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Correspondence: Yoshinobu Kanda, Division of Haematology, Saitama Medical Centre, Jichi Medical University, 1-847, Amanuma-cho, Omiya-ku, Saitama-shi, Saitama 330-8503, Japan.
E-mail: ycanda-tky@umin.ac.jp

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 $n = 1966$	A $n = 187$	B $n = 31$	C $n = 524$	DRB1 $n = 295$	*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.

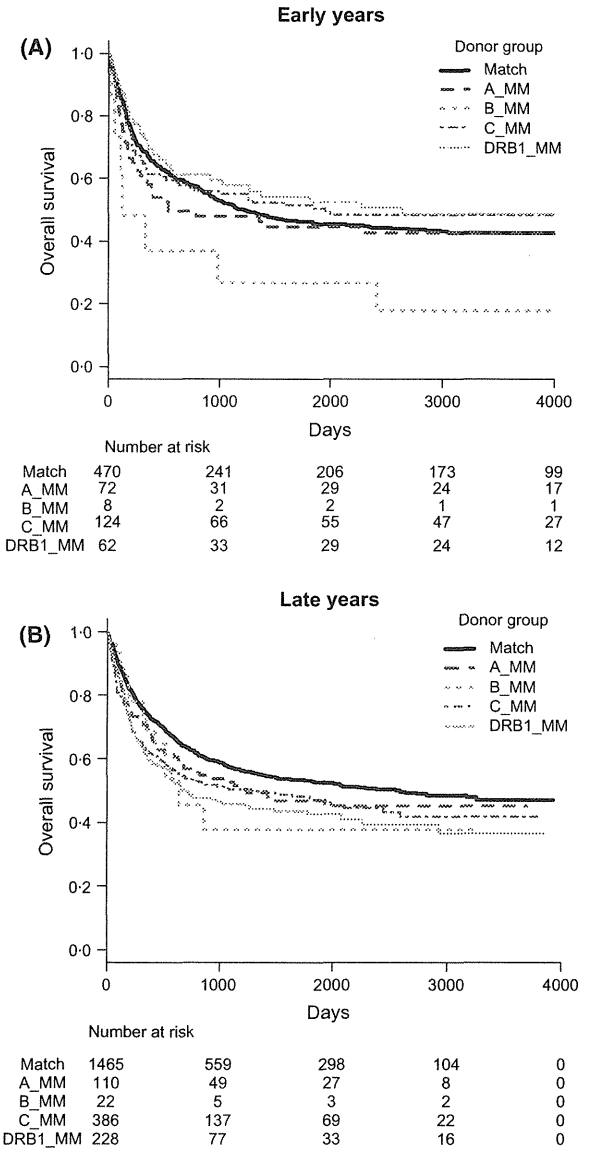


Fig 1. Overall survival grouped according to the human leucocyte antigen mismatch between the donor and recipient in the (A) early (1993–1999) and (B) late (2000–2009) time periods. The survival curves were adjusted for other significant factors by the mean of covariates method, in which average values 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, $P = 0·71$).

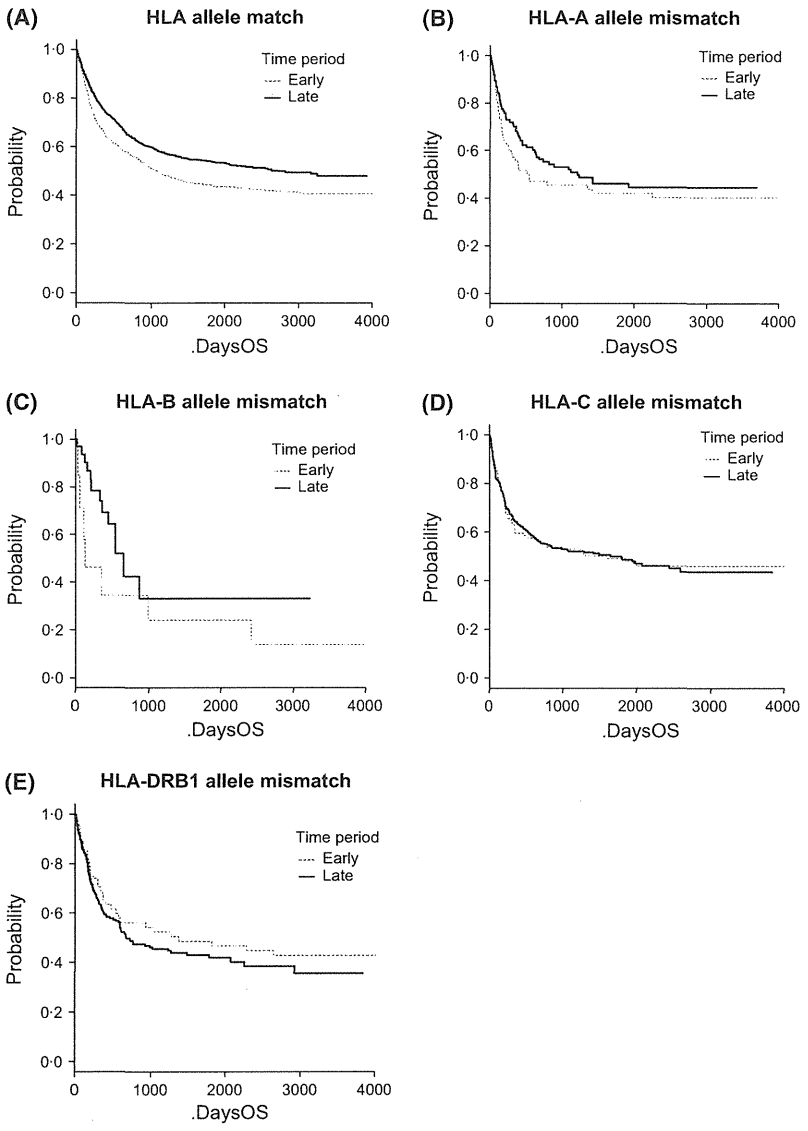


Fig 2. Overall survival grouped according to the transplantation time period in the human leucocyte antigen (HLA) match (A), HLA-A allele mismatch (B), HLA-B allele mismatch (C), HLA-C allele mismatch (D), and HLA-DRB1 allele mismatch (E) groups. The survival curves were adjusted for other significant 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

