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西田徹也	同種造血幹細胞移植後ウイルス感染に対するドナーリンパ球を用いた養子免疫療法	日本アフェレーシス学会雑誌	31	223-228	2012
Minako Iida, Ritsuro Suzuki, Takashi Yoshida, Ken Ishiyama, Hiroyasu Kaya, Jun Taguchi, Atsushi Kikuta, Kaoru Kahata, Eiji Ohno, Koichi Miyamura, Yoshihisa Kodera	Outpatient peripheral blood stem cell collection in Japan: Donor follow-up survey by the Japan Society for Hematopoietic Cell Transplantation (Outpatient PBSC collection in Japan)	日本造血細胞移植学会雑誌	1(2)	52-58	2012
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VI. 研究成果の刊行物

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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.*

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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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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 0.5×10^9/l for 3 consecutive days following transplantation. Platelet recovery was considered to have occurred when the absolute platelet count exceeded 50×10^9/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/m^2 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
Recipient sex			
Female	1004 (44%)	239 (46%)	0.494
Male	1284 (56%)	286 (54%)	
Disease			
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%)	
Duration from diagnosis to transplant			
Median time (range), months	7.9 (0.2–768.5)	7.6 (0–251.7)	0.233
Disease risk			
Standard	959 (42%)	249 (47%)	0.050
High	1217 (53%)	257 (49%)	
Unknown	112 (5%)	19 (4%)	
Source of stem cells			
Bone marrow	—	251 (48%)	—
Peripheral blood	—	274 (52%)	
Cord blood	2288 (100%)	—	
HLA compatibility in the graft-versus-host direction			
Matched	225 (10%)	—	<0.001
One-antigen mismatch	753 (33%)	525 (100%)	
Two-antigen mismatch	1310 (57%)	—	
HLA compatibility in the host-versus-graft direction			
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%)	
Conditioning regimen			
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%)	
GVHD prophylaxis			
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%)	
Use of in vivo *T-cell depletion*			
No	2258 (99%)	472 (90%)	<0.001
Yes	30 (1%)	53 (10%)	
Year at transplant			
1998–2004	760 (33%)	260 (50%)	<0.001
2005–2009	1528 (67%)	265 (50%)	
Follow-up of survivors			
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).

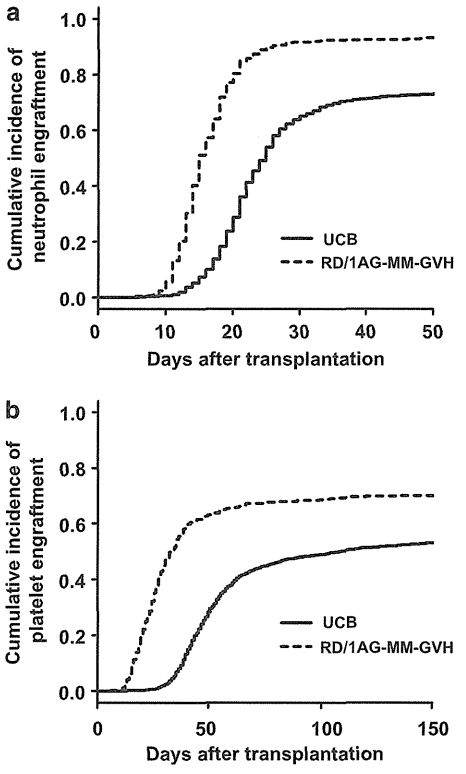

Figure 1. Neutrophil (**a**) and platelet engraftment (**b**).

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 GHVD, 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

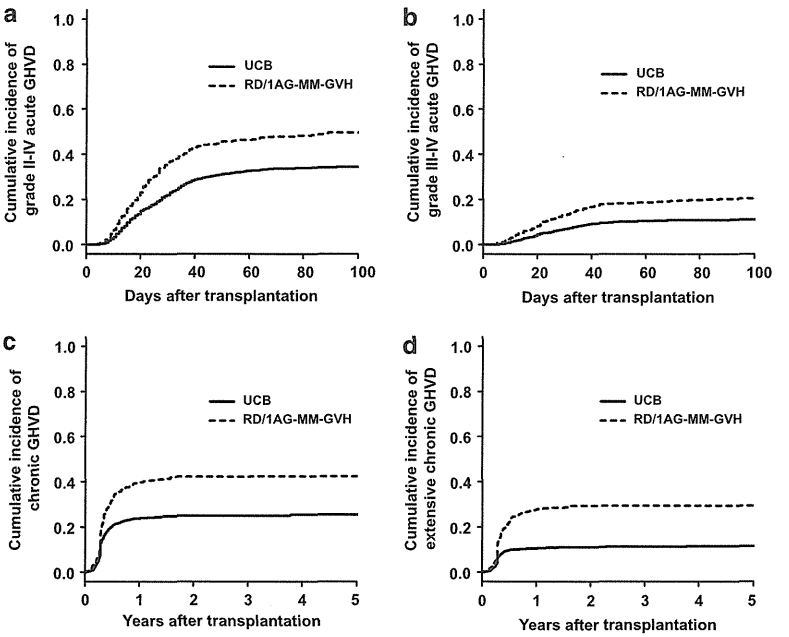

Figure 2. Acute and chronic GVHD. Cumulative incidences of grade II–IV (**a**) and grade III–IV acute GVHD (**b**) and chronic (**c**) and extensive chronic GVHD (**d**) are shown.

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. [a]Other 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; $P=0.038$). [b]Other 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; $P=0.319$). [c]Other 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, $P=0.002$).

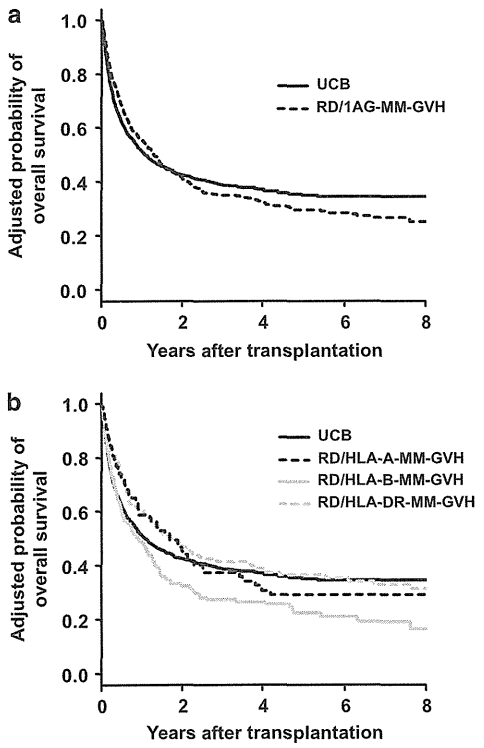

Figure 3. Overall survival. Overall survival rates in the transplantation using an unrelated cord blood vs a related donor with a 1-antigen mismatch at the HLA-A, HLA-B or HLA-DR locus in the GVH direction (**a**) or with an HLA-A, -B, or -DR antigen mismatch in the GVH direction (**b**) are shown.

