that in the UCB group

(Supplementary Figure 1 and Supplementary Table 4), whereas the incidences of neutrophil and platelet engraftment were significantly higher in the RD/1AG-MM-GVH group using *in vivo* T-cell depletion than in the UCB group (neutrophil engraftment, HR 5.52, 95% CI, 3.36–9.05, $P<0.001$; platelet engraftment, HR 2.01, 95% CI, 1.26–3.21, $P<0.001$). Compared to the UCB group, the RD/1AG-MM-GVH group with T-cell depletion showed lower overall and NRM, albeit these differences were not significant, which suggests that the use of *in vivo* T-cell depletion may improve the outcome of transplantation from an RD/1AG-MM-GVH (Figure 5, Supplementary Table 5). It is interesting to note that the adverse impact of an HLA-B mismatch vs HLA-A or -DR

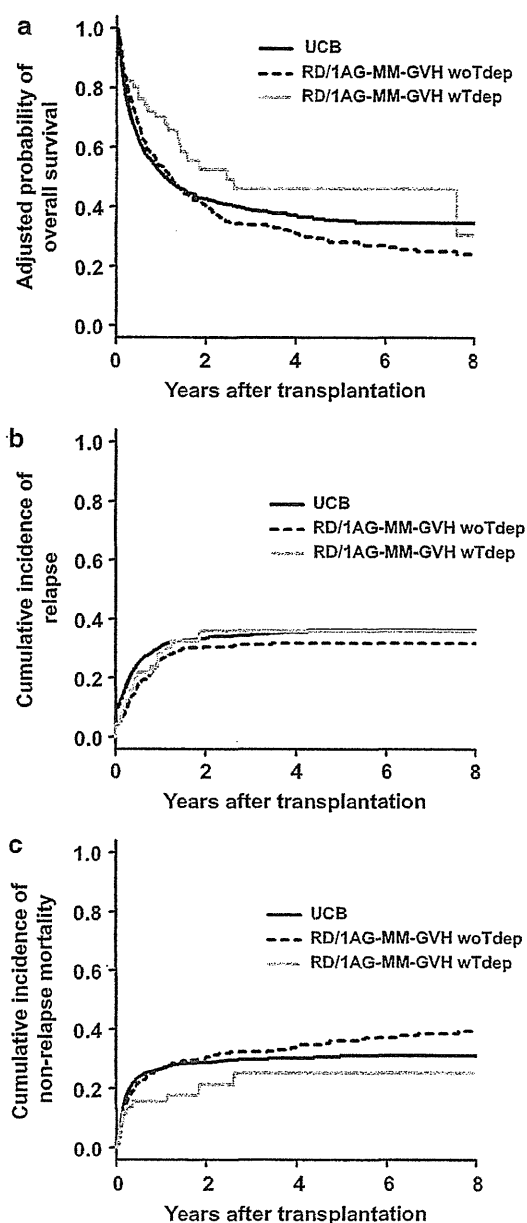


Figure 5. OS (a), relapse (b) and NRM (c) according to the use of *in vivo* T-cell depletion in the RD/1AG-MM-GVH group.

mismatch in the RD/1AG-MM-GVH group disappeared with the use of *in vivo* T-cell depletion (with *in vivo* T-cell depletion; HLA-B vs HLA-A/DR mismatch; HR 1.08, 95% CI, 0.45–2.62, $P=0.864$, without *in vivo* T-cell depletion; HLA-B vs HLA-A/DR mismatch; HR 1.59, 95% CI, 1.25–2.01, $P<0.001$).

With regard to the effect of stem cell source, the incidence of acute and chronic GVHD in the RD/1AG-MM-GVH group using BM was lower than that with PB but higher than that with UCB (Supplementary Figure 2). The use of PB or BM did not affect OS, relapse, or NRM (Supplementary Table 5).

DISCUSSION

In this nationwide retrospective study, we found that the survival rate in the UCB group was comparable to that in the RD/1AG-MM-GVH group regardless of the disease risk. The RD/1AG-MM-GVH

group with an HLA-B mismatch showed significantly higher overall and NRM, whereas the RD/1AG-MM-GVH group with an HLA-A or HLA-DR mismatch showed an OS comparable to that in the UCB group. Neutrophil and platelet engraftment in the RD/1AG-MM-GVH group were significantly faster than those in the UCB group, whereas the incidence of acute or chronic GVHD in the RD/1AG-MM-GVH group was significantly higher. However, the incidence of acute or chronic GVHD in the RD/1AG-MM-GVH group with *in vivo* T-cell depletion was comparable to that in the UCB group, which translated into a better, but not significantly better, OS than that in the UCB group.

In Japan, unrelated BM donor coordination (from donor search to transplantation) takes a median of 4 months, whereas much less time is required for UCB or RD/1AG-MM-GVH transplantation if there is a candidate. This was reflected in the longer duration from diagnosis to transplantation in unrelated BM transplantation.³² In contrast, UCB and RD/1AG-MM-GVH transplantation show a similar and shorter duration (Table 1; 7.9 months vs 7.6 months). Therefore, in cases where both UCB and RD/1AG-MM-GVH are available, donors should be chosen based on their advantages and disadvantages. Compared with UCB, the use of RD/1AG-MM-GVH has a great advantage in neutrophil and platelet engraftment, which is not inconsistent with a previous finding that engraftment in the UCB group was significantly delayed comparing with that in MUD.³³ This translated into a lower rate of death from graft failure or infection in the RD/1AG-MM-GVH group. However, these advantages were offset by a substantial increase in the incidence of acute and chronic GVHD in the RD/1AG-MM-GVH group. The risk of grade III–IV acute GVHD and extensive chronic GVHD in the RD/1AG-MM-GVH group was twice that in the UCB group. If UCB units containing adequate total nucleated cell doses (ex. $>2.5 \times 10^7/\text{kg}$) are available,³⁴ the selection of UCB would be appropriate to avoid the risk of chronic GVHD. In contrast, RD/1AG-MM-GVH would be more appropriate when early neutrophil engraftment should be prioritized, such as for a patient with an active infectious disease at transplantation.

The high incidences of GVHD and GVHD-related death in the RD/1AG-MM-GVH group indicate the need for stronger immunosuppression to improve the clinical outcome. The use of T-cell depletion, mostly by ATG, was significantly associated with a lower incidence of grade III–IV acute GVHD and extensive chronic GVHD in the RD/1AG-MM-GVH group. Although this effect was not statistically significant, the RD/1AG-MM-GVH group with *in vivo* T-cell depletion showed lower overall and treatment-related mortality, which would outweigh a possible increased risk of relapse. These findings in our cohort suggest that ATG may be effective, and the addition of ATG in the RD/1AG-MM-GVH group should be assessed in a prospective study.