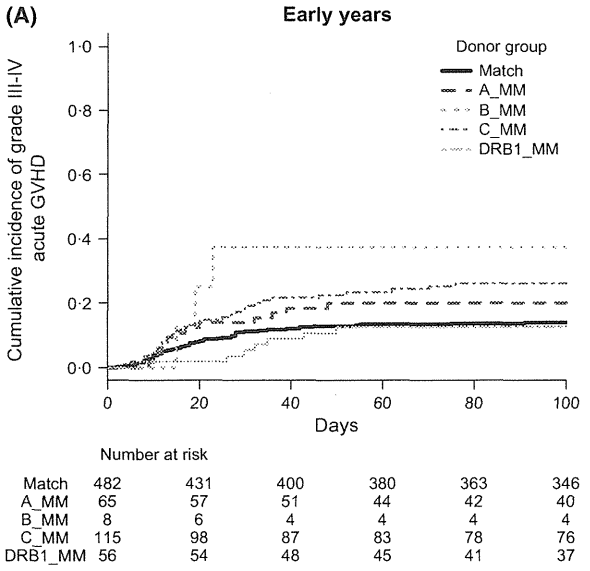
This study re-evaluated the effect of a single HLA allele mismatch on the outcome of unrelated BMT in the recent cohort. We chose 2000 as the cutoff of time period, as high-resolution typing for HLA-A and -B became a common practice in Japan after 2000. In contrast to our previous findings (Sasazuki *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



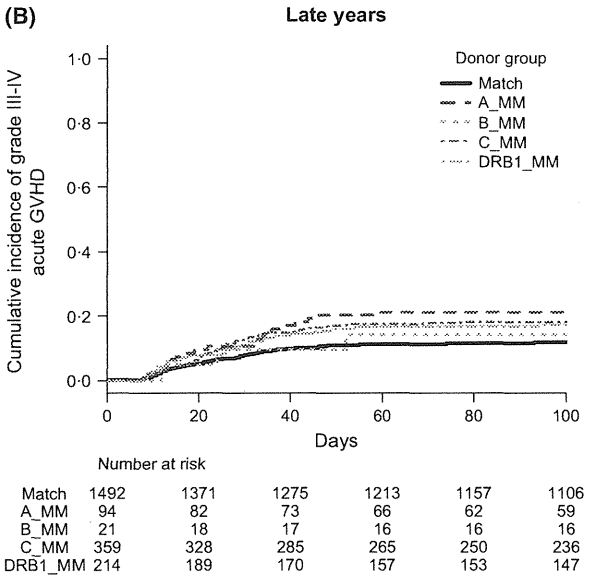
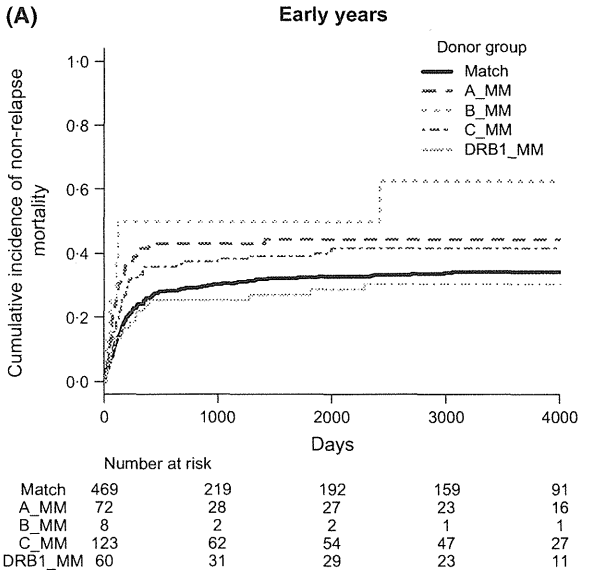


Fig 3. The cumulative incidence of grade III-IV acute graft-versus-host disease (GVHD) grouped according to the human leucocyte antigen mismatch between the donor and recipient in the (A) early (1993–1999) and (B) late (2000–2009) time periods.



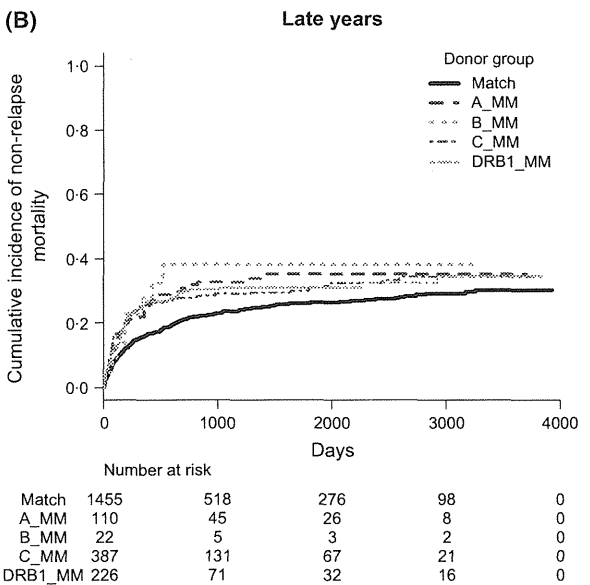


Fig 4. The cumulative incidence of non-relapse mortality grouped according to the human leucocyte antigen mismatch between the donor and recipient in the (A) early (1993–1999) and (B) late (2000–2009) time periods.

(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

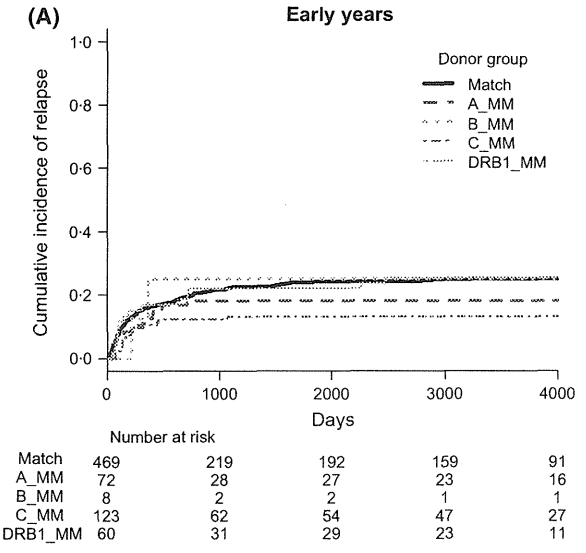


Fig 5. The cumulative incidence of relapse grouped according to the human leucocyte antigen mismatch between the donor and recipient in the (A) early (1993–1999) and (B) late (2000–2009) time periods.

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.

Acknowledgements

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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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ORIGINAL ARTICLE

The role of HLA-matched unrelated transplantation in adult patients with Ph chromosome-negative ALL in first remission. A decision analysis

S Kako[1], S Morita[2], H Sakamaki[3], H Iida[4], M Kurokawa[5], K Miyamura[6], H Kanamori[7], M Hara[8], N Kobayashi[9], Y Morishima[10], K Kawa[11], T Kyo[12], T Sakura[13], I Jinnai[14], J Takeuchi[15], Y Miyazaki[16], S Miyawaki[17], K Ohnishi[18], T Naoe[19] and Y Kanda[1]

The efficacy of unrelated transplantation for patients with ALL who lack an HLA-matched sibling remains unclear. We performed a decision analysis to determine the efficacy of myeloablative transplantation from a genetically HLA-A, -B, -DRB1 allele-matched unrelated donor for patients with Ph chromosome-negative ALL aged 21–54 years. The transition probabilities were estimated from the Japan Adult Leukemia Study Group studies (ALL93; $n=80$, ALL97; $n=82$), and the Japan Marrow Donor Program database (transplantation in first CR (CR1): $n=177$). The primary outcome measure was the 10-year survival probability with or without quality of life (QOL) adjustment. Subgroup analyses were performed according to risk stratification based on the WBC count and cytogenetics, and according to age stratification. In all patients, unrelated transplantation in CR1 was shown to be superior in analyses both with and without QOL adjustment (40.8 vs 28.4% and 43.9 vs 29.0%, respectively). A similar tendency was observed in all subgroups. The decision model was sensitive to the probability of leukemia-free survival following chemotherapy and the probability of survival after transplantation in standard-risk and higher-aged patients. Unrelated transplantation in CR1 improves the long-term survival probability in patients who lack an HLA-matched sibling. However, recent improvements in treatment strategies may change this result.