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.

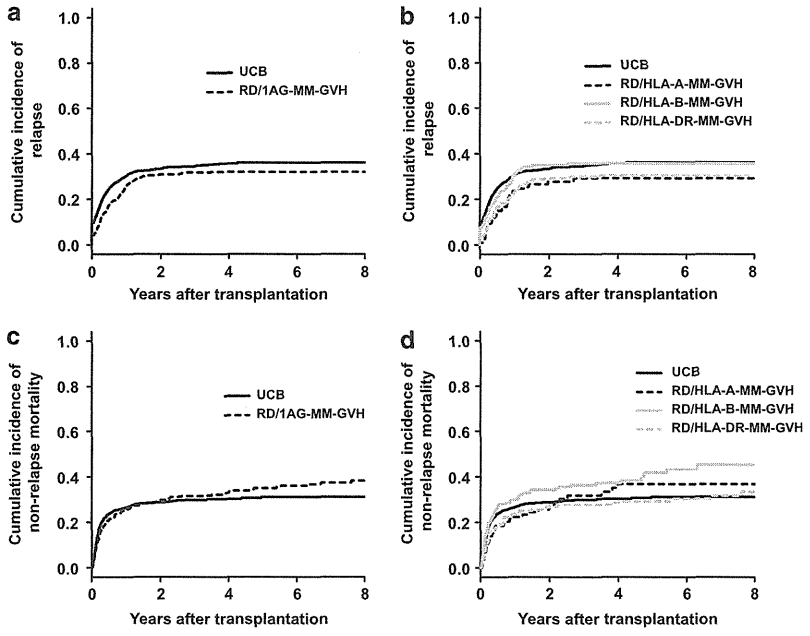

Figure 4. Relapse and non-relapse mortality. Cumulative incidence of relapse and non-relapse mortality after transplantation using an unrelated cord blood vs a related donor with a 1-antigen mismatch at the HLA-A, HLA-B or HLA-DR locus in the GVH direction (**a**, **c**) or with an HLA-A, -B, or -DR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

Table 3. Multivariate analysis of relapse and non-relapse mortality

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. [a]Other 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; $P=0.825$). [b]Other 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

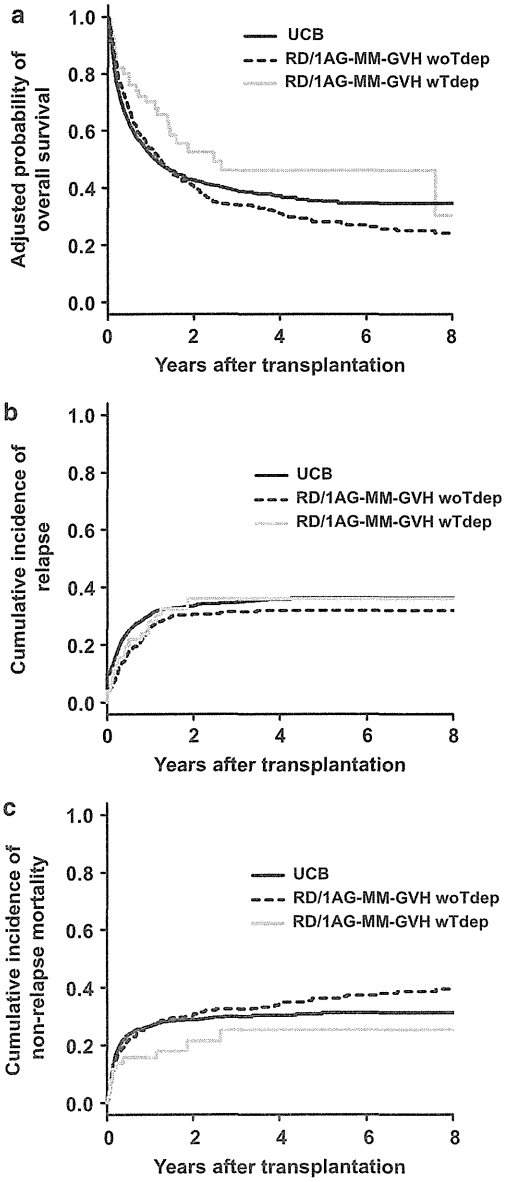

Figure 5. OS (**a**), relapse (**b**) and NRM (**c**) according to the use of *in vivo* T-cell depletion in the RD/1AG-MM-GVH group.

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. $>2.5 \times 10^7$/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 morality 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.

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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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ORIGINAL ARTICLE

Allogeneic hematopoietic stem cell transplantation for intermediate cytogenetic risk AML in first CR

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Allogeneic hematopoietic SCT (allo-HCT) from matched sibling donor (MSD) is recommended for younger patients with intermediate cytogenetic risk AML in first CR (CR1), whereas the role of alternative donor transplants in these patients is unknown. We retrospectively analyzed 605 patients with intermediate-risk AML, who received myeloablative allo-HCT in CR1. The 4-year OS for MSD ($n = 290$) and matched unrelated donor (MUD; $n = 141$) was 65% and 68% ($P = 0.50$), respectively. In multivariate analysis, MUD had a similar risk of overall mortality as MSD (hazard ratio = 0.90; 95% confidence interval, 0.62–1.30; $P = 0.58$), whereas older age, female donor/male recipient (FDMR) combination, and requiring more than one course of induction chemotherapy to achieve CR1 were poor prognostic factors for OS. Thus, OS after MUD HCT with sex combinations other than FDMR was significantly higher than that after MSD HCT from female donors to male recipients (4-year OS 72% versus 55%, $P = 0.04$). These results suggest that HCT, not only from MSD, but also from MUD, should be considered in younger patients with intermediate-risk AML in CR1, and that the donor–recipient sex combination is more important than the donor type in donor selection.

