essential when deciding whether to use allo-HCT in the first CR. Based on the idea of risk-adapted allo-HCT, MRD-positive patients may be candidates for allo-HCT.[66,67] To determine whether adults receiving pediatric protocols have better outcomes will require a randomized trial.

Indication for reduced-intensity conditioning (RIC) allo-HCT for Ph(−) ALL

Most studies described above were conducted for patients with myeloablative conditioning (MAC). As the GVL effect is considered to be weak for ALL, there have been few analyses of RIC for ALL.[68] Several studies showed results of RIC for high-risk ALL,[69,70] and the utility of RIC for ALL has been recognized. As the GVL effect on Ph(−) ALL in the first CR was established in UKALL XII/ECOG 2993, there has been a potent rationale for evaluating RIC for Ph(−) ALL in CR. A retrospective comparison of mRD and URD allo-HCT between 93 RIC and 1428 MAC cases from CIBMTR revealed that the statistically adjusted survival between them was comparable (3-year survival: 45% (RIC) vs 51% (MAC) in the first CR and 28% (RIC) vs 33% (MAC) in the second CR).[71] From the European Group for Blood and Marrow Transplantation, a retrospective comparison of mRD allo-HCT between RIC ($n = 127$) and MAC ($n = 449$) for ALL patients in CR aged ⩾45 (including Ph(+) ALL patients) indicated that comparable survival rates for patients who underwent RIC and MAC suggest the utility of RIC for ALL in CR.[72] Attention should be given to the interpretation of results of these studies, as they were both statistically adjusted retrospective comparisons and, therefore, some biases based on different backgrounds might affect their outcomes.[12] Older age is known to be a strong adverse prognostic factor for ALL, and the results of conventional chemotherapy for elderly ALL patients have been dismal.[73] The utility and indication of RIC for Ph(−) ALL, especially UCBT,[74,75] await further studies, given that results of only a few retrospective studies on RIC for Ph(−) ALL have been reported. Although we wait for randomized study results, RIC allo-HCT could be considered a reasonable option for Ph(−) ALL CR patients of advanced age.

CONCLUSIONS

It is unlikely that randomized head-to-head comparisons of the three alternative sources (URD, UCB, and haplo-RD) will be performed, and therefore clinical decisions will be based on observational studies or registry retrospective comparison data. Putting emphasis on the quality and quantity of the existing data, HLA-matched URD would be the first alternative source for Ph(−) ALL. HLA 0–2 antigen-mismatched UCBT would be the second choice, especially for young patients (<45 years) without an HLA-matched URD or patients who are in urgent need of allo-HCT. Haplo-RD allo-HCT would be considered for ALL in CR as a third alternative source. An algorithm of donor selection is shown in Figure 2. As the three alternative sources have different characteristics, it may be difficult to make a definite hierarchy among these sources. Flexible consideration based on the latest data would be warranted according to patient status and appropriate timing of allo-HCT.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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bjh research paper

Impact of a single human leucocyte antigen (HLA) allele mismatch on the outcome of unrelated bone marrow transplantation over two time periods. A retrospective analysis of 3003 patients from the HLA Working Group of the Japan Society for Blood and Marrow Transplantation

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Summary

A previous Japanese study revealed that a human leucocyte antigen (HLA)-A or -B allele mismatch was associated with higher overall mortality, whereas an HLA-C or -DRB1 allele mismatch did not affect mortality after serologically matched unrelated bone marrow transplantation (BMT). This study reanalysed 3003 adult patients who underwent unrelated BMT from a serologically HLA-A, -B, or -DR matched unrelated donor between 1993 and 2009 using the latest database, that included 1966 HLA-matched unrelated BMT and 187, 31, 524, and 295 unrelated BMT with a single HLA-A, -B, -C, or -DRB1 allele mismatch, respectively. As opposed to our previous findings, HLA-C and -DRB1 mismatches had a significant negative impact [hazard ratio (HR) 1·35, $P < 0·001$, and HR 1·45, $P < 0·001$] on survival in the period 2000–2009. The negative impact of each single HLA allele mismatch was not significantly different among the HLA-A, -B, -C, and -DRB1 mismatches ($P = 0·79$). An interaction test revealed that the effects of single HLA-C and -DRB1 allele mismatches significantly differed over the two time periods ($P = 0·032$ and $P = 0·0072$, respectively). In conclusion, the impact of a single HLA allele mismatch changed over time. In the recent cohort, the negative impact of HLA-DRB1 and -C mismatches became apparent.

Keywords: allogeneic haematopoietic stem cell transplantation, human leucocyte antigen, graft-versus-host disease, human leucocyte antigen mismatch, unrelated donor.

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Haematopoietic stem cell transplantation (HSCT) from an unrelated donor has been investigated for patients who lack a human leucocyte antigen (HLA)-matched sibling donor. However, the outcome of serologically HLA-matched unrelated HSCT has been shown to be inferior to that of HSCT from an HLA-matched sibling due to the development of graft failure or severe graft-versus-host disease (GVHD), which resulted partly from the presence of an HLA mismatch at the genetic level (allele mismatch). High-resolution typing is needed to detect an allele mismatch, whereas a serological HLA mismatch (antigen mismatch) requires only low-resolution typing. A retrospective study by the Japan Marrow Donor Program (JMDP) revealed that an HLA-A or -B allele mismatch was associated with higher overall mortality, whereas an HLA-C or -DRB1 allele mismatch did not affect mortality after serologically HLA-A, -B, and -DR matched unrelated bone marrow transplantation (BMT; Sasazuki *et al*, 1998). Subsequently, Morishima *et al* (2002) analysed the impact of a single allele mismatch by including only patients who were matched for all other loci. They confirmed that an HLA-A and/or -B allele mismatch, but not an HLA-C or -DRB1 allele mismatch, was associated with worse survival. However, studies from the National Marrow Donor Program (NMDP) and the Fred Hutchinson Cancer Research Center have shown conflicting results with regard to the impact of single HLA allele mismatches (Flomenberg *et al*, 2004; Petersdorf *et al*, 2004; Lee *et al*, 2007). These discrepancies could be explained by differences in the study population or study designs (Bray *et al*, 2008). For example, there are differences in the inclusion criteria for disease, phase of disease, and HLA matching (Bray *et al*, 2008).

The present study focused on the potential effect of the difference between HLA mismatches that were known and not known by the attending physicians before HSCT. In 1994, while high-resolution typing for HLA-DRB1 was started as a routine test in JMDP, only low-resolution typing was performed for HLA-A and -B until high-resolution typing for these loci became routine in 2003. More accurately, high-resolution typing for HLA-A and -B was available as an option after 1996, and these tests were gradually ordered more frequently after JMDP published the first retrospective analysis using frozen samples, which showed that HLA-A and -B allele mismatches were more important than an HLA-DRB1 allele mismatch (Sasazuki *et al*, 1998), and it has become a common practice since 2000. Therefore, in the 1990's, physicians only had information on an HLA-DRB1 allele mismatch before BMT, and this may have influenced the strategies against GVHD in patients with an HLA-DRB1 allele mismatch. In contrast, in the 2000's, physicians had information about HLA-A and -B mismatches and therefore strategies against GVHD in patients with an HLA-A or -B allele mismatch may have been more intense than those in patients with an HLA-DRB1 allele mismatch, as the latter was shown to have little effect on the incidence of severe acute GVHD (Sasazuki *et al*, 1998). With regard to HLA-C antigen, both high- and low-resolution tests for HLA-C were optional until they became routine in 2009. The intensity of immunosuppression for GVHD prophylaxis may also affect the incidence of graft failure.

We hypothesized that the availability of information about an HLA allele mismatch may affect the impact of single HLA-mismatches on survival, and reanalysed the impact of a mismatch in each single allele in the recent cohort (i.e. those who underwent BMT between 2000 and 2009). We also analysed the statistical interaction between single HLA allele mismatches and the time periods when BMT was performed.

Methods

Patients

Patients aged at least 16 years with acute myeloid leukaemia (AML), acute lymphoblastic leukaemia (ALL), myelodysplastic syndrome (MDS), or chronic myeloid leukaemia (CML) who underwent a first BMT from a serologically HLA-A, -B and -DR matched unrelated donor between 1993 and 2009, and who had full HLA-A, -B, -C, and -DRB1 allele data, were included in this study. Clinical data for these patients were obtained from the Transplant Registry Unified Management Program (TRUMP; Atsuta *et al*, 2007). We excluded patients who lacked data on survival status, those with more than 1 allele or antigen mismatch, those who received a reduced-intensity conditioning regimen, and those who received *ex vivo* or *in vivo* T-cell depletion. Finally, 3003 patients were included in this study. The study was planned by the HLA working group of the Japan Society for Haematopoietic Cell Transplantation and was approved by the data management committees of TRUMP and by the institutional review board of Saitama Medical Centre, Jichi Medical University.

Histocompatibility

Histocompatibility data for serological and genetic typing for the HLA-A, HLA-B, HLA-C, and HLA-DR loci were obtained from the TRUMP database, which includes HLA allele data determined retrospectively by the JMDP using frozen samples (Morishima *et al*, 2002; Kawase *et al*, 2007). The extent of HLA testing was exon 2 and 3 for HLA class I and exon 2 for HLA class II, and exon 4 and exon 3 were additionally analysed for class I and class II, respectively, if required. An HLA mismatch in the GVHD was defined as when recipient antigens or alleles were not shared by the donor, and a mismatch in the host-versus-graft direction was defined as when donor antigens or alleles were not shared by the recipient. The direction of mismatch was considered in the analysis of engraftment and GVHD (Morishima *et al*, 2002; Lee *et al*, 2007).

Statistical analyses

The primary endpoint was overall survival after unrelated BMT. Secondary endpoints included the incidences of engraftment, grade III–IV acute GVHD, non-relapse mortality, and relapse. While the follow-up duration differed between patients in the two time periods [early (1993–1999) and late (2000–2009)], for the primary endpoint, we used the data obtained at last contact (Gooley *et al*, 2010). Then, we confirmed that there were no changes in the major findings, when surviving patients were censored at 5 years after BMT.

The chi-square test or Fisher's exact test was used to compare categorical variables and Student's *t*-test or an analysis of variance test was used for continuous variables. Overall survival was estimated according to the Kaplan–Meier method, and compared among groups with the log-rank test. The probabilities of non-relapse mortality, relapse, acute GVHD, and neutrophil engraftment were calculated while treating relapse, death without relapse, relapse or death without GVHD, and death without engraftment, respectively, as competing events, and compared using Gray's test (Gray, 1988).

