target CsA concentration for GVHD prophylaxis at our center (17) might counteract the effect of delayed lymphocyte recovery on the development of acute GVHD. Alemtuzumab-containing regimen was also associated with high L-index(100) values, because alemtuzumab strongly inhibited T lymphocyte for approximately 2 months (16).

Another purpose of this study was to investigate the effect of lymphopenia indexes, including L-index, on CMV reactivation. According to Einsele et al., lymphopenia parameters of ALC < 300/μl, $CD4^+$ cell count < 100/μl, and $CD8^+$ cell count < 100/μl at day 49 after HSCT were significant risk factors for the development of CMV disease in patients with polymerase chain reaction (PCR)-proven CMV viremia (10). Kim et al. showed that cumulative incidence of CMV reactivation in patients with ALC < 350/μl at day 21 after HSCT was significantly higher than that in high ALC group (≥ 350/μl) (2). The result of this study showed that L-index(30) might be more closely associated with CMV reactivation than ALC at day 30. L-index(30) was identified as an independent factor in multivariate analysis when it was dealt as dichotomous variable with a cut-off value of 22,318 determined by ROC curve analysis. This finding suggested that not only the intensity but also the duration of lymphopenia was an important risk factor for CMV reactivation. Furthermore, the area under the lymphocyte curve in the same period did not show statistically significant association with CMV reactivation. Hence, the extent of lymphocyte deficit might be more closely

associated with CMV reactivation than the simple sum of area under the lymphocyte curve. The difference between these 2 parameters becomes important when ALC ≥ 700/μl is achieved early after HSCT. In fact, in this study, ALC exceeded 700/μl at least temporarily within 30 days after HSCT in approximately 20% of patients. On the other hand, L-index(100) was not related to CMV reactivation, probably because CMV antigenemia was detected in more than 3 cells at a median of 29 days after transplantation, and therefore, the cumulative L-index until reactivation might have a significant effect. The limitation of the L-index(30) was that it could be obtained only after 30 days from HSCT, when half of the patients in CMV-AG ≥ 3 group had already developed CMV reactivation. Therefore, the L-index(30) might be less useful as a predictor of CMV reactivation. With regard to lymphocyte subset analysis, $CD4^+$ cell count was significantly lower in CMV-AG ≥ 3 group than in CMV-AG < 3 group. According to the study by Kim et al., the incidence of CMV reactivation was not affected by $CD4^+$ cell count at 3 months after transplantation (28). However, our result suggested that $CD4^+$ cell count at the early phase after HSCT might play an important role in preventing CMV reactivation. The ability of the L-index(30) for predicting CMV reactivation was considered to be almost the same as that of $CD4^+$ cell count at day 30 because the area under the curve of these two indexes were almost equal in the ROC curve analyses. Our result also showed that $CD8^+$ cell count on day 90

after HSCT in CMV-AG ≥ 3 group was significantly higher than that in CMV-AG < 3 group, which agreed with the result reported by Heining et al. (9), because CMV reactivation enhanced immune function and significantly improved $CD8^+$ T cell recovery (9, 11).

Advanced age has been known as a risk factor of CMV disease (29, 30). The present study showed that patients' age older than 41 years old was identified as an independent significant factor on CMV reactivation, which suggested that patients' age affect not only CMV diseases but also reactivation. Other well-recognized risk factors for CMV infection include seropositivity for CMV before HSCT, unrelated donor status, development of aGVHD, and corticosteroid use (31-33). In this study, all patients in CMV-AG ≥ 3 group were donor or/and recipients who were seropositive for CMV, though there was no significant difference as compared with those in CMV < 3 group. The percentage of unrelated donor status was significantly higher in CMV-AG ≥ 3 group than in CMV < 3 group in univariate analysis, but it was not identified as an independent factor in multivariate analysis. The development of acute GVHD and corticosteroid use did not differ between the 2 groups.

In conclusion, our present study showed that both the intensity and the duration of lymphopenia in early phase after HSCT evaluated as the L-index(30) were significantly associated with CMV reactivation. However, L-index(30), which became available only after 30 days from transplantation, might be less useful as a predictor of CMV reactivation.

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310-318.