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Keywords: ALL; decision analysis; first remission; unrelated SCT

INTRODUCTION

The outcome of chemotherapy for Ph chromosome (Ph)-negative ALL in adult patients is inferior to that in children. Although about 90% of patients achieve CR, most of them eventually relapse, and leukemia-free survival is only 30–40%.[1] Therefore, allogeneic hematopoietic SCT (HSCT) in first CR (CR1) has been investigated to decrease the relapse rate. The efficacy of this approach has been evaluated through clinical studies using genetic randomization, in which patients with a HLA-matched sibling donor are allocated to the allogeneic HSCT arm, and those without a donor are placed in the chemotherapy or autologous HSCT arm.[2–10] These studies, as well as a meta-analysis of seven similar studies, confirmed that the donor group had a superior outcome compared with the no-donor group, and that autologous HSCT was not superior to chemotherapy in patients with adult ALL in CR1.[11] However, the efficacy of unrelated HSCT in patients with ALL in CR1, who lack an HLA-matched sibling, is still unclear. Although retrospective studies have reported a similar outcome for related and unrelated HSCT for ALL, a major problem was that the duration between the achievement of remission and HSCT was considered to be longer in unrelated HSCT due to the coordination process.[12,13] Therefore, patients who relapsed early after achieving remission might have been excluded in the unrelated HSCT group. On the other hand, it is practically difficult to perform a prospective clinical trial, in which patients with ALL in CR1, who lack an HLA-matched sibling but who have an HLA-matched unrelated donor, are randomly assigned to receive unrelated HSCT or chemotherapy alone.

A decision analysis is a statistical technique that aids the clinical decision making process under conditions of uncertainty. We previously demonstrated through a decision analysis that allogeneic HSCT is superior to chemotherapy alone in CR1 for adult patients with Ph-negative ALL who have an HLA-matched sibling, even after adjusting for quality of life (QOL).[14] In the

[1]Division of Hematology, Department of Internal Medicine, Saitama Medical Center, Jichi Medical University, Saitama, Japan; [2]Department of Biostatistics and Epidemiology, Yokohama City University, Kanagawa, Japan; [3]Hematology Division, Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital, Tokyo, Japan; [4]Department of Hematology, Meitetsu Hospital, Aichi, Japan; [5]Department of Hematology and Oncology, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; [6]Department of Hematology, Japanese Red Cross Nagoya First Hospital, Aichi, Japan; [7]Department of Hematology, Kanagawa Cancer Center, Kanagawa, Japan; [8]Division of Hematology, Ehime Prefectural Central Hospital, Ehime, Japan; [9]Department of Hematology, Sapporo Hokuyu Hospital, Hokkaido, Japan; [10]Department of Hematology and Cell Therapy, Aichi Cancer Center Hospital, Aichi, Japan; [11]Department of Pediatrics, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan; [12]Department of Internal Medicine, Hiroshima Red Cross Hospital and Atomic Bomb Survivors Hospital, Hiroshima, Japan; [13]Department of Hematology, Saiseikai Maebashi Hospital, Gunma, Japan; [14]Department of Hematology, Saitama Medical University, Saitama, Japan; [15]Department of Hematology and Rheumatology, Division of Medicine, Nihon University School of Medicine, Tokyo, Japan; [16]Department of Hematology and Molecular Medicine Unit, Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan; [17]Department of Hematology, Tokyo Metropolitan Ohtsuka Hospital, Tokyo, Japan; [18]Oncology Center, Hamamatsu University School of Medicine, Shizuoka, Japan and [19]Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Aichi, Japan. Correspondence: Dr Y Kanda, Division of Hematology, Department of Internal Medicine, Saitama Medical Center, Jichi Medical University, 1-847 Amanuma, Omiya-ku, Saitama 330-8503, Japan.
E-mail: ycanda-tky@umin.ac.jp
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present study, we performed a decision analysis to evaluate the efficacy of unrelated myeloablative HSCT for adult patients with Ph-negative ALL in CR1 who lack an HLA-matched sibling. We used a decision tree based on the results of prospective studies by the Japan Adult Leukemia Study Group (JALSG) (ALL93[3] and ALL97[15]), in which conventional-intensity regimens were used, the database of the Japan Marrow Donor Program (JMDP),[16] and the literature. Patients with Ph-positive ALL were not included in our analysis, because the outcome of treatment in these patients has improved dramatically as tyrosine kinase inhibitors became available.[17] In addition, patients aged less than 21 years were excluded from this analysis because the outcome of treatment in these patients has also improved greatly using intensified chemotherapy based on a pediatric regimen.[18]

MATERIALS AND METHODS

Model structure

We constructed a decision tree (Figure 1) to identify the optimal treatment strategy for adult patients with Ph-negative ALL in CR1, who lack an HLA-matched sibling, but who have an HLA-matched unrelated donor. At a decision node, we can decide to either proceed to unrelated HSCT or continue chemotherapy in CR1. Each decision is followed by chance nodes, which have possible outcomes with a transition probability (TP), and every branch finally ends with terminal nodes, which have utilities according to different health states. The sum of the products of the transition probabilities and utilities of all branches following each chance node become the expected value of each chance node, and the expected value of each decision is calculated as the sum of the expected values in all of the chance nodes following each decision. The following analyses were performed using TreeAge Pro 2009 software (Williamstown, MA, USA). This study was approved by the Institutional Review Boards of JMDP and Jichi Medical University.

Data sources

Outcomes after continuing chemotherapy in CR1 were estimated from JASLG studies (ALL93[3] and ALL 97[15]). Patients with Ph-negative ALL aged 21–54 years were included, and those who never achieved remission with chemotherapy were excluded. The data from 80 patients in ALL93 and 82 patients from ALL97 were analyzed separately and then combined by weighting the number of patients. Outcomes after unrelated HSCT in various disease statuses were estimated from the database of JMDP. Patients with Ph-negative ALL aged 21–54 years who underwent a first myeloablative allogeneic HSCT from a genetically HLA-A, -B, -DRB1 allele-matched unrelated donor between 1993 and 2008 were included. Of these, 177, 45 and 62 patients were in first remission, second remission and non-remission, respectively, at unrelated HSCT. All patients received BM graft.