The impacts of single HLA allele mismatches, the time period when BMT was performed, and the interaction between them were evaluated using multivariate models; Cox proportional hazards model for overall survival and Fine and Gray's proportional hazards model for the other endpoints (Fine & Gray, 1999). Potential confounding factors that were considered in these analyses included recipient/donor age, recipient/donor sex, sex mismatch, ABO major/minor mismatch, the use of total body irradiation (TBI) in the conditioning regimen, cell dose in the bone marrow graft, the use of ciclosporin (CSA) or tacrolimus (TAC) as GVHD prophylaxis, background disease, and disease risk. We divided GVHD prophylaxis regimens into only CSA-based and TAC-based regimens, because more than 95% of the patients received a combination of a calcineurin inhibitor and methotrexate. Acute leukaemia in first or second remission, CML in first or second chronic phase, CML in accelerated phase, and MDS of refractory anaemia or refractory anaemia with excess blasts were considered low-risk diseases, and other conditions were considered high-risk diseases. All of these potential confounding factors were included in the multivariate analyses and then deleted in a stepwise manner from the model to exclude factors with a *P*-value of 0·05 or higher. Finally, each single HLA allele mismatch and the time periods were added to the model to evaluate the effects of these factors adjusted for the other significant factors with or without interaction terms between the BMT time period and each single HLA allele mismatch. The model without interaction terms evaluated the impact of each single HLA allele mismatch adjusted for the BMT time period and the other significant factors. On the other hand, the model with interaction terms evaluated whether the impact of each single HLA allele mismatch was different between the two time periods, as well as the impact of each single HLA allele mismatch in each time period. Significant interaction means that the impact of the single HLA allele mismatch differs over the two time periods.

All *P*-values were two sided and *P*-values of 0·05 or less were considered statistically significant. All statistical analyses were performed with EZR (Saitama Medical Centre, Jichi Medical University; http://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmedEN.html; Kanda, 2012), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria, version 2.13.0). More precisely, it is a modified version of R commander (version 1.6-3) that was designed to add statistical functions frequently used in biostatistics.

Results

Patients

The patients characteristics are summarized in Table I. The total number of patients was 3003, and 751 and 2252 BMTs were performed in the early and late time periods, respectively. Of these, 1966 patients received a graft from an HLA-A, -B, -C, and -DRB1 allele matched donor, whereas 187, 31, 524, and 295 patients, respectively, underwent single HLA-A, -B, -C, and DRB1 allele-mismatched BMT. Only the HLA-C mismatch group included HLA mismatch at a serological (antigen) level. Bone marrow was exclusively used as the stem cell source.

Overall survival

To adjust the impact of HLA mismatch for possible confounding factors, we identified the following independently significant factors for overall survival: recipient age, disease, disease risk, and GVHD prophylaxis. After we adjusted for these factors, all single allele mismatches were significantly

Table I. Patient characteristics.

		1 allele mismatch				
	Match	A	B	C	DRB1	
	n = 1966	n = 187	n = 31	n = 524	n = 295	P value
Transplantation time period						
1993–1999	480	74	8	126	63	<0·001
2000–2009	1486	113	23	398	232	
Antigen mismatch						
No	1966 [480/1486]	187 [74/113]	31 [8/23]	38 [7/31]	295 [63/232]	<0·001*
Yes	0	0	0	486 [119/367]	0	
Mismatch in GVH direction						
No	1966 [480/1486]	22 [6/16]	1 [0/1]	38 [9/29]	11 [3/8]	0·0068*
Yes	0	165 [68/97]	30 [8/22]	486 [117/369]	284 [60/224]	
Mismatch in HVG direction						
No	1966 [480/1486]	13 [3/10]	0	43 [10/33]	18 [4/14]	0·29*
Yes	0	174 [71/103]	31 [8/23]	481 [116/365]	277 [59/218]	
Age, years						
Median (range)	37 (16–70) [30/39]	34 (16–56) [30/37]	34 (17–59) [28·5/35]	36 (16–67) [30/38]	37 (16–64) [26/39]	0·21
Age (donor), years						
Median (range)	34 (20–55) [34/34]	35 (20–55) [33/36]	35 (23–49) [29/37]	34 (20–54) [33/34]	34 (20–53) [34/34]	0·90
Sex						
Female	747 [183/564]	76 [31/45]	16 [4/12]	233 [57/176]	117 [30/87]	0·055
Male	1219 [297/922]	111 [43/68]	15 [4/11]	291 [69/222]	178 [33/145]	
Sex (donor)						
Female	651 [159/492]	62 [25/37]	14 [4/10]	218 [45/173]	119 [21/98]	0·016
Male	1307 [317/990]	124 [49/75]	17 [4/13]	303 [81/222]	175 [42/133]	
N.A.	8 [4/4]	1 [0/1]	0	3 [0/3]	1 [0/1]	
Sex mismatch						
Match	1241 [287/954]	101 [36/65]	21 [6/15]	310 [70/240]	159 [28/131]	0·077
Female to Male	311 [83/228]	36 [16/20]	4 [1/3]	99 [22/77]	69 [13/56]	
Male to Female	406 [106/300]	49 [22/27]	6 [1/5]	112 [34/78]	66 [22/44]	
N.A.	8 [4/4]	1 [0/1]	0	3 [0/3]	1 [0/1]	
ABO blood type						
Match	1119 [248/871]	91 [38/53]	13 [6/7]	190 [45/145]	135 [25/110]	<0·001
Minor mismatch	375 [92/283]	44 [14/30]	7 [1/6]	149 [34/115]	69 [17/52]	
Major mismatch	300 [93/207]	23 [8/15]	10 [1/9]	120 [32/88]	56 [13/43]	
Bidirectional mismatch	156 [37/119]	27 [13/14]	1 [0/1]	60 [12/48]	31 [7/24]	
N.A.	16 [10/6]	2 [1/1]	0	5 [3/2]	4 [1/3]	
Disease						
AML	876 [161/715]	64 [15/49]	13 [1/12]	216 [38/178]	136 [22/114]	0·029
ALL	563 [139/424]	58 [21/37]	9 [2/7]	136 [32/104]	81 [20/61]	
CML	321 [142/179]	44 [33/11]	7 [3/4]	94 [41/53]	53 [17/36]	
MDS	206 [38/168]	21 [5/16]	2 [2/0]	78 [15/63]	25 [4/21]	
Disease risk						
Low	1302 [327/975]	120 [51/69]	19 [6/13]	336 [79/257]	180 [36/144]	0·58
High	593 [136/457]	63 [22/41]	10 [1/9]	166 [41/125]	105 [25/80]	
N.A.	71 [17/54]	4 [1/3]	2 [1/1]	22 [6/16]	10 [2/8]	
Cell dose (cells/kg)						
Median	2·80 [3·07/2·70]	2·99 [2·97/2·99]	2·71 [3·10/2·58]	2·79 [3·15/2·60]	2·78 [3·10/2·61]	0·40
GVHD prophylaxis						

Table I. (*Continued*)

		1 allele mismatch				
	Match $n = 1966$	A $n = 187$	B $n = 31$	C $n = 524$	DRB1 $n = 295$	*P* value
CSA-based	918 [377/541]	93 [62/31]	14 [7/7]	243 [100/143]	115 [47/68]	0·17
TAC-based	1017 [93/924]	89 [10/79]	16 [1/15]	267 [24/243]	175 [15/160]	
N.A.	31 [10/21]	5 [2/3]	1 [0/1]	14 [2/12]	5 [1/4]	
Conditioning regimen						
TBI regimen	1634 [467/1167]	168 [74/94]	29 [8/21]	430 [121/309]	249 [63/186]	0·21
Non-TBI regimen	257 [10/247]	14 [0/14]	1 [0/1]	68 [5/63]	37 [0/37]	
N.A.	75 [3/72]	5 [0/5]	1 [0/1]	26 [0/26]	9 [0/9]	

Numbers in the square brackets show the data separated according to the time periods.
HVG, host-versus-graft; GVH (D), graft-versus-host (disease); AML, acute myeloid leukaemia; ALL, acute lymphoblastic leukaemia; CML, chronic myeloid leukaemia; MDS, myelodysplastic syndrome; N.A., not available; CSA, ciclosporin; TAC, tacrolimus, TBI, total body irradiation.
*Comparison excluding the HLA-matched group.

associated with inferior survival except that the effect of HLA-A allele mismatch was nearly significant [HR 1·22, 95% confidence interval (CI) 1·00–1·51, $P = 0·055$, HR 1·60, 95% CI 1·03–2·49, $P = 0·038$, HR 1·23, 95% CI 1·07–1·41, $P = 0·00037$, and HR 1·26, 95% CI 1·07–1·49, $P = 0·0068$ for HLA-A, -B, -C, and -DRB1 mismatch, respectively]. However, when the effects of single HLA allele mismatches were evaluated separately in the early and late BMT time periods by adding interaction terms between HLA allele mismatches and time periods, only an HLA-B allele mismatch was associated with significantly inferior survival (HR 2·47, 95% CI 1·16–5·24, $P = 0·019$) in the early time period, whereas HLA-A, -C and -DRB1 mismatches did not exhibit a significant effect (HR 1·16, 95% CI 0·84–1·59, $P = 0·37$, HR 0·96, 95% CI 0·73–1·26, $P = 0·77$, and HR 0·83, 95% CI 0·58–1·19, $P = 0·32$, Table II). On the other hand, HLA-C and -DRB1 mismatches were associated with significantly inferior survival in the late time period (HR 1·35, 95% CI 1·15–1·59, $P < 0·001$, and HR 1·45, 95% CI 1·20–1·75, $P < 0·001$). The effects of HLA-A and -B allele mismatches were not statistically significant in the late time period, but the HR values (HR 1·24, 95% CI 0·95–1·62, $P = 0·12$, and HR 1·36, 95% CI 0·78–2·35, $P = 0·28$) were almost equivalent to those of HLA-C and -DRB1 mismatches. In fact, the negative impact of each single HLA allele mismatch was not significantly different among the HLA-A, -B, -C, and -DRB1 mismatches ($P = 0·79$ by the Wald test). Fig 1 shows the survival curves adjusted for other significant factors. In the early time period, the survival curves of the HLA-C and -DRB1 mismatch groups were at least equivalent to that of the HLA matched group, whereas that of the HLA-B mismatch group was separate from those of the other groups (Fig 1A). On the other hand, in the late time period, the survival curves of all of the single HLA allele mismatch groups were close to each other (Fig 1B).