As shown in our previous study,²³ overall mortality in the RD/1AG-MM-GVH group involving an HLA-B mismatch was significantly higher than that in the RD/1AG-MM-GVH group with an HLA-A or -DR mismatch, probably because of an additional HLA-C antigen mismatch as expected from linkage disequilibrium between HLA-B and HLA-C and available data on HLA-C antigen.^{23,35} The incidence of grade III–IV acute GVHD in the HLA-B mismatch group was higher than that in the HLA-DR mismatch group, but was comparable to that in the HLA-A mismatch group. In addition, the incidence of death from GVHD was similar in the HLA-B and HLA-A/DR mismatch groups (data not shown). Therefore, the reason for the lower overall mortality in the RD/1AG-MM-GVH group with an HLA-B mismatch remains unclear. However, the adverse effect of an HLA-B mismatch disappeared when *in vivo* T-cell depletion was used, which suggests that an immunological effect is involved in this mechanism.

This study has several limitations. First, in clinical practice in Japan, matching of HLA-DR is counted at a low resolution, as with HLA-A and HLA-B, whereas it is counted at a high resolution in the

United States and Europe. To evaluate the impact of this difference, we divided patients in the UCB group with two antigen mismatches into two groups by using available HLA-DRB1 allele information: a group with two antigen mismatches with one additional HLA-DRB1 allele mismatch ($n=609$) and another group with two antigen mismatches without an additional HLA-DRB1 mismatch ($n=295$). We did not find a significant difference in OS between these two groups ($P=0.758$), which suggests that HLA-matching using HLA-DR antigen or allele information will not affect OS in the present study. Second, the findings in the present study are based on Asian cohort who received a 'single' UCB or RD/1AG-MM-GVH transplantation. Lighter body weight in Asian population than Caucasian population may make it easy to find a suitable single UCB unit that contains adequate total nucleated cell doses. In addition, as suggested by Oh *et al.*,³⁶ limited heterogeneity of Japanese population may affect the outcomes of transplantation. Therefore, the findings should be externally validated in the non-Asian cohort or transplantation using double UCB units. Third, information on the dose and type of ATG was missing in two-third of the patients who received ATG. However, the available data showed that the median dose of thymoglobulin (2.5 mg/kg) or ATG-F (8 mg/kg) was equivalent to the dose that is widely used in our daily practice. Lastly, heterogeneous backgrounds may have resulted in a bias, although we tried to adjust for possible confounders by multivariate analyses. Lastly, the effect of multiple testing should be taken into account for the interpretation of secondary end points.

In conclusion, our findings suggest that both UCB and RD/1AG-MM-GVH are suitable as alternative donors for patients without an HLA-matched sibling or unrelated donor. However, the presence of an HLA-B-antigen mismatch in the GVH direction has an adverse effect on OS because of treatment-related complications. Neutrophil and platelet engraftment in the RD/1AG-MM-GVH group were significantly faster than those in the UCB group, whereas the incidence of acute and chronic GVHD in the RD/1AG-MM-GVH group was significantly higher, which translated into a high incidence of death from GVHD. Donor selection between UCB and RD/1AG-MM-GVH should be determined based on the presence of an HLA-B mismatch in RD/1AG-MM-GVH and from the risks and benefits derived from the risk of graft failure and infection in the UCB group and acute or chronic GVHD in the RD/1AG-MM-GVH group. Additional immune suppression using *in vivo* T-cell depletion may improve the clinical outcome in the RD/1AG-MM-GVH group by decreasing the incidences of GVHD and NRM and may also overcome the adverse effect of an HLA-B mismatch. This approach should be assessed in a prospective study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We are indebted to all of the physicians and data managers who contributed valuable data on transplantation to the Japan Society for Hematopoietic Cell Transplantation and the Japan Cord Blood Bank Network. We also thank the members of the data management committees of the Japan Society for Hematopoietic Cell Transplantation and the Japan Cord Blood Bank Network for managing data. JK is a research fellow of the Japan Society for the Promotion of Science. This work was supported in part by Grant-in-Aid for JSPS Fellows (JK).

AUTHOR CONTRIBUTIONS

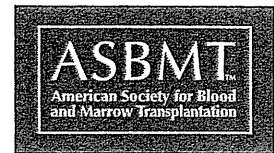
JK and YK designed the research, organized the project and wrote the manuscript; JK, YA, and YK performed the statistical analysis and analyzed the data; KK and TN-I collected data from JCBBN; and all of the authors interpreted the data and reviewed and approved the final manuscript.

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Supplementary Information accompanies the paper on the Leukemia website (<http://www.nature.com/leu>)



Comparison of Unrelated Cord Blood Transplantation and HLA-Mismatched Unrelated Bone Marrow Transplantation for Adults with Leukemia

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Recent advances in unrelated cord blood transplantation (UCBT) and high-resolution typing of human leukocyte antigen (HLA) from an unrelated donor have increased choices in alternative donor/stem cell source selection. We assessed HLA-mismatched locus-specific comparison of the outcomes of 351 single-unit UCB and 1,028 unrelated bone marrow (UBM) adult recipients 16 years old or older at the time of transplantation who received first stem cell transplantation with myeloablative conditioning for acute leukemia or myelodysplastic syndromes. With adjusted analyses, HLA 0 to 2 mismatched UCBT showed similar overall mortality (relative risk [RR] = 0.85, 95% confidence interval [CI], 0.68-1.06; $P = .149$) compared with that of single-HLA-DRB1-mismatched UBMT. UCBT showed inferior neutrophil recovery (RR = 0.50, 95% CI, 0.42-0.60; $P < .001$), lower risk of acute graft-versus-host disease (RR = 0.55, 95% CI, 0.42-0.72; $P < .001$), and lower risk of transplantation-related mortality (RR = 0.68, 95% CI, 0.50-0.92; $P = .011$) compared with single-HLA-DRB1-mismatched UBMT. No significant difference was observed for risk of relapse (RR = 1.28, 95% CI, 0.93-1.76; $P = .125$). HLA 0 to 2 antigen-mismatched UCBT is a reasonable second alternative donor/stem cell source with a survival outcome similar to that of single-HLA-DRB1-mismatched or other 7 of 8 UBMT.

Biol Blood Marrow Transplant 18: 780-787 (2012) © 2012 American Society for Blood and Marrow Transplantation

KEY WORDS: Unrelated cord blood transplantation, HLA-mismatched unrelated bone marrow transplantation

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Financial disclosure: See Acknowledgments on page 786.