Table 1 Clinical and epidemiological characteristics of the study patients

Clinical and epidemiological factors	Total (n = 50)	CMV-AG ≥ 3 (n = 30)	CMV-AG < 3 (n = 20)	*P* value
Median age, years (range)	41 (15-63)	47 (15-61)	36 (15-63)	0.062
Sex male/female	27/23	14/16	13/7	0.203
Underlying disease				0.460
Acute myeloblastic leukemia	19 (38%)	10 (33%)	9 (45%)	
Acute lymphoblastic leukemia	5 (10%)	2 (7%)	3 (15%)	
Lymphoma	4 (8%)	4 (13%)	0	
Adult T cell leukemia/lymphoma	6 (12%)	4 (13%)	2 (10%)	
Myelodysplastic syndrome	5 (10%)	3 (10%)	2 (10%)	
Aplastic anemia	8 (16%)	6 (20%)	2 (10%)	
Others	3 (6%)	1 (3%)	2 (10%)	
Lymphoid malignancy	15 (30%)	10 (33%)	5 (25%)	0.529
Conditioning regimen				0.556
Myeloablative regimen	30 (60%)	17 (57%)	13 (65%)	
Reduced-intensity regimen	20 (40%)	13 (43%)	7 (35%)	
Fludarabine containing	20 (40%)	13 (43%)	7 (35%)	0.556
ATG containing	7 (14%)	5 (17%)	2 (10%)	0.506
Alemtuzumab containing	4 (8%)	4 (13%)	0	0.083
Donor Related/Unrelated	29/21	14/16	15/5	0.047
HLA Match/Mismatch	35/15	20/10	15/5	0.350
Graft				0.704
BM	30 (60%)	18 (60%)	12 (60%)	
PBSC	19 (38%)	11 (37%)	8 (40%)	
CB	1 (2%)	1 (3%)	0	
GVHD prophylaxis				0.032
CsA300 + sMTX	19 (38%)	15 (50%)	4 (20%)	
CsA500 + sMTX	30 (60%)	14 (47%)	16 (80%)	
FK506 + sMTX	1 (2%)	1 (3%)	0	
CMV serostatus				0.162
Donor and recipient seronegative	2 (4%)	0	2 (10%)	
Donor or/and recipient seropositive	48 (96%)	30 (100%)	18 (90%)	
Acute GVHD				
Grade II–IV	16 (32%)	10 (33%)	6 (30%)	0.804
Grade III–IV	7 (14%)	5 (17%)	2 (10%)	0.506
Corticosteroid use	11 (22%)	5 (17%)	6 (30%)	0.265
Chronic GVHD				
Total	18 (36%)	9 (30%)	9 (45%)	0.399
Extensive type	13 (26%)	7 (23%)	6 (30%)	0.368

CMV-AG, cytomegalovirus antigenemia; BM, bone marrow; PBSC, peripheral blood stem cell; CB, cord blood; GVHD, graft-versus-host disease; CsA, cyclosporine A; CsA300, target CsA concentration of 300 ng/ml during continuous intravenous infusion; CsA500, target CsA concentration of 500 ng/ml during continuous intravenous infusion; sMTX, short-term methotrexate.

Table 2 Factors associated with high L-index(30) and L-index(100) values

	Median value	*P* value
L-index(30)		
Univariate analysis		
Female sex (vs. male)	22,264 vs. 18,950	0.048
Non-lymphoid disease (vs. lymphoid malignancy)	21,684 vs. 16,552	0.009
ATG-containing regimen (yes vs. no)	22,299 vs. 19,268	0.157
Alemtuzumab-containing regimen (yes vs. no)	24,956 vs. 19,461	0.001
Unrelated donor (vs. related donor)	22,264 vs. 19,268	0.023
HLA-mismatched donor (vs. HLA-matched donor)	22,962 vs. 19,038	0.015
BMT (vs. PBSCT)	21,953 vs. 17,110	0.089
Multivariate analysis		
HLA-mismatched donor (vs. HLA-matched donor)		0.010
Female sex (vs. male)		0.019
Non-lymphoid disease (vs. lymphoid malignancy)		0.042
L-index(100)		
Univariate analysis		
Female sex (vs. male)	34,406 vs. 23,711	0.142
Non-lymphoid disease (vs. lymphoid malignancy)	34,935 vs. 16,757	0.017
ATG-containing regimen (yes vs. no)	45,394 vs. 28,455	0.069
Alemtuzumab-containing regimen (yes vs. no)	52,621 vs. 27,872	0.020
HLA-mismatch donor (vs. HLA-matched donor)	39,535 vs. 23,711	0.008
BMT (vs. PBSCT)	31,249 vs. 19,933	0.134
Grade III–IV aGVHD (yes vs. no)	45,937 vs. 28,185	0.078
Multivariate analysis		
Grade III–IV aGVHD (yes vs. no)		0.003
Alemtuzumab-containing regimen (yes vs. no)		0.002
Non-lymphoid disease (vs. lymphoid malignancy)		0.003

ATG, anti-thymoglobulin; BMT, bone marrow transplantation; PBSCT, peripheral blood stem cell transplantation; GVHD, graft-versus-host disease.

Table 3 Association between lymphopenia indexes and CMV infection

Lymphopenia indexes	Total (n = 50)	CMV-AG ≥ 3 (n = 30)	CMV-AG < 3 (n = 20)	*P* value
L-index(30), median (range)	21,081 (8,757-26,512)	22,030 (10,062-26,512)	19,038 (8,757-24,527)	0.050
L-index(30)				
≥ 22,318	18	15	3	0.016
< 22,318	32	15	17	
L-index(100), median (range)	29,987 (8,757-65,789)	31,453 (10,062-65,789)	29,585 (8,757-60,624)	0.476
ALC, median (range)				
Day 30	366 (21-3,774)	326 (21-1,453)	418 (58-3,774)	0.607
Day 60	598 (52-4,308)	589 (106-2,705)	630 (52-4,308)	0.843
Day 90	754 (0-5,261)	859 (0-5,261)	724 (67-2,822)	0.411
$CD4^+$ cell count, median (range)				
Day 30	97 (4-902)	77 (4-587)	174 (17-902)	0.023
Day 60	130 (7-702)	60 (7-544)	176 (91-702)	0.263
Day 90	193 (6-1,005)	189 (6-1,005)	289 (30-401)	0.739
$CD8^+$ cell count, median (range)				
Day 30	142 (5-2,264)	86 (5-1,027)	170 (41-2,264)	0.189
Day 60	295 (22-3,132)	215 (22-1,563)	300 (155-3,132)	0.441
Day 90	383 (25-2,994)	622 (25-2,994)	205 (28-383)	0.041

CMV-AG, cytomegalovirus antigenemia; ALC, absolute lymphocyte count.