An interaction test between the BMT time period and each single HLA allele mismatch revealed that the effects of single HLA-C and -DRB1 allele mismatches significantly differed over the two time periods ($P = 0·032$ and $P = 0·0072$, Table II). The major reason for these significant interactions was that, while overall survival in the HLA match group significantly improved from the early to the late time periods (HR 0·75, 95% CI 0·64–0·90, $P = 0·0011$), overall survival in the HLA-C and -DRB1 mismatch groups did not improve (HR 1·00, 95% CI 0·73–1·36, $P = 0·98$ and HR 1·20, 95% CI 0·79–1·82, $P = 0·40$, Fig 2). Similarly, overall survival in the HLA-A and -B mismatch groups did not change significantly between the two time periods (HR 0·81, 95% CI 0·49–1·34, $P = 0·41$ and HR 0·55, 95% CI 0·15–2·00, $P = 0·36$).

Engraftment and acute GVHD

The achievement of engraftment was significantly improved over the two time periods (HR 1·13, $P = 0·023$) after adjusting for other significant factors. None of the single HLA allele mismatches in the host-versus-graft direction affected the incidence of engraftment in either the early or late time periods, except for HLA-B allele mismatch in the late time period (HR 0·70, $P = 0·037$, Table III). The HR for engraftment was decreased, from 1·06 to 0·95 in the HLA-A mismatch group and from 1·03 to 0·89 in the HLA-DRB1 mismatch group, but the interaction tests were not significant.

With regard to the incidence of grade III–IV acute GVHD, single HLA-C allele mismatch in the graft-versus-host direction was associated with a significantly higher incidence of severe acute GVHD in the early time period (HR 2·02, $P = 0·0029$). In the late time period, single HLA-A and DRB1 allele mismatches, in addition to the HLA-C allele mismatch, were associated with a significantly higher incidence of grade III–IV acute GVHD (HR 1·72, $P = 0·025$, HR 1·51, $P = 0·0067$, and HR 1·45, $P = 0·045$ for HLA-A, -C, and -DRB1 mismatches, respectively), but the interactions between the time period and HLA-A and DRB1 allele mismatches were not statistically significant (Table III, Fig 3).

Table II. Multivariate analysis to evaluate the impact of single HLA allele mismatches, transplantation time periods, and their interaction on overall survival.

Factor	Hazard ratio	*P* value
Main effects		
Age	1·01 (1·01–1·02)	<0·001
Disease		
AML	1	
ALL	1·16 (1·02–1·32)	0·024
CML	0·90 (0·77–1·07)	0·23
MDS	0·56 (0·47–0·68)	<0·001
Disease risk		
Low	1	
High	2·98 (2·66–3·35)	<0·001
N.A.	2·40 (1·85–3·11)	<0·001
GVHD prophylaxis		
CSA-based	1	
TAC-based	0·94 (0·84–1·06)	0·30
HLA (early years)		
Match	1	
A mismatch	1·16 (0·84–1·59)	0·37
B mismatch	2·47 (1·16–5·24)	0·019
C mismatch	0·96 (0·73–1·26)	0·77
DRB1 mismatch	0·83 (0·58–1·19)	0·32
HLA (late years)		
Match	1	
A mismatch	1·24 (0·95–1·62)	0·12
B mismatch	1·36 (0·78–2·35)	0·28
C mismatch	1·35 (1·15–1·59)	0·0003
DRB1 mismatch	1·45 (1·20–1·75)	0·0001
Transplantation time period		
Early period	1·00	
Late period	0·74 (0·63–0·86)	0·00016
Interactions		
Time period * A mismatch	1·07 (0·70–1·63)	0·75
Time period * B mismatch	0·55 (0·22–1·40)	0·21
Time period * C mismatch	1·41 (1·03–1·93)	0·032
Time period * DRB1 mismatch	1·74 (1·16–2·61)	0·0072

GVHD, graft-versus-host disease; HLA, human leucocyte antigen; AML, acute myeloid leukaemia; ALL, acute lymphoblastic leukaemia; CML, chronic myeloid leukaemia; MDS, myelodysplastic syndrome; N.A., not available; CSA, ciclosporin; TAC, tacrolimus.

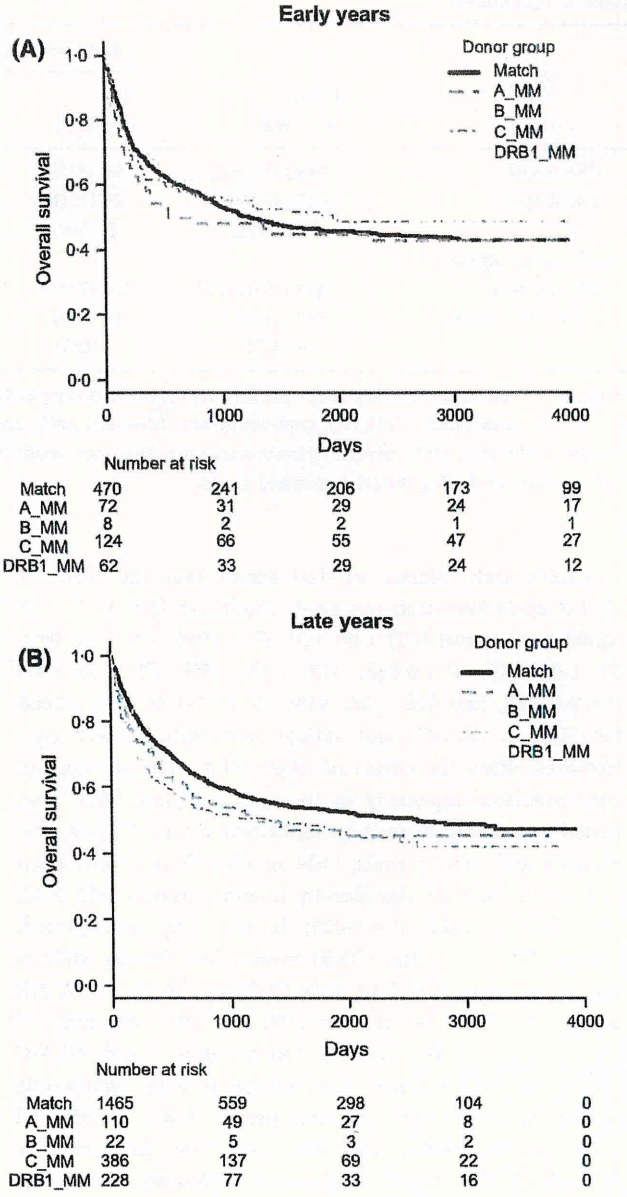


Fig 1. Overall survival grouped according to the human leucocyte antigen mismatch between the donor and recipient in the (A) early (1993–1999) and (B) late (2000–2009) time periods. The survival curves were adjusted for other significant factors by the mean of covariates method, in which average values of covariates are entered into the Cox proportional hazards model.

Non-relapse mortality and relapse

The incidence of non-relapse mortality was higher in the HLA-B allele mismatch group with borderline significance in the early time period (HR 2·48, $P = 0·069$, Table III, Fig 4). In the late time period, single HLA-A and -C allele mismatches were associated with a significantly higher incidence of non-relapse mortality (HR 1·47, $P = 0·027$ and HR 1·33, $P = 0·011$). While the HR for non-relapse mortality was highest in the HLA-B allele mismatch group (HR 1·72, $P = 0·10$), the effect was not statistically significant, probably due to the small sample size.

In the early period, a single HLA-C allele mismatch was associated with a significantly lower incidence of relapse (HR 0·46, $P = 0·0063$, Table III, Fig 5). However, an HLA-C mismatch did not have a significant relationship with the relapse rate in the late time period. There was a significant interaction between the BMT time period and an HLA-C allele mismatch ($P = 0·0094$).

Non-relapse mortality was significantly decreased from the early to late time period (HR 0·69, $P = 0·00078$), whereas the incidence of relapse was not changed (HR 0·96, $P = 0·71$).

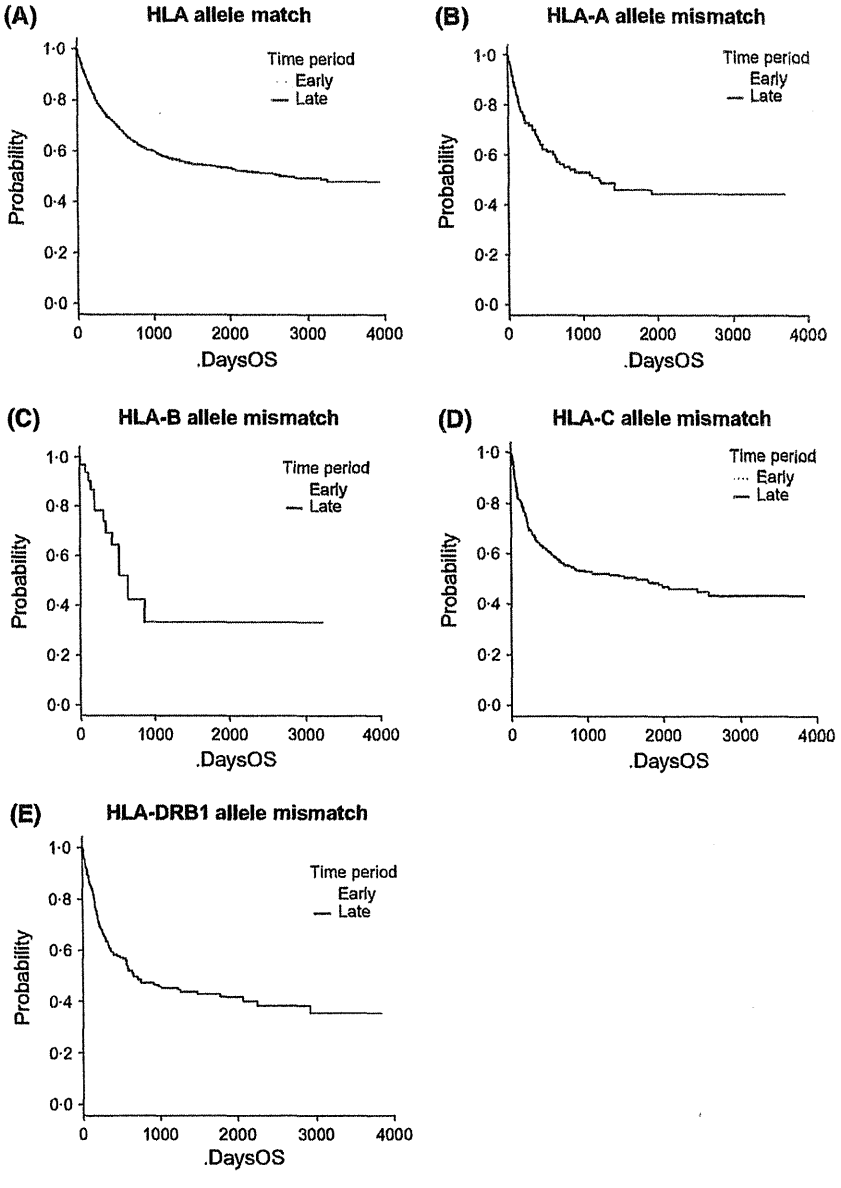


Fig 2. Overall survival grouped according to the transplantation time period in the human leucocyte antigen (HLA) match (A), HLA-A allele mismatch (B), HLA-B allele mismatch (C), HLA-C allele mismatch (D), and HLA-DRB1 allele mismatch (E) groups. The survival curves were adjusted for other significant confounding factors by the mean of covariates method, in which average values of covariates are entered into the Cox proportional hazards model. Early, transplanted between 1993 and 1999; Late, transplanted between 2000 and 2009.