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Received July 20, 2011; accepted October 9, 2011

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doi:10.1016/j.bbmt.2011.10.008

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is a widely used, curative treatment for hematologic malignancies. When available, a human leukocyte antigen (HLA)-identical sibling is the donor of choice. However, only about 30% of candidates eligible for allogeneic HSCT will have such a donor. In addition, older patients with older siblings have more difficulty finding such a donor capable of stem cell donation. High-resolution donor-recipient HLA matching has contributed to the success of unrelated donor marrow transplantation, and the current first recommended alternative donor after an HLA-matched sibling for HSCT is an HLA-A, -B, -C, and -DRB1 8 of 8-allele-matched unrelated donor [1-4]. However, there are still a significant number of patients for which finding an HLA 8 of 8-matched unrelated donor is difficult and for whom a second alternative donor/stem cell source should be found.

The effect of HLA mismatches after bone marrow transplantation from unrelated donors (UBMT) has been well studied, and single mismatched UBM donors are usually selected as a second alternative donor/stem cell source [1-4]. Lee et al. [3] showed that a single mismatch, antigen-level, or high-resolution, at HLA-A, -B, -C, or -DRB1 loci was associated with higher mortality and decreased survival. However, the reduction in survival may be acceptable in comparison with the survival rates for currently available alternative treatments. Analyses from the Japan Marrow Donor Program (JMDP) showed better survival in HLA class II mismatched recipients; thus, single-DRB1-mismatched UBM donor is currently a second alternative in Japan [1,2,5].

Recent advances in unrelated cord blood transplantation (UCBT) have provided patients with increased choices for a second alternative donor/stem cell source [6]. Clinical comparison studies of cord blood transplantation and HLA-A, -B, and -DRB1 6 of 6 allele-matched bone marrow transplantation for leukemia from unrelated donors in adult recipients showed comparable results [7-9]. More recently, promising outcomes of UCBT were shown compared with HLA-A, -B, -C, and -DRB1 8 of 8 allele-matched UBMT, the current first alternative donor/stem cell source [10-12].

The aim of this study was to determine the utility of UCBT as a second-alternative donor source in adult patients with acute leukemia or myelodysplastic syndromes. It is common today to perform high-resolution typing of HLA for donor selection of unrelated donors; thus, we performed mismatched-allele-specific analyses for comparison of HLA-mismatched UBMT and UCBT in terms of overall survival (OS) and other HSCT outcomes, setting single-DRB1-mismatched UBMT, the current second alternative, as the reference.

PATIENTS AND METHODS

Collection of Data and Data Source

The recipients' clinical data were provided by the Japan Cord Blood Bank Network (JCBBN) and the JMDP [13]. Peripheral blood stem cell donation from unrelated donors was not permitted in Japan during the study period. All 11 cord blood banks in Japan are affiliated with JCBBN. Both JCBBN and JMDP collect recipients' clinical information at 100 days posttransplantation. Patients' information on survival, disease status, and long-term complications including chronic graft-versus-host (cGVHD) disease and second malignancies is renewed annually using follow-up forms. This study was approved by the institutional review board of Nagoya University Graduate School of Medicine.

Patients

The subjects were adult patients of at least 16 years of age with acute myeloid leukemia, acute lymphoblastic leukemia, and myelodysplastic syndromes, who were recipients of first UBMT or UCBT with myeloablative conditioning. All patients in the UCBT cohort received a single-unit CB. Transplantation years were between 1996 and 2005 for UBMT and between 2000 and 2005 for UCBT to avoid the first 3 years of a pioneering period (1993-1995 for UBMT and 1997-1999 for UCBT). There were no statistically significant differences between UBMT in 1996-1999 and UBMT in 2000-2005 in probabilities of OS (41% versus 44%, at 3 years posttransplantation; $P = .86$) and in relapse-free survival (RFS) (40% versus 40%, at 3 years posttransplantation; $P = .93$).

Among 2,253 UBMT recipients with complete HLA high-resolution data, the following recipients with HLA -A, -B, -C, and -DRB1 8 of 8 allele match ($n = 1,079$) and more than three mismatches (5 of 8 allele match [$n = 117$], 4 of 8 allele match [$n = 24$], 3 of 8 allele match [$n = 4$], 2 of 8 allele match [$n = 1$]) were excluded. There were no statistically significant differences in risk of mortality or treatment failure (RFS) associated with single high-resolution (allele) versus single low-resolution (antigen) mismatches (data not shown), so in the analyses, allele and antigen mismatches were considered equivalent. HLA matching of cord blood was performed using low-resolution molecular typing methods for HLA-A and -B, and high-resolution molecular typing for HLA-DRB1. Of 557 recipients of CB with complete HLA data, 105 recipients with three mismatches and nine recipients with four mismatches were excluded. A total of 1,028 UBMT recipients (248 HLA class II locus mismatched, 424 HLA class I locus mismatched, and 356 HLA 2 loci mismatched) and 351 UCBT recipients (20 HLA-A, -B, low-resolution and -DRB1 matched, 87

locus mismatched, and 244 2 loci mismatched) were the subjects for analyses. Both host-versus-graft and graft-versus-host directions were accounted for in terms of HLA mismatch.

HLA Typing

Alleles at the HLA-A, -B, -C, and -DRB1 with unrelated bone marrow donor-recipient pairs and for HLA-DRB1 for unrelated cord blood donor-recipient pairs were identified by the methods described previously [1,5,14]. Serologic or antigen-level typing was performed with a standard two-stage complement-dependent test of microcytotoxicity or low-resolution DNA-based typing usually by collapsing the four-digit typing result back to its first two digits in part.

Definitions

The primary outcome of the analyses was OS, defined as time from transplantation to death from any cause. A number of secondary endpoints were also analyzed. Neutrophil recovery was defined by an absolute neutrophil count of at least 500 cells per cubic millimeter for three consecutive points; platelet recovery was defined by a count of at least 50,000 platelets per cubic millimeter without transfusion support. Diagnosis and clinical grading of acute GVHD (aGVHD) were performed according to the established criteria [15,16]. Relapse was defined as a recurrence of underlying hematologic malignant diseases. Transplantation-related death was defined as death during a continuous remission. RFS was defined as survival in a state of continuous remission.