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Keywords: AML; first CR; allogeneic hematopoietic SCT

INTRODUCTION

The current standard treatment strategy for young patients with AML consists of induction chemotherapy and subsequent post-remission therapy. The post-remission therapy includes intensive consolidation chemotherapy and allogeneic hematopoietic SCT (allo-HCT). Although the toxicity of consolidation chemotherapy is relatively low, a substantial proportion of patients relapse, and the risk of relapse depends on cytogenetic risk.[1,2] On the other hand, allo-HCT as a post-remission therapy is associated with the lowest relapse rates. However, this benefit is limited by the high nonrelapse mortality (NRM) and the donor type has a significant impact on NRM.[3] The risk of NRM associated with allo-HCT needs to be balanced with the risk of relapse, and hence, the indication for allo-HCT among patients with AML in the first CR (CR1) depends on the cytogenetic risk and available donor type.[4]

Regarding those patients with favorable cytogenetic risk AML, who achieved CR1, the long-term disease-free survival after intensive consolidation chemotherapy of approximately 60% is reported, and they did not benefit from allo-HCT in CR1.[5–7] Thus, these patients are not considered candidates for allo-HCT in CR1.[8]

As for patients with unfavorable cytogenetic risk AML in CR1, previous prospective studies that assigned allo-HCT versus alternative post-remission therapies, on an intent-to-treat donor versus no-donor basis showed significant disease-free survival and OS benefit with allo-HCT, not only from a matched sibling donor (MSD), but also from a matched unrelated donor (MUD).[5–7,9] Accordingly, allo-HCT in CR1 from MSD or MUD is recommended for unfavorable risk AML.[8]

The indication for allo-HCT in CR1 depends on the available donor type in patients with intermediate cytogenetic risk AML. As meta-analyses of prospective studies showed that allo-HCT in CR1 from MSD offered significant disease-free survival and OS benefit,[5,6] allo-HCT in CR1 from MSD is recommended. In contrast, the indication for allo-HCT from alternative donors among these patients is unknown, because higher NRM may offset therapeutic benefits.[3] Although several studies reported comparable outcome after MUD or MSD transplantation,[10–13] these studies included only a small number of patients with intermediate-risk AML in CR1, and information regarding the outcome of allo-HCT from alternative donors in this group of patients is limited. Collectively, further investigation of the outcome of allo-HCT from alternative donors in patients with intermediate-risk AML in CR1 is warranted. In the present study, we retrospectively analyzed the impact of donor type on

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transplant outcomes among patients with intermediate-risk AML in CR1.

MATERIALS AND METHODS

Collection of data and data source

The recipients' clinical data were provided by the Japan Society for Hematopoietic Cell Transplantation (JSHCT) and the Japan Marrow Donor Program (JMDP). The registry data is managed using the 'Transplant Registry Unified Management Program' system.[14] Both JSHCT and JMDP collect recipients' clinical data at 100 days after allo-HCT. The patient's data on survival, disease status and long-term complications, including chronic GVHD and second malignancies, are renewed annually by follow-up forms. This study was approved by the data management committees of JSHCT. Informed consent was provided according to the Declaration of Helsinki.

Patients

Between January 1996 and December 2008, a total of 682 adult patients aged 16 to 70 years, with intermediate cytogenetic risk AML in CR1, received first BM or PBSC transplantation with myeloablative conditioning regimens. Excluding 66 patients without complete HLA data and 11 patients whose follow-up data were not available, we analyzed 605 patients. Only BM grafts were used in unrelated HCT, because the PBSC donation from unrelated donors was not permitted in Japan. HLA compatibility was determined by serological typing for HLA-A, -B and -DR in related donor (RD) HCT, and by high-resolution typing for HLA-A, -B, -C and -DRB1 in unrelated donor HCT. A MSD was defined as a serologically MSD, whereas other RDs were defined as RDs other than MSD. A MUD was defined as an eight/eight identical unrelated donor, whereas a mismatched unrelated donor (MMUD) was defined as an unrelated donor who had at least one locus mismatch.

Definitions

Neutrophil recovery was defined by an ANC of at least 500 cells per mm^3 for three consecutive points. Acute and chronic GVHD were diagnosed and graded according to defined criteria.[15,16] Relapse was defined as a recurrence of underlying hematological malignant diseases. NRM was defined as death during continuous remission. For OS, failure was death due to any cause, and surviving patients were censored at the last follow-up. The date of transplantation was the starting time point for calculating all outcomes. Cytogenetic risk-group assignment was done according to the Southwest Oncology Group/Eastern Cooperative Oncology Group classification.[2]

Statistical analysis

The two-sided χ^2-test was used for categorical variables, and the two-sided Wilcoxon rank sum test was used for continuous variables. OS was calculated using the Kaplan–Meier method. The log-rank test was used for group comparisons. Cumulative incidence curves were used in a competing-risks setting to calculate the probability of acute and chronic GVHD, relapse and NRM.[17] For GVHD, death without GVHD and relapse were the competing events; for relapse, death without relapse was the competing event; and for NRM, relapse was the competing event. Gray's test was used for group comparison of cumulative incidence.[18] The Cox proportional hazards regression model was used to test the statistical significance of several potential prognostic factors for relapse, NRM and OS. Variables with a significance level less than 0.1 in univariate analysis were entered into multivariable models and sequentially eliminated in a stepwise backward fashion. Each step of model building contained the main effect of donor type. Factors with a significance level less than 0.05 were kept in the final model. The median value was used as a cut-off point for year of transplant. For WBC counts at diagnosis, 50×10^9/L was used as a cut-off point according to the previous report.[10] All *P*-values were two-sided, and *P*-values of less than 0.05 were considered statistically significant.

RESULTS

Patient characteristics

Characteristics of the patients are summarized in Table 1. Among the 605 patients analyzed, 290 had MSD HCT, 53 had other RD HCT, 141 had MUD HCT and 121 had MMUD HCT. Of 53 patients with other RD, HLA was matched in 14 and mismatched in 39 patients. Of 121 patients with MMUD, 69 were one locus mismatched and 52 were two or more loci mismatched. The median age of patients was 37 (range, 16–59) years, and median time from diagnosis to HCT was 7.43 (range, 0.43–54.3) months. The median follow-up period of survivors was 4.2 (range, 0.1–13) years. The proportions of male patients, normal karyotype, conditioning regimens, including TBI, and BMT were significantly higher, whereas those of M1/M2/M3/M4/M5 FAB classification and CYA-based GVHD prophylaxis were significantly lower in the unrelated HCT than in the related HCT. The time from diagnosis to HCT was longer in the unrelated HCT compared with related HCT. Other characteristics were not significantly different between related and unrelated HCT.

Acute and chronic GVHD

The unadjusted cumulative incidences of grade II–IV acute GVHD for the MSD and MUD HCT were 26% and 25% at 100 days ($P = 0.89$), respectively, and those of grade III–IV acute GVHD were 10% and 7% at 100 days ($P = 0.46$), respectively (Table 2). The unadjusted cumulative incidences of chronic GVHD for the MSD and MUD HCT were 45% and 44% at 2 years ($P = 0.98$), respectively, and those of extensive chronic GVHD were 28% and 23% at 2 years ($P = 0.37$), respectively (Table 2).