Discussion

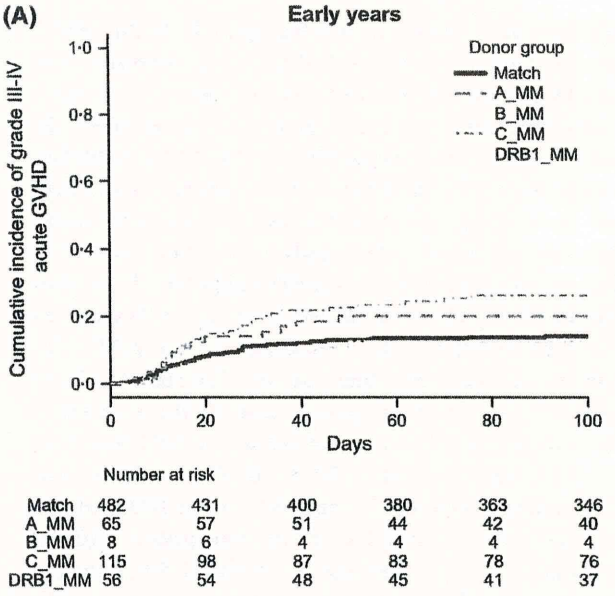
This study re-evaluated the effect of a single HLA allele mismatch on the outcome of unrelated BMT in the recent cohort. We chose 2000 as the cutoff of time period, as high-resolution typing for HLA-A and -B became a common practice in Japan after 2000. In contrast to our previous findings (Sasazuki *et al*, 1998), only the effects of single HLA-C and -DRB1 mismatches were statistically significant in the recent time period, but the negative impact of each single HLA allele mismatch was not significantly different among the HLA-A, -B, -C, and -DRB1 mismatches. Previous JMDP studies showed that HLA-A and -B allele mismatches were associated with higher overall mortality, whereas HLA-C or -DRB1 allele mismatches did not affect mortality after unrelated BMT (Sasazuki *et al*, 1998). In contrast, Petersdorf *et al* (2004) reported that a single HLA-A, -B, -C or -DRB1 allele mismatch had no significant relationship with survival in patients with leukaemia other than chronic myeloid leukaemia in chronic phase. The recent NMDP study analysed the effect of a single allele mismatch on survival in 1840 HLA-matched and 985 one-allele mismatched unrelated HSCT and showed that a single mismatch at HLA-B or -C had smaller relationship with survival than single mismatch at HLA-A or -DRB1 (Lee *et al*, 2007). These discrepancies could be explained by the difference in study population or study designs (Bray *et al*, 2008). For example, the distribution of HLA alleles is different between the US and Japanese populations. Several HLA allele mismatch combinations have been shown to have higher risk for severe acute GVHD compared to other mismatch combinations (Kawase *et al*, 2007). The proportion of high-risk mismatch combinations may affect

Table III. Multivariate analysis to evaluate the impact of single human leucocyte antigen (HLA) allele mismatches, transplantation time periods, and their interaction on the incidences of neutrophil engraftment, grade III–IV acute GVHD, non-relapse mortality, and relapse.

Factor	Hazard ratio	*P* value
Engraftment		
Main effects		
HLA (early years)		
Match	1	
A mismatch	1·06 (0·87–1·29)	0·59
B mismatch	0·65 (0·28–1·54)	0·33
C mismatch	0·93 (0·77–1·11)	0·42
DRB1 mismatch	1·03 (0·79–1·36)	0·80
HLA (late years)		
Match	1	
A mismatch	0·95 (0·77–1·18)	0·66
B mismatch	0·70 (0·50–0·98)	0·037
C mismatch	0·95 (0·73–1·08)	0·4
DRB1 mismatch	0·89 (0·77–1·03)	0·12
Transplantation time period		
Early period	1	
Late period	1·13 (1·02–1·25)	0·023
Interactions		
Time period * A mismatch	0·90 (0·68–1·21)	0·49
Time period * B mismatch	1·07 (0·43–2·67)	0·89
Time period * C mismatch	1·02 (0·82–1·27)	0·85
Time period * DRB1 mismatch	0·86 (0·63–1·17)	0·33
Grade III-IV acute GVHD		
Main effects		
HLA (early years)		
Match	1	
A mismatch	1·46 (0·79–2·69)	0·22
B mismatch	1·74 (0·22–13·55)	0·60
C mismatch	2·02 (1·27–3·20)	0·0029
DRB1 mismatch	0·80 (0·37–1·74)	0·58
HLA (late years)		
Match	1	
A mismatch	1·72 (1·07–2·77)	0·025
B mismatch	1·26 (0·42–3·79)	0·68
C mismatch	1·51 (1·12–2·02)	0·0067
DRB1 mismatch	1·45 (1·01–2·09)	0·045
Transplantation time period		
Early period	1	
Late period	1·01 (0·75–1·36)	0·96
Interactions		
Time period * A mismatch	1·18 (0·54–2·55)	0·68
Time period * B mismatch	0·73 (0·07–7·44)	0·79
Time period * C mismatch	0·75 (0·43–1·29)	0·30
Time period * DRB1 mismatch	1·81 (0·77–4·25)	0·17
Non-relapse mortality		
Main effects		
HLA (early years)		
Match	1	
A mismatch	1·41 (0·93–2·12)	0·11
B mismatch	2·48 (0·93–6·57)	0·069
C mismatch	1·20 (0·87–1·67)	0·27
DRB1 mismatch	0·86 (0·52–1·41)	0·55
HLA (late years)		
Match	1	
A mismatch	1·47 (1·05–2·07)	0·027
B mismatch	1·72 (0·90–3·29)	0·1
C mismatch	1·33 (1·07–1·66)	0·011
DRB1 mismatch	1·22 (0·93–1·60)	0·15
Transplantation time period		
Early period	1	
Late period	0·69 (0·56–0·86)	0·00078
Interactions		
Time period * A mismatch	1·05 (0·61–1·79)	0·86
Time period * B mismatch	0·69 (0·21–2·25)	0·54
Time period * C mismatch	1·11 (0·74–1·64)	0·62
Time period * DRB1 mismatch	1·42 (0·81–2·50)	0·23
Relapse		
Main effects		
HLA (early years)		
Match	1	
A mismatch	0·79 (0·45–1·39)	0·42
B mismatch	1·97 (0·57–6·76)	0·28
C mismatch	0·46 (0·27–0·81)	0·0063
DRB1 mismatch	0·90 (0·54–1·51)	0·70
HLA (late years)		
Match	1	
A mismatch	0·71 (0·44–1·14)	0·15
B mismatch	1·10 (0·49–2·49)	0·81
C mismatch	1·04 (0·81–1·33)	0·79
DRB1 mismatch	1·27 (0·95–1·68)	0·10
Transplantation time period		
Early period	1	
Late period	0·96 (0·76–1·20)	0·71
Interactions		
Time period * A mismatch	0·89 (0·43–1·87)	0·77
Time period * B mismatch	0·56 (0·13–2·46)	0·44
Time period * C mismatch	2·23 (1·22–4·08)	0·0094
Time period * DRB1 mismatch	1·40 (0·78–2·52)	0·26

Factors used for adjustment included donor sex, ABO major mismatch, ABO minor mismatch, cell dose, GVHD prophylaxis, and disease risk in analysis for engraftment, donor age, donor sex, female to male transplantation, cell dose, disease, and disease risk in analysis for GVHD, recipient age, donor age, donor sex, female to male transplantation, ABO major mismatch, disease, disease risk, and GVHD prophylaxis in analysis for non-relapse mortality, and donor age, disease, disease risk, and the use of TBI in analysis for relapse.

the effect of each single HLA allele mismatch. With regard to the study designs, the inclusion criteria for disease, phase of disease, and HLA matching were different among studies (Bray *et al*, 2008). Japanese studies included HLA-A, -B, and -DR antigen matched transplantation only, whereas the other studies included one-antigen mismatched transplantation. Earlier studies reported that an allele mismatch and an antigen mismatch had similar effects on mortality, although the risk of graft failure was higher with an antigen mismatch



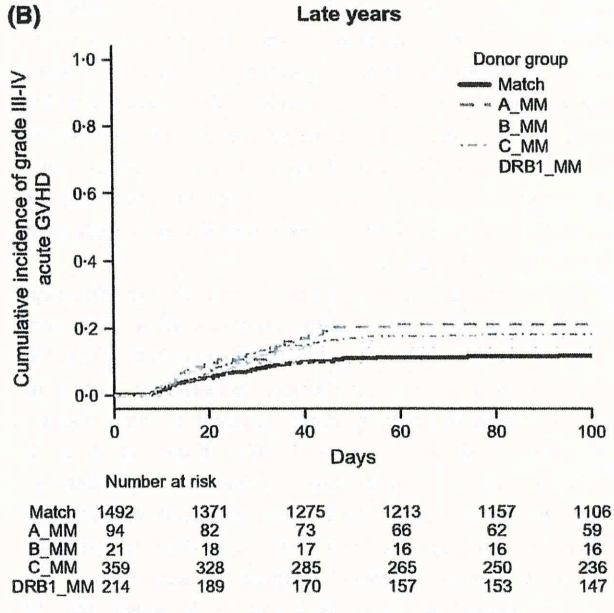
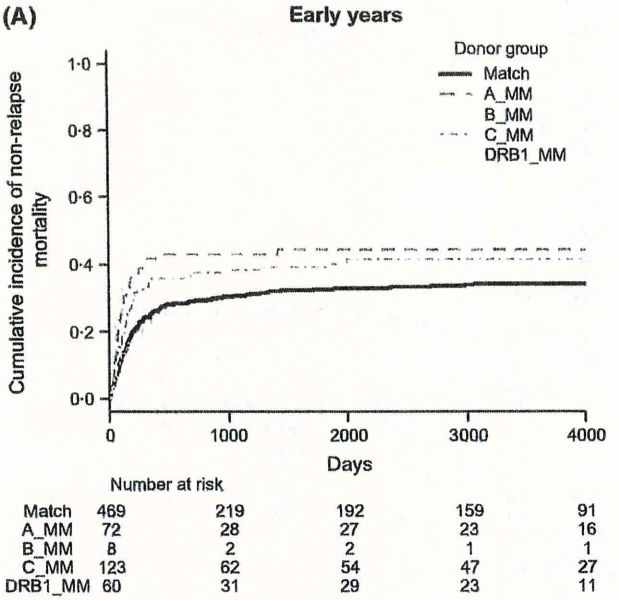


Fig 3. The cumulative incidence of grade III-IV acute graft-versus-host disease (GVHD) grouped according to the human leucocyte antigen mismatch between the donor and recipient in the (A) early (1993–1999) and (B) late (2000–2009) time periods.