Statistical Analysis

Descriptive statistical analysis was performed to assess patient baseline characteristics, diagnosis, disease status at conditioning, donor-patient ABO mismatches, preparative regimen, and GVHD prophylaxis. Medians and ranges are provided for continuous variables and percentages for categorical variables. Cumulative incidence curves were used in a competing-risks setting to calculate the probability of aGVHD and cGVHD, relapse, and transplantation-related mortality (TRM) [17]. Gray's test was used for group comparison of cumulative incidences [18]. Adjusted comparison of the groups on OS and RFS was performed with the use of the Cox proportional-hazards regression model [19]. For other outcomes with competing risks, Fine and Gray's proportional-hazards model for subdistribution of a competing risk was used [20]. For neutrophil and platelet recovery, death before neutrophil or platelet recovery was the competing event; for GVHD, death without GVHD and relapse were the competing events; for relapse, death without relapse was the competing

event; and, for TRM, relapse was the competing event [21]. Adjusted probabilities of OS and RFS were estimated using the Cox proportional-hazards regression model, with consideration of other significant clinical variables in the final multivariate models. The variables considered were the patient's age at transplantation, patient's sex, donor-patient sex mismatch, donor-patient ABO mismatch, diagnosis, disease status at conditioning, the conditioning regimen, and the type of prophylaxis against GVHD. Factors differing in distribution between CB and BM recipients and factors known to influence outcomes were included in the final models. Variables with more than two categories were dichotomized for the final multivariate model. Variables were dichotomized as follows: patient age >40 or <40 years at transplantation, recipient's sex, sex-mismatched donor-patient pair versus sex-matched pair, donor-recipient ABO major mismatch versus others for ABO matching, advanced versus standard (first and second complete remission of acute myeloid leukemia, first complete remission of acute lymphoblastic leukemia, or refractory anemia or refractory anemia with ring sideroblasts of myelodysplastic syndromes) risk of the disease, cyclophosphamide, and total-body irradiation (TBI) or busulfan and cyclophosphamide or others for conditioning regimen, and cyclosporine-based versus tacrolimus-based prophylaxis against GVHD. No significant interactions were identified between each variable and HLA disparity/stem cell source groups. All *P* values were two-sided.

RESULTS

Patient Characteristics

Table 1 shows characteristics of patients, their disease, and transplantation regimens. Proportions of females, sex-mismatched donor-recipient pairs, and ABO mismatched donor recipient pairs were larger in cord blood recipients ($P < .001$, $P < .001$, and $P < .001$, respectively). UCB recipients were older than recipients of UBM (median age, 37 years versus 34 years; $P < .001$). A preparative regimen with TBI and cyclophosphamide was used in the majority of patients in all groups, and cytosine arabinoside was supplemented for CB recipients in addition to TBI and cyclophosphamide in about half the recipients with cyclophosphamide and TBI. For GVHD prophylaxis, tacrolimus and short-term methotrexate was used preferentially in BM recipients (61% of DRB1-one-mismatched BM recipients), while cyclosporine A and short-term methotrexate was used preferentially in CB recipients (61%). The median follow-up period for survivors was 2.1 years (range, 0.1-6.2) for CB recipients and 5.5 (range, 0.3-11.6) years for BM recipients.

Table I. Patient, Disease, and Transplantation Characteristics According to Stem Cell Source and Number of Mismatched Loci

	Bone Marrow Transplant			
	Class II One Locus Mismatch	Class I One Locus Mismatch	Two Loci Mismatch	Cord Blood Transplantation
	N (%)	N (%)	N (%)	N (%)
Number of transplantations	248	424	356	351
Patient age at transplantation				
Median (range)	36 (16-60)	34 (16-67)	34 (16-59)	37 (16-58)
Patient sex				
Male	151 (61)	241 (57)	210 (59)	162 (46)
Female	97 (39)	183 (43)	146 (41)	189 (54)
Sex matching				
Matched	145 (58)	268 (63)	217 (61)	170 (48)
Male to female	52 (21)	82 (19)	73 (21)	97 (28)
Female to male	50 (20)	71 (17)	64 (18)	84 (24)
Unknown	1 (<1)	3 (1)	2 (1)	0 (0)
Diagnosis				
AML	135 (54)	204 (48)	172 (48)	193 (55)
ALL	78 (31)	149 (35)	135 (38)	113 (32)
MDS	35 (14)	71 (17)	49 (14)	45 (13)
Disease status				
Standard	124 (50)	214 (50)	168 (47)	147 (42)
Advanced	114 (46)	195 (46)	169 (47)	174 (50)
Unknown	10 (4)	15 (4)	19 (5)	30 (9)
ABO matching				
Matched	119 (48)	184 (43)	153 (43)	114 (32)
Minor mismatch	53 (21)	108 (25)	85 (24)	99 (28)
Major mismatch	67 (27)	116 (27)	97 (27)	73 (21)
Bidirectional	8 (3)	12 (3)	14 (4)	64 (18)
Unknown	1 (<1)	4 (1)	7 (2)	1 (<1)
HLA-mismatched number and direction				
Matched				20 (6)
One locus mismatched				87 (25)
HVG direction	16 (6)	38 (9)		8 (9)
GVH direction	17 (7)	30 (7)		8 (9)
Both directions	215 (87)	356 (84)		71 (82)
Two loci mismatched				244 (70)
Two HVG direction			4 (1)	2 (1)
One HVG direction and one GVH direction			6 (2)	4 (2)
Two GVH direction			4 (1)	3 (1)
One both directions and one HVG direction			42 (12)	40 (16)
One both directions and one GVH direction			29 (8)	28 (11)
Two both directions			271 (76)	167 (68)
No. of nucleated cells infused ($\times 10^7$ /kg)				
Median	25.0	24.5	23	2.46
Range	2.40-59.8	2.10-97.5	1.5-66.0	1.41-6.01
Preparative regimen				
CY + TBI	94 (38)	168 (40)	151 (42)	109 (31)
CY + CA + TBI	46 (19)	78 (18)	74 (21)	124 (35)
CY + BU + TBI	20 (8)	39 (9)	27 (8)	15 (4)
Other TBI regimen	45 (18)	70 (17)	61 (17)	80 (23)
BU + CY	34 (14)	54 (13)	30 (8)	21 (6)
Other non-TBI regimen	9 (4)	15 (4)	13 (4)	2 (1)
GVHD prophylaxis				
Cyclosporine A + sMTX	87 (35)	221 (52)	150 (42)	213 (61)
Cyclosporine A \pm other	1 (<1)	5 (1)	5 (1)	24 (7)
Tacrolimus + sMTX	152 (61)	191 (45)	193 (54)	76 (22)
Tacrolimus \pm other	8 (3)	5 (1)	6 (2)	35 (10)
Others	0 (0)	2 (<1)	2 (<1)	3 (1)

ALL indicates acute lymphoblastic leukemia; AML, acute myelogenous leukemia; BU, oral busulfan; CA, citarabine; CY, cyclophosphamide; GVH, graft-versus-host; HVG, host-versus-graft; MDS, myelodysplastic syndromes; sMTX, short-term methotrexate.