Survival

OS rates for the MSD and MUD HCT were 65% and 68% at 4 years, respectively ($P = 0.50$; Table 2, Figure 1a). Univariate analysis of risk factors for overall mortality showed that the following factors were significant at the 0.1 level: patient age $\geqslant$40 years, female donor/male recipient (FDMR) combination, and requiring more than one course of induction chemotherapy to achieve CR1 (Table 3). In multivariate analysis, MUD was not a significant factor for overall mortality (hazard ratio (HR) = 0.90; 95% confidence interval (CI), 0.62–1.30; $P = 0.58$). Significant factors for overall mortality were patient age $\geqslant$40 years (HR = 1.55; 95% CI, 1.17–2.06; $P < 0.01$), FDMR combination (HR = 1.42; 95% CI, 1.03–1.95; $P = 0.03$) and requiring more than one course of induction chemotherapy to achieve CR1 (HR = 1.81; 95% CI, 1.36–2.41; $P < 0.01$) (Table 4). As the donor–recipient sex combination, but not donor type, was a significant factor for overall mortality, OS after MUD HCT with sex combinations other than FDMR was significantly higher than that after MSD HCT from female donors to male recipients (4-year OS 72% versus 55%, $P = 0.04$) (Figure 1b).

Nonrelapse mortality

The cumulative incidences of NRM for the MSD and MUD HCT were 17% and 19% at 4 years, respectively ($P = 0.52$) (Table 2, Figure 2a). Univariate analysis of risk factors for NRM showed that the following factors were significant at the 0.1 level: patient age $\geqslant$40 years, FDMR combination and MMUD (Table 3). In multivariate analysis, MUD HCT was not a significant factor for NRM compared with MSD HCT (HR = 1.26; 95% CI, 0.77–2.06; $P = 0.35$; Table 4). Significant factors for higher NRM were patient age $\geqslant$40 years (HR = 1.71; 95% CI, 1.17–2.50; $P < 0.01$), FDMR combination (HR = 1.68; 95% CI, 1.12–2.52; $P = 0.01$) and MMUD (HR = 1.83; 95% CI, 1.16–2.86; $P < 0.01$).

Relapse

The cumulative incidences of relapse for the MSD and MUD HCT were 24% and 19% at 4 years, respectively ($P = 0.25$; Table 2, Figure 2b). Univariate analysis of risk factors for relapse showed that the following factors were significant at the 0.1 level: longer interval between diagnosis and transplantation, peripheral blood

Table 1. Patient characteristics

Characteristics	*MSD*	*Other RD*	*MUD*	*MMUD*	P-*values*[a]
No. of patients	290	53	141	121	
Median patient age at HCT, years	39	36	35	37	0.09
Range	16–58	17–58	16–59	16–59	
Patient sex, n *(%)*					0.02
Male	155 (53)	24 (45)	86 (61)	75 (62)	
Female	135 (47)	29 (55)	55 (39)	46 (38)	
Sex matching, n *(%)*					0.61
Others	202 (77)	45 (87)	112 (79)	98 (81)	
Female to male	61 (23)	7 (13)	29 (21)	23 (19)	
Not available	27	1	0	0	
FAB classification, n *(%)*					<0.01
M1–M5	227 (82)	39 (80)	90 (70)	83 (74)	
M0, M6, M7	51 (18)	10 (20)	39 (30)	29 (26)	
Others, not available	12	4	12	9	
Prior myelodysplastic syndrome, n *(%)*					0.52
No	279 (97)	49 (92)	134 (98)	116 (96)	
Yes	10 (3)	4 (8)	3 (2)	5 (4)	
Not available	1	0	4	0	
Cytogenetics, n *(%)*					0.03
Normal	272 (94)	49 (92)	138 (98)	117 (97)	
+8, +6, -Y, del(12p)	18 (6)	4 (8)	3 (2)	4 (3)	
Conditioning regimen					<0.01[b]
CY + TBI	94 (32)	25 (47)	65 (46)	64 (53)	
CY + CA + TBI	40 (14)	3 (6)	18 (13)	10 (8)	
CY + BU + TBI	12 (4)	1 (2)	13 (9)	5 (4)	
Other TBI regimen	36 (12)	8 (15)	12 (9)	16 (13)	
BU + CY	102 (35)	12 (23)	31 (22)	17 (14)	
Other non-TBI regimen	6 (2)	4 (8)	2 (1)	9(7)	
GVHD prophylaxis, n *(%)*					<0.01[c]
CsA-based	268 (94)	29 (55)	55 (39)	40 (34)	
FK-based	9 (3)	21 (40)	79 (56)	69 (59)	
Others[d]	9 (3)	3 (6)	7 (5)	8 (9)	
Not available	4	0	0	4	
Time from diagnosis to HCT[e]					
Median	5.79	7.60	8.62	10.2	<0.01
Range	0.43–47.6	2.83–27.6	2.50–54.3	3.49–27.7	
<6 months	153 (54)	17 (33)	20 (14)	10 (8)	<0.01
6 to < 9 months	97 (34)	21 (41)	53 (38)	35 (29)	
9 months or longer	34 (12)	13 (25)	68 (48)	75 (63)	
Not available	6	2	0	1	
Year of transplant, n *(%)*					0.76
1996–2003	156 (54)	23 (43)	74 (52)	66 (55)	
2004–2008	134 (46)	30 (57)	67 (48)	55 (45)	
Stem cell source, n *(%)*					<0.01
BM	175 (60)	33 (62)	141 (100)	121 (100)	
Peripheral blood	115 (40)	20 (38)	0 (0)	0 (0)	
WBC counts at diagnosis, $\times 10^9$*/L*					
<50	196 (71)	36 (75)	108 (79)	82 (75)	0.14
⩾50	79 (29)	12 (25)	29 (21)	27 (25)	
Not available	15	5	4	12	
No. of induction courses to achieve CR, n *(%)*					0.43
1	187 (68)	31 (62)	88 (67)	68 (60)	
⩾2	88 (32)	19 (38)	43 (33)	45 (40)	
Not available	15	3	10	8	

Abbreviations: CA = cytarabine; FK = tacrolimus; HCT = hematopoietic SCT; MMUD = mismatched unrelated donor; MSD = matched sibling donor; MUD = matched unrelated donor; RD = related donor. [a]*P*-value between related and unrelated donors. [b]*P*-value between TBI regimen and non-TBI regimen. [c]*P*-value between CsA-based prophylaxis and FK-based prophylaxis. [d]Others include T-cell depletion. [e]The median time from diagnosis to transplant was 7.43 months for the whole group.