The characteristics of the patients included in this study are summarized in Table 1. There was no significant difference in baseline characteristics among the JALSG studies and the JMDP data. To determine the following transition probabilities, OS and leukemia-free survival with a 95% confidence interval (CI) were calculated using the Kaplan–Meier method, whereas the cumulative incidences of non-relapse mortality and relapse with 95% CI were calculated using Gray's method,[19] where the other event was considered a competing risk. Probabilities that we could not estimate from these data were estimated from the literature.

Transition probabilities and utilities

Transition probabilities of the entire population were determined as summarized in Table 2. Each TP has a baseline value and a plausible range. Baseline decision analyses were performed based on the baseline value.

Patients may have been precluded from the undergoing unrelated HSCT due to early relapse or comorbidities even if they decided to undergo HSCT, and therefore the TP of actually undergoing unrelated HSCT in CR1 after the decision branch to undergo HSCT was determined as follows. First, the median duration between the achievement of CR1 and HSCT without relapse was calculated as 270 days based on the JMDP data. Next, leukemia-free survival rates at 270 days after achieving CR1 were calculated using the data for all patients who achieved remission in the JALSG studies, and the combined leukemia-free survival was 0.70 (95% CI: 0.64–0.77). We considered this to be the TP for actually receiving HSCT in CR1, and assigned a baseline value of 0.70 and 95% CI to the plausible range. The TP of undergoing unrelated HSCT in second remission (CR2) after the patient had a relapse following a decision to continue chemotherapy could not be calculated from our data. We assigned a plausible range of 0.5–0.70; the former value was the only available rate in a large study,[20] and the latter was the TP calculated above. The median of this range was taken as the baseline value. Probabilities regarding the actual rate of receiving HSCT in other disease statuses could not be obtained, even in the literature. Therefore, a baseline value of 0.5 was assigned with a wide plausible range of 0.3–0.7. The TP values for 'Alive at 10 years' following HSCT in various disease statuses were determined based on the JMDP data. We assigned 95% CI to the plausible ranges. Recently, results of HSCT in more specific disease statuses, such as HSCT following an early or late relapse after chemotherapy[21] and HSCT following

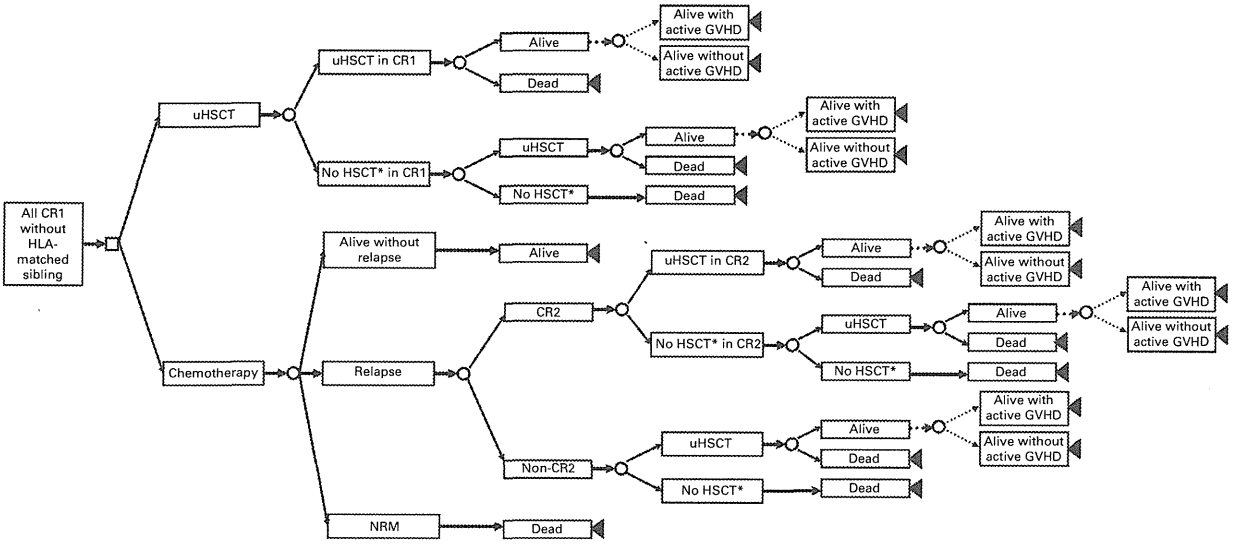

Figure 1. Decision tree used in this study. Decision analysis was performed based on this decision tree. A square indicates a decision node and open circles indicate chance nodes. In analyses with a QOL adjustment, 'Alive' after transplantation was followed by two branches with or without active chronic GVHD (dotted arrow). *Unrelated hematopoietic SCT (uHSCT) was not performed due to early relapse, death and so on. ALL, acute lymphoblastic leukemia; CR, complete remission; NRM = non-relapse mortality.

Table 1. Patient characteristics in the three data sources

	Chemotherapy in CR1		*HSCT in CR1*	P-*value*[a]
	JALSG ALL 93	*JALSG ALL97*	*JMDP*	
No. of patients	80	82	177	
Median age (range) (years)	38 (21–54)	37 (21–54)	35 (21–54)	0.58
No. of males/females	44/36	33/49	97/80	0.07
No. of each phenotype T/B/other	8/49/11	13/66/3	32/104/26	0.19
Median WBC count at diagnosis (range) ($\times 10^9$/L)	8.8 (0.7–301.1)	10.4 (1.3–398.0)	9.0 (0.6–480.0)	0.49
Karyotype standard: high[b], ratio	12:1	8:1	12: 1	0.66

Abbreviations: CR1 = first CR; HSCT = hematopoietic SCT; JALSG = Japan Adult Leukemia Study Group; JMDP = Japan Marrow Donor Program. [a]Statistical analyses were performed using the Kruskal-Wallis test for continuous variables and the Fisher's exact test for categoric variables. [b]t(4;11) and complex karyotype (five or more chromosomal abnormalities) were classified as high-risk karyotypes, and other karyotypes were classified as standard-risk.