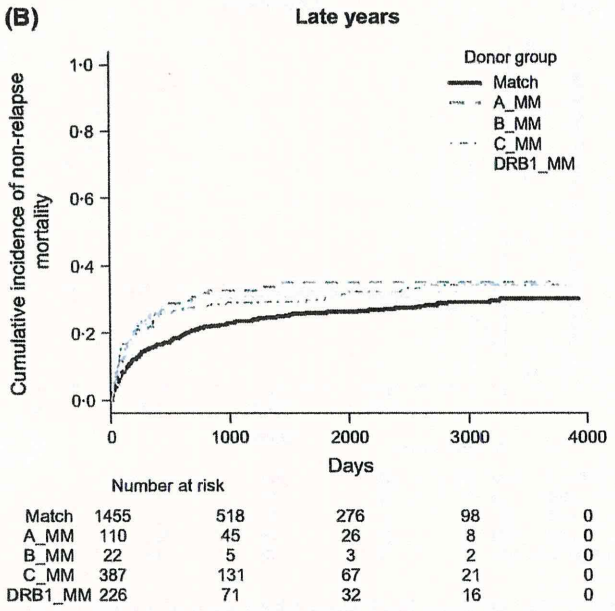


Fig 4. The cumulative incidence of non-relapse mortality grouped according to the human leucocyte antigen mismatch between the donor and recipient in the (A) early (1993–1999) and (B) late (2000–2009) time periods.

(Petersdorf *et al*, 2001, 2004). A subsequent report from NMDP confirmed that there was no significant difference in the effect on survival between a single antigen mismatch and a single allele mismatch (Lee *et al*, 2007). In the current study, only patients who underwent unrelated BMT from an HLA-A, -B, and -DR antigen matched donor were included, as such a donor can be found in more than 90% of patients in Japan. Therefore, only the HLA-C mismatch group included patients with HLA-mismatch at an antigen level. The effect of HLA-C antigen mismatch and HLA-C allele mismatch on survival was equivalent (HR 1·33 vs. 1·28) in the current cohort, although the number of patients with HLA-C allele mismatch was limited.

The second important finding is the positive interaction test that revealed the statistically significant change in the effects of HLA-C and -DRB1 mismatches from the early to the late time period. These significant interactions resulted from the fact that survival after HLA-matched BMT was significantly improved in the late time period, while there was no such improvement after HLA-C or -DRB1 mismatched

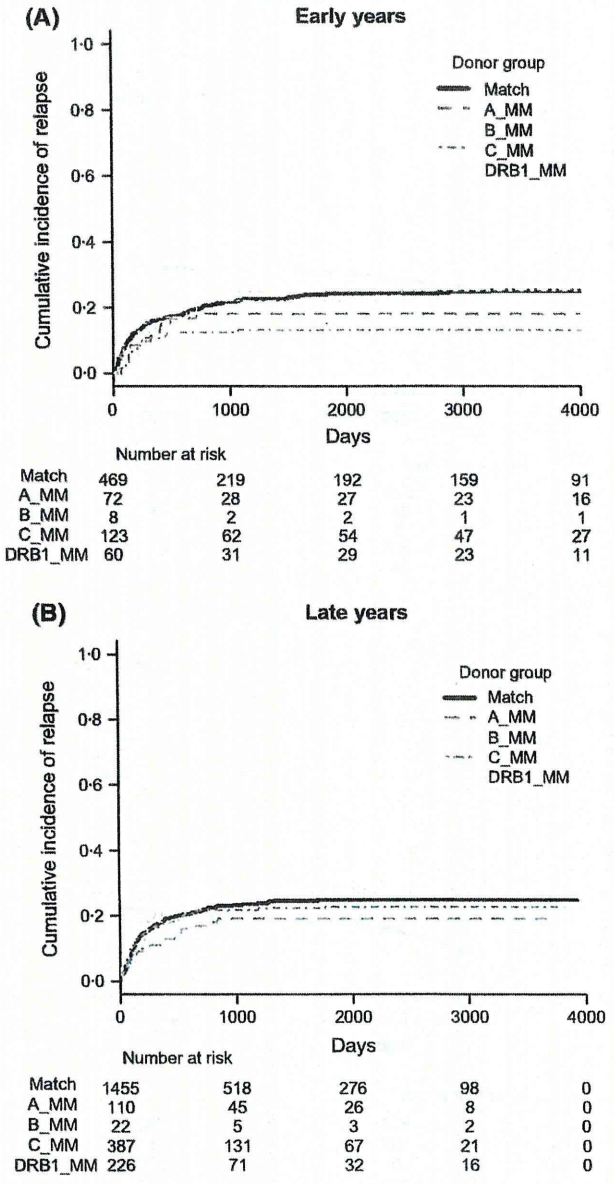


Fig 5. The cumulative incidence of relapse grouped according to the human leucocyte antigen mismatch between the donor and recipient in the (A) early (1993–1999) and (B) late (2000–2009) time periods.

BMT (Fig 2). The improvement in survival in the HLA match group is probably due to the progress in transplantation procedures, including strategies against GVHD and infectious complications. The incidence of grade III–IV acute GVHD in the HLA match group decreased from 13·9% to 11·9% over the two time periods, and furthermore, the incidence of transplant-related mortality among patients who developed grade III–IV acute GVHD decreased, from 25·4% to 15·9%. While such progress should also be reflected in the HLA-C and -DRB1 mismatch groups, other factors may have counterbalanced this benefit. With regard to HLA-DRB1 allele mismatch, significant interaction could be explained by the difference in the availability of information about HLA allele mismatch between the two time periods. In the 1990's, only the presence of an HLA-DRB1 allele mismatch was noted by physicians before BMT, whereas both HLA-A and -B mismatches were also tested before BMT in the 2000's. In addition, the landmark paper from the JMDP was published in 1998 (Sasazuki *et al*, 1998), and the presence of an HLA-A or -B mismatch was recognized as a risk factor for severe acute GVHD. These backgrounds might have induced a trend toward more intensive GVHD prophylaxis in patients with an HLA-DRB1 allele mismatch in the 1990's and in those with an HLA-A or -B mismatch in the 2000's. For example, in the early time period, TAC-based GVHD prophylaxis was most frequently used in the HLA-DRB1 mismatch group (odds ratios for the use of TAC were 0·65, 0·58, 0·97 and 1·29 for the HLA-A, -B, -C, and -DRB1 mismatch groups, respectively, compared to the HLA-matched group). On the other hand, in the late time period, TAC was used almost equally in the HLA-A, -B, and -DRB1 mismatch groups (odds ratios for the use of TAC were 1·49, 1·25, 1·00 and 1·38 for the HLA-A, -B, -C, and -DRB1 mismatch groups, respectively, compared to the HLA-matched group). The statistical interaction was significant even after an adjustment for the use of TAC, and therefore this is not the major reason for the interaction. The target blood concentrations of CSA or TAC and the dose of methotrexate in GVHD prophylaxis may also have been affected by the availability of information about HLA allele mismatch, but such data were not included in the database.

Another bias that may have been caused by the difference in the availability of information about the HLA allele mismatch is the trend to avoid HLA-mismatched BMT for patients with less advanced diseases, because the impact of HLA mismatch is generally more apparent in such diseases (Petersdorf *et al*, 2004; Lee *et al*, 2007; Kanda *et al*, 2003, 2012). In fact, the proportion of patients with low-risk disease in the HLA-DRB1 allele mismatch group was less than that in the other groups (57·1% vs. 62·7–75%) in the early time period, while equivalent proportions were seen in the late time period (62·1% vs. 56·5–65·6%). However, the HR value for HLA-DRB1 allele mismatch increased from 0·79 in the early period to 1·42 in the late period even when we only analysed patients with low-risk disease, although the interaction was not statistically significant ($P = 0·069$). The proportion of patients with a high-risk HLA allele mismatch may also affect the impact of each single HLA-allele mismatch on survival (Kawase *et al*, 2007), but the proportions were similar in the early and late time periods (6·3% and 7·3%). Therefore, this cannot explain the significant interaction between the time period and HLA-DRB1 allele mismatch.

With regard to the interaction between the time period and HLA-C allele mismatch, there was no difference in the availability of information, because HLA-C typing was not routinely performed until 2009. The significant interaction probably resulted from the increased incidence of relapse in the late time period in the HLA-C allele mismatched group.

The proportion of patients with a killer immunoglobulin-like receptor ligand mismatch in the graft-versus-host direction (KIR_L_MM_G) may affect the incidence of relapse (Dupont & Hsu, 2004; Morishima *et al*, 2007). However, the interaction test for relapse was significant even when we excluded patients with a KIR_L_MM_G mismatch ($P = 0{\cdot}022$). Therefore, we could not find a clear explanation for this interaction.

