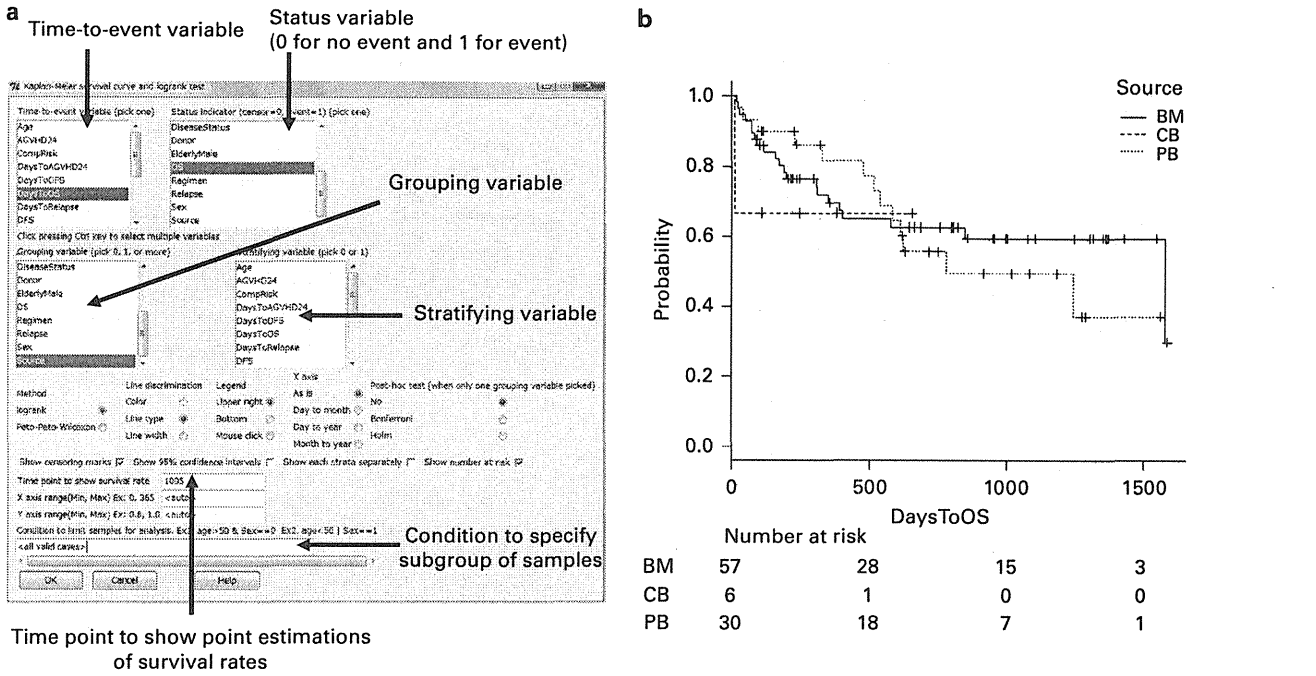


Figure 2. (**a**) Dialog for plotting KM curves and performing a log-rank test. (**b**) KM curves of overall survival grouped according to the stem cell source.

command reflects the full model and not the model after stepwise selection of explanatory variables. Survival curves adjusted for other factors by the mean of covariates method, in which average values of covariates are entered into the Cox proportional hazards model, can be drawn by selecting **'Graphs'>'Adjusted survival curve'**.

A TD covariate can be incorporated in the Cox proportional hazards regression in EZR. However, the function is limited to a simple TD covariate. EZR can handle only one TD covariate, which is initially 0 and may change to a value of 1 thereafter. For example, if a user wants to evaluate the impact of grade II–IV acute GVHD on survival, it is not appropriate to treat the development of acute GVHD as if it were known before transplantation, as patients who died or relapsed before the development of GVHD would be included in the 'no GVHD group'. A variable whose value may change after transplantation should be treated as a TD covariate, and this can be performed in EZR by selecting **'Statistical analysis'>'Survival analysis'>'Cox proportional hazard regression with time-dependent covariate'**. In this case, 'AGVHD24', which has a value of 1 for patients who developed grade II–IV acute GVHD, should be selected in the 'TD covariate' list. 'DaysToAGVHD24', which is the time from transplantation to the development of grade II–IV acute GVHD for patients who developed grade II–IV acute GVHD or the time to the last evaluation for patients who did not develop grade II–IV acute GVHD, should be specified in the 'Time when TD covariate changes from 0 to 1' list. Other explanatory variables should be specified in the same manner with Cox proportional hazard regression, as described above. In the **'Output window'**, the effect of grade II–IV acute GVHD will be shown in the row 'covariate_td'.

COMPETING RISK ANALYSIS

A competing risk analysis is an important statistical function in studies on hematopoietic SCT. For example, if an investigator wants to analyze the cumulative incidence of relapse after transplantation, death without relapse (non-relapse mortality) precludes the occurrence of relapse. Previously, one minus the Kaplan–Meier (1 – KM) method of relapse while treating deaths without relapse as censored observations has been used to estimate the incidence of relapse. However, this analysis overestimates the incidence of relapse, as it attempts to predict the incidence of relapse when patients who actually die would have relapsed. As a result, the sum of the incidence of relapse, the incidence of non-relapse mortality and the probability of relapse-free survival exceeds 100%. A more appropriate estimate can be obtained using the cumulative incidence function. This method subdivides the probability of failure into the probability corresponding to each competing event and provides an accurate incidence for each event. The statistical significance of the difference in the cumulative incidences of competing events among groups can be assessed by Gray's test.[9] In addition, regression models for competing risks data have been proposed by Fine and Gray,[10] and by Klein and Anderson.[11]

These competing risk analyses can be provided by adding the 'cmprsk' package to R.[6] Excellent instructions for the use of this package have been provided in this journal by Scrucca *et al.* in 2007 and 2010.[12,13] EZR makes it possible to access these analyses in a point-and-click manner. For example, the cumulative incidences of relapse and non-relapse mortality can be plotted and compared among groups by selecting **'Statistical analysis'>'Survival analysis'>'Cumulative incidence of competing events and Gray test'** (Figure 4b). Users have to specify a time-to-event variable ('DaysToDFS' in this case, which indicates the time to the earliest event or time to the last evaluation for patients without any events), a status indicator ('CompRisk', which has a value of 1 for relapse, 2 for non-relapse mortality and 0 for no event), and grouping variables, if required ('Source' in this case). The **'Output window'** shows the results of Gray's test following the point estimations with 95% confidence intervals of the cumulative incidences of each event. If a user wants to plot cumulative incidence curves for only one of the

a

Point estimations, standard errors, and 95% confidence intervals of survival rate at each time point

```
> summary(km)
Call: survfit(formula = Surv(DaysToOS, OS) ~ Source, data = Dataset,
    na.action = na.omit, conf.type = "log-log")

                Source=BM
 time n.risk n.event survival std.err lower 95% CI upper 95% CI
   22     57       1    0.982  0.0174        0.8819        0.998
   25     56       1    0.965  0.0244        0.8669        0.991
   30     55       1    0.947  0.0296        0.8456        0.983
   54     54       1    0.930  0.0338        0.8237        0.973
   74     53       1    0.912  0.0375        0.8020        0.963
   76     52       1    0.895  0.0406        0.7806        0.951
   84     51       1    0.877  0.0435        0.7596        0.939
   95     48       1    0.859  0.0462        0.7376        0.927
  120     45       1    0.840  0.0490        0.7146        0.913
  160     44       1    0.821  0.0515        0.6921        0.899
  176     43       1    0.802  0.0537        0.6701        0.885
  190     42       1    0.783  0.0557        0.6485        0.870
  201     41       1    0.763  0.0575        0.6272        0.855
  308     34       1    0.741  0.0601        0.6010        0.838
  313     33       1    0.719  0.0623        0.5754        0.821
```