Table 2. Transition probabilities of the overall population and all subgroups

	baseline value (plausible range)				
	All patients	*Standard-risk*	*High-risk*	*Lower age*	*Higher age*
HSCT in CR1	0.70 (0.64–0.77)	0.74 (0.65–0.83)	0.58 (0.44–0.72)	0.72 (0.63–0.81)	0.69 (0.60–0.78)
Alive at 10 years following HSCT in CR1	0.58 (0.49–0.66)	0.55 (0.41–0.66)	0.71 (0.53–0.83)	0.61 (0.49–0.72)	0.54 (0.39–0.67)
HSCT after failure of HSCT in CR1	0.5 (0.3–0.7)	0.5 (0.3–0.7)	0.5 (0.3–0.7)	0.5 (0.3–0.7)	0.5 (0.3–0.7)
Alive at 10 years following HSCT after failure of HSCT in CR1[a]	0.22 (0.15–0.29)	0.27 (0.25–0.28)	0.16 (0.08–0.23)	0.23 (0.19–0.26)	0.23 (0.11–0.35)
Alive at 10 years without relapse following CTx,	0.20 (0.13–0.28)	0.24 (0.11–0.37)	0.13 (0.01–0.16)	0.14 (0.03–0.25)	0.25 (0.16–0.35)
NRM at 10 years following CTx	0.09 (0.04–0.14)	0.08 (0.01–0.16)	0.12 (0.01–0.24)	0.06 (0–0.12)	0.11 (0.05–0.18)
Achievement of CR2 after relapse following CTx	0.4 (0.3–0.5)	0.4 (0.3–0.5)	0.4 (0.3–0.5)	0.4 (0.3–0.5)	0.4 (0.3–0.5)
HSCT in CR2	0.6 (0.5–0.7)	0.62 (0.5–0.74)	0.54(0.5–0.58)	0.61 (0.5–0.72)	0.60 (0.5–0.69)
Alive at 10 years following HSCT in CR2	0.29 (0.16–0.44)	0.28 (0.09–0.51)	0.23 (0.04–0.51)	0.26 (0.10–0.45)	0.35 (0.13–0.59)
HSCT after failure of HSCT in CR2	0.5 (0.3–0.7)	0.5 (0.3–0.7)	0.5 (0.3–0.7)	0.5 (0.3–0.7)	0.5 (0.3–0.7)
Alive at 10 years following HSCT after failure of HSCT in CR2[b]	0.15 (0.07–0.26)	0.25 (0.08–0.45)	0.08 (0.01–0.24)	0.19 (0.07–0.35)	0.11 (0.03–0.26)
HSCT in non-CR after relapse following CTx	0.5 (0.3–0.7)	0.5 (0.3–0.7)	0.5 (0.3–0.7)	0.5 (0.3–0.7)	0.5 (0.3–0.7)
Alive at 10 years following HSCT in non-CR after relapse	0.15 (0.07–0.26)	0.25 (0.08–0.45)	0.08 (0.01–0.24)	0.19 (0.07–0.35)	0.11 (0.03–0.26)
Rate of active GVHD at 10 years[c]	0.18 (0.1–0.25)	0.18 (0.1–0.25)	0.18 (0.1–0.25)	0.18 (0.1–0.25)	0.18 (0.1–0.25)

Abbreviations: HSCT = hematopoietic SCT; CTx = chemotherapy; NRM = non-relapse mortality. [a]This rate was estimated from the survival rate following HSCT in CR2 and HSCT in non-CR. [b]The same rate of survival following HSCT in non-CR was used. [c]The same baseline value and plausible range were used as the rate of active GVHD at 10 years following HSCT in various disease statuses, but one-way sensitivity analyses were performed separately for each status.

a relapse after first HSCT,[22] have been reported, but sufficient data for this decision analysis were not provided in these reports.

The transition probabilities for 'Alive without relapse at 10 years' and non-relapse mortality following chemotherapy in CR1 were determined based on the JALSG studies, and the TP of relapse following chemotherapy was determined by subtracting the sum of these TPs from one. The TP of achieving CR2 after relapse in patients who decided to continue chemotherapy in CR1 was estimated to have a baseline value of 0.4 with a plausible range of 0.3–0.5 based on the literature.[10,20,23]

Utilities were calculated based on a 10-year survival probability, which was the primary outcome measure, with or without adjusting for QOL. The survival curve nearly reaches a plateau after 5 years, and therefore 'Alive at 10 years' reflects 'Cure of leukemia', which is the primary goal of HSCT. In an analysis without an adjustment for QOL, we considered only two kinds of health states, 'Alive at 10 years' and 'Dead', and assigned utility values of 100 to the former and 0 to the latter. On the other hand, in an analysis with an adjustment for QOL, 'Alive after chemotherapy without relapse at 10 years', 'Alive with active GVHD at 10 years' and 'Alive without active GVHD at 10 years' were considered as different health states. The proportion of patients with active GVHD among those who were alive at 10 years was determined based on the literature.[24–26] We assigned a value of 100 to the utility for being alive without relapse at 10 years after chemotherapy alone, and a value of 0 to the utility for being dead in all situations. We assigned a fixed value of 98 to the utility for being alive without active GVHD at 10 years following HSCT because a part of patients had suffered from complications other than active GVHD, such as cataract.[27] Moreover, we assigned a value of 70 with a wide plausible range of 0–98 to the utility for being alive with active GVHD at 10 years. These utilities were determined based on the opinions of 10 doctors who were familiar with HSCT and the literature.[28,29]

Subgroup analyses were also performed according to risk stratification based on the WBC count and cytogenetics, and according to age stratification with a cutoff of 35 years. This cutoff value is based on the age used in the Medical Research Council/Eastern Cooperative Oncology Group trial for risk stratification.[9] Patients with a high WBC count (more than 30×10^9/L for B lineage and more than 100×10^9/L for T lineage) and/or with t(4;11) or complex karyotype (5 or more chromosomal abnormalities) were classified as high-risk, and all other patients were classified as standard-risk. It was difficult to perform other subgroup analyses regarding the possible prognostic factors like phenotypes, due to the limited number of patients involved. All transition probabilities, based on the JALSG studies and the JMDP data, were recalculated using the data for patients in each subgroup (Table 2).

Sensitivity analyses

To evaluate the robustness of the decision model, we performed one-way sensitivity analyses for all transition probabilities, in which the decision tree was recalculated by varying each TP value in its plausible range, and confirmed whether or not the decision of the baseline analyses changed. In analyses with an adjustment for QOL, the utility for being alive with

active GVHD at 10 years was also subjected to a one-way sensitivity analysis.

We also performed a probabilistic sensitivity analysis using a Monte Carlo simulation[30], in which the uncertainties of all transition probabilities were considered simultaneously. The distribution of the random variables for each TP was determined to follow a normal distribution, with 95% of the random variables included in the plausible range. One thousand simulations were performed based on the decision tree, and the mean and s.d. of the expected value for each decision were calculated.

RESULTS

Baseline analysis

The baseline analysis in the overall population without adjusting for QOL revealed an expected 10-year survival of 43.9% for the decision to perform unrelated HSCT in CR1, which was better than the value (29.0%) for the decision to continue chemotherapy. The decision to perform unrelated HSCT was superior even after adjusting for QOL (40.8% for HSCT vs 28.4% for chemotherapy, Table 3).

Sensitivity analysis

First, we performed one-way sensitivity analyses for all transition probabilities in the decision model without adjusting for QOL. A better expected survival for the decision to perform HSCT was consistently demonstrated in all transition probabilities within the plausible ranges. In the probabilistic sensitivity analysis, the mean value and s.d. of the expected survival probability for HSCT were 44.0 and 3.5% (Figure 2a), and those for chemotherapy were 29.1 and 3.9% (Figure 2b), respectively.