Table 4 Factors associated with CMV infection

A. Univariate analyses			
Factors	CMV-AG ≥ 3 (n = 30)	CMV-AG < 3 (n = 20)	P value
Age			
< 41	10	13	0.043
≥ 41	20	7	
Donor			
Related	14	15	0.047
Unrelated	16	5	
GVHD prophylaxis			
CsA300 + sMTX	15	4	0.032
CsA500 or FK + sMTX	15	16	
L-index(30)			
≥ 22,318	15	3	0.016
< 22,318	15	17	
B. Multivariate analyses			
Factors	Odds ratio	95% CI	P value
Age	4.45	1.190 - 16.60	0.0263
L-index(30)	6.71	1.470 - 30.70	0.0141

CsA300, target CsA concentration of 300 ng/ml during continuous intravenous infusion; CsA500, target CsA concentration of 500 ng/ml during continuous intravenous infusion; sMTX, short-term methotrexate.

Figure legends

Fig.1. Area over the lymphocyte curve (L-index) in a hypothetical patient. Lymphopenia (absolute lymphocyte count; ALC < 700/μl) developed 7 days before transplantation in the patient. The L-index (Ae–Ao) was calculated as the difference between the expected lymphocyte area (shaded area, Ae) and the observed area under the curve (striped area, Ao), which was calculated by the trapezoidal method. If the area under the lymphocyte curve until day 30 is 4,550, the L-index(30) = 37 × 700 – 4,550 = 21,350. In the same way, if the area under the lymphocyte curve until day 100 is 43,928, the L-index(100) = 107 × 700 – 43,928 = 30,972.

Fig.2. Receiver operating characteristic (ROC) curves comparing L-index(30) with $CD4^+$ cell count at day 30. The area under the ROC curve was 0.68 and 0.71 for the L-index(30) and $CD4^+$ cell count at day 30, respectively. The sum of the sensitivity and specificity reached the maximum when the thresholds for the L-index(30) and CD4+ cell count at day 30 were 22,318 and 59, respectively. With the use of these cut-off values, the sensitivity and specificity for predicting CMV infection were 50.0% and 85.0%, and 47.8% and 88.9%, respectively.

Fig.1. Area over the lymphocyte curve (L-index) in a hypothetical patient

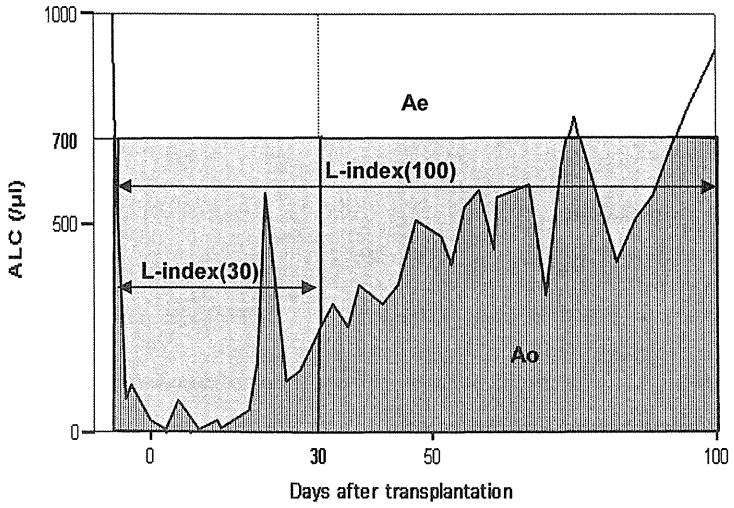
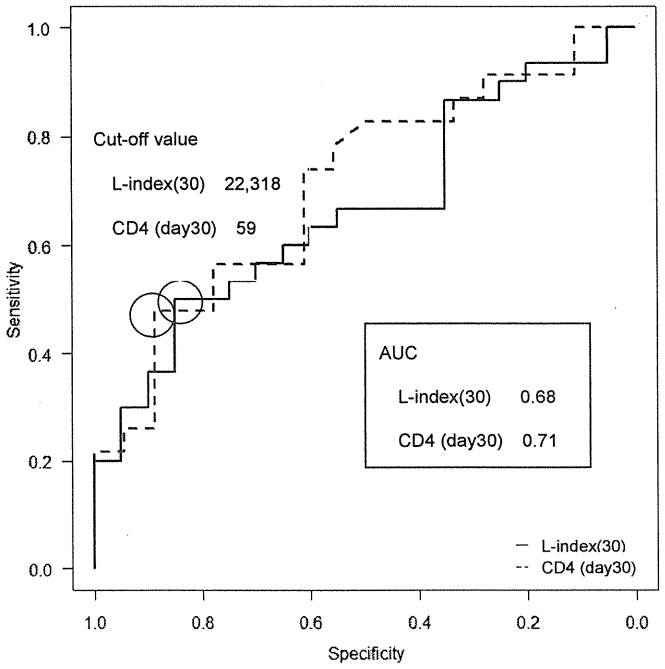


Fig.2. Receiver operating characteristic (ROC) curves comparing L-index(30) with $CD4^+$ cell count at day 30.