c

	A	B	C	D	E	F
1	Factor	Group	n	probability	median	p.value
2	Disease	ALL	16	0.397 (0.127–0.661)	479 (231–NA)	0.647
3		AML	62	0.606 (0.455–0.727)	1578 (781–NA)	
4		MDS	15	0.503 (0.187–0.755)	NA (176–NA)	
5	DiseaseRisk	High	30	0.191 (0.044–0.416)	331 (97–613)	2.09E-06
6		Low	63	0.709 (0.561–0.815)	1578 (1242–NA)	
7	Donor	R	35	0.554 (0.353–0.715)	1242 (584–NA)	0.921
8		U	58	0.566 (0.405–0.699)	NA (404–NA)	
9	Source	BM	57	0.595 (0.439–0.721)	1578 (404–NA)	0.705
10		CB	6	NA NA	NA (13–NA)	
11		PB	30	0.494 (0.275–0.681)	781 (539–NA)	

b

Numbers of patients, observed events, and expected events based on the null hypothesis of each group

```
> (res <- survdiff(Surv(DaysToOS,OS)~Source, data=Dataset, rho=0, na.action =
+   na.omit))
Call:
survdiff(formula = Surv(DaysToOS, OS) ~ Source, data = Dataset,
    na.action = na.omit, rho = 0)

              N Observed Expected (O-E)^2/E (O-E)^2/V
Source=BM    57       21    22.76    0.1354     0.376
Source=CB     6        2     1.24    0.4689     0.490
Source=PB    30       13    12.01    0.0822     0.126

 Chisq= 0.7  on 2 degrees of freedom, p= 0.705
```

P value

Figure 3. (**a**) Point estimations, s.e. and 95% confidence intervals of survival rate at each time point. (**b**) The results of log-rank test. (**c**) Summary of the survival analyses copied to the clipboard and then pasted into a spreadsheet.

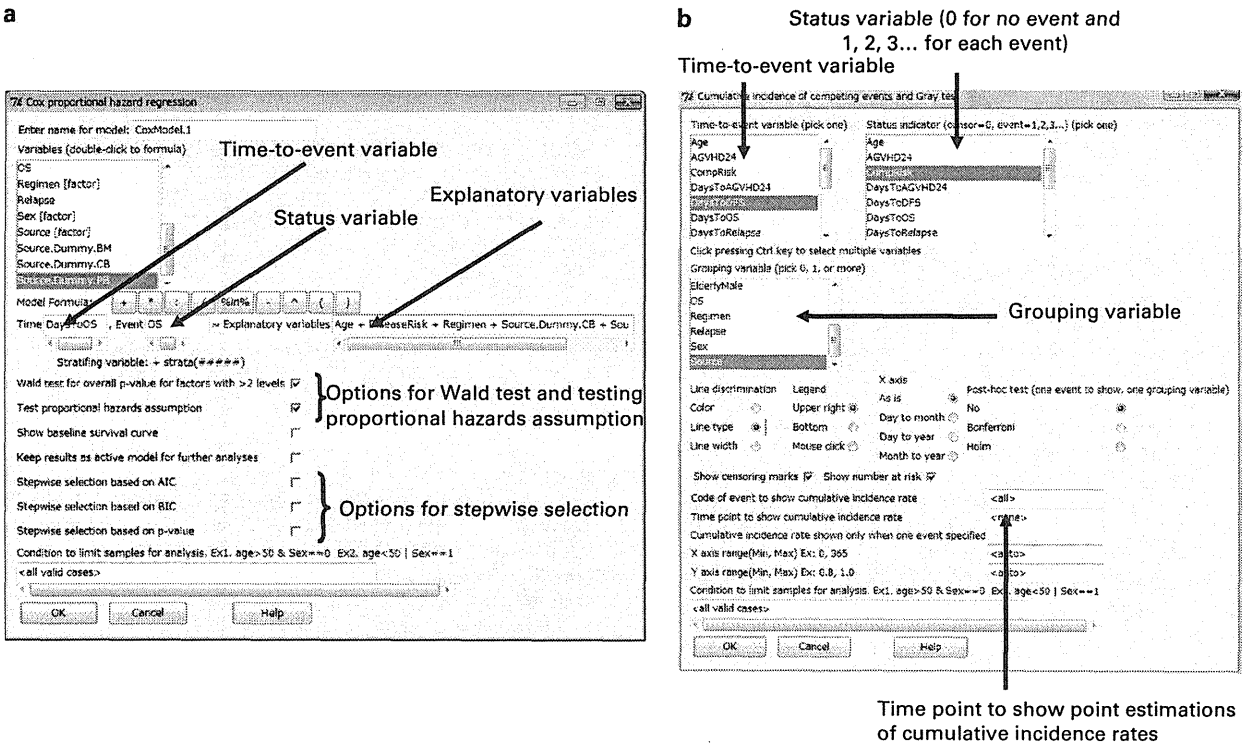


Figure 4. (**a**) Dialog for performing a Cox proportional hazards regression. (**b**) Dialog for plotting cumulative incidence curves and performing Gray's test.