Next, we performed one-way sensitivity analyses for all transition probabilities and for the utility for being alive with active GVHD at 10 years in the decision model adjusted for QOL. Even in these analyses, the results of the baseline analysis were not reversed for any of the transition probabilities. In addition, a higher expected survival probability for HSCT was retained in a sensitivity analysis, in which the utility for being alive with active GVHD was changed between 0 and 98 (Figure 3a). In the probabilistic sensitivity analysis, the mean value and s.d. of the expected survival probability for HSCT were 40.9 and 3.4% (Figure 2c), and those for chemotherapy were 28.4 and 3.9% (Figure 2d), respectively.

Table 3. Expected 10-year survival probabilities with and without adjusting for quality of life (QOL)

	Expected survival probability without a QOL adjustment		*Expected survival probability with a QOL adjustment*	
	HSCT	*Chemotherapy*	*HSCT*	*Chemotherapy*
All patients	43.9%	29.0%	40.8%	28.4%
Standard-risk patients	44.2%	35.1%	41.1%	34.3%
High-risk patients	44.5%	19.1%	41.4%	18.7%
Lower-aged patients	47.1%	24.8%	43.8%	24.1%
Higher-aged patients	40.8%	33.1%	38.0%	32.5%

Abbreviation: HSCT = hematopoietic SCT.

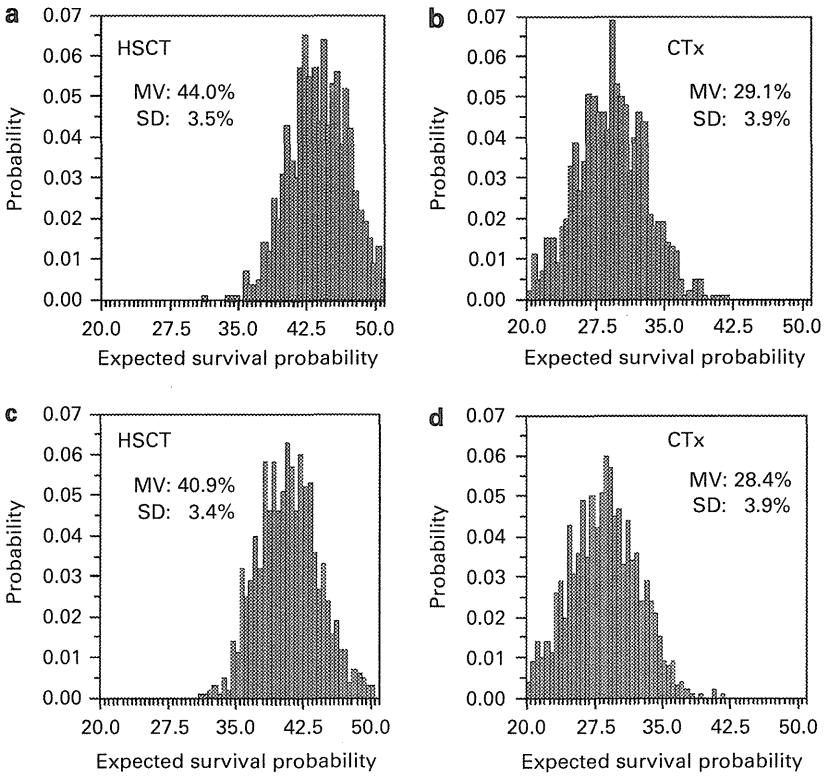

Figure 2. Probabilistic sensitivity analysis (PSA) using a Monte Carlo simulation. We performed a PSA using a Monte Carlo simulation. In the analysis without a QOL adjustment, the mean value (MV) and s.d. of the expected survival probability for unrelated HSCT were 44.0 and 3.5% (**a**), and those for chemotherapy (CTx) were 29.1 and 3.9% (**b**), respectively. In the analysis with a QOL adjustment, the MV and s.d. of the expected survival probability for HSCT were 40.9 and 3.4% (**c**), and those for CTx were 28.4 and 3.9% (**d**), respectively.

Subgroup analyses

In subgroup analyses both with and without adjusting for QOL, a better expected survival probability for HSCT was consistently observed in all of the subgroups (Table 3).

We also performed one-way sensitivity analyses in all of the subgroups. In high-risk and lower-aged patients, the results of baseline analyses were not affected when each TP value was varied within its plausible range in the decision models both with and without adjusting for QOL. In standard-risk patients, the results reversed in favor of chemotherapy if the probability of leukemia-free survival at 10 years without relapse following chemotherapy was higher than 0.35 (Figure 3b) or the probability of OS at 10 years following HSCT in CR1 was lower than 0.42 (Figure 3c) in the decision model without adjusting for QOL. In the decision model with adjusting for QOL, the results reversed in favor of chemotherapy if the probability of leukemia-free survival at 10 years without relapse following chemotherapy was higher than 0.32 (Figure 3f) or the probability of OS at 10 years following HSCT in CR1 was lower than 0.45 (Figure 3g). In older patients, the decision models both with and without adjusting for QOL were also sensitive to both the probability of leukemia-free survival at 10 years without relapse following chemotherapy and the probability of OS at 10 years following HSCT in CR1 (Figures 3d, e, h and i). We also performed one-way sensitivity analyses for a utility for being alive with active GVHD within the range of 0–98. A higher expected survival probability for HSCT was retained in all of the subgroups.

DISCUSSION

About two-thirds of patients with adult ALL lack an HLA-matched sibling, and for these patients, allogeneic HSCT from an HLA-matched unrelated donor might be an alternative treatment. Several studies have suggested that unrelated HSCT may be effective for high-risk adult ALL patients in various disease statuses.[31,32] In addition, two retrospective studies showed no difference between related and unrelated HSCT for adult ALL patients, including those in CR1,[12,13] and the recent evidence-based review from the American Society for Blood and Marrow Transplantation supported this.[33,34] However, patients who undergo unrelated HSCT in CR1 are a select population of patients who have maintained their remission status during the donor-coordination process. We performed a decision analysis to identify the optimal strategy for patients with ALL in CR1, who lack an HLA-matched sibling but who have an HLA-matched unrelated donor. We tried to exclude selection bias in patients who underwent unrelated transplantation by considering patients who did not undergo unrelated HSCT in CR1 due to early relapse or comorbidities even if they decided to undergo unrelated HSCT.

We used data from JALSG prospective studies to estimate outcomes after continuing chemotherapy. On the other hand, we used the database of JMDP to estimate outcomes after unrelated HSCT, due to the limited number of patients who underwent unrelated HSCT in the JALSG prospective studies. The outcomes after unrelated HSCT in CR1 were not significantly different among the JALSG prospective studies and the JMDP database. (OS at 10 years in patients who underwent unrelated HSCT in CR1 was 54.2, 50 and 58.2% in JALSG ALL93 study, JALSG ALL97 study, and the JMDP database, respectively ($P = 0.56$ in log-rank test)).