Table 2. Clinical outcomes

	MSD	*Other RD*		*MUD*		*MMUD*	
	% (95% CI)	*% (95% CI)*	P-*values*[a]	*% (95% CI)*	P-*values*[a]	*% (95% CI)*	P-*values*[a]
Acute GVHD, grades II–IV at 100 days	26 (21–31)	38 (25–51)	0.04	25 (18–32)	0.89	51 (42–59)	<0.01
Acute GVHD, grades III–IV at 100 days	10 (6–13)	15 (7–26)	0.19	7 (4–12)	0.46	14 (9–21)	0.16
Chronic GVHD at 2 years	45 (39–51)	48 (33–62)	0.75	44 (35–53)	0.98	41 (32–51)	0.55
Extensive chronic GVHD at 2 years	28 (23–34)	31 (18–44)	0.73	23 (16–31)	0.37	23 (15–31)	0.25
OS at 4 years	65 (59–71)	53 (37–68)	0.26	68 (59–76)	0.50	61 (51–70)	0.25
Nonrelapse mortality at 4 years	17 (12–22)	18 (9–30)	0.73	19 (13–27)	0.52	25 (18–34)	<0.01
Relapse at 4 years	24 (19–29)	29 (17–42)	0.45	19 (13–27)	0.25	12 (7–19)	0.02

Abbreviations: CI = confidence interval; MSD = matched sibling donor; RD = related donor; MUD = matched unrelated donor; MMUD = mismatched unrelated donor. [a]*P*-values for comparison with MSD.

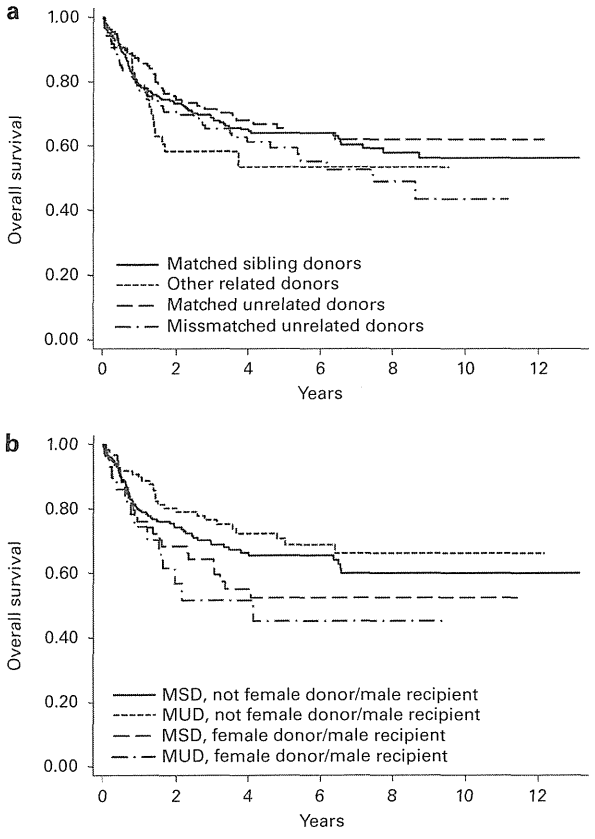

Figure 1. OS. (**a**) Comparison of MSD, other RD, MUD and MMUD transplantation. (**b**) Comparison according to the donor–recipient sex combination and donor type among patients with MSD and MUD.

as stem cell source, WBC counts at diagnosis $\geqslant 50 \times 10^9$/L, requiring more than one course of induction chemotherapy to achieve CR1, and MMUD (Table 3). In multivariate analysis, MUD HCT was not a significant factor for relapse compared with MSD HCT (HR = 0.98; 95% CI, 0.58–1.64; $P = 0.93$; Table 4). Significant factors for relapse were WBC counts at diagnosis $\geqslant 50 \times 10^9$/L (HR = 1.77; 95% CI, 1.20–2.63; $P < 0.01$) and requiring more than one course of induction chemotherapy to achieve CR1 (HR = 2.24; 95% CI, 1.54–3.27; $P < 0.01$), and 9 months or longer interval between diagnosis and transplantation (HR = 0.56; 95% CI, 0.32–0.98; $P = 0.04$).

DISCUSSION

We retrospectively analyzed the impact of donor type on transplant outcomes among patients with intermediate-risk AML in CR1. We observed comparable survival after MSD or MUD HCT, but the donor–recipient sex combination had a significant impact on transplant outcomes. The prognosis of older patients was poorer than that of younger patients because of higher NRM. These findings have important implications for the treatment of intermediate-risk AML in CR1.

The prognosis of younger patients with intermediate-risk AML could be improved by performing allo-HCT in CR1 when MSD is available.[5,6] On the other hand, it is unknown whether these patients without MSD may benefit from alternative donor transplantation, because higher NRM associated with alternative donor transplantation may offset therapeutic benefits.[3] In our study, NRM for a MUD HCT was 19% at 4 years, which was similar to that for a MSD HCT and appeared acceptable. The comparable outcomes after a MSD or a MUD HCT observed in our study suggest that HCT, not only from MSD, but also from MUD, should be considered in younger patients with intermediate-risk AML in CR1.

The FDMR combination had a crucial negative impact on transplant outcome in the present study, whereas it had no or a modest effect on transplant outcome in other studies.[19–21] We suggest two possible explanations for this discrepancy. First, it has been reported that the negative effect of the FDMR combination on survival was more pronounced in the standard-risk disease group than in the high-risk disease group, because the negative impact of the FDMR combination on NRM was stronger in the former than in the latter group, whereas the GVL effect associated with the FDMR combination becomes less important in the standard-risk disease group.[21,22] In the current study, subjects were restricted to patients with intermediate-risk AML in CR1. This may have resulted in a pronounced impact of the FDMR combination on transplant outcome in the current study. Second, as the impact of the FDMR combination on NRM is reported to be at least partially independent from that of GVHD on NRM,[21] and Japanese patients have lower incidence of GVHD,[23] the impact of sex combination on transplant outcome may be more evident in the Japanese than in the western populations.[22] The results of the present study suggest that the donor–recipient sex combination is a more important factor than the donor type in donor selection, in a certain subgroup of patients. As this may alter the current strategies in donor selection, verification in future studies is warranted.

Regarding older patients with intermediate-risk AML, a recent retrospective study showed that patients who underwent allo-HCT in CR1 had better survival than those who were treated with conventional chemotherapy alone, because the latter patients were associated with high relapse rates.[24] On the other hand, previous prospective studies, including patients with AML of all