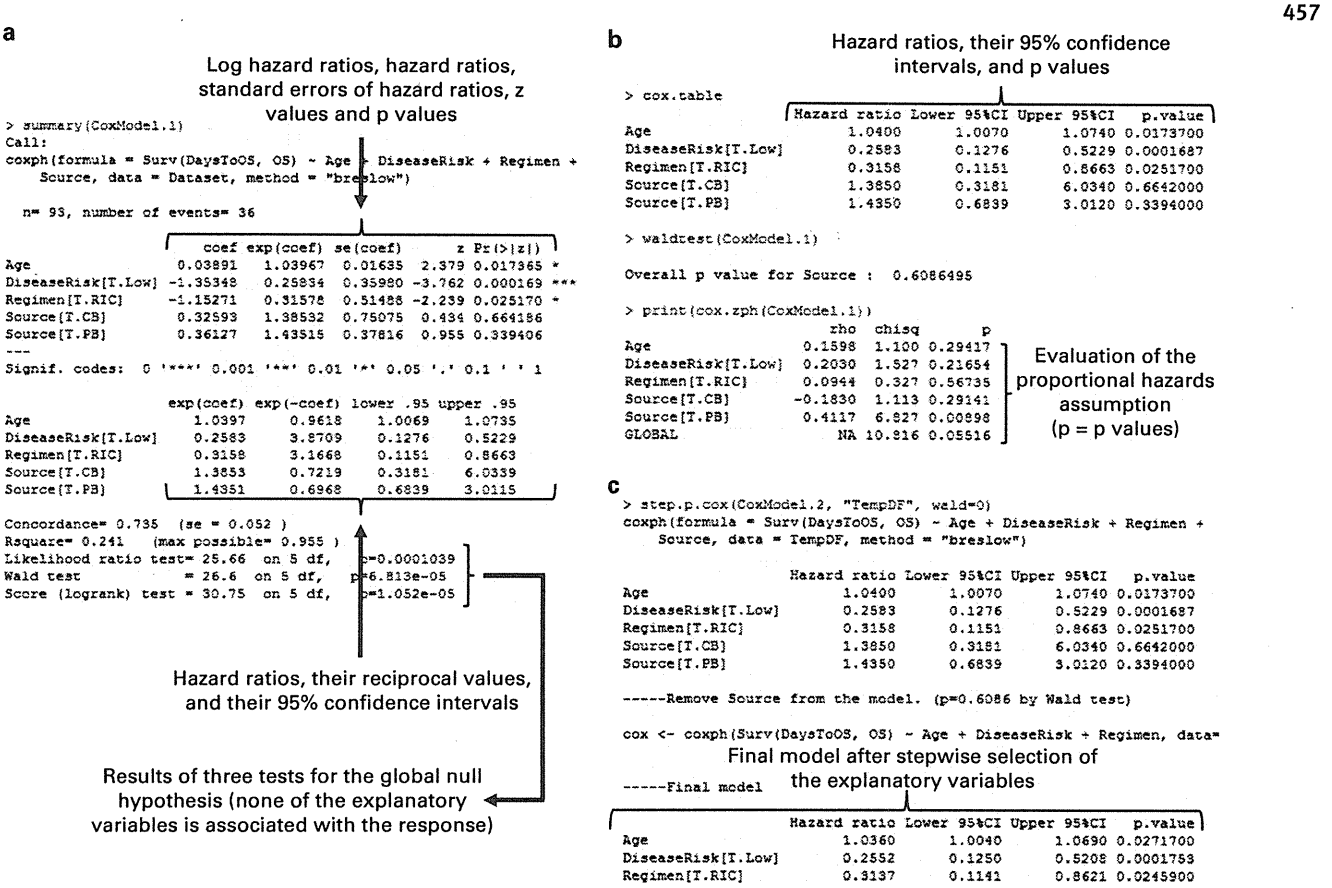


Figure 5. (**a**) Main result of Cox proportional hazard regression that includes the hazard ratios, their 95% confidence intervals and *P*-values for each explanatory variable, followed by the results of three tests for the global null hypothesis. (**b**) Summary of proportional hazards regression analysis, the results of Wald test and the results of testing the proportional hazards assumption. (**c**) Results of stepwise selection of explanatory variables.

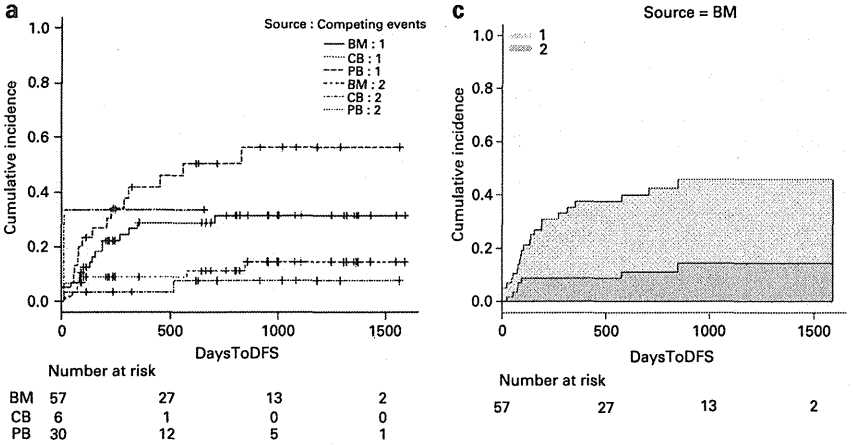
competing events, the number of event that corresponds to the event of interest should be specified in the 'Code of event to show cumulative incidence rate' column in the dialog. If more than 1 grouping variable is specified and only one of the events is specified in the 'Code of event to show cumulative incidence rate' column, a summary table will be shown, which can be copied to the clipboard by the w.ci() command (Figure 6b). A graph that shows the cumulative incidences in a stacked manner can be plotted by selecting **'Graphs'>'Stacked cumulative incidences'** (Figure 6c).

Fine and Gray regression modeling can be performed from the menu, **'Statistical analysis'>'Survival analysis'>'Fine-Gray proportional hazard regression for competing events'**. Users have to specify a time-to-event variable, a status variable, the number of event corresponding to the event of interest and explanatory variables. The results of a regression analysis can be copied to the clipboard by the w.multi(crr.table) command. When we consider the sample file, if the effect of the use of PB or CB compared with BM on the incidence of relapse is evaluated by adjusting for age, disease risk and conditioning regimen, the use of PB as stem cell graft is significantly associated with an increased incidence of relapse with a subdistribution hazard ratio of 2.37 (95% confidence interval: 1.11–5.04; $P=0.025$). However, this result should be considered with caution, as the overall *P*-value for stem cell graft was 0.070 by the Wald test, which can be calculated by checking this option in the dialog.

I should note that the log-rank test and Cox proportional hazards regression are also valid analyses of competing risks data. In these analyses, cause-specific hazard function is evaluated instead of the cumulative incidence function, censoring events other than the event of interest. Therefore, the time-to-event variable should indicate the time to the earliest event or time to the last evaluation for patients without any events, and the status variable should have a value of 1 for event of interest and 0 for other events or no event. The choice and interpretation of these statistical tests for competing risks data are discussed elsewhere.[14]

FINAL REMARKS

In addition to the functions introduced above, EZR enables the analysis of diagnostic tests in the **'Statistical analysis'>'Accuracy of diagnostic test'** menu, matched-pair analysis in the **'Statistical analysis'>'Matched-pair analysis'** menu, meta-analysis in the **'Statistical analysis'>'Metaanalysis and metaregression'** menu and a sample size calculation in the **'Statistical analysis'>'Calculate sample size'** menu. A variety of graphs can be accessed in the **'Graphs'** menu and the statistical functions that were included in the original R commander can be found in the **'Original menu'**. Created graphs can be copied to the clipboard from the menu of the graph window, **'File'>'Copy to the clipboard'**, either as a bitmap or as a metafile. I hope that EZR will help researchers to perform statistical analyses, especially in clinical studies on hematopoietic SCT.



b

	A	B	C	D	E	F
1	Factor	Group	n	Incidence	median	pvalue
2	Disease	ALL	16	0.727 (0.344-0.859)	272	0.016
3		AML	62	0.312 (0.190-0.431)	NA	
4		MDS	15	0.400 (0.155-0.637)	NA	
5	DiseaseRisk	High	30	0.473 (0.261-0.643)	NA	3.51E-02
6		Low	63	0.359 (0.227-0.492)	NA	
7	Regimen	MAC	74	0.389 (0.269-0.507)	NA	0.842
8		RIC	19	NA (NA- NA)	NA	
9	Source	BM	57	0.313 (0.169-0.445)	NA	0.17
10		CB	6	NA (NA- NA)	NA	
11		PB	30	0.542 (0.333-0.739)	558	

Figure 6. (**a**) Cumulative incidence curves of relapse (event = 1) and non-relapse mortality (event = 2) grouped according to the stem cell source. (**b**) Summary of the cumulative incidence analyses copied to the clipboard and then pasted into a spreadsheet. (**c**) Stacked cumulative incidence graph. The light gray area indicates the incidence of relapse (event = 1) and the dark gray area indicates the incidence of non-relapse mortality (event = 2).

CONFLICT OF INTEREST

The author declares no conflict of interest.

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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).

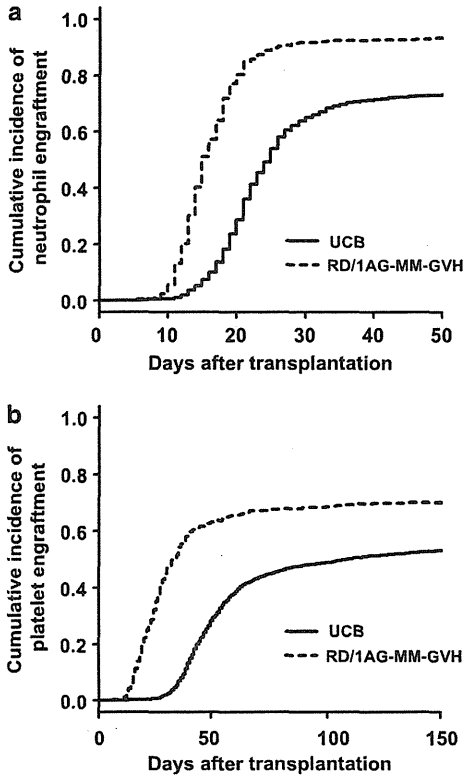

Figure 1. Neutrophil (**a**) and platelet engraftment (**b**).