In our baseline analysis both with and without adjusting for QOL, unrelated HSCT in CR1 was shown to give a superior outcome in both the overall population and in all of the subgroups. In the overall population, probabilistic sensitivity analysis using a Monte Carlo simulation also supported this result (Figure 2). However, in a one-way sensitivity analysis, the decision model was sensitive to the probability of leukemia-free survival following chemotherapy in CR1 in both standard-risk and older patients (Figures 3b, d, f and h). The adaptation of high-intensified chemotherapy, especially the adaptation of chemotherapy according to pediatric regimens up to young adult, has led to improved outcomes in recent trials,[1,9,10] but the JALSG studies in this analysis included less-intensified regimens. Therefore, this improvement in chemotherapy might change our result. In a one-way sensitivity analysis, the decision model was also sensitive to the probability of OS at 10 years following HSCT in CR1 in both standard-risk and older patients (Figures 3c, e, g and i). This study only included data on unrelated HSCT from a genetically HLA-A, -B, -DRB1 allele-matched donor. It has been

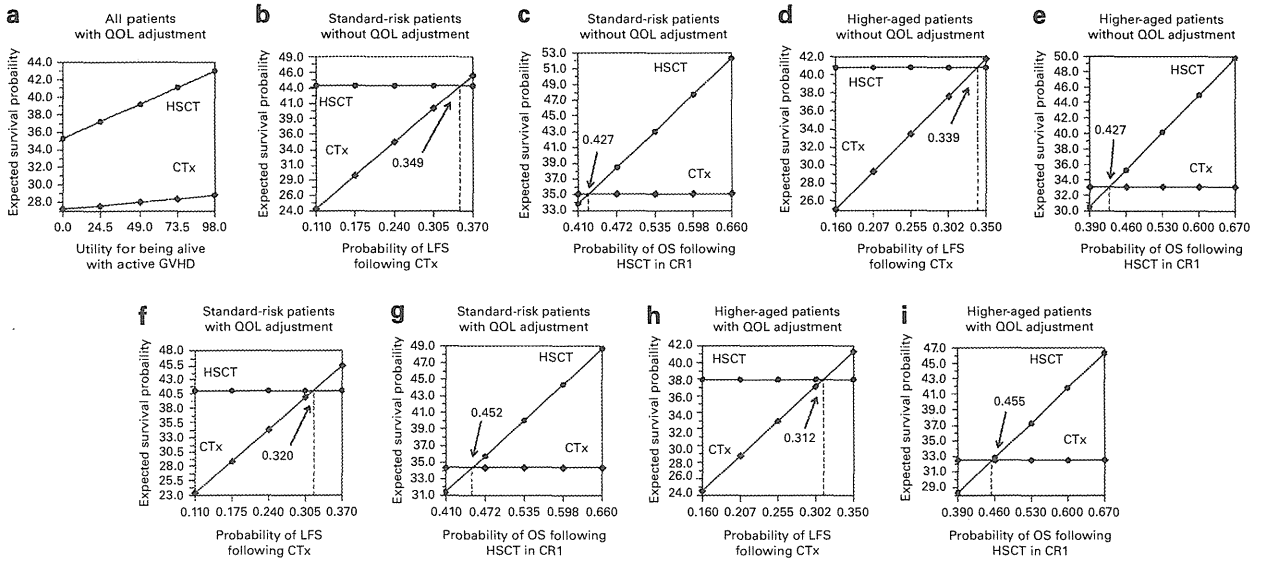

Figure 3. One-way sensitivity analysis. We performed a one-way sensitivity analysis. The superiority of unrelated HSCT compared with CTx was consistently observed with a wide plausible range of the utility for being alive with active GVHD in the overall population (**a**). On the other hand, the models both without adjusting for QOL were sensitive to the probability of leukemia-free survival at 10 years following CTx and the probability of OS at 10 years following HSCT in CR1 in standard-risk (**b**, **c**, **f**, **g**) and older patients (**d**, **e**, **h**, **i**).

reported that the presence of an HLA allele mismatch, especially in some specific combinations, significantly affects the outcome of serologically HLA-matched unrelated HSCT.[35] Therefore, the indications for HSCT from an unrelated donor with an HLA allele mismatch should be considered with great caution, especially in standard-risk and older patients.

Recently, minimal residual disease assays are increasingly involved in the evaluation of treatment response for ALL,[36] and the prevalence of minimal residual disease after the induction therapy or early consolidation therapy has been demonstrated as an important prognostic factor. In the current study, we considered only hematological response, and minimal residual disease status was not included in risk stratification. Minimal residual disease status should be taken into account in the future analysis.

In this study, the median duration from achieving CR1 to unrelated HSCT without relapse was 270 days, which precluded HSCT in CR1 in 30% of patients after a decision to perform HSCT (mainly due to early relapse). This duration was 4 months longer than the duration from achieving CR1 to related HSCT without relapse in our previous study, as the coordination process for an unrelated donor through JMDP requires a longer duration. A meta-regression analysis by Yanada *et al.*[11] showed that the proportion of patients who actually underwent allogeneic HSCT among patients with a donor was positively correlated with survival. The coordination process for a JMDP donor is currently getting shorter, and, as a consequence, the efficacy of unrelated HSCT in CR1 may increase.

The low incidence of severe GVHD has been demonstrated in Japanese patients,[37,38] and this might have influenced the superior outcome of unrelated HSCT in CR1 in our analysis. Therefore, caution should be paid when the current results are applied to patients of other origins.

In conclusion, to improve the probability of long-term survival, myeloablative HSCT from a genetically HLA-A, -B, -DRB1 allele-matched unrelated donor in CR1 is recommended for patients, aged 21–54 years, who lack an HLA-matched sibling donor. Even when we considered QOL, the superiority of unrelated HSCT was confirmed in the overall population and in all of the subgroups. However, recent improvements in treatment strategies, like high-intensified chemotherapy, may change this result.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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In vivo T-cell depletion with alemtuzumab in allogeneic hematopoietic stem cell transplantation: Combined results of two studies on aplastic anemia and HLA-mismatched haploidentical transplantation

Yoshinobu Kanda,[1]* Kumi Oshima,[1] Shinichi Kako,[1] Takahiro Fukuda,[2] Naoyuki Uchida,[3] Koichi Miyamura,[4] Yukio Kondo,[5] Shinji Nakao,[5] Koji Nagafuji,[6] Toshihiro Miyamoto,[7] Mineo Kurokawa,[8] Yasushi Okoshi,[9] Shigeru Chiba,[9] Yasuo Ohashi,[10] Yoichi Takaue,[11] and Shuichi Taniguchi[3]