The major limitation of this study is the sample size in the HLA-B mismatch groups, especially in the early time period. Although the major object of this study was to reevaluate the impact of a mismatch in each single allele in the late time period, there were only 23 patients in the HLA-B mismatch group even in the late period, and therefore we could not conclude that the effects of all single HLA mismatches were equivalent, despite that there was no significant difference in the negative impact on survival among the HLA-A, -B, -C, and -DRB1 mismatches. Another limitation of this study was the exclusion of HLA-DQ mismatch in the analyses, as the allele data for HLA-DQ was available only in 493 of the 3003 patients in this study. However, when we included HLA-DQ in the multivariate analysis for overall survival, the effect of HLA-DQ mismatch on survival was not significant (HR 0·93, 95% CI 0·63–1·38, $P = 0{\cdot}73$) and the HRs for HLA-A, -B, -C, and -DRB1 did not obviously change after the addition of HLA-DQ in the model (data not shown).

In conclusion, this retrospective study revealed that the impact of single HLA allele mismatches might have changed after HLA-A and -B mismatch information became available to physicians before BMT. In the recent cohort (BMT between 2000 and 2009), the negative impact of HLA-C and -DRB1 mismatches became apparent. We should reconsider the algorithm for unrelated donor selection in Japan.

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Author contributions

Y.K. and Y.M. designed the study. Y.K., J.K., Y.A., and S.M. analysed the data. Y.M., T.I., K.O., T.F., K.M., H.I., T.M., K.I., T.E., and K.K. gathered the data. Y.K. wrote the first draft of the paper and all other authors contributed to the final version.

Disclosure of conflicts of interest

We declare that we have no conflicts of interest.

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Double-Unit Cord Blood Transplantation after Myeloablative Conditioning for Patients with Hematologic Malignancies: A Multicenter Phase II Study in Japan

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A B S T R A C T

We analyzed the outcomes of 61 patients with hematologic malignancies who underwent double-unit cord blood transplantation (dCBT) after myeloablative conditioning performed as part of a prospective multicenter phase II study. The conditioning regimen for dCBT included total body irradiation, cyclophosphamide, and granulocyte colony-stimulating factor combined with cytosine arabinoside for myeloid malignancies and with total body irradiation and cyclophosphamide for lymphoid malignancies. The cumulative incidence of neutrophil engraftment after dCBT was 85% (95% confidence interval [CI], 73%-92%). All 51 of the patients who engrafted had complete chimerism derived from a single donor by day +60. Only the degree of HLA disparity in the host-versus-graft direction had an impact on unit dominance. The cumulative incidence of grade II-IV acute graft-versus-host disease was 25% (95% CI, 15%-37%), and that of chronic graft-versus-host disease was 32% (95% CI, 20%-44%). The 1-year cumulative incidence of relapse was 23% (95% CI, 13%-34%), and that of transplantation-related mortality was 28% (95% CI, 17%-39%). With a median follow-up of 41 months, event-free survival was 48% (90% CI, 37%-58%) at 1 year and 46% (90% CI, 35%-56%) at 3 years. Event-free survival at 3 years was 67% (95% CI, 46%-81%) for patients with standard risk and 29% (95% CI, 15%-45%) for those with advanced risk. This study suggests that dCBT after myeloablative conditioning is a promising alternative for adults and large children with hematologic malignancies who need stem cell transplantation but lack a suitable adult donor or an adequate single-unit cord blood graft.

INTRODUCTION

Cord blood (CB) is being increasingly used as an alternative source of hematopoietic stem cells for adults with hematologic malignancies requiring hematopoietic stem cell transplantation (HSCT) [1-5]. Although CB has advantages, including rapid availability [6] and low risk of severe acute graft-versus-host disease (GVHD) despite HLA mismatches, the low cell dose in a single CB unit contributes to high rates of graft failure and transplantation-related mortality (TRM), especially in adults and large children [7-9]. Double-unit CB transplantation (dCBT) was introduced to overcome these obstacles [10] and is becoming more widely applied [11-15]. We conducted a prospective multicenter Phase II study assessing the safety and efficacy of dCBT for patients with high-risk hematologic malignancies. We used relatively standard myeloablative conditioning regimens: total body irradiation (TBI) plus cyclophosphamide (CY) for lymphoid malignancies and TBI, CY, and granulocyte colony-stimulating factor (G-CSF) combined with cytosine arabinoside (ara-C) for myeloid malignancies. We used cyclosporine A (CyA) and short-term methotrexate (MTX) for GVHD prophylaxis.

PATIENTS AND METHODS

Thirty-nine centers participated in this study after approval by each pertinent institutional Review Board (trial identifier: UMIN: C000000359, C-SHOT 0507). Written informed consent was obtained from all patients before transplantation.

Eligibility Criteria

Inclusion criteria were as follows: (1) age <55 years with a high-risk hematologic malignancy; (2) no HLA-matched or single antigen-mismatched related donor available; (3) no HLA-matched unrelated donor available, or requiring urgent transplantation even if an HLA-matched unrelated donor were available; (4) no 4-6/6 HLA-A, -B, or -DR serologically antigen-matched single CB unit containing a cell dose >2.5 × 10^7/kg; (5) no previous stem cell transplantation; (6) no active infection at the start of conditioning chemoradiotherapy; and (7) HIV-negative status. Patients with an Eastern Cooperative Oncology Group performance status ≥2, ejection fraction <50%, SaO_2 (arterial oxygen saturation) <93% in room air, serum creatinine of ≥1.3 mg/dL, total bilirubin ≥1.6 mg/dL, or glutamic-oxaloacetic transaminase ≥2 times the normal value were excluded. Patients with Down syndrome or Fanconi anemia were also excluded.

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CB Unit Selection Criteria

CB units were obtained from CB banks belonging to the Japan Cord Blood Bank Network. The criterion for CB unit selection was 4-6/6 HLA-A, -B, and -DR antigens matched to the recipient. One of the 2 units should contain a cell dose of at least 1.5×10^7/kg. The total cell dose of the 2 units had to be $>2.5 \times 10^7$/kg, and transplantation of 2 units each with a cell dose $>2 \times 10^7$/kg was not allowed.

Treatment

All patients received a myeloablative preparative conditioning regimen of 12 Gy TBI fractionated in 4 or 6 doses. Ara-C was given at a dose of 3 g/m^2 every 12 hours for 2 days (days -5 and -4). Recombinant human G-CSF was given by continuous infusion at a dose of 5 μg/kg/day; infusion was started at 12 hours before the first dose of ara-C and stopped at the completion of the last dose. CY was administered i.v. at 60 mg/kg/day for 2 days (days -3 and -2). A regimen of TBI, CY, and G-CSF combined with ara-C was used for patients with myeloid leukemias and myelodysplastic syndrome (MDS) [1]. TBI plus CY was used for those with lymphoid malignancies. At 2 days after completion of conditioning, CB units were thawed and then infused sequentially in an arbitrary order and nonmandatory time interval after premedication with hydrocortisone (100 mg) and hydroxyzine hydrochloride (25 mg).

GVHD prophylaxis was provided with CyA plus short-term MTX. CyA was given by continuous infusion at a dose of 3 mg/kg/day starting on day -1. MTX was given at 15 mg/m^2 i.v. on day +1 and at 10 mg/m^2 on days +3 and +6. Once oral intake could be tolerated, oral CyA was started at a dose ratio of 1:2.5 in 2 divided doses per day based on the last i.v. dose. In the absence of GVHD after day +60, CyA was tapered by 10% to 20% per week until it could be discontinued. The supportive care regimen, including prophylaxis for infection, was similar to that for single-unit CBT in each transplantation center. All patients received G-CSF starting on day 5 and continuing until the absolute neutrophil count (ANC) reached 5000/μL.

HLA Typing and Chimerism Analysis

HLA typing of the recipient and CB unit was determined by low-resolution (2 digits) and/or high-resolution (4 digits) DNA typing for HLA-A, -B, -C, and -DRB1. Donor chimerism was determined serially for bone marrow and/or blood at days +14, +30, +60, and +100 after dCBT, and at additional time points as needed. The analytic method used was based on the quantitative amplification of informative polymorphic short tandem repeat regions in the recipient and the donor.

Definitions

Patients who underwent dCBT in first or second remission of acute myelogenous leukemia (AML), in first remission of acute lymphoblastic leukemia (ALL) and malignant lymphoma (ML), or in the chronic phase of chronic myelogenous leukemia (CML) and refractory anemia of MDS were classified as standard risk. All others were classified as advanced risk.

Neutrophil recovery was defined as achievement of an absolute neutrophil count (ANC) of ≥500/μL for 3 consecutive days; platelet recovery was defined as a count of ≥50,000/μL without transfusion support. Primary engraftment failure was defined as the absence of donor-derived myeloid cells on the day of death or day +60 in patients surviving beyond day +2+8 after dCBT, or when a second stem cell transplantation was required for donor-derived myeloid recovery. Diagnosis and clinical grading of acute GVHD (aGVHD) were performed according to established criteria [16]. Relapse was defined as recurrence of the underlying hematologic malignancy. TRM was defined as death during a continuous remission. Disease-free survival (DFS) was defined as survival in a state of continuous remission. Event-free survival (EFS) was defined as survival in a state of remission without engraftment failure.

Statistical Analyses

The primary endpoint of this study was 1-year EFS; secondary endpoints were neutrophil and platelet engraftment, incidence of aGVHD and chronic GVHD (cGVHD), toxicity within 28 days, incidence of TRM and relapse, DFS, and overall survival (OS). The expected and threshold EFS at 1 year were estimated as 60% and 40%, respectively. With a statistical power of 90% and a 1-sided type I error of 5%, the number of eligible patients required for this study was calculated as 56 using a binomial analysis method. The projected sample size was 70 patients, assuming that 20% of patients would be ineligible. Primary endpoint analysis was performed using the Kaplan-Meier method to calculate the probability of EFS. Treatment was considered effective if the lower limit of the 90% confidence interval (CI) exceeded the threshold EFS (ie, 40%).