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

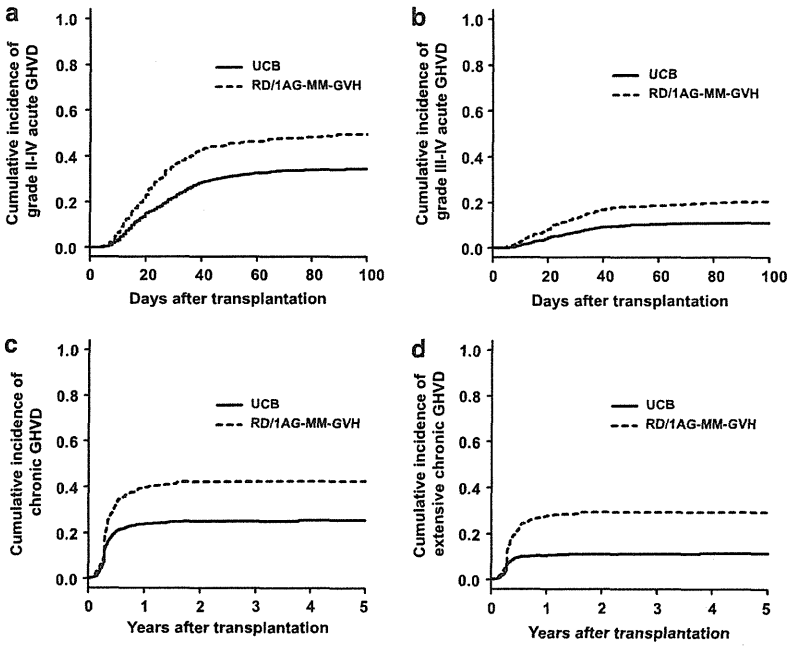

Figure 2. Acute and chronic GVHD. Cumulative incidences of grade II–IV (**a**) and grade III–IV acute GVHD (**b**) and chronic (**c**) and extensive chronic GVHD (**d**) are shown.

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, $P=0.002$).

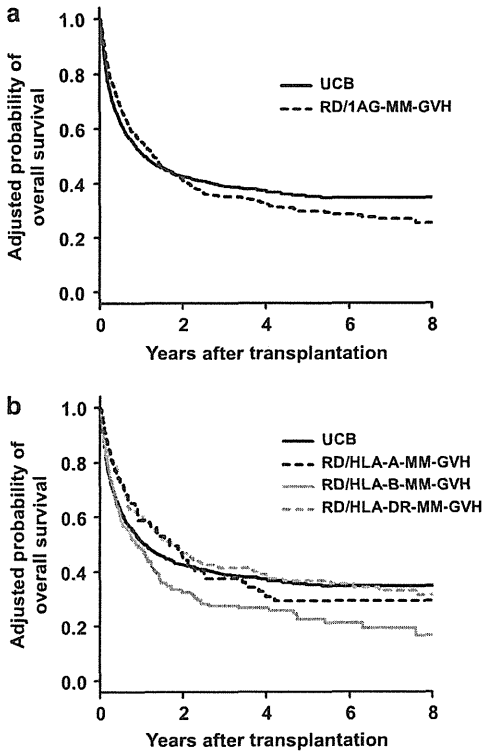

Figure 3. Overall survival. Overall survival rates in the transplantation using an unrelated cord blood vs a related donor with a 1-antigen mismatch at the HLA-A, HLA-B or HLA-DR locus in the GVH direction (**a**) or with an HLA-A, -B, or -DR antigen mismatch in the GVH direction (**b**) are shown.

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.

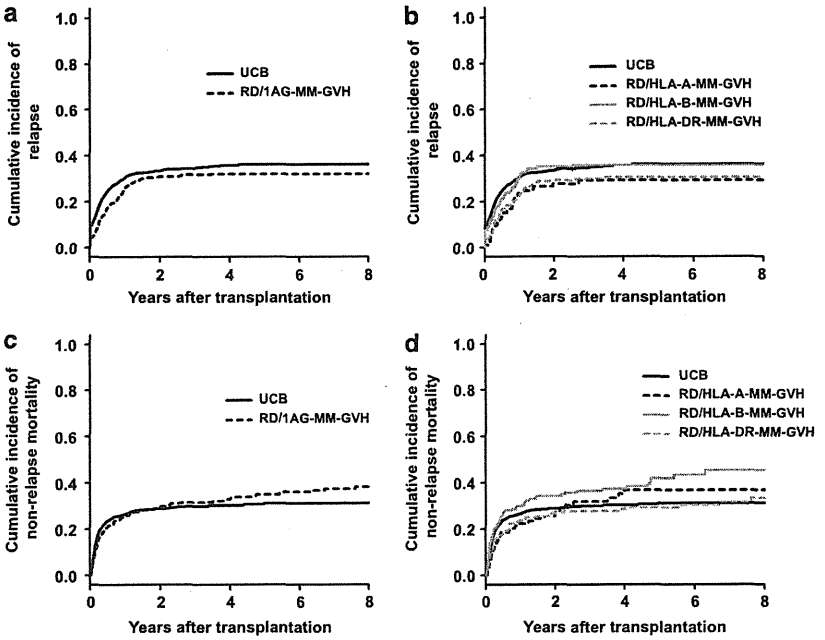

Figure 4. Relapse and non-relapse mortality. Cumulative incidence of relapse and non-relapse mortality after transplantation using an unrelated cord blood vs a related donor with a 1-antigen mismatch at the HLA-A, HLA-B or HLA-DR locus in the GVH direction (**a**, **c**) or with an HLA-A, -B, or -DR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

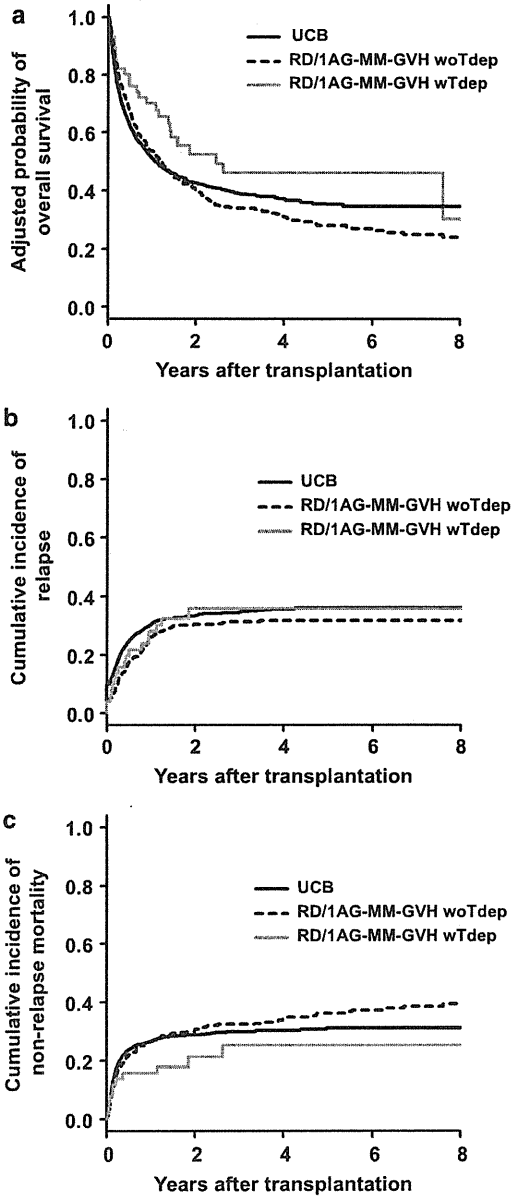

Figure 5. OS (**a**), relapse (**b**) and NRM (**c**) according to the use of *in vivo* T-cell depletion in the RD/1AG-MM-GVH group.