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Related transplantation with HLA 1-antigen mismatch in the graft-versus-host direction and HLA 8/8-allele-matched unrelated transplantation: a nationwide retrospective study

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Related transplantation with HLA 1-antigen mismatch in the graft-versus-host direction and HLA 8/8-allele-matched unrelated transplantation: A nationwide retrospective study

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Running head: Transplant from HLA 1-AG mismatched RD vs 8/8 MUD

ABSTRACT

To clarify whether we should prefer a related donor with an HLA-1-antigen mismatch at the HLA-A, HLA-B, or HLA-DR loci in the graft-versus-host direction (RD/1AG-MM-GVH) or an HLA 8/8-allele (HLA-A, HLA-B, HLA-C, and HLA-DRB1) matched unrelated donor (8/8 MUD), we evaluated 779 patients with acute leukemia, chronic myelogenous leukemia, or myelodysplastic syndrome who received a T-cell-replete graft from an RD/1AG-MM-GVH or 8/8 MUD. The use of an RD/1AG-MM-GVH was significantly associated with a higher overall mortality rate than an 8/8 MUD in a multivariate analysis (hazard ratio, 1.49; $P<0.001$), and this impact was statistically significant only in patients with standard-risk diseases ($P=0.001$). Among patients with standard-risk diseases who received transplantation from an RD/1AG-MM-GVH, the presence of an HLA-B-antigen mismatch was significantly associated with a lower overall survival rate than an HLA-DR-antigen mismatch due to an increased risk of treatment-related mortality (TRM). The HLA-C-antigen mismatch or multiple allelic mismatches were frequently observed in the HLA-B-antigen-mismatched group, and were possibly associated with the poor outcome. In conclusion, an 8/8 MUD should be prioritized over an RD/1AG-MM-GVH during donor selection. In particular, an HLA-B-antigen mismatch in the GVH direction has an adverse effect on overall survival and TRM in patients with standard-risk diseases.

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Introduction

An HLA-matched unrelated donor (MUD) is considered to be an alternative donor in hematopoietic stem cell transplantation (SCT) for patients who lack an HLA-identical sibling. However, it is difficult to find an MUD for patients with rare HLA haplotypes. SCT from a related donor with a 1-antigen mismatch at HLA-A, HLA-B, or HLA-DR loci in the graft-versus-host (GVH) direction results in a higher but acceptable incidence of acute GVHD and outcomes comparable to that of SCT from a matched related donor (MRD) in patients with high-risk diseases; this is because it reduces the risk of relapse via a graft-versus-leukemia (GVL) effect.[1-3] In previous studies, HLA mismatches in the HVG direction were associated with higher graft failure and lower overall survival.[1,2,4] However, strategies to reduce the risk of graft failure might have been improved by the use of conditioning regimens that strongly suppress recipient immune system.[5] Therefore, in current clinical practice in Japan, SCT from a related donor with 1-antigen mismatch in the GVH direction is being performed accepting multiple antigen mismatches in the HVG direction without specific stem cell manipulation,[1,2] although such an approach has not yet been evaluated in a large cohort.

Our previous study showed that SCT from an HLA 1-antigen-mismatched donor in the GVH or host-versus-graft (HVG) direction is comparable to that from an HLA-A, HLA-B, and HLA-DR antigen-matched unrelated donor.[11] However, this cohort was relatively old (1991–2000) and may not reflect our current practice. Furthermore, the analysis was mainly performed based on serological matching, since information on HLA allele matching in unrelated transplantation was insufficient. The importance of allele-matching in HLA-A, HLA-B, and HLA-DRB1 loci in unrelated donor transplantation has been established.[6-8] In addition, the importance of allele-matching in the HLA-C locus has been highlighted in several recent studies regarding unrelated transplantation, although HLA-C matching is, in general, still not considered in related

transplantation.[9-12] Therefore, we conducted a nationwide retrospective study to compare the clinical outcomes of transplantation from a related donor with 1-antigen mismatch in the GVH direction (RD/1AG-MM-GVH) to those from an 8/8 MUD.