Table 3. Univariate analysis of OS, nonrelapse mortality and relapse

Variables	N	*OS*		*NRM*		*Relapse*	
		HR (95% CI)	P-*values*	*HR (95% CI)*	P-*values*	*HR (95% CI)*	P-*values*
Patient age							
20–39	290	1.00		1.00		1.00	
<20	45	0.83 (0.47–1.46)	0.52	0.67 (0.29–1.57)	0.36	1.05 (0.53–2.06)	0.89
⩾40	270	1.47 (1.11–1.95)	<0.01	1.65 (1.14–2.41)	<0.01	1.13 (0.78–1.65)	0.52
Sex matching							
Others	457	1.00		1.00		1.00	
Female to male	120	1.39 (1.01–1.91)	0.04	1.68 (1.12–2.53)	0.01	0.80 (0.49–1.31)	0.38
FAB classification							
M1–M5	439	1.00		1.00		1.00	
M0, M6, M7	129	0.89 (0.63–1.25)	0.51	1.01 (0.65–1.56)	0.97	0.87 (0.56–1.37)	0.55
Prior MDS							
No	578	1.00		1.00		1.00	
Yes	22	0.67 (0.28–1.64)	0.39	0.46 (0.11–1.86)	0.28	0.70 (0.22–2.19)	0.54
Cytogenetics							
Normal	576	1.00		1.00		1.00	
+8, +6, -Y, del(12p)	29	0.72 (0.35–1.46)	0.36	1.11 (0.52–2.38)	0.80	0.31 (0.08–1.25)	0.10
TBI							
Yes	422	1.00		1.00		1.00	
No	183	1.06 (0.80–1.42)	0.68	1.01 (0.69–1.50)	0.94	1.01 (0.68–1.49)	0.97
GVHD prophylaxis							
CsA-based	392	1.00		1.00		1.00	
FK-based	178	1.13 (0.84–1.53)	0.42	1.14 (0.77–1.71)	0.51	1.10 (0.73–1.64)	0.65
Others	27	1.19 (0.63–2.27)	0.59	1.06 (0.43–2.63)	0.89	1.48 (0.68–3.20)	0.32
Time from diagnosis to HCT							
< 6 months	200	1.00		1.00		1.00	
6 to <9 months	206	0.86 (0.62–1.20)	0.37	0.92 (0.58–1.48)	0.74	0.77 (0.51–1.17)	0.23
9 months or longer	190	0.88 (0.63–1.22)	0.45	1.26 (0.81–1.96)	0.31	0.48 (0.29–0.77)	<0.01
Year of transplant							
2004–2008	286	1.00		1.00		1.00	
1996–2003	319	0.91 (0.69–1.21)	0.53	1.08 (0.73–1.59)	0.69	0.83 (0.57–1.19)	0.31
Stem cell source							
BM	470	1.00		1.00		1.00	
Peripheral blood	135	1.08 (0.78–1.49)	0.64	0.76 (0.47–1.23)	0.27	1.64 (1.11–2.42)	0.01
WBC counts at diagnosis							
$<50 \times 10^9$/L	422	1.00		1.00		1.00	
$\geqslant 50 \times 10^9$/L	147	1.15 (0.84–1.57)	0.38	0.77 (0.49–1.24)	0.28	1.86 (1.27–2.74)	<0.01
No. of induction courses							
1	374	1.00		1.00		1.00	
⩾2	195	1.76 (1.32–2.33)	<0.01	1.36 (0.92–2.01)	0.12	2.25 (1.55–3.26)	<0.01
Donor							
MSD	290	1.00		1.00		1.00	
Other RD	53	1.34 (0.84–2.15)	0.23	1.17 (0.58–2.39)	0.66	1.31 (0.73–2.33)	0.36
MUD	141	0.88 (0.61–1.26)	0.49	1.12 (0.69–1.79)	0.65	0.77 (0.48–1.23)	0.28
MMUD	121	1.21 (0.86–1.71)	0.27	1.73 (1.11–2.67)	0.02	0.56 (0.32–0.99)	0.046

Abbreviations: CI = confidence interval; FK = tacrolimus; HCT = hematopoietic SCT; HR = hazard ratio; MDS = myelodysplastic syndrome; MSD = matched sibling donor; MMUD = mismatched unrelated donor; MUD = matched unrelated donor; NRM = nonrelapse mortality; RD = related donor.

cytogenetic risk groups, showed that the beneficial effect of allo-HCT in CR1 on OS was absent in patients older than 35–40 years, because the benefits of the reduced relapse rate were offset by a higher NRM.[6,25] In accordance with these prospective studies, older patients had higher NRM and overall mortality than younger patients in the current study. Our study revealed that a substantial number of older patients received allo-HCT in CR1, but the results of our study and others indicate that prospective studies to evaluate the efficacy of allo-HCT in CR1 for older patients with intermediate-risk AML are necessary before it becomes a general practice.

The proportion of patients who received TBI regimens tended to be lower in the older patients than in the younger patients in the current study (data not shown), perhaps in an attempt to

Table 4. Significant factors in multivariate analysis for OS, nonrelapse mortality and relapse

Variables	N	*OS*		*NRM*		*Relapse*	
		HR (95% CI)	*P-values*	*HR (95% CI)*	*P-values*	*HR (95% CI)*	*P-values*
Patient age							
20–39	290	1.00		1.00		—	—
<40	45	0.85 (0.48–1.50)	0.58	0.67 (0.28–1.57)	0.35	—	—
⩾40	270	1.55 (1.17–2.06)	<0.01	1.71 (1.17–2.50)	<0.01	—	—
Sex matching							
Others	457	1.00		1.00		—	—
Female to male	120	1.42 (1.03–1.95)	0.03	1.68 (1.12–2.52)	0.01	—	—
WBC counts at diagnosis							
$<50 \times 10^9$/L	422	—	—	—	—	1.00	
$\geqslant 50 \times 10^9$/L	147	—	—	—	—	1.77 (1.20–2.63)	<0.01
No. of induction courses							
1	374	1.00	—	—	—	1.00	
⩾2	195	1.81 (1.36–2.41)	<0.01	—	—	2.24 (1.54–3.27)	<0.01
Time from diagnosis to HCT							
<6 months	200	—	—	—	—	1.00	
6 to <9 months	206	—	—	—	—	0.85 (0.55–1.31)	0.45
9 months or longer	190	—	—	—	—	0.56 (0.32–0.98)	0.04
Donor							
MSD	290	1.00		1.00		1.00	
Other RD	53	1.35 (0.84–2.18)	0.21	1.31 (0.64–2.68)	0.47	1.44 (0.80–2.61)	0.22
MUD	141	0.90 (0.62–1.30)	0.58	1.26 (0.77–2.06)	0.35	0.98 (0.58–1.64)	0.93
MMUD	121	1.17 (0.83–1.67)	0.37	1.83 (1.16–2.86)	<0.01	0.71 (0.38–1.32)	0.28

Abbreviations: CI = confidence interval; HCT = hematopoietic SCT; HR = hazard ratio; MMUD = mismatched unrelated donor; MSD = matched sibling donor; MUD = matched unrelated donor; NRM = nonrelapse mortality; RD = related donor.