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. $>2.5\times10^7$/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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Related transplantation with HLA-1 Ag mismatch in the GVH direction and HLA-8/8 allele-matched unrelated transplantation: a nationwide retrospective study

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To clarify which is preferable, a related donor with an HLA-1 Ag mismatch at the HLA-A, HLA-B, or HLA-DR loci in the graft-versus-host (GVH) 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 donor was significantly associated with a higher overall mortality rate than the use of an 8/8-MUD in a multivariate analysis (hazard ratio, 1.49; $P < .001$), and this impact was statistically significant only in patients with standard-risk diseases ($P = .001$). Among patients with standard-risk diseases who received transplantation from an RD/1AG-MM-GVH donor, the presence of an HLA-B Ag mismatch was significantly associated with a lower overall survival rate than an HLA-DR Ag mismatch because of an increased risk of treatment-related mortality. The HLA-C Ag mismatch or multiple allelic mismatches were frequently observed in the HLA-B Ag-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 donor during donor selection. In particular, an HLA-B Ag mismatch in the GVH direction has an adverse effect on overall survival and treatment-related mortality in patients with standard-risk diseases. (*Blood.* 2012;119(10):2409-2416)

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 1 Ag 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 because it reduces the risk of relapse via a graft-versus-leukemia (GVL) effect.[1-3] In previous studies, HLA mismatches in the host-versus-graft (HVG) direction were associated with higher graft failure and lower overall survival (OS).[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 Ag mismatch in the GVH direction and accepting multiple Ag mismatches in the HVG direction without specific stem cell manipulation is being performed,[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 Ag-mismatched donor in the GVH or HVG direction is comparable to that from an HLA-A, HLA-B, or HLA-DR Ag-MUD.[1] However, this study is relatively old (1991-2000) and may not reflect current practice. Furthermore, the analysis was mainly performed based on serological matching, because information on HLA allele matching in unrelated transplantation was insufficient at that time. The importance of allele matching at the HLA-A, HLA-B, and HLA-DRB1 loci in unrelated donor transplantation has been established previously.[6-8] In addition, the importance of allele matching at the HLA-C locus has been highlighted in several recent studies of unrelated transplantation, although HLA-C matching is, in general,

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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 an HLA-1 Ag mismatch at the HLA-A, HLA-B, or HLA-DR loci in the GVH direction (RD/1AG-MM-GVH) with an HLA 8/8-allele (HLA-A, HLA-B, HLA-C, and HLA-DRB1)–MUD (8/8-MUD).

Methods

Data collection

Data for patients 16-70 years of age with acute myelogenous leukemia (AML), acute lymphoblastic leukemia (ALL), myelodysplastic syndrome (MDS), or chronic myelogenous leukemia (CML) who received a first allogeneic transplantation from a related donor or HLA-6/6–Ag-MUD between January 1, 2001 and December 31, 2008 were obtained from the Transplant Registry Unified Management Program,[13] which includes data from the Japan Society for Hematopoietic Cell Transplantation and the Japan Marrow Donor Program. Our analysis included 344 patients who received a graft from an RD/1AG-MM-GVH donor and 453 patients who received a graft from an 8/8-MUD. 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 BM 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 donor and 452 patients who received a graft 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 OS rate. The study was approved by the data management committees of Transplant Registry Unified Management Program and by the institutional review board of Saitama Medical Center (Jichi Medical University, Saitama, Japan), 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-GVH donors was assessed by serological data for HLA-A, HLA-B, and HLA-DR loci, whereas that in 8/8-MUD was assessed by genomic data for HLA-A, HLA-B, HLA-C, and HLA-DR loci. When the recipient's Ags or alleles were not shared by the donor, this was considered an HLA mismatch in the GVH direction; when the donor's Ags or alleles were not shared by the recipient, this was considered a mismatch in the HVG direction. SCT from a related donor with 1 Ag mismatch in the GVH direction has been performed by accepting multiple Ag mismatches in the HVG direction,[1,2] and therefore was included in this study.

End points and statistical analyses

The primary end point 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 standard 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 after transplantation.

Descriptive statistics were used to summarize variables related to patient characteristics. Comparisons between groups were performed with the χ^2 statistic or extended Fisher exact test as appropriate for categorical variables and the Mann-Whitney U test or the 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 a Gray test.[17] Cox proportional-hazards regression was used to evaluate variables that may affect OS, whereas the Fine and Gray 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 reclassified the conditioning regimen as either myeloablative or reduced-intensity according to the National Marrow Donor Program/Center for International Blood and Marrow Transplant Research 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 (0-1 or 2-4), disease (AML, ALL, CML, or MDS), disease status before transplantation (standard- or high-risk), type of conditioning regimen (myeloablative or reduced-intensity), type of GVHD prophylaxis (cyclosporine-based, tacrolimus-based, or other), use of antithymocyte globulin or alemtuzumab, and the time from diagnosis to transplantation (< 6 months or ≥ 6 months). In addition, a variable of graft source (BM or peripheral blood) was also considered in the analysis specific to related donors. Factors with $P < .10$ 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. We added donor type to the final model. All tests were 2-sided, and $P < .05$ was considered to indicate statistical significance. All statistical analyses were performed with STATA Version 11 software (StataCorp) and R Version 2.12.0 software (The R Foundation for Statistical Computing).

Results

Patient characteristics

Compared with recipients of an 8/8-MUD, recipients of an RD/1AG-MM-GVH were more likely to be younger, to be male receiving a transplantation from a female donor, to have a shorter duration from diagnosis to transplantation, to have a high-risk disease, to receive cyclosporine for GVHD prophylaxis, to receive antithymocyte globulin or alemtuzumab, and to 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 BM. In the RD/1AG-MM-GVH group, the number of Ag mismatches in the HVG direction was 0 in 11%, 1 in 67%, 2 in 20%, and 3 in 2%. HLA-A, HLA-B, and HLA-DRB1 allelic information in both recipients and donors was available in 148 of 327 transplantations from an RD/1AG-MM-GVH donor and information on HLA-C Ag mismatch in either the GVH or HVG direction was available in 123 of 327.