Outcome

OS and RFS

OS and RFS for CB recipients were similar when compared with that of single-HLA-DRB1-mismatched BM recipients (relative risk [RR] = 0.85, 95% confidence interval [CI], 0.68-1.06; $P = .149$ for OS and $RR = 0.97$, 95% CI, 0.92-1.35; $P = .747$) (Table 2).

The adjusted probabilities of survival at 3 years posttransplantation of CB recipients (47%) were not

different from those of single HLA-DRB1 mismatched BM recipients (41%; $P = .19$) or single HLA class I-mismatched BM recipients (47%; $P = .96$), but superior to those of 6 of 8 BM recipients (38%; $P = .014$) (Figure 1A). Figure 1B shows adjusted RFS curves (42% for CB recipients, 36% for single HLA-DRB1-mismatched BM, 44% for single HLA class I-mismatched BM, and 36% for 6 of 8 BM recipients, at 3 years posttransplant) (P values of comparison between CB and single HLA-DRB1-mismatched BM, CB, and single HLA

Table 2. Multivariate Analyses of Overall Survival, Relapse-Free Survival, Relapse, and Transplant-Related Mortality

Degree of HLA Mismatch	N	Overall Survival			Relapse-Free Survival			Relapse			Transplant-Related Mortality		
		RR	(95% CI)	P value	RR	(95% CI)	P value	RR	(95% CI)	P value	RR	(95% CI)	P value
Bone marrow transplant	248	1.00			1.00			1.00			1.00		
Single DRB1 (7/8)	137	0.84	(0.64-1.11)	.216	0.82	(0.63-1.08)	.158	0.65	(0.41-1.01)	.056	1.07	(0.77-1.49)	.698
Single A or B (7/8)	287	0.89	(0.72-1.12)	.324	0.86	(0.69-1.07)	.170	0.60	(0.41-0.87)	.007	1.13	(0.86-1.48)	.391
Single C (7/8)	144	0.97	(0.74-1.27)	.831	0.95	(0.73-1.24)	.726	0.76	(0.49-1.17)	.208	1.10	(0.78-1.55)	.600
C + DRB1 (6/8)	122	1.22	(0.94-1.59)	.143	1.15	(0.88-1.49)	.300	0.70	(0.44-1.10)	.12	1.42	(1.03-1.96)	.032
A/B + C (6/8)	90	1.25	(0.92-1.68)	.146	1.13	(0.84-1.53)	.409	0.60	(0.35-1.02)	.061	1.48	(1.03-2.13)	.035
Other two loci (6/8)	351	0.85	(0.68-1.06)	.149	0.97	(0.92-1.35)	.747	1.28	(0.93-1.76)	.125	0.68	(0.50-0.92)	.011
Cord blood transplant													

RR indicates relative risk; CI, confidence interval.

Adjusted by patient age at transplantation >40 versus ≤40, patient sex, donor-patient sex mismatch versus matched, ABO major mismatch versus others, advanced versus standard disease status at transplantation, cyclophosphamide and total-body irradiation or busulfan and cyclophosphamide for conditioning versus other conditioning regimen, and cyclosporine-based versus tacrolimus-based prophylaxis against graft-versus-host disease.

class I-mismatched BM, and CB and 6 of 8 BM recipients were 0.80, 0.12, and 0.43, respectively).

Relapse and TRM

There was no significant increase of relapse rates among CB recipients when compared with DRB1 single-mismatched BM recipients (RR = 1.28, 95% CI, 0.93-1.76; P = .125). The risk of TRM was lower in CB recipients compared with that of single HLA-DRB1-mismatched BM recipients (RR = 0.68, 95% CI, 0.50-0.92; P = .011) (Table 2). The risk of TRM was also lower in CB recipients when compared with 6 of 8 BM recipients (RR = 0.52, 95% CI, 0.39-0.68; P < .001).

Hematologic recovery

Neutrophil and platelet recovery was inferior in CB recipients, as shown in Table 3 (RR = 0.50, 95% CI, 0.42-0.60; P < .001 for neutrophil recovery, RR = 0.52, 95% CI, 0.42-0.63; P < .001 for platelet recovery).

Acute GVHD and chronic GVHD

The risk of grade 2 to 4 or severe (grades 3-4) aGVHD was lower in CB recipients than that of single HLA-DRB1-mismatched BM recipients (RR = 0.55, 95% CI, 0.42-0.72; P < .001 for grade 2 to 4 aGVHD and RR = 0.43, 95% CI, 0.27-0.58; P < .001 for severe aGVHD) (Table 4). Unadjusted cumulative incidence of severe aGVHD was 9% for CB, 19% for single HLA-DRB1-mismatched BM, 18% for single HLA

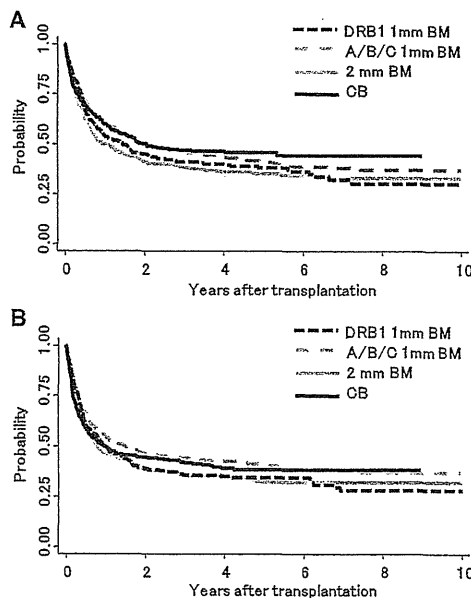


Figure 1. Adjusted probabilities of OS (A) and RFS (B). The adjusted 3-year probabilities of OS for unrelated cord blood recipients, single-HLA-DRB1-mismatched unrelated bone marrow (UBM) recipients, single-HLA-class-I-mismatched UBM, and 6 of 8 UBM recipients were 47%, 41%, 47%, and 38%, respectively (A). The adjusted 3-year probabilities of RFS were 42%, 36%, 44%, and 36%, respectively (B).

Table 3. Multivariate Analyses of Neutrophil and Platelet Recovery

	Degree of HLA Mismatch	N	Neutrophil Recovery			Platelet Recovery		
			RR	(95% CI)	P value	RR	(95% CI)	P value
Bone marrow transplantation	Single DRB1 (7/8)	248	1.00			1.00		
	Single A or B (7/8)	137	1.31	(1.04-1.65)	.021	1.31	(1.01-1.70)	.039
	Single C (7/8)	287	1.19	(0.98-1.43)	.069	0.98	(0.79-1.21)	.840
	C + DRB1 (6/8)	144	0.96	(0.77-1.20)	.735	0.79	(0.62-1.02)	.065
	A/B + C (6/8)	122	1.14	(0.89-1.45)	.307	0.84	(0.63-1.13)	.255
	Other two loci (6/8)	90	0.89	(0.68-1.14)	.346	0.80	(0.58-1.10)	.174
Cord blood transplantation		351	0.50	(0.42-0.60)	<.001	0.52	(0.42-0.63)	<.001

RR indicates relative risk; CI, confidence interval.