Methods

Data collection

Data for patients (age: 16–70 years) with acute myelogenous leukemia (AML), acute lymphoblastic leukemia (ALL), myelodysplastic syndrome (MDS), and chronic myelogenous leukemia (CML) who received a first allogeneic transplantation from a related donor or HLA-6/6-antigen-MUD between January 1, 2001 and December 31, 2008 were obtained from the Transplant Registry Unified Management Program (TRUMP),[13] which includes data from the Japan Society for Hematopoietic Cell Transplantation (JSHCT) and Japan Marrow Donor Program (JMDP). Our analysis included 344 patients who received a graft from an RD/1AG-MM-GVH (RD/1AG-MM-GVH group) and 453 patients who received a graft from an 8/8 MUD (8/8 MUD group). The following patients were excluded: 11 patients who lacked data on survival status, survival date, sex of recipient and donor, stem cell source, GVHD prophylaxis, or performance status; 2 patients who received both bone marrow and peripheral blood in related transplantation; and 5 patients who received stem cells manipulated by *ex vivo* T-cell depletion or CD34 selection. Finally, 327 patients who received a graft from an RD/1AG-MM-GVH and 452 from an 8/8 MUD fulfilled the criteria. The data on 2318 patients who received transplantation from an MRD were also collected on the basis of similar inclusion and exclusion criteria to compare the overall survival (OS) rate. The study was approved by the data management committees of TRUMP and by the institutional review board of Saitama Medical Center, Jichi Medical University, where this study was organized.

Histocompatibility

Histocompatibility data for serological and genomic typing for the HLA-A, HLA-B, HLA-C, and HLA-DR loci were obtained from reports obtained from the institution at which the transplantation was performed. To reflect current practice in Japan, HLA matching in RD/1AG-MM-GVHs was assessed by serological data for HLA-A, HLA-B, and HLA-DR loci, while that in 8/8-MUD was assessed by genomic data for HLA-A, HLA-B, HLA-C, 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; on the other hand, a mismatch in the HVG direction was defined as when the donor's antigens or alleles were not shared by the recipient. SCT from a related donor with a 1-antigen mismatch in the GVH direction has been performed by accepting multiple antigen mismatches in the HVG direction,[1,2] and therefore was included in this study.

Endpoints and statistical analyses

The primary endpoint of the study was to compare OS rates between the RD/1AG-MM-GVH and 8/8 MUD groups. For exploratory purposes, OS, treatment-related mortality (TRM), relapse, acute and chronic GVHD, and cumulative incidences of neutrophil engraftment were analyzed in a subset of cohorts. The physicians who performed transplantation at each center diagnosed and graded acute and chronic GVHD according to the traditional criteria.[14,15] The incidence of chronic GVHD was evaluated in patients who survived for at least 100 days. Neutrophil recovery was considered to have occurred when the absolute neutrophil count exceeded 0.5×10^9/L for 3 consecutive days following transplantation.

Descriptive statistics were used to summarize variables related to the patient characteristics. Comparisons between groups were performed with the chi-square statistic

or extended Fisher's exact test as appropriate for categorical variables and the Mann-Whitney *U*-test or Kruskal-Wallis test as appropriate 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 probabilities of TRM, relapse, acute and chronic GVHD, and neutrophil engraftment were estimated on the basis of cumulative incidence curves to accommodate the following competing events:[16] death for relapse, relapse for TRM, death without GVHD for acute and chronic GVHD, and death without engraftment for neutrophil engraftment; the groups were compared with Gray's test.[17] Cox proportional-hazards regression was used to evaluate variables that may affect OS, while Fine and Gray's proportional-hazard model was used to evaluate variables that may affect TRM, relapse, acute and chronic GVHD, and neutrophil engraftment.[18] For patients for whom conditioning intensity (myeloablative or reduced-intensity) was not reported, we re-classified the conditioning regimen as either myeloablative or reduced-intensity according to the NMDP/CIBMTR operational definitions.[19] To be consistent with our previous study, acute leukemia in the first or second remission, CML in the first or second chronic phase, and MDS without leukemic transformation were defined as standard-risk diseases, and others were defined as high-risk diseases.[1] The following variables were considered: the recipient's age group (≤50 years or >50 years at transplantation), recipient's sex, presence of female (donor) to male (recipient) sex mismatch, performance status (PS) (0–1 or 2–4), disease (AML, ALL, CML, or MDS), disease status prior to transplantation (standard- or high-risk), type of conditioning regimen (myeloablative or reduced-intensity), type of GVHD prophylaxis (cyclosporine- or tacrolimus-based, or other), use of antithymocyte globulin (ATG) or alemtuzumab, and the time from diagnosis to transplantation (<6 months or ≥6 months). In addition, a variable of graft source (bone marrow or peripheral blood) was also considered in the analysis specific to related donors. Factors with $P < 0.10$ in the univariate analysis were

used in the first multivariate model without donor type and deleted in a stepwise manner from the model by backward selection. Then, we added donor type to the final model. All tests were 2-sided, and $P < 0.05$ was considered to indicate statistical significance. All statistical analyses were performed with STATA version 11 (Stata Corp., College Station, TX) and R, Version 2.12.0 (The R Foundation for Statistical Computing, Vienna, Austria) software.

Results

Patient characteristics

Compared to recipients of an 8/8 MUD, recipients of an RD/1AG-MM-GVH were more likely to be younger, be male receiving a transplant from a female donor, have a shorter duration from diagnosis to transplantation, have a high-risk disease, receive cyclosporine for GVHD prophylaxis, use ATG or alemtuzumab, and have a longer follow-up period (Table 1). Approximately half of the recipients in the RD/1AG-MM-GVH group received peripheral blood stem cells, whereas, during this period in Japan, the source of transplantation from an MUD was restricted to bone marrow. In the RD/1AG-MM-GVH group, the number of antigen mismatches in the HVG direction was 0 in 11%, 1 in 67%, 2 in 20%, and 3 in 2%. HLA-A, -B and -DRB1 allelic information in both recipients and donors was available in 148 of 327 transplantations from an RD/1AG-MM-GVH, and information on HLA-C antigen mismatch in either the GVH or HVG direction was available in 123 of 327 transplantations from an RD/1AG-MM-GVH.