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; $P < .001$; Figure 1A). Multivariate analysis revealed that, compared with 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], 1.49; 95% CI,

Table 1. Patient characteristics

Variable	RD/1AG-MM-GVH (n = 327)	8/8 MUD (n = 452)	P
Median age at transplantation, y (range)	45 (16-69)	48 (16-68)	.043
Recipient sex, n (%)			
Male	184 (56%)	267 (59%)	.434
Female	143 (44%)	185 (41%)	
Sex combination of donors and recipients, n (%)			
Female to male	91 (28%)	73 (16%)	< .001
Other combinations	236 (72%)	379 (84%)	
Performance status, n (%)			
0/1	298 (91%)	415 (92%)	.736
2/3/4	29 (9%)	37 (8%)	
Disease, n (%)			
AML	167 (51%)	249 (55%)	.512
ALL	90 (28%)	107 (24%)	
CML	19 (6%)	21 (5%)	
MDS	51 (16%)	75 (17%)	
Duration from diagnosis to transplantation, n (%)			
< 6 mo	124 (38%)	102 (23%)	< .001
≥ 6 mo	191 (58%)	350 (77%)	
Unknown	12 (4%)	0 (0%)	
Disease risk, n (%)			
Standard	175 (54%)	317 (70%)	< .001
High	133 (41%)	129 (29%)	
Unknown	19 (6%)	6 (1%)	
Source of stem cells, n (%)			
BM	142 (43%)	452 (100%)	< .001
Peripheral blood	185 (57%)		
HLA compatibility in the HVG direction, n (%)*			
Matched	36 (11%)	452 (100%)	< .001
1-antigen mismatch	218 (67%)		
2-antigen mismatch	65 (20%)		
3-antigen mismatch	8 (2%)		
HLA compatibility in the GVH direction, n (%)*			
Matched	0 (0%)	452 (100%)	< .001
1-allele mismatch	111 (34%)		
2-allele mismatch	36 (11%)		
3-allele mismatch	1 (0%)		
Uncertain/missing	179 (55%)		
Conditioning regimen, n (%)			
Myeloablative	243 (74%)	338 (75%)	.883
Reduced-intensity	84 (26%)	114 (25%)	
GVHD prophylaxis, n (%)			
Cyclosporine-based	113 (35%)	108 (24%)	0.004
Tacrolimus-based	209 (64%)	338 (75%)	
Others	5 (2%)	6 (1%)	
Use of ATG/alemtuzumab, n (%)			
Yes	33 (10%)	13 (3%)	< .001
No	294 (90%)	439 (97%)	
Median follow-up of survivors, mo (range)	36.2 (3.0-95.7)	13.5 (1.7-62.8)	< .001

*HLA compatibility was defined according to the HLA-A, HLA-B, and HLA-DR loci.

1.19-1.86; $P < .001$; Table 2). Age > 50 years, performance status ≥ 2, and high-risk disease were also found to be significant adverse factors, whereas other variables, such as the time from diagnosis to transplantation, were not.

Because our previous study showed that the impact of an HLA-1 Ag mismatch in a related transplantation on OS differed according to whether patients had standard-risk or high-risk diseases,[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 ($P = .003$), whereas there was no significant difference in high-risk patients ($P = .090$; Figure 1B-C). Although the interaction between the donor type and disease risk did not reach statistical significance ($P = .140$), multivariate analyses in each disease-risk group showed that the adverse impact of the use of an RD/1AG-MM-GVH donor was significant in standard-risk patients (HR, 1.72; 95% CI, 1.24-2.39; $P = .001$), 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 (Figure 1). The OS curve of transplantation from an MRD

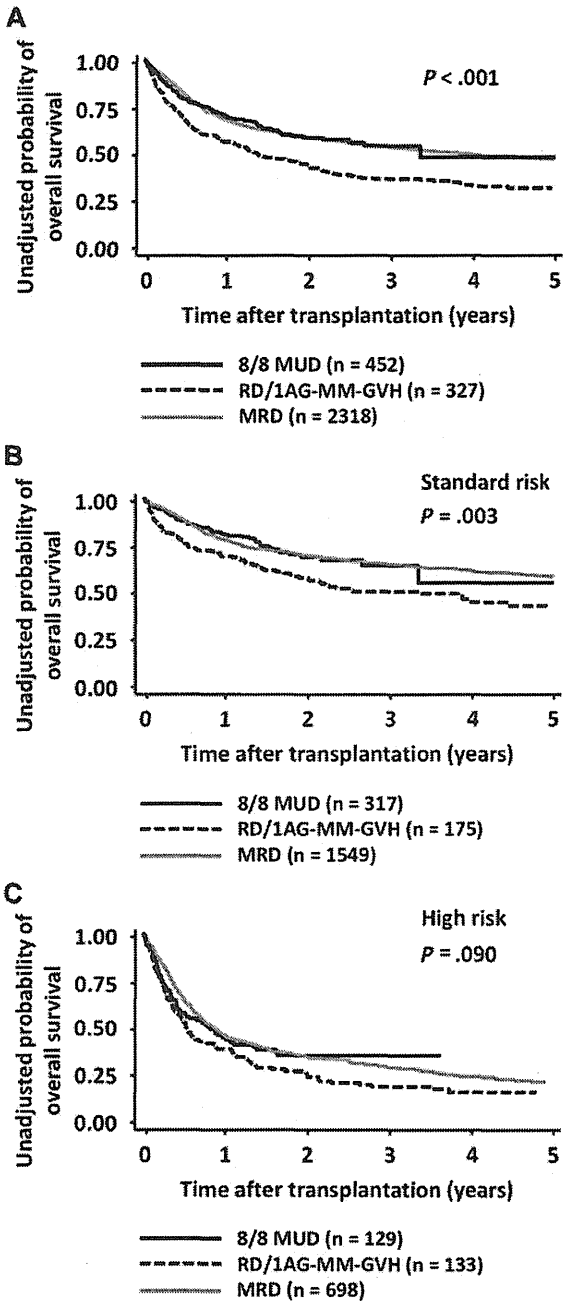

Figure 1. OS according to donor type and risk of disease. OS after transplantation from an RD/1AG-MM-GVH donor, an 8/8-MUD, and HLA-MRD in patients with *both-risk (A), standard-risk (B), or high-risk diseases (C). Survival rates in the* 8/8-MUD and RD/1AG-MM-GVH groups were compared with the log-rank test.

Table 2. Multivariate analysis of OS

	Total (n = 779)		Standard-risk (n = 492)		High-risk (n = 262)	
Variable	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Donor type						
8/8 MUD	1.00		1.00		1.00	
RD/1AG-MM-GVH	1.49 (1.19-1.86)	< .001	1.72 (1.24-2.39)	.001	1.30 (0.96-1.76)	.095
Age, y						
≤ 50	1.00		1.00			
> 50	1.44 (1.16-1.79)	.001	1.55 (1.13-2.15)	.007		
Performance status						
0/1	1.00				1.00	
2/3/4	1.79 (1.30-2.48)	< .001			1.76 (1.24-2.52)	.002
Disease risk						
Standard	1.00					
High	2.41 (1.92-3.03)	< .001				
Unknown	1.38 (0.82-2.33)	.227				

Only variables that remained after backward selection in the multivariate analysis are shown.

was superimposed on that from an MUD in both standard- and high-risk patients (MRD vs MUD: standard-risk group, $P = .895$, and high-risk group, $P = .581$). Multivariate analysis confirmed that OS in the MRD group was comparable to the MUD group (MRD vs MUD: standard-risk group, HR, 1.02; 95% CI, 0.79-1.32; $P = .878$; high-risk group, HR, 0.98; 95% CI, 0.76-1.26; $P = .865$).