Cumulative incidence curves were used in a competing-risks setting to calculate the probabilities of neutrophil and platelet recovery, aGVHD, cGVHD, relapse, and TRM. For neutrophil and platelet recovery, death before recovery was the competing event; for GVHD, death without GVHD and relapse was the competing events; for relapse, death without evidence of relapse was the competing event; and for TRM, relapse was the competing event. OS, DFS, and EFS were calculated by the Kaplan-Meier method. The log-rank test was used for univariate comparisons. For multivariate analysis of prognostic variables affecting transplant outcomes, a Fine-Gray model was used to analyze transplantation outcomes with competing risks. A Cox proportional hazard regression model was used to analyze other outcomes. The following variables were considered: recipient cytomegalovirus (CMV) serology (positive versus negative), recipient age at enrollment (age ≥40 years versus <40 years; cutoff point was around the median), degree of ABO matching between recipient and engrafting unit (major mismatch versus matched or minor mismatch), sex matching between recipient and engrafting unit (mismatched versus matched), degree of HLA matching between donor and recipients (2 antigen- mismatched versus 0 or 1 antigen-mismatched, with HLA matching defined by the worst-matched of the 2 units), disease status at transplantation (advanced versus standard), cryopreserved TNC dose (median, $<3.52 \times 10^7$/kg versus $\geq 3.52 \times 10^7$/kg), CD34$^+$ cell dose (median, <1.04/kg $\times 10^5$/kg versus $\geq 1.04 \times 10^5$/kg), and cell dose difference [(TNC of large unit − TNC of smaller unit)/(TNC of large unit, ≥15% versus <15%)], and degree of HLA mismatch between the 2 units (≥3 antigen mismatches versus ≤2 antigen mismatches).

Variables found to affect outcome with a P value <.20 on univariate analyses were selected for the multivariate analyses. Variables were selected in a backward stepwise manner with a variable retention criterion of $P < .05$ for the final model. The Wilcoxon signed-rank test was used to evaluate the effect of cell dose and HLA compatibility on engraftment of a predominant single CB unit, and the McNemar test was used for evaluation of categorical factors. The median duration of follow-up of survivors was 41 months (range, 12 to 57.4 months). Results are reported as of March 2011. Calculations were performed using Stat View J version 5.0 and Stata version 11.1 (StataCorp, College Station, TX).

RESULTS

Patient and Graft Characteristics

A total of 70 patients were enrolled between April 2006 and January 2010. Nine patients did not undergo dCBT, 7 because of disease progression and 2 because they received a graft from another source. Patient and graft characteristics are summarized in Table 1. The 61 patients who underwent dCBT included 8 females and 53 males, with a median age of 37 years (range, 10 to 54 years) and a median body weight of 70.5 kg (range, 50.1 to 129.8 kg). Antibodies against CMV were detected in 75.4% of the patients; CMV antibody was not tested in 3 patients. The underlying malignancy was AML in 30 patients, ALL in 17 patients, CML in 6 patients, MDS in 5 patients, and ML in 3 patients. Disease status at dCBT was classified as standard risk in 27 patients and as advanced risk in 34 patients. The median TNC and CD34$^+$ cell doses (both units combined) at cryopreservation were 3.52×10^7/kg (range, 2.25 to 4.43×10^7/kg) and 1.04×10^5/kg (range, 0.39 to 2.67×10^5/kg), respectively. The median TNC doses of the larger and smaller units were 1.90×10^7/kg (range, 1.47 to 2.48×10^7/kg) and 1.60×10^7/kg (range, 0.74 to 1.97×10^7/kg), respectively. In 1 patient, the TNC dose of the larger unit was decreased from $>1.5 \times 10^7$/kg at registration to 1.47×10^7/kg at dCBT because of weight gain. Because 1.47 rounded off to 1 decimal place is 1.5, we decided to include this case in the analyses.

HLA matching for HLA-A, -B, and -DRB1 low- and high-resolution types between recipients and donors and between donors is described in Table 1. When the graft with fewest HLA mismatches was counted for each recipient, only 2 patients (3%) received a graft that contained at least 1 unit in which HLA-A, -B, and -DRB1 were matched at a low-resolution level to the recipient; 21 patients (34%) received a graft with at least 1 unit 5/6 HLA-matched to the recipient; and 38 patients (62%) received a graft with both units 4/6 HLA-matched to the recipient. Among the 58 patients with HLA-DRB1 typed by high-resolution DNA typing, 2 patients

Table 1
Patient and Graft Characteristics

Characteristic	Value
Number of patients	61
Sex, male/female, n	53/8
Age, years, median (range)	37 (10-54)
Body weight, kg, median (range)	70.5 (50.1-129.8)
Diagnosis and disease status at CBT, n	
ALL	17 (CR1, 8; CR2, 6; relapse, 3)
AML	30 (CR1, 6; CR2, 11; CR3, 1; relapse, 6; PIF, 4; no induction therapy, 2)
CML	6 (AP, 1; BC, 5)
MDS	5 (RA, 2; RAEB2, 3)
Non-Hodgkin lymphoma	3 (refractory, 3)
CMV antibody, positive/negative/unknown, n	46/12/3
Performance status, 0/1, n	48/13
Cell dose at cryopreservation, median (range)	
TNC, $\times 10^7$/kg	
Total	3.52 (2.25-4.43)
Large unit	1.90 (1.47-2.48)
Small unit	1.60 (0.74-1.97)
$CD34^+$ cells, $\times 10^5$/kg	
Total	1.04 (0.39-2.67)
Large unit	0.50 (0.12-2.41)
Small unit	0.46 (0.02-1.42)
GM-CFU, $\times 10^3$/kg	
Total	27.42 (0.42-100.9)
Large unit	11.94 (0.17-39.6)
Small unit	12.85 (0.25-88.98)
ABO compatibility, large unit/small unit, n	
Major mismatches	23/20
Minor mismatches	16/16
Matches	22/25
HLA compatibility, n	
-A, -B, and -DRB1 low resolution	
5/6 + 6/6	1
5/6 + 5/6	6
4/6 + 6/6	1
4/6 + 5/6	15
4/6 + 4/6	38
-A and -B low resolution, -DRB1 high resolution	
5/6 + 5/6	3
4/6 + 6/6	1
4/6 + 5/6	10
4/6 + 4/6	17
3/6 + 6/6	1
3/6 + 5/6	4
3/6 + 4/6	13
3/6 + 3/6	4
2/6 + 4/6	1
2/6 + 3/6	4
HLA compatibility to each unit, n	
-A, -B, and -DRB1 low resolution	
6/6	1
5/6	14
4/6	13
3/6	23
2/6	10
-A and -B low resolution, -DRB1 high resolution	
6/6	0
5/6	8
4/6	12
3/6	20
2/6	15
1/6	6

AP indicates accelerated phase; BC, blast crisis; CR, complete remission; PIF, primary induction failure; RA, refractory anemia; RAEB, refractory anemia with excess blasts.

(3%) received a graft that contained at least one 6/6 HLA-matched unit, 17 (29%) received at least one 5/6 HLA-matched unit, 31 (53%) received at least one 4/6 HLA-matched unit, 8 (14%) received at least one 3/6 HLA-matched unit. Three, 17, and 4 patients received a graft with both units 5/6, 4/6, and 3/6 HLA-matched to the recipient, respectively. The units were 6/6 HLA-A–, -B–, and DRB1–matched at low resolution to each another in 1 patient, 5/6 matched in 14 patients, 4/6 matched in 13 patients, 3/6 matched in 23 patients, and 2/6 matched in 10 patients. When HLA was typed by HLA-A and -B low-resolution and -DRB1 high-resolution typing, the units were 5/6 matched to each other in 8 patients, 4/6 matched in 12 patients, 3/6 matched in 20 patient, 2/6 matched in 15 patients, and 1/6 matched in 6 patients.

Survival

The median follow-up for survivors (n = 32) was 41 months. EFS at 1 year was 48% (90% CI, 37%-58%) (Figure 1A). One-year EFS at 1 year was 67% (95% CI, 46%-81%) in patients with standard risk and 32% (95% CI, 18%-48%) in patients with advanced risk at dCBT, and 3-year EFS was 67% (95% CI, 46%-81%) in patients with standard risk and 29% (95% CI, 15%-45%) in those with advanced risk ($P = .023$) (Figure 1B). One-year DFS was 49% (95% CI, 36%-61%), and 1-year OS was 57% (95% CI, 44%-69%). Three-year DFS was 47% (95% CI, 34%-59%), and 3-year OS was 54% (95% CI, 40%-65%). Disease status at transplantation was the sole prognostic factor affecting EFS (relative risk [RR], 2.71; $P = .011$). No other variable considered had a significant effect on EFS.

Toxicity Within 28 Days after dCBT

Toxicities occurring within 28 days after dCBT were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3. The most frequent grade 3-4 toxicity was infection, occurring in 43 of 61 patients (70.5%); other grade 3-4 toxicities included nausea/vomiting (17 patients; 27.9%), oral mucosa lesions (16; 26.2%), diarrhea (13; 21.3%), cardiac events (6; 9.8%), liver toxicity (6; 9.8%), bleeding (5; 8.2%), neurologic events (3; 4.9%), renal/urinary events (3; 4.9%), skin toxicity (3; 4.9%), lung toxicity (2; 3.3%), and thrombotic thrombocytopenic purpura/hemolytic uremic syndrome (1; 1.6%). Grade 4 toxicities involving infections were seen in 5 patients (8.2%), and those involving the heart occurred in 2 patients (3.3%). Other grade 4 toxicities included bleeding and neurologic, lung, and liver toxicities, which were seen in 1 patient each.

Hematopoietic Recovery and Chimerism

The cumulative incidence of neutrophil recovery was 67% (95% CI, 53%-77%) at day +28 and 85% (95% CI, 73%-92%) at day +50 (Figure 2). The cumulative incidence of platelet recovery at day +180 was 77% (95% CI, 66%-89%). The median time to neutrophil recovery was 25 days (range, 17 to 49 days). A greater degree of HLA matching between the 2 units (≥3 antigen mismatches with increased risk of no neutrophil recovery compared with ≤2 antigen mismatches; RR, 0.53; $P = .023$) was the sole risk factor affecting neutrophil recovery.

Three patients (5%) died too early to allow evaluation of engraftment (2 patients on day +7 and 1 patient on day +12). Failure of primary engraftment occurred in 7 patients; 6 of these 7 patients underwent a second transplantation (single-unit CBT in 3, autologous peripheral blood stem cell transplantation in 2, haploidentical peripheral

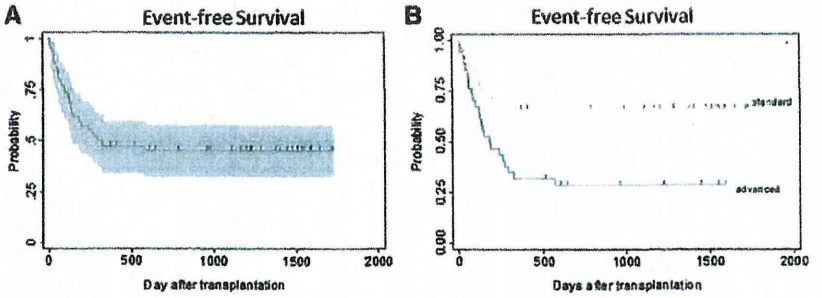

Figure 1. Kaplan-Meier analysis of EFS in patients with dCBT after myeloablative conditioning (A) and according to disease status (B). Patients with standard risk had significantly better posttransplantation survival than those with advanced risk ($P = .023$, log-rank test).

blood stem cell transplantation in 1) between day +27 and day +49. Only 1 of these patients survived beyond 1 year after dCBT.