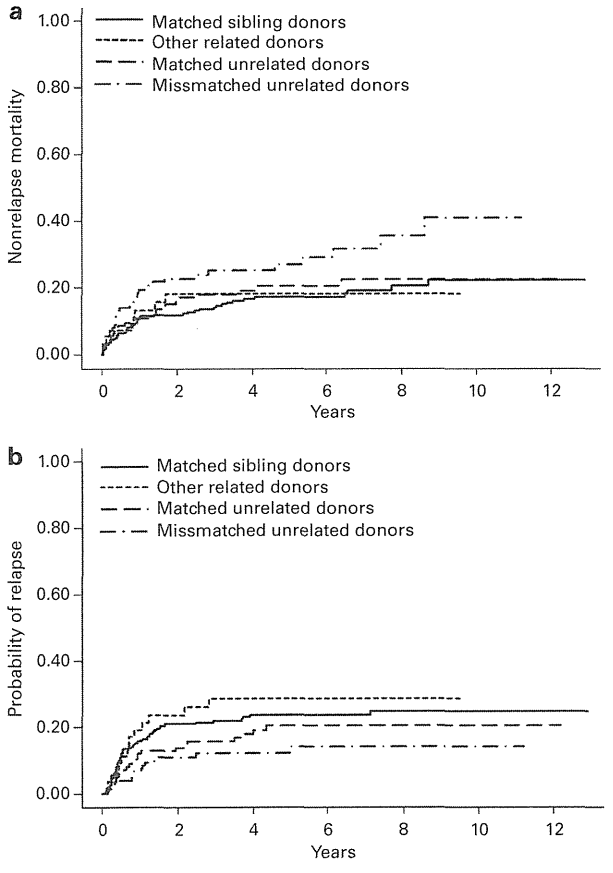

Figure 2. Comparison of MSD, other RD, MUD, and MMUD transplantation. (**a**) Cumulative incidence of NRM. (**b**) Cumulative incidence of relapse.

reduce toxicity. However, there was no significant difference in NRM between TBI and non-TBI regimens among older patients (data not shown). Recently, reduced toxicity myeloablative regimens, such as the combination of fludarabine with myeloablative doses of BU, were developed with an aim to decrease toxicity without compromising antileukemic effects.[26] These regimens might be beneficial for older patients, especially for those with standard-risk disease.[27] The optimal conditioning regimens for older patients need to be determined in the future studies.

OS after other RD and MMUD HCT did not differ significantly from that after MSD HCT in the current study, but these results need to be interpreted with caution. First, the small number of patients with other RD limited the power to detect significant differences in survival between MSD and other RD HCT. Second, other RD and MMUD included donors with various degrees of HLA incompatibilities. Thus, it is difficult to draw firm conclusions regarding the role of other RD and MMUD HCT from this study. Nonetheless, considering that other RD and MMUD HCT yielded a 4-year OS of 53% and 61%, respectively, allo-HCT from these donors might be an option for patients with unfavorable features. For example, as patients who required more than one course of induction therapy to achieve CR1 have poor outcomes with conventional chemotherapy,[8] they might benefit from allo-HCT from other RD or MMUD, when MSD and MUD are not available.

Our study has several limitations. First, this is a non-randomized, retrospective observational study using registry data, which would allow for the introduction of bias. To minimize bias, we conducted multivariate analyses to adjust for baseline differences. However, some factors which might have influenced transplant outcomes (such as performance score and extramedullary disease) could not be included in the Cox proportional hazards regression model due to a high frequency of missing values. Second, a time-censoring effect might have influenced the results.[28] Patients who undergo transplantation late after achievement of CR may be at a lower risk of relapse, by virtue of having remained in remission a time long enough for a transplantation to be performed.[28] This effect might have favorably affected the outcome of unrelated donor HCT. However, there was no significant difference in OS between MSD

and MUD HCT, even when the time from diagnosis to transplantation was included in the final model of multivariate analyses (data not shown). Third, although the role of allo-HCT according to genetic mutations, such as *FLT3-ITD*, *NPM1* and *CEBPA*, is now being explored,[29] the information about these mutations was not available and this was beyond the scope of the present study. However, the results of our study do support the inclusion of not only MSD HCT, but also MUD HCT, in the prospective studies, which evaluate the role of allo-HCT according to these genetic mutations.

In conclusion, the results of our study suggest that HCT, not only from MSD, but also from MUD ,should be considered in younger patients with intermediate-risk AML in CR1, and that the donor–recipient sex combination is more important than the donor type in donor selection. Prospective studies to evaluate the role of allo-HCT in CR1 for older patients are warranted.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

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ORIGINAL ARTICLE

Clinical significance of hemophagocytosis in BM clot sections during the peri-engraftment period following allogeneic hematopoietic SCT

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The effects of macrophage activation on the outcome of allogeneic hematopoietic SCT (allo-HSCT) have yet to be fully examined. A total of 70 adult patients who received a first allo-HSCT for hematological diseases were studied. We counted the number of hemophagocytic cells in BM clot sections on day +14 ± 7, and analyzed its impact on subsequent outcome. In all, 23 patients were diagnosed as having increased numbers of hemophagocytic cells (HP group), whereas 47 were not (non-HP group). The HP group was not associated with an increased incidence of acute or chronic GVHD, but was associated with worse hematopoietic recovery than the non-HP group. The 2-year OS for the HP group and the non-HP group was 30 and 65% ($P<0.01$), respectively, and 2-year non-relapse mortality was 48% and 27% ($P<0.01$), respectively. Multivariate analysis confirmed that the HP group was associated with a lower OS (hazard ratio (HR) = 2.3; 95% confidence interval (CI), 1.0–5.4; $P=0.048$) and higher non-relapse mortality (HR = 4.0; 95% CI, 1.6–9.9; $P<0.01$). The HP group had higher incidences of death due to graft failure ($P<0.01$) and endothelial complications, such as sinusoidal obstruction syndrome and transplant-associated microangiopathy ($P=0.01$). Macrophage activation is a previously unrecognized complication with negative impact on outcome of allo-HSCT.
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Keywords: hemophagocytosis; macrophages; SCT; non-relapse mortality

Introduction

Macrophages have an indispensable role in both innate and acquired immunity and they have at least 3 major functions: antigen presentation, phagocytosis and immunomodulation.[1,2] Following allogeneic hematopoietic SCT (allo-HSCT), macrophages contribute to the development of acute GVHD by producing pro-inflammatory cytokines.[3] In addition to aGVHD, pro-inflammatory cytokine release is implicated in the pathogenesis of various early complications after allo-HSCT, such as sinusoidal obstruction syndrome, engraftment syndrome (ES) and capillary leakage syndrome.[4–7] Although the role of macrophages in these complications is undetermined, macrophages have an ability to secrete significant amounts of pro-inflammatory cytokines.[2] Furthermore, fatal outcomes of hemophagocytic syndrome after allo-HSCT have been described in case reports.[8] This evidence suggests that activation of macrophages has a significant impact on post-transplantation outcome. However, there are only a few clinical studies that have analyzed the effects of macrophage activation on outcome of allo-HSCT.[9]

Measuring the levels of cytokines or chemokines produced by activated macrophages, such as IL-1, IL-6, IL-12, TNF-α and macrophage inflammatory protein-1, may be a possible method to evaluate the activation of macrophages.[1,2,10] However, as these cytokines and chemokines are produced by many cell types, their elevated levels are not specific to macrophage activation.[1,10–12] An alternative method for evaluating the activation of macrophages is to assess the morphological change associated with macrophage activation, namely phagocytosis. Although phagocytosis reflects only a part of macrophage activation, the increased number of phagocytic cells provides direct evidence that macrophages are activated.[2] In addition, assessment of hemophagocytosis can be carried out easily using BM clot sections. Thus, hemophagocytosis serves as a specific and simple marker of macrophage activation. We assessed hemophagocytosis in BM clot sections during the early post-transplantation period, and analyzed its impact on subsequent outcome.