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 with those in the 8/8-MUD group, we evaluated the impact of each HLA-A, HLA-B, or HLA-DR Ag mismatch in the GVH direction and the number of Ag 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 Ag mismatch in the GVH direction and that from a donor with 2 or 3 Ag mismatches in the HVG direction were significantly lower than those in other groups (log-rank test for HLA-A Ag mismatch vs HLA-B Ag mismatch vs HLA-DR Ag mismatch in the GVH direction, $P < .001$, and 0-1 mismatches vs 2-3 mismatches in the HVG direction, $P = .003$; Figure 2). However, multivariate analysis revealed that only the presence of an HLA-B Ag mismatch in the GVH direction (HR, 1.57; 95% CI, 1.13-2.18; $P = .007$) 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 (Figure 2). Although the interaction between the presence of an HLA-B Ag mismatch and disease risk did not reach statistical difference ($P = .232$), the adverse impact of an HLA-B Ag mismatch in the GVH direction was observed in the standard-risk group (HR, 1.86 95% CI, 1.14-3.01; $P = .012$), but not in the high-risk group (Table 3). Conversely, the survival curve for the HLA-A Ag or HLA-DR Ag-mismatched group was almost superimposed on that for 8/8-MUDs (Figure 2; standard-risk group: for the HLA-A Ag-mismatched group vs the 8/8-MUD group, HR, 1.26; 95% CI, 0.73-2.19; $P = .411$; for the HLA-DR Ag-mismatched group vs the 8/8-MUD group, HR, 1.37; 95% CI, 0.89-2.11; $P = .154$; high-risk group: for the HLA-A Ag-mismatched group vs the 8/8-MUD group, HR, 1.26; 95% CI, 0.80-2.00; $P = .320$; and for the HLA-DR Ag-mismatched group vs the 8/8-MUD group, HR, 1.03; 95% CI, 0.67-1.59]; $P = .880$). The impact of 2 or 3 Ag 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

Our findings showed that an HLA-B Ag 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 with the 8/8-MUD group. Therefore, we evaluated the impact of an HLA-B Ag 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 Ag mismatch in the GVH direction (supplemental Table 1, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). Two or 3 Ag mismatches in the HVG direction were observed more frequently in the HLA-B Ag-mismatched group (28%) than in the HLA-A Ag-mismatched group (2%) or the HLA-DR Ag-mismatched group (17%). Although there was no information available on allelic mismatch or HLA-C Ag mismatch in more than half of the patients, an HLA-C Ag mismatch in either the GVH or HVG direction was observed more frequently in the HLA-B Ag-mismatched group (61% among the available data) than in the HLA-A Ag-mismatched group (25%) or the HLA-DR Ag-mismatched group (17%).

The incidence of TRM was higher in the HLA-B Ag-mismatched group (3-year mortality rate: HR, 0.47; 95% CI, 0.32-0.60) than in the HLA-A Ag-mismatched group (HR, 0.28; 95% CI, 0.14-0.44) or the HLA-DR Ag-mismatched group (HR, 0.27; 95% CI, 0.17-0.38; Figure 3A; log-rank test, $P = .030$). The presence of an HLA-B Ag mismatch in the GVH direction was an independent significant adverse factor that affected TRM in the RD/1AG-MM-GVH group (Table 4). Conversely, the incidence of relapse did not significantly differ among the 3 groups (Figure 3B and Table 4).

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

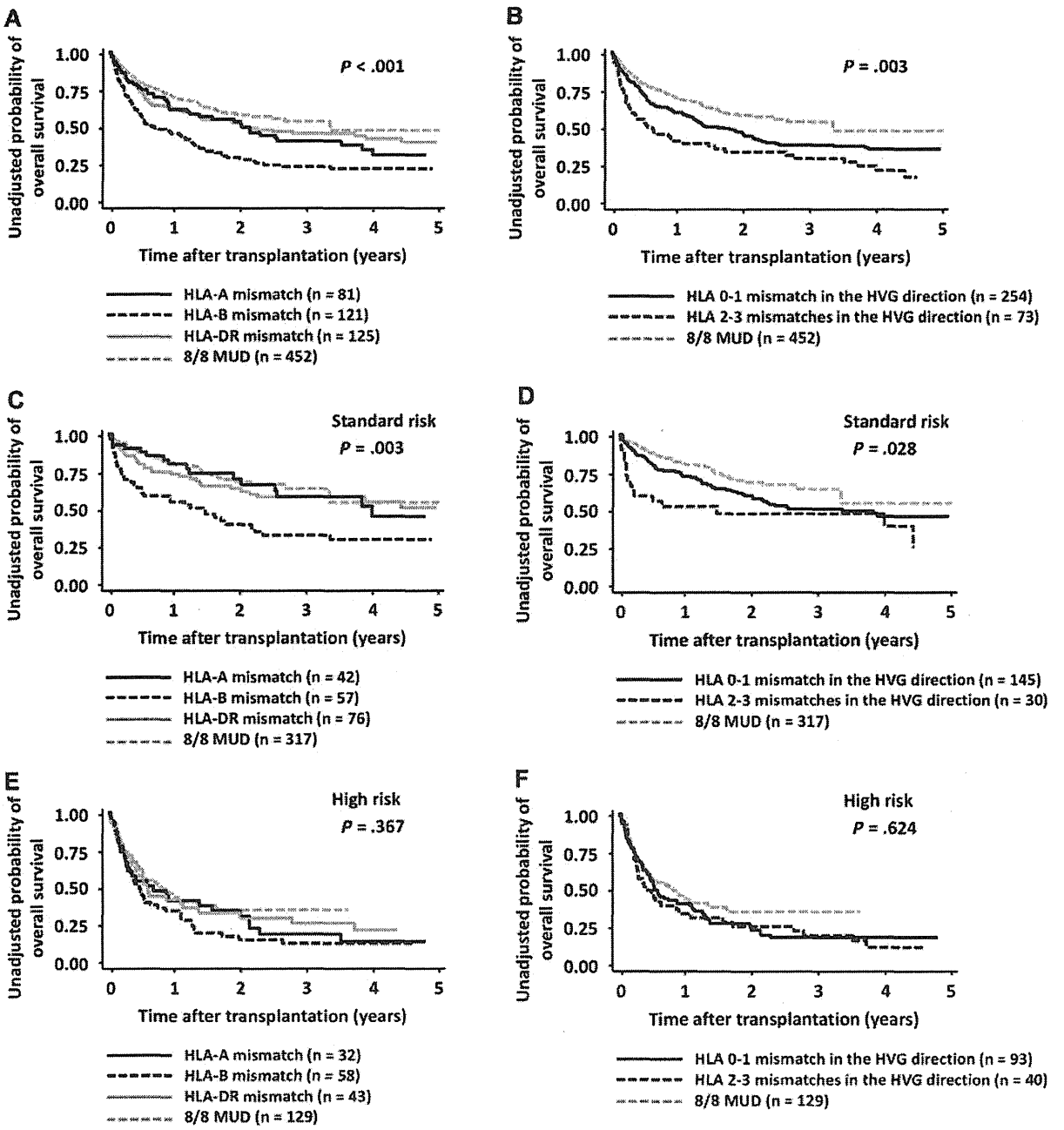

Figure 2. OS in patients with both-risk, standard-risk, or high-risk diseases according to the locus of HLA mismatch in the GVH direction and the number of mismatches in the HVG direction. Survival rates in patients with HLA-A, HLA-B, and HLA-DR Ag mismatches in the GVH direction were compared with the log-rank test (A,C,E). Survival rates in patients with 0-1 and 2-3 mismatches in the HVG direction were compared with the log-rank test (B,D,F). Survival rates of the 8/8-MUD group are shown for visual comparison.

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 reported previously that transplantation from a related donor with 1 Ag mismatch in the GVH or HVG direction gave a clinical outcome comparable to that of transplantation from a 6/6-Ag-MUD in patients with either standard-risk or high-risk diseases.[1] However, because HLA matching at the allelic level in unrelated transplantation significantly reduces the risk of GVHD, in the present study, 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.