OS

The 2-year OS rates in the 8/8 MUD and RD/1AG-MM-GVH groups were 0.59 (95% confidence interval [CI], 0.53–0.64) and 0.44 (95% CI, 0.38–0.49), respectively (log-rank test; $P < 0.001$) (Fig. 1A). Multivariate analysis revealed that, compared to the use of an

8/8 MUD, the use of an RD/1AG-MM-GVH was a significant adverse factor for OS (hazard ratio (HR) [95% CI], 1.49 [1.19–1.86], $P < 0.001$) (Table 2). Age > 50 years, PS ≥ 2, and high-risk disease were also found to be significant adverse factors, while other variables, such as the time from diagnosis to transplantation, were not.

Since our previous study showed that the impact of an HLA 1-antigen mismatch in a related transplantation on OS differed according to whether patients had either standard-risk or high-risk disease,[1] the survival rates were compared separately in each disease-risk group. The OS rates of patients with standard-risk diseases in the 8/8 MUD group were significantly higher than those in the RD/1AG-MM-GVH group ($P = 0.003$), while there was no significant difference in high-risk patients ($P = 0.090$) (Fig. 1B and 1C). Although the interaction between the donor type and disease risk did not reach statistical significance ($P = 0.140$), multivariate analyses in each disease-risk group showed that the adverse impact of the use of an RD/1AG-MM-GVH was significant in standard-risk patients (HR [95% CI], 1.72 [1.24–2.39], $P = 0.001$), but not in high-risk patients (Table 2).

To visually compare MRDs and other stem cell sources, the OS rate for MRDs was layered on those for MUDs and RD/1AG-MM-GVHs (Fig. 1). The OS curve of transplantation from an MRD was superimposed on that from an MUD in both standard- and high-risk patients (MRD vs. MUD; standard-risk group, $P = 0.895$, and high-risk group, $P = 0.581$). Multivariate analysis confirmed that overall survival in the MRD group was comparable to the MUD group (MRD vs. MUD; standard-risk group, HR [95% CI], 1.02 [0.79–1.32], $P = 0.878$, and high-risk group, HR [95% CI], 0.98 [0.76–1.26], $P = 0.865$).

Effect of HLA mismatches on OS

To identify factors that may contribute to the inferior OS in standard-risk patients in the RD/1AG-MM-GVH group compared to those in the 8/8 MUD group, we evaluated the impact of each HLA-A, HLA-B, or HLA-DR-antigen mismatch in the GVH direction and the number of antigen mismatches in the HVG direction on OS rates in the RD/1AG-MM-GVH group.

In the RD/1AG-MM-GVH group, the OS rate for patients who received a transplantation from a related donor with an HLA-B-antigen mismatch in the GVH direction and that from a donor with 2- or 3-antigen mismatches in the HVG direction were significantly lower than those in other groups (log-rank test for HLA-A-antigen mismatch vs. HLA-B-antigen mismatch vs. HLA-DR-antigen mismatch in the GVH direction; $P < 0.001$ and 0-1 mismatch vs. 2-3 mismatches in the HVG direction; $P = 0.003$) (Fig. 2). However, a multivariate analysis revealed that only the presence of an HLA-B-antigen mismatch in the GVH direction (HR [95% CI], 1.57 (1.13–2.18), $P = 0.007$) was significantly associated with a lower OS (Table 3).

The OS rates were also compared separately in the standard-risk and high-risk disease groups (Fig. 2). Although the interaction between the presence of HLA-B-antigen mismatch and disease risk did not reach statistical difference ($P = 0.232$), the adverse impact of an HLA-B-antigen mismatch in the GVH direction was observed in the standard-risk group (HR [95% CI], 1.86 (1.14–3.01), $P = 0.012$), but not in the high-risk group (Table 3). On the other hand, the survival curve for HLA-A- or HLA-DR-antigen-mismatched group was almost superimposed on that for 8/8 MUDs (Fig. 2) (standard-risk group; HR [95% CI] for HLA-A-antigen mismatched group vs. 8/8 MUD, 1.26 [0.73–2.19], $P = 0.411$ and HR [95% CI] for HLA-DR-antigen mismatched group vs. 8/8 MUD, 1.37 [0.89–2.11], $P = 0.154$, high-risk group; HR [95% CI] for HLA-A-antigen mismatched group vs. 8/8 MUD, 1.26 [0.80–2.00], $P = 0.320$ and HR [95% CI] for HLA-DR-antigen mismatched group vs. 8/8 MUD, 1.03 [0.67–1.59], $P = 0.880$). The impact of 2- or 3-antigen mismatches in the HVG direction was not

significant in either the standard-risk or high-risk group (Table 3).