Adjusted by patient age at transplantation >40 versus <40, patient sex, donor-patient sex mismatch versus matched, ABO major mismatch versus others, advanced versus standard disease status at transplant, cyclophosphamide, and total-body irradiation or busulfan and cyclophosphamide for conditioning versus other conditioning regimen, and cyclosporine-based versus tacrolimus-based prophylaxis against graft-versus-host disease.

class I-mismatched BM, and 22% for 6 of 8 BM at 100 days posttransplantation ($P < .001$ between CB and single HLA-DRB1-mismatched BM) (Figure 2A).

Among recipients who survived at least 100 days posttransplantation, the risk of developing cGVHD and extensive-type cGVHD was not significantly increased in all HLA disparity groups of CB recipients when compared with that of HLA-DRB1-allele/antigen-mismatched BM recipients (RR = 1.36, 95% CI, 0.99-1.88; $P = .057$ for cGVHD, and RR = 0.86, 95% CI, 0.55-1.34; $P = .500$ for extensive-type cGVHD). The unadjusted cumulative incidence of extensive-type cGVHD was 17% for CB recipients, 20% for single HLA-DRB1-mismatched BM, 25% for single HLA class I-mismatched BM, and 30% for 6 of 8 BM recipients at year posttransplantation ($P = .34$ between CB and single HLA-DRB1-mismatched BM) (Figure 2B).

DISCUSSION

Our main objective was to compare OS after transplantation of UCBT and single-HLA-mismatched UBMT and to provide useful data for selection of an appropriate donor and graft source in second stem cell source/donor selection for adults with hematologic malignancy. To the best of our knowledge, this is the first study to involve mismatched allele/antigen-specific analyses including CB for the process of donor selection. Our results suggest that 0 to 2 HLA-mismatched UCB is a reasonable second alternative of choice for adult patients with leukemia, with similar survival to that of single DRB1-mismatched or other 7 of 8 UBMT recipients, the current first choice for second alternative donor/stem cells.

Neutrophil and platelet recovery was slower in CB recipients than BM recipients, consistent with the results of previous reports [7-10,12]. This is the major limitation of the use of UCB, and several strategies have been studied to reduce the neutropenic period, such as screening for patients' pretransplantation anti-HLA antibodies and their specificity, transplantation of 2 UCB units if a single UCB unit with an ade-

quate cell dose is not available, or direct infusion of UCB into bone marrow [22-26].

Despite higher HLA disparity at the antigen level (69% 2 antigen mismatch, 25% antigen mismatch, and 6% matched), UCB recipients showed lower incidence of severe aGVHD than single DRB1-mismatched UBMT recipients, consistent with other reports that compared UCB with single-mismatched UBMT (7 of 8) [8,11,12]. In our study, tacrolimus and short-term methotrexate were used preferentially in BM recipients, whereas cyclosporine A was used in 68% of CB recipients. Prior studies have shown reduced severe aGVHD with tacrolimus, and this difference may have underscored the improved aGVHD control of UCB over mismatched BM in unadjusted analyses [27,28]. It is likely that decreased risk of grade 2 to 4 aGVHD in UCB recipients contributed to decreased risk of TRM among UCB recipients.

Increasing the number of HLA mismatches from 7 of 8 to 6 of 8 was associated with an approximately 10% reduction in survival in UBMT recipients, which was quite similar to the results from the National Marrow Donor Program [3]. Because we eliminated data from the first 3 pioneering years of unrelated BMT, most of the bone marrow recipients and donors were allele-typed for at least HLA-A, -B, and -DRB1 before transplantation. Survival outcomes of single class I mismatch were not significantly different from those of single class II mismatch in the current analyses. We believe that allele typing of HLA-A, -B, and -DRB1 before transplantation led to better selection of the donor compared with that in the first several years of UBMT. This study includes a large number of fully typed BM and CB recipients, but there are limitations. The choice of stem cell source is influenced by many unmeasured factors that can affect outcome. It is also influenced by the availability of acceptable HLA disparity for unrelated donors and mainly cell dose for cord blood units. Although we have adjusted for known risk factors and disparities between groups, we cannot rule out the influence of potential selection bias, which can only be excluded in a randomized controlled trial. Transplantation years

Table 4. Multivariate Analyses of Acute (Grades 2 to 4 and Grades 3 to 4), Chronic, and Extensive-Type Chronic Graft-versus-Host Disease

Degree of HLA Mismatch	Grade 2-4 acute GVHD			Grade 3-4 acute GVHD			Chronic GVHD			Extensive cGVHD				
	N	RR	(95% CI)	P-value	RR	(95% CI)	P-value	N	RR	(95% CI)	P-value	RR	(95% CI)	P value
Bone marrow transplantation														
Single DRB1 (7/8)	248	1.00			1.00			199	1.00			1.00		
Single A or B (7/8)	137	0.76	(0.55-1.06)	.103	0.91	(0.56-1.47)	.698	111	0.91	(0.61-1.36)	.646	0.89	(0.52-1.50)	.651
Single C (7/8)	287	0.93	(0.72-1.20)	.584	0.91	(0.61-1.35)	.635	227	1.56	(1.15-2.10)	.004	1.79	(1.22-2.63)	.003
C + DRB1 (6/8)	144	0.85	(0.60-1.18)	.320	0.88	(0.54-1.44)	.610	109	1.44	(1.01-2.05)	.041	1.47	(0.93-2.32)	.097
A/B + C (6/8)	122	1.40	(1.04-1.90)	.028	1.90	(1.25-2.87)	.003	87	1.64	(1.14-2.34)	.007	2.26	(1.46-3.50)	<.001
Other two loci (6/8)	90	0.88	(0.60-1.28)	.501	0.65	(0.34-1.22)	.183	60	1.35	(0.86-2.12)	.191	1.15	(0.62-2.13)	.652
Cord blood transplantation	351	0.55	(0.42-0.72)	<.001	0.43	(0.27-0.58)	<.001	252	1.36	(0.99-1.88)	.057	0.86	(0.55-1.34)	.500

GVHD indicates graft-versus-host disease; cGVHD, chronic graft-versus-host disease.