Patients and methods

Patients

We reviewed 96 consecutive adult patients who received their first allo-BM or PBSCT between December 2005 and December 2008 at the Japanese Red Cross Nagoya First Hospital. As our purpose was to examine the impact of

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hemophagocytosis in BM clot sections on day $+14\pm7$ on subsequent outcome, two patients who died within the first 21 days after transplantation were excluded. Although BM aspiration is routinely performed on day $+14\pm7$ in our institution, it was not performed in 17 patients. In addition, specimens were insufficient for evaluation in seven patients. As a result, 70 patients were included in the analysis, all of whom received T cell-replete grafts. Standard risk diseases were defined as AML in first or second CR, ALL in first CR, CML in first chronic phase, myelodysplastic syndrome as refractory anemia, malignant lymphoma in CR, chronic active EB-virus infection, aplastic anemia and paroxysmal nocturnal hemoglobinuria, whereas high-risk diseases were defined as the others. This study was approved by the institutional review board. All patients provided written informed consent.

Transplantation procedure

Conditioning included myeloablative and reduced-intensity regimens. The myeloablative regimens were mainly CY/TBI based, whereas the reduced-intensity conditioning regimens were mainly fludarabine 125 mg/m^2 plus melphalan 135–180 mg/m^2. Antithymocyte globulin was added in two patients who received HLA-mismatched transplants, and alemtuzumab was added in one patient with aplastic anemia. For GVHD prophylaxis, CYA and short-term MTX were used for allo-HSCT from a related donor, and tacrolimus and short-term MTX for allo-HSCT from an unrelated donor. All patients were cared for in laminar airflow units and received oral gut decontamination. Standard prophylaxis against *Pneumocystis carinii*, fungal infections and herpes simplex virus was given. G-CSF was administered after transplantation in all patients until engraftment was confirmed. Engraftment was defined as an ANC of more than 500/μL for 3 consecutive days. Primary graft failure was said to have occurred when engraftment was not seen in patients surviving more than 21 days after transplantation.[13] Secondary graft failure was defined as loss of neutrophil engraftment as determined by an ANC of less than 500/μL for 3 consecutive days after having achieved neutrophil engraftment, and no evidence of disease progression in the marrow.[14] ES was diagnosed, if patients presented with two or more of the following symptoms within 96 h of the start of neutrophil recovery (ANC >100/μL): (1) fever (temperature $>=38.5$°C) without an identifiable infectious cause; (2) weight gain $>=5\%$ over the pre-transplantation baseline weight; (3) erythematous rash not attributable to a medication; and (4) hypoxia, pulmonary infiltrates or both not attributable to infection or cardiac disease.[15,16] Acute GVHD was evaluated by established criteria.[17] Chronic GVHD was evaluated in patients who survived beyond day $+100$ without a relapse according to the traditional Seattle criteria.[18]

BM examination

BM aspiration was routinely performed on day $+14\pm7$. All specimens were fixed in formalin solution, embedded in paraffin and stained with hematoxylin-eosin. BM clot sections were reviewed retrospectively and the total number of hemophagocytic cells in three fields at a 200-fold magnification was counted (Figure 1).

Statistical considerations

Chi-square, Fisher's exact and Mann–Whitney tests were used to compare clinical and patient characteristics. The probability of survival was calculated using the Kaplan–Meier method, and the differences between groups were compared using log-rank statistics. Probabilities of non-relapse mortality (NRM) and relapse were calculated using the cumulative incidence function.[19] For NRM, relapse was the competing event, and for relapse, death in the absence of persistent or recurrent disease was the competing event. As our purpose was to examine the impact of hemophagocytosis in BM clot sections on day $+14\pm7$ on subsequent outcomes, all time-to-event comparisons were made from day $+21$ after transplantation. The Cox proportional hazards regression model was used to test the statistical significance of several potential prognostic factors for relapse, NRM and OS. Variables with a significance level less than 0.1 in univariate analysis

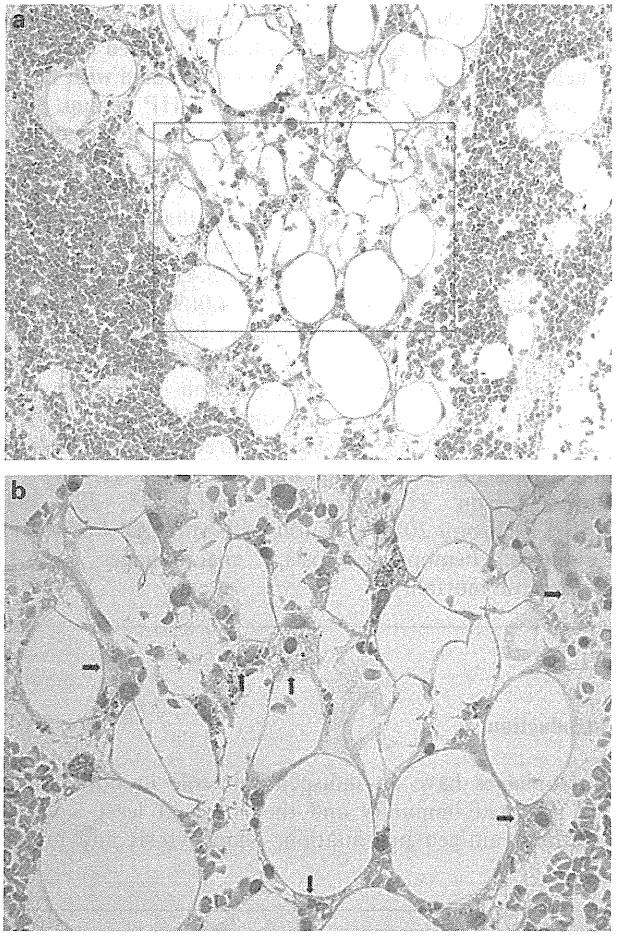

Figure 1 BM clot section stained with hematoxylin-eosin. (**a**) The specimen is from a representative patient who had an increased number of hemophagocytic cells. The indicated region is magnified in (**b**). Original magnification ×200. (**b**) Arrow indicates hemophagocytosis. Original magnification ×400.