Effect of an HLA-B mismatch on TRM, relapse, GVHD, and neutrophil engraftment in patients with standard-risk diseases

These findings showed that an HLA-B-antigen mismatch in the GVH direction strongly contributed to the low survival rate in standard-risk patients, which can explain the inferior survival rates in the RD/1AG-MM-GVH group compared to the 8/8 MUD group. Therefore, we evaluated the impact of an HLA-B-antigen mismatch in the GVH direction on other outcomes in patients with standard-risk diseases in the RD/1AG-MM-GVH group.

First, we compared the characteristics of patients with standard-risk diseases who received transplantation from a related donor with an HLA-A, HLA-B, and HLA-DR-antigen mismatch in the GVH direction (Supplemental Table 1). Two- or three-antigen mismatches in the HVG direction were observed more frequently in the HLA-B-antigen-mismatched group (28%) than in the HLA-A-antigen- (2%) or HLA-DR-antigen-mismatched group (17%). Although there was no information available on allelic mismatch or HLA-C-antigen mismatch in more than half of the patients, an HLA-C-antigen mismatch in either the GVH or HVG direction was observed more frequently in the HLA-B-antigen-mismatched group (61% among the available data) than in the HLA-A-antigen- (25%) or HLA-DR-antigen-mismatched group (17%).

The incidence of TRM was higher in the HLA-B-antigen-mismatched group (3-year mortality rate; 0.47 [95% CI, 0.32-0.60]) than in the HLA-A- (0.28 [95% CI, 0.14-0.44]) or HLA-DR-antigen-mismatched group (0.27 [95% CI, 0.17-0.38]) (Fig. 3A; log-rank test, $P = 0.030$). The presence of an HLA-B-antigen mismatch in the GVH direction was an independent significant adverse factor that affected TRM in the RD/1AG-MM-GVH group (Table 4). On the other hand, the incidence of relapse did not

significantly differ among the 3 groups (Fig. 3B, Table 4).

The incidence of grade 2–4 acute GVHD in the HLA-B-antigen-mismatched group was higher than that in the HLA-A-antigen-mismatched group, but comparable to that in the HLA-DR-antigen-mismatched group (Supplemental Figure 1, Supplemental Table 2). There was no significant difference in the incidence of grade 3-4 acute GVHD among the 3 groups. Regarding neutrophil engraftment, a multivariate analysis showed that an HLA-B-antigen mismatch was significantly associated with inferior neutrophil engraftment, and 2- or 3-antigen mismatches in the HVG direction were associated with inferior neutrophil engraftment with marginal significance (Supplemental Table 2).

Discussion

In this nationwide retrospective study, we found that the survival rate of the RD/1AG-MM-GVH group was significantly inferior to that of the 8/8 MUD group, and this significant difference was observed only in patients with standard-risk diseases, although the interaction between donor type and disease risk did not reach statistical significance. We previously reported that transplantation from a related donor with a 1-antigen mismatch in the GVH or HVG direction gave a clinical outcome comparable to that of transplantation from a 6/6-antigen-MUD in patients with either standard-risk or high-risk diseases.[1] However, since HLA matching at the allelic level in unrelated transplantation significantly reduced the risk of GVHD, the survival curve of transplantation from an 8/8 MUD was substantially improved, and could be superimposed on a curve corresponding to that from an MRD in the current study. Consistent with our findings, several studies have shown that the clinical outcomes of transplantation from an 8/8–10/10 MUD are comparable to those from an MRD.[20,21] The significant difference in survival rates between transplantation from an RD/1AG-MM-GVH and 8/8 MUD disappeared in patients with high-risk diseases,

probably because the adverse impact of acute GVHD on survival might be offset by the potential GVL effect in transplantation from an RD/1AG-MM-GVH.[1,2,22]

We evaluated factors that may contribute to the inferior OS in patients with standard-risk diseases in the RD/1AG-MM-GVH group, and found that, compared to the presence of an HLA-DR-antigen mismatch, the presence of an HLA-B-antigen mismatch in the GVH direction was significantly associated with lower OS and higher TRM. On the other hand, the rates of OS and TRM in the HLA-A- or HLA-DR-mismatched group were superimposed on those in the MUD group. However, HLA-A, HLA-B, and HLA-DR-antigen mismatches had similar effects on the incidence of severe acute GVHD; consequently, the causal relationship between an HLA-B-antigen mismatch in the GVH direction and higher TRM remains unknown. In contrast to our findings, Valcarcel et al.[23] reported that there was no significant difference in OS between the use of 1-antigen-mismatched related donors (n = 89) and 8/8 MUDs (n = 700) in transplantation for AML and ALL during the first or second complete remission. The difference from our results can be partly explained by the fact that the MUD group in their study included a significantly smaller number of ALL patients with low-risk cytogenetics. In addition, genetic homogeneity in the Japanese population might affect the lower incidence of severe acute GVHD in MUD transplantation in our study, due to the less frequent mismatches in minor histocompatibility antigens.[24,25]

The frequency of an HLA-C-antigen mismatch was substantially higher in the HLA-B-antigen-mismatched group than in the HLA-A or HLA-DR-mismatched group. This finding may represent linkage disequilibrium between the HLA-B and HLA-C genes; they are located at a very close physical proximity within the major histocompatibility complex.[26,27] Therefore, the impact of HLA-B-antigen might be

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affected by the co-presence of HLA-C-antigen mismatch. We could not evaluate the impact of HLA-C antigen mismatch on OS rates due to the limited information on HLA-C antigen mismatch; therefore, an analysis with larger cohorts with complete HLA-C antigen information will be needed to evaluate the impact of HLA-C and/or HLA-B mismatch in transplantation from an RD/1AG-MM-GVH. Accordingly, we could not evaluate the impact of the KIR ligand mismatch. Although the impact of KIR ligand mismatch is still controversial, several studies analyzing T-cell-replete transplantation showed that KIR ligand mismatch is associated with lower over survival.[12,28,29] The analysis of KIR matching would be helpful to elucidate the mechanism underlying the adverse effect of HLA-B mismatch in T-cell-replete transplantation from an RD/1AG-MM-GVH.