Adjusted by patient age at transplantation >40 versus <40, patient sex, donor-patient sex mismatch versus matched, ABO major mismatch versus others, advanced versus standard disease status at transplantation, cyclophosphamide, and total-body irradiation or busulfan and cyclosporine-based for conditioning versus other conditioning regimen, and cyclosporine-based versus tacrolimus-based prophylaxis against graft-versus-host disease.

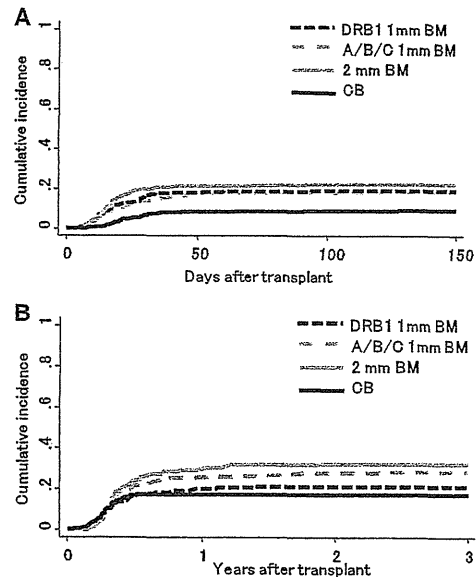


Figure 2. Cumulative incidence of grade 3 to 4 aGVHD (A) and extensive-type cGVHD (B). The cumulative incidences of grade 3 to 4 aGVHD at 100 days posttransplantation for unrelated cord blood recipients, single HLA-DRB1-mismatched unrelated bone marrow (UBM) recipients, and single HLA class I-mismatched UBM were 9%, 19%, 18%, and 22% (A). The cumulative incidences of extensive-type cGVHD at 1-year posttransplantation were 17%, 20%, 25%, and 30% (B).

of UBM recipients included from 1996 and 1999, for which there were no significant outcome differences between UBMT performed in 1996 to 1999 and after 2000. In these periods, there were advances including in supportive care and nutritional management, introduction of new antifungal agents, and more frequent use of tacrolimus, which may have affected transplantation outcomes [27-32].

In conclusion, we suggest that 0 or 2 HLA-mismatched UCB is a comparable second alternative for adult patients with leukemia in the absence of the first alternative, an 8 of 8 UBM donor, with survival similar to that of single DRB1-mismatched or other 7 of 8 UBM recipients. UCB may be preferred over single mismatched UBM when a transplantation is needed urgently, considering the short time needed for UCBT.

ACKNOWLEDGMENTS

The authors are grateful for the assistance and cooperation of all the staff members of the collaborating institutes of the Japan Cord Blood Bank Network and Japan Marrow Donor Program. This work was supported by a Research Grant for Tissue Engineering (H17-014), a Research Grant for Allergic Disease and Immunology (H20-015), a Research Grant for Cancer (H19-1), and a Research Grant for Allergic Disease and Immunology (H23-010) from the Japanese Ministry of Health, Labor, and Welfare.

Financial disclosure: The authors have nothing to disclose.

AUTHORSHIP STATEMENT

Contributions: Y.A., Y.M., R.S., and S. Kato designed the study, and wrote the article; Y.A. analyzed results and created the figures; T.N.I., H.A., and M. Takanashi reviewed and cleaned the Japan Cord Blood Bank Network data, and reviewed the results; S. Taniguchi, S. Takahashi, S. Kai., H.S., Y. Kouzai, N.K., T.M., T.F., and Y. Kodera submitted and cleaned the data; M. Tsuchida, K.K., T.K., and Y.M. reviewed and cleaned the Japan Marrow Donor Program data, and reviewed the results.

SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at doi:10.1016/j.bbmt.2011.10.008.

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ORIGINAL ARTICLE

Allogeneic cord blood transplantation for adult acute lymphoblastic leukemia: retrospective survey involving 256 patients in Japan

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We investigated the efficacy of cord blood transplantation (CBT) for adult acute lymphoblastic leukemia (ALL) by reviewing medical records of 256 patients reported to the Japan Cord Blood Bank Network between June 1997 and August 2006.

Cumulative incidence of neutrophil engraftment at day 100 was 78%. Infused CD34-positive cell dose ($>1 \times 10^5$ cells/kg) was associated with successful neutrophil engraftment. Cumulative incidence of grade II–IV acute graft-versus-host disease (GVHD) at day 100 was 37%. A 2-year disease-free and overall survival (OS) rates were 36% and 42%, respectively. Multivariate analysis showed that age (51 or older vs younger than 50) (hazard ratio 1.9, 95% confidence interval (CI), 1.3–2.8, $P=0.001$), disease status (non-remission vs remission) (hazard ratio 2.2, 95% CI, 1.5–3.2, $P<0.0001$), grade III–IV acute GVHD (hazard ratio 2.0, 95% CI, 1.2–3.2, $P=0.006$) and absence of chronic GVHD (hazard ratio 2.4, 95% CI, 1.1–5.1, $P=0.02$) were negatively associated with OS. CBT is effective for some patients with advanced ALL. It is worth considering for further evaluation.

Leukemia (2012) 26, 1482–1486; doi:10.1038/leu.2012.11

Keywords: cord blood transplantation; adult acute lymphoblastic leukemia; graft-versus-host disease; graft-versus-leukemia effect; engraftment

INTRODUCTION

Adult patients with acute lymphoblastic leukemia (ALL) achieved complete remission with induction chemotherapy in the high rate of 85–90%, although most of them relapse and finally die of disease progression. The indication and timing of allogeneic hematopoietic cell transplantation continue to be debated for adult ALL.^{1–5}

Umbilical cord blood is a promising alternative for allogeneic transplantation. It has a great advantage over bone marrow and peripheral blood because of its immediate availability and lack of invasive interventions to donors. The value of cord blood transplantation (CBT) has been intensively evaluated in previous studies.^{6–9}

Graft-versus-leukemia effects are associated with graft-versus-host disease (GVHD) in allogeneic transplantation for hematological malignancies.¹⁰ Some clinical studies suggested the presence of graft-versus-leukemia effects after CBT in pediatric and adult patients,^{7,11–14} most of them were small sized. At present, limited information is available on the graft-versus-leukemia effects after CBT for adult ALL.

We conducted a retrospective nation-wide study to investigate the usefulness of CBT for adult ALL.

PATIENTS AND METHODS

Data collection

The recipient's clinical data were provided by the Japan Cord Blood Bank Network (JCBBN). All 11 CB banks in Japan are affiliated to JCBBN. The data

management committee of JCBBN collects all the recipients' clinical information at day 100, then 1–5 years after CBT. The numbers of nucleated and CD34-positive cells were provided by the CB banks. The numbers of these cells were measured before cryopreservation. The information on the number of CD3-positive cells in the cord blood was not available.