All but 1 of the 51 patients with donor engraftment had complete chimerism derived from a single donor (median, 100%; range, 91.2% to 100%) by day 30 after dCBT. One patient demonstrated mixed chimerism from both donors (81.6% and 11.5%) at day +30, but this changed to complete chimerism of single-donor origin by day +60.

Predicting Factors Responsible for Unit Dominance

The degree of HLA disparity in the host-versus-graft (HVG) direction was associated with unit dominance (Table 2). Twenty of the 51 patients with donor engraftment received 2 units with varying degrees of HLA disparity (HLA-A, -B, and -DR antigen-level typing). Of these, the unit that was better HLA-matched to the recipient engrafted in 15 patients, whereas the more poorly matched unit predominated in 5 patients ($P = .0218$). Twenty-seven of 49 engrafted patients typed by HLA-A or -B antigen-level and -DRB1 high-resolution typing received 2 units with different degrees of HLA disparity; of these, the better-matched unit engrafted in 21 patients ($P = .0056$).

There was no correlation between unit dominance and cell dose (cryopreserved TNCs, $P = .4589$; cryopreserved $CD34^+$ cells, $P = .3823$; cryopreserved granulocyte macrophage colony-forming units (GM-CFU), $P = .6854$; infused TNCs, $P = .6114$; infused $CD34^+$ cells, $P = .3875$; infused GM-CFU, $P = .8405$). Other factors, including sex match ($P = .7003$), ABO match ($P = 1.0$), order of infusion ($P = .4838$), and graft viability ($P = .6152$), were not associated with unit dominance.

GVHD

aGVHD developed in 33 of the 61 patients (54%), classified as grade I in 18 patients, grade II in 11, grade III in 3, and grade IV in 1 (25% grade II-IV and 7% grade III-IV). cGVHD was observed in 18 of the 50 evaluable patients who survived for >100 days, and was extensive in 9 patients. The cumulative incidence of grade II-IV aGVHD was 25% (95% CI, 15%-37%), and that of cGVHD at 1 year was 32% (95% CI, 20%-44%) (Figure 3A and B). No risk factors for the development of grade II-IV aGVHD were identified in univariate and multivariate analyses including HLA disparities ($P = .327$).

Relapse

Relapse occurred in 15 patients, between 57 and 573 days (median, 135 days) after dCBT. The cumulative incidence of relapse at 1 year was 23% (95% CI, 13%-34%) (Figure 3C). Seven of 17 patients with ALL relapsed, compared with only 8 of 41 patients with myeloid malignancies (AML, 6 of 29; CML, 1 of 6; MDS, 1 of 6). In terms of disease status at transplantation, relapse occurred in 4 of 27 patients with standard risk and in 11 of 34 patients with advanced risk. No risk factors for relapse were identified by univariate and multivariate analyses, including disease status at CBT ($P = .291$) and HLA disparities ($P = .156$).

TRM and Cause of Death

The cumulative incidence of TRM was 15% (95% CI, 7%-25%) at day +100 and 28% (95% CI, 17%-39%) at 1 year (Figure 3D). No risk factors for TRM were identified on univariate and multivariate analyses. The causes of death are listed in Table 3. Disease progression was the leading cause of death. Of the 29 patients who died between 7 and 1368 days (median, 188 days) after dCBT, 15 died from causes other than relapse: graft failure in 5 (of whom 3 died from infection and 1 died from hepatic veno-occlusive disease after a second transplantation), infection without graft failure in 2, organ failure in 3, acute respiratory distress syndrome/interstitial pneumonia in 3, and cGVHD and bleeding in 1.

DISCUSSION

The present study is the first reported analysis of dCBT in Japan. In this multicenter Phase II study, greater HLA disparities between recipient and donor and between each of the 2 units were found compared with those reported in previous studies of dCBT, because we selected the 4-6/6 HLA-

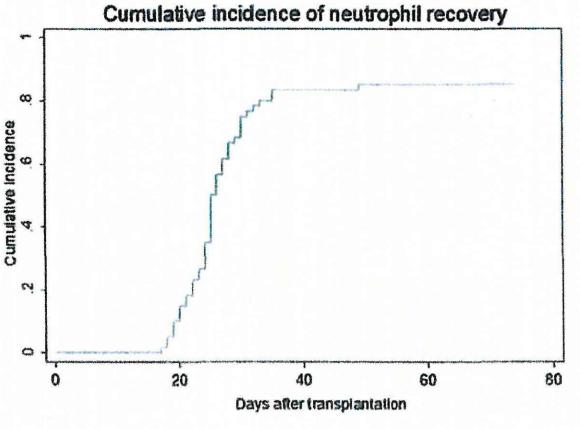

Figure 2. Cumulative incidence of neutrophil engraftment after myeloablative conditioning and subsequent dCBT.

Table 2
Degree of HLA Disparity in the HVG Direction and Unit Dominance

Number of HLA Mismatches in the HVG Direction for Winner/Loser	Difference in HLA Mismatches	Number of Patients with Sustained Engraftment	
		HLA-A, -B, and -DR at Low Resolution Level	HLA-A and -B at Low Resolution Level, HLA-DRB1 at High Resolution Level
1/1	0	11	3
2/2		20	15
3/3		0	4
0/1	−1	1	1
1/2		13	7
2/3		0	6
3/4		0	1
0/2	−2	1	1
1/3		0	4
2/4		0	1
1/0	1	1	0
2/1		4	2
3/2		0	2
4/3		0	1
3/0	3	0	1
		$P = .0218$	$P = .0056$

Analyses were performed with the Wilcoxon signed-rank test.

matched CB unit for the recipient by matching at the low-resolution DNA typing level of HLA-A, -B, and -DRB1, with no consideration of unit–unit match. The lower limit of the 90% CI did not exceed the threshold EFS by 3% in primary endpoint analyses. The threshold and expected EFS was estimated prior to study initiation according to survival results of single-unit CBT (EFS of 40% at 1 year; unpublished data, Japan Cord Blood Bank Network, 2005) and dCBT [17] (EFS of 64% at 1 year) for adults. In these studies, 21% and 36% of patients were received CBT in advanced-risk disease status, respectively, whereas 54% of patients in this study were in advanced-risk disease status at the time of dCBT. Our survival data are comparable to earlier reports of dCBT after myeloablative conditioning [10-15]; thus, we can confirm that dCBT after myeloablative conditioning is a promising alternative option for adults and large children with hematologic malignancies who need HSCT but do not have a suitable related/unrelated donor or an adequate single-unit CB graft. We have also shown that HLA disparity in the HVG direction helps determine which unit was engrafted. These data may provide clinically useful information to aid in the selection of CB units for dCBT.

Our cumulative incidence of neutrophil engraftment of 85% and median time to neutrophil recovery of 25 days are comparable to previously reported values for dCBT with myeloablative conditioning (ie, cumulative incidence of neutrophil engraftment, 80%-94%; median time to neutrophil recovery, 23-25 days) [10,12-15]. The degree of HLA disparity between the 2 units was the sole factor associated with neutrophil engraftment. On the other hand, unit–unit HLA match reportedly had no significant effect on sustained engraftment and speed of neutrophil recovery [18]. Further studies are needed to investigate the influence of cross-immunologic reactions between the 2 units on neutrophil engraftment.

Our results are in agreement with previously reported data, which indicated that 1 CB unit becomes predominant and supports sustained hematopoiesis in dCBT. The parameters that determine unit dominance have not yet been elucidated. In our analysis, only the degree of HLA disparity in the HVG direction was correlated with unit dominance. To our knowledge, this is the first report suggesting that host-versus-graft immune reactions play a role in determining the engrafting unit. There was no correlation between dominance and the doses of TNCs, $CD34^+$ cells, and GM-CFU or ABO, sex mismatch, cell viability, or order of infusion. Previous reports have implied that $CD3^+$, GM-CFU, and $CD34^+$ cell doses and the viability of $CD34^+$ cells were associated with the unit dominance [14,18-21], and that the presence of graft-versus-graft reactions mediated by $CD8^+$ T cells expanding from the dominant unit play a critical role

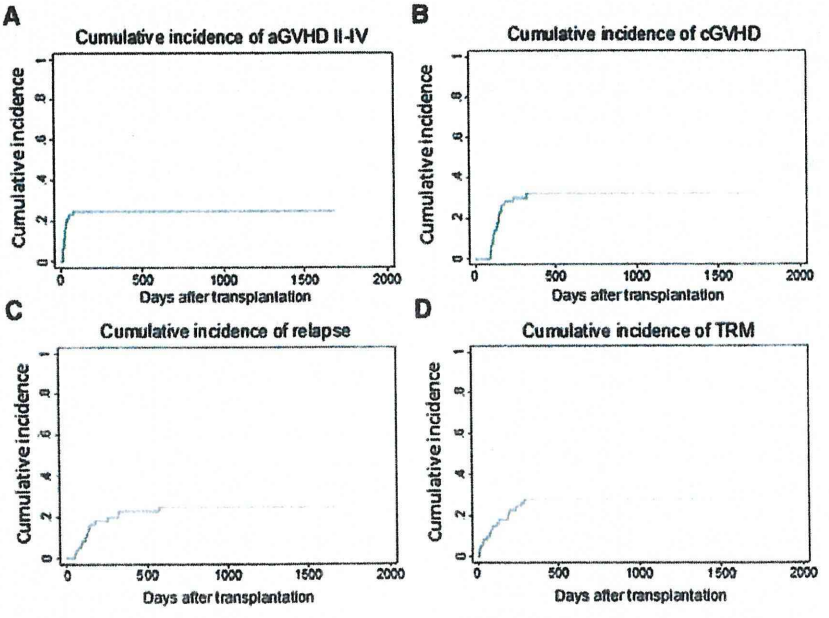

Figure 3. Cumulative incidence of grade II-IV aGVHD (A), cGVHD (B), relapse (C), and TRM (D).