Table 3. Multivariate analysis of OS in patients receiving transplantation from a related donor with a 1-antigen mismatch in the GVH direction

	Total (n = 327)		Standard-risk (n = 175)		High-risk (n = 133)	
Variable	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
HLA mismatch in the GVH direction						
HLA-DR mismatch	1.00		1.00		1.00	
HLA-A mismatch	1.07 (0.73-1.56)	.737	0.98 (0.54-1.81)	.966	1.11 (0.65-1.89)	.701
HLA-B mismatch	1.57 (1.13-2.18)	.007	1.86 (1.14-3.01)	.012	1.36 (0.86-2.17)	.193
HLA mismatch in the HVG direction						
0-1 mismatches	1.00		1.00		1.00	
2-3 mismatches	1.27 (0.91-1.76)	.154	1.67 (0.98-2.85)	.061	1.06 (0.69-1.61)	.799
Age, y						
≤ 50	1.00		1.00			
> 50	1.52 (1.14-2.03)	.004	1.87 (1.21-2.91)	.005		
Disease risk						
Standard	1.00					
High	2.06 (1.53-2.78)	< .001				
Unknown	1.00 (0.53-1.89)	.989				

Only variables that remained after backward selection in the multivariate analysis are shown.

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 donor and an 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 donor.[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 with the presence of an HLA-DR Ag mismatch, the presence of an HLA-B Ag mismatch in the GVH direction was significantly associated with lower OS and higher TRM. Conversely, the rates of OS and TRM in the HLA-A Ag- or HLA-DR Ag-mismatched group were superimposed on those in the MUD group. However, HLA-A, HLA-B, and HLA-DR Ag mismatches had similar effects on the incidence of severe acute GVHD; consequently, the causal relationship between an HLA-B Ag mismatch in the GVH direction and higher TRM remains unknown. In contrast to our findings, Valcarcel et al reported that there was no significant difference in OS between the use of 1-Ag–mismatched related donors (n = 89) and 8/8-MUDs (n = 700) in transplantation for AML and ALL during the first or second complete remission.[23] This difference from our results can be partly

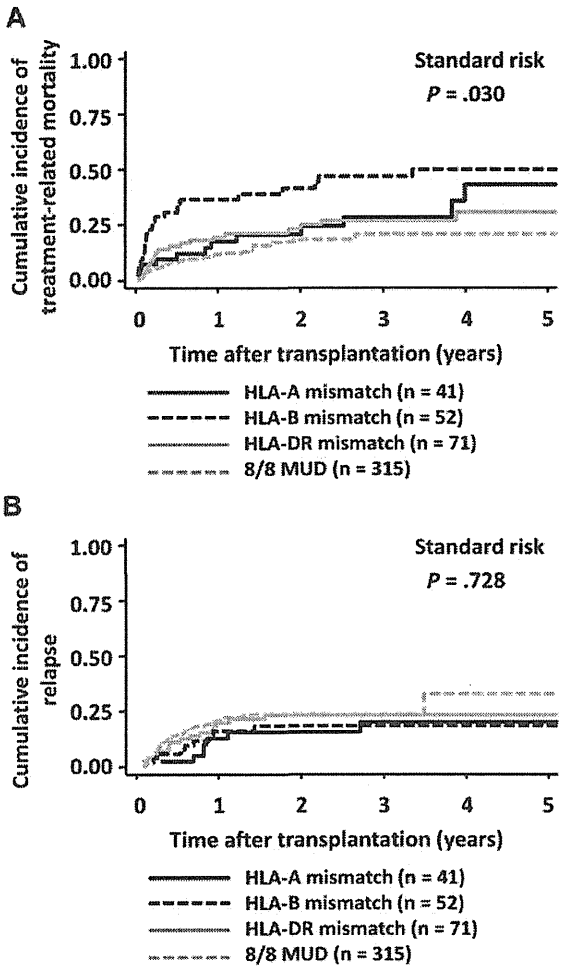

Figure 3. Cumulative incidence according to the locus of HLA mismatch in the GVH direction in patients with standard-risk diseases. Cumulative incidences in the related transplantation groups were compared with the Gray test. (A) TRM. (B) Relapse.

Table 4. Multivariate analysis of TRM and relapse in patients with standard-risk diseases receiving transplantations from a related donor with a 1-antigen mismatch in the GVH direction

	TRM (n = 164)		Relapse (n = 164)	
Variable	HR (95% CI)	P	HR (95% CI)	P
HLA mismatch in the GVH direction				
HLA-DR mismatch	1.00		1.00	
HLA-A mismatch	1.22 (0.59-2.52)	.587	0.70 (0.29-1.67)	.418
HLA-B mismatch	2.00 (1.09-3.65)	.025	0.80 (0.34-1.87)	.605
HLA mismatch in the HVG direction				
0-1 mismatches	1.00		1.00	
2-3 mismatches	2.21 (1.14-4.28)	.019	0.67 (0.23-1.98)	.467
Age, y				
≤ 50	1.00			
> 50	2.08 (1.18-3.65)	.011		
Duration from diagnosis to transplantation				
< 6 mo	1.00			
≥ 6 mo	2.40 (1.19-4.82)	.014		
Unknown	2.23 (0.77-6.48)	.140		

Only variables that remained after backward selection in the multivariate analysis are shown.

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, in our study, genetic homogeneity in the Japanese population might affect the lower incidence of severe acute GVHD in MUD transplantation because of the less frequent mismatches in minor histocompatibility Ags.[24,25]

The frequency of an HLA-C Ag mismatch was substantially higher in the HLA-B Ag-mismatched group than in the HLA-A or HLA-DR Ag-mismatched groups. This finding may represent linkage disequilibrium between the HLA-B and HLA-C genes, which are located at a very close physical proximity within the major histocompatibility complex.[26,27] Therefore, the impact of HLA-B-Ag might be affected by the co-presence of HLA-C Ag mismatch. We could not evaluate the impact of HLA-C Ag mismatch on OS rates because of the limited information on HLA-C Ag mismatch; therefore, an analysis with larger cohorts with complete HLA-C Ag information will be needed to evaluate the impact of HLA-C and/or HLA-B mismatch in transplantation from an RD/1AG-MM-GVH donor. 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 OS.[12,28,29] The analysis of KIR matching would be helpful in elucidating the mechanism underlying the adverse effect of HLA-B mismatch in T cell–replete transplantation from an RD/1AG-MM-GVH donor.

Whether the presence of allelic mismatches in addition to the 1-Ag mismatch (2 or more allelic mismatches in total) affects transplantation outcome is also an important clinical question in transplantation from an RD/1AG-MM-GVH donor. A high frequency of 2-allele mismatches in the GVH direction was seen in the HLA-B Ag-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 donor, possibly because of the small sample size.

Our study has several limitations. First, because several months are required to arrange unrelated transplantations, patients at low risk for relapse may more often be selected for these procedures. 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. In particular, the stem-cell source in unrelated transplantation was exclusively BM. However, the analysis of OS in the subgroup of patients who received a BM graft from an RD/1AG-MM-GVH donor or an 8/8-MUD showed similar results. Third, because we have incomplete Ag and allele information on the HLA-C and -DQB1 loci, we may have underestimated the degree of mismatching in transplantation from an RD/1AG-MM-GVH donor. 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 because of 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 donor for patients without an MRD if an immediate transplantation is not necessary. In particular, the presence of an HLA-B Ag mismatch in the GVH direction has an adverse effect on OS because of treatment-related complications. This may be because of the high frequencies of additional mismatches of HLA-C Ag or allele in the HLA-B Ag-mismatched group. To elucidate the mechanism of the adverse outcomes in RD/1AG-MM-GVH donors with an HLA-B Ag mismatch, HLA Ag/allele matching including HLA-C should be performed in transplantations from an RD/1AG-MM-GVH donor.

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Authorship

Contribution: Y.K. designed the research and organized the project; J.K., H. Saji, and Y.K. reviewed and analyzed the data and wrote the manuscript; J.K. and Y.K. performed the statistical analysis; H. Sakamaki, J.T., R.S., and Y.A. collected data from Japan Society for Hematopoietic Cell Transplantation; K.K. and Y.M. collected data from Japan Marrow Donor Program; and all authors interpreted the data and reviewed and approved the final manuscript.

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