Between March 1998 and June 2006, 424 adult patients with ALL received CBT and were registered to JCBBN. All recipients received a single cord blood unit. We excluded 77 patients with a history of any types of allogeneic transplantation before CBT. We also excluded adult T-cell leukemia/lymphoma because of its different disease entity. Finally, a total of 256 patients met the criteria. Some of them were reported previously in other studies.^{15–18}

Approval for this study was obtained from the JCBBN institutional review board. CB units were provided with written informed consent in accordance with the Declaration of Helsinki Principles, which approved by the institutional review board of each participating institution.

Definitions and endpoints

Day of neutrophil engraftment was defined as the first of 3 consecutive days on which absolute neutrophil count was >500 cells/ μ l. Graft failure was diagnosed when neutrophil recovery was not achieved within 60 days of transplantation. GVHD was graded according to the criteria published previously.¹⁹ Relapse was defined as presence of ALL cells based on morphological evaluation of the bone marrow or other sites. Patients who had never achieved complete remission after CBT were considered to have

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Received 12 April 2011; revised 3 January 2012; accepted 9 January 2012; accepted article preview online 17 January 2012; advance online publication, 31 January 2012

progression on day 0. Treatment-related mortality (TRM) was defined as deaths without progression of ALL. Reduced-intensity regimens were defined as reported previously.^{20,21}

Statistical analysis

The available data were as of October 2006. The data sets were fixed in March 2008, and we analyzed them between April 2008 and October 2010. The probabilities of overall survival (OS) and disease-free survival (DFS) were estimated by the Kaplan–Meier method. Cumulative incidence curves were used in a competing-risk setting to calculate the probability of engraftment, acute and chronic GVHD, relapse and TRM. For neutrophil and platelet engraftment, death before neutrophil and platelet recovery within 60 days of transplant was the competing event; for GVHD, disease relapse and engraftment failure without GVHD, and deaths within 60 days of transplant without GVHD were the competing events; for relapse, death without relapse was the competing event; and for TRM, death with disease relapse was the competing event.²²

Associations between potential prognostic factors and outcomes were evaluated using the Cox's proportional hazard regression models. The following variables were considered as covariates: age, body weight, human leukocyte antigen mismatch, blood-type mismatch, sex mismatch, infused nucleated cell dose and CD34-positive cell dose, status of underlying disease at transplantation, chromosomal abnormality, preparative regimens and GVHD prophylaxis. Occurrence of acute GVHD was added to the models as a time-dependent covariate. SAS version 9.1.3 (SAS Institute Inc., Cary, NC, USA) was used for all statistical analyses.

RESULTS

Patients' characteristics

Patients' characteristics are shown in Table 1. The median follow-up of the surviving patients was 20.5 months (range, 1.1–86.4). Ph-positive ALL comprised 39% of the population analyzed. No patients received tyrosine kinase inhibitors for maintenance following CBT.

Engraftment

Of the 256 patients, 190 (74%) achieved primary neutrophil engraftment at a median of day 24 (range 11–51) and 44 (17%) died without engraftment. Median time to platelet recovery (>20 000/ μ l) was day 46 (range 20–179).

Of the remaining 22, who survived without neutrophil engraftment, 14 received second transplantation as stem-cell rescue. The other eight survived without neutrophil engraftment. Their disease status at last follow-up was remission after autologous recovery ($n=4$) and non-remission ($n=4$). The diagnosis of primary and secondary graft failure was established in 22 and 10 patients, respectively.

Cumulative incidence of neutrophil engraftment at day 100 was 78% (95% confidence interval (CI), 73–84%). The cumulative incidence of platelet recovery to 20 000/ μ l at day 100 was 64% (95% CI, 58–70%). The prognostic factors for neutrophil and platelet engraftment were shown in Table 2.

GVHD

The cumulative incidence of grade II–IV acute GVHD at day 100 is 37% (95% CI, 30–43%). No prognostic factors were identified in multivariate analysis for grade II–IV acute GVHD, whereas the number of infused nucleated cells, conditioning regimens, GVHD prophylaxis and the number of human leukocyte antigen disparities were examined.

Chronic GVHD was diagnosed in 41 of the 180 evaluable patients, who survived longer than 100 days. The cumulative incidence of chronic GVHD at 2 years after CBT was 24% (95% CI, 21–28%). Of the 41 patients, 27 and 14 developed limited and extensive diseases, respectively. The presence of grade III–IV acute GVHD and the number of CD34-positive cells were the prognostic factors of chronic GVHD (Table 2).

Table 1. Patient characteristics

Variables	n
Patients	256
Male/female	120/136
Median age, years (range)	40 (16–74)
Median body weight, kg (range)	54 (23.5–81.5)
Median duration from diagnosis to transplantation, months (range)	7 (2–127)
Disease lineage	
B cell/T cell/other/unknown	145/29/42/39
Chromosomal abnormality	
t(9;22)	100
t(4;11)	8
Complex karyotype	36
Other abnormality	21
Normal	53
Not available	38
Disease status at transplant	
First remission	125
Second remission	44
Third remission	8
Primary refractory	20
Relapse	58
Unknown	1
Preparative regimens	
Myeloablative	
TBI (10–14 Gy) containing regimens	184
Non-TBI regimens	6
Reduced intensity regimens	
TBI (2–8 Gy) containing regimens	50
Non-TBI regimens	16
GVHD prophylaxis	
CyA+MTX	115
CyA+PSL	5
CyA only	38
FK+MTX	45
FK only	42
Others	6
Not available/unspecified	5
Infused cord blood	
Median number of nucleated cells, 10 ⁷ /kg (range)	2.50 (1.51–5.00)
Median number of CD34-positive cells, 10 ⁵ /kg (range)	0.78 (0.08–5.80)
Number of HLA-A, B and DRB1 mismatches	
GVHD direction 0/1/2/3	27/82/143/2
Rejection direction 0/1/2/3	27/86/140/1
ABO compatibility	
Match	81
Major/minor mismatch	111/62

Abbreviations: CyA, cyclosporin; FK, tacrolimus; GVHD, graft-versus-host disease; HLA, human leukocyte antigen; MTX, methotrexate; PSL, prednisolone; TBI, total-body irradiation.

Complications other than GVHD

Infections were documented in 129 patients (50%). They included bacterial ($n=85$), fungal ($n=16$) and viral infection ($n=52$). Cytomegalovirus antigenemia was detected in 111 patients (43%).

Non-infectious complications other than GVHD occurred in 41 patients (16%); interstitial pneumonitis ($n=13$), acute respiratory distress syndrome ($n=12$), hepatic veno-occlusive disease