Whether the presence of allelic mismatches in addition to the 1-antigen mismatch (2 or more allelic mismatches in total) affects the transplantation outcome is also an important clinical question in transplantation from an RD/1AG-MM-GVH. A high frequency of 2-allele mismatches in the GVH direction was seen in the HLA-B-antigen-mismatched group, suggesting a possible association between 2-allele mismatches and low OS. However, we did not observe a significant effect of the number of allelic mismatches on OS after transplantation from an RD/1AG-MM-GVH, possibly due to the small sample size.

This study has several limitations. First, since several months are required to arrange unrelated transplantation, patients at low risk for relapse may more often be selected for unrelated transplantation. To minimize this bias, we included the duration from diagnosis to transplantation in the multivariate analysis; however, this variable did not have a significant effect in the multivariate analysis. Second, heterogeneous

backgrounds may have resulted in a bias. Particularly, the stem cell source in unrelated transplantation was exclusively bone marrow. However, the analysis of OS in the subgroup of patients who received a bone marrow graft from an RD/1AG-MM-GVH or 8/8 MUD showed similar results. Third, because we have incomplete antigen information on HLA-C and -DQB1 loci as well as allelic information, we may have underestimated the degree of mismatching in transplantation from an RD/1AG-MM-GVH. Fourth, the difference in the impact of donor type between standard- and high-risk diseases should be cautiously interpreted, because the interaction between the donor type and disease risk did not reach statistical significance. This may be partly due to the lower statistical power to detect the interaction than the main effect.

In conclusion, our findings suggest that an 8/8 MUD, if available, should be prioritized over an RD/1AG-MM-GVH for patients without an MRD if an immediate transplantation is not necessary. In particular, the presence of an HLA-B-antigen mismatch in the GVH direction has an adverse effect on OS because of treatment-related complications. This may be due to the high frequencies of additional mismatches of HLA-C-antigen or allele in the HLA-B-antigen-mismatched group. To elucidate the mechanism of the adverse outcomes in RD/1AG-MM-GVHs with an HLA-B-antigen mismatch, HLA antigen/allele matching including HLA-C should be performed in transplantation from an RD/1AG-MM-GVH.

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Authorship

Contributions: Y.K. designed the research and organized the project; J.K., H.Saji., and Y.K. reviewed data, analyzed data, and wrote the paper; J.K. and Y.K. performed statistical analysis; H.Sakamaki., J.T., R.S., and Y.A. collected data from JSHCT; K.K. and Y.M. collected data from JMDP; all authors interpreted data, reviewed and approved final manuscript.

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Table 1. Patient characteristics

Variable		RD/1AG-MM-GVH (n = 327)	8/8 MUD (n = 452)	P
Age at transplant, median (range)		45 (16-69)	48 (16-68)	0.043
Recipient sex	Male	184 (56%)	267 (59%)	0.434
	Female	143 (44%)	185 (41%)	
Sex combination of donors and recipients	Female to male	91 (28%)	73 (16%)	<0.001
	Other combinations	236 (72%)	379 (84%)	
Performance status	0/1	298 (91%)	415 (92%)	0.736
	2/3/4	29 (9%)	37 (8%)	
Disease	AML	167 (51%)	249 (55%)	0.512
	ALL	90 (28%)	107 (24%)	
	CML	19 (6%)	21 (5%)	
	MDS	51 (16%)	75 (17%)	
Duration from diagnosis to transplant	<6 months	124 (38%)	102 (23%)	<0.001
	>=6 months	191 (58%)	350 (77%)	
	Unknown	12 (4%)	0 (0%)	
Disease risk	Standard	175 (54%)	317 (70%)	<0.001
	High	133 (41%)	129 (29%)	
	Unknown	19 (6%)	6 (1%)	
Source of stem cells	Bone marrow	142 (43%)	452 (100%)	<0.001
	Peripheral blood	185 (57%)	-	
HLA compatibility in the HVG direction*	Matched	36 (11%)	452 (100%)	<0.001
	One-antigen mismatch	218 (67%)	-	
	Two-antigen mismatch	65 (20%)	-	
	Three-antigen mismatch	8 (2%)	-	
HLA compatibility in the GVH direction*	Matched	0 (0%)	452 (100%)	<0.001
	One-allele mismatch	111 (34%)	-	
	Two-allele mismatch	36 (11%)	-	
	Three-allele mismatch	1 (0%)	-	
	Uncertain/missing	179 (55%)	-	