We evaluated the efficacy of *in vivo* T-cell depletion with alemtuzumab in two prospective studies according to the International Conference on Harmonisation (ICH)—Good Clinical Practice (ICH–GCP) guidelines; one was for patients with aplastic anemia (AA study) and the other was for patients who were undergoing hematopoietic stem cell transplantation (HSCT) from a 2- or 3-antigen-mismatched haploidentical donor (MM study). The final dose of alemtuzumab in these studies was 0.16 mg/kg/day for 6 days. At this dose, all of the 12 and 11 patients in the AA and MM studies, respectively, achieved initial engraftment and the incidences of Grade II–IV acute graft-versus-host disease (GVHD) were 0% and 18%. While cytomegalovirus (CMV) frequently reactivated, none of the patients developed fatal CMV disease. Transplantation-related mortality within 1 year after HSCT was observed in only two and one patients, respectively. The numbers of CD4+ and CD8+ T-cells and T-cell receptor rearrangement excision circles remained low within 1 year after HSCT. These findings suggest that the use of alemtuzumab at this dose in a conditioning regimen enables safe allogeneic HSCT even from a 2- or 3-antigen-mismatched donor. However, the use of a lower dose of alemtuzumab should be explored in future studies to accelerate immune recovery after HSCT. Am. J. Hematol. 88:294–300, 2013. © 2013 Wiley Periodicals, Inc.

Introduction

Graft-versus-host disease (GVHD) is an important complication after allogeneic hematopoietic stem cell transplantation (HSCT), especially in the presence of an HLA-mismatch between the donor and recipient. Alemtuzumab is a humanized monoclonal antibody against CD52, and the use of alemtuzumab in the conditioning regimen before HSCT has been shown to decrease the incidences of acute and chronic GVHD through the *in vivo* depletion of donor T cells [1]. A growing body of evidence supports the efficacy of alemtuzumab at preventing acute GVHD in a variety of HSCT settings [2–9]. In addition, we and others have reported that alemtuzumab enables HLA-mismatched haploidentical HSCT without the *ex vivo* depletion of donor T cells [10,11]. We administered alemtuzumab at 0.2 mg/kg/day for 6 days before allogeneic HSCT from a 2- or 3-anitigen-mismatched related donor in 12 patients [10]. All patients achieved neutrophil engraftment and the cumulative incidence of Grade III to IV acute GVHD was only 9%. Rizzieri et al. used alemtuzumab at a total dose of 100 mg spread over 5 days in reduced-intensity haploidentical HSCT without *ex vivo* T-cell depletion [11]. The incidences of Grade III–IV acute GVHD and transplant-related mortality were 8% and 10.2%, respectively. Therefore, *in vivo* T-cell depletion using alemtuzumab enables haploidentical HSCT without excessive GVHD.

With regard to allogeneic HSCT for aplastic anemia (AA), alemtuzumab has been incorporated into the conditioning regimen to achieve sustained engraftment with minimal toxicity and GVHD [12]. The incidence of graft failure was 9.5% for HSCT from an HLA-matched sibling donor and 14.5% for HSCT from an unrelated donor. Acute GVHD was observed in only 13.5% of the patients and there was no Grade III–IV acute GVHD.

However, the use of alemtuzumab as a conditioning regimen for allogeneic HSCT has not been approved in Japan or in any other countries. Therefore, we performed two pivotal trials according to the International Conference on Harmonisation (ICH)—WHO Good Clinical Practice (GCP) guidelines to obtain approval from the Pharmaceuticals and Medical Devices Agency (PMDA); one was for HSCT for AA study and the other was for HSCT from an HLA-mismatched donor (MM study). These studies were approved by the Institutional Review Board of each participating center and were registered in the UMIN Clinical Trial Registry (C000000356 and C000000357).

Patients and Methods

Patients

The common inclusion criteria of the two studies were age between 20 and 65, ECOG performance status <2, the absence of severe organ dysfunctions (for example, $SaO_2 < 94\%$ or ejection fraction

[1]Division of Hematology, Saitama Medical Center, Jichi Medical University, Saitama, Japan; [2]Stem Cell Transplantation Division, National Cancer Center Hospital, Tokyo, Japan; [3]Department of Hematology, Toranomon Hospital, Minato-Ku, Tokyo, Japan; [4]Department of Hematology, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan; [5]Cellular Transplantation Biology, Kanazawa University Graduate School of Medical Science, Japan; [6]Division of Hematology and Oncology, Department of Medicine, Kurume University School of Medicine, Japan; [7]Department of Medicine and Biosystemic Science, Kyushu University Graduate School of Medical Sciences, Fukuoka, Japan; [8]Department of Hematology and Oncology, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; [9]Department of Hematology, University of Tsukuba, Tsukuba, Japan; [10]Department of Biostatistics, School of Public Health, University of Tokyo, Tokyo, Japan; [11]Institute for Research, St. Luke's International Hospital, Tokyo, Japan

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*Correspondence to: Yoshinobu Kanda, MD, PhD, Division of Hematology, Saitama Medical Center, Jichi Medical University, 1-847 Amanuma-cho, Omiya-ku, Saitama-shi, Saitama 330-8503, Japan. E-mail: ycanda-tky@umin.ac.jp

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<55%) and the absence of active infection. All patients provided their written informed consent before being enrolled in these studies.

The AA study included patients with very severe, severe, or transfusion-dependent moderate AA. The donor was either an HLA-matched related donor, one-antigen-mismatched related donor, matched unrelated donor, or HLA-DRB1 one-allele-mismatched unrelated donor. Patients who underwent HSCT from a donor other than HLA-matched related donor must have received immunosuppressive treatment and were refractory to or relapsed after such treatment.

The MM study included patients with relapsed or refractory acute leukemia, high-risk acute leukemia such as Philadelphia chromosome-positive acute lymphoblastic leukemia in first complete remission, chronic myelogenous leukemia after blastic transformation, myelodysplastic syndrome with severe cytopenia or a bone marrow blast ratio of at least 20%, and lymphoma that was refractory to chemotherapy or that relapsed after autologous HSCT. Patients who had an available HLA-matched related donor, one-antigen-mismatched related donor, matched unrelated donor, or HLA-DRB1 one-allele-mismatched unrelated donor were excluded.

Conditioning regimens

The conditioning regimen in the AA study (FLU-CY) consisted of fludarabine at 30 mg/kg/day for 4 days (days -6 to -3) and cyclophosphamide at 25 mg/kg/day for 4 days (days -6 to -3). Total body irradiation (TBI) at 2 Gy was added on day-1 in HSCT from a donor other than an HLA-matched related donor. Alemtuzumab was added to this regimen for 6 days (days -10 to -5).

The conditioning regimen in the MM study included myeloablative and reduced-intensity regimens. Patients who were aged at least 55 years or those who had previously undergone autologous HSCT received a reduced-intensity regimen (FLU-BU) that consisted of fludarabine at 30 mg/kg/day for 6 days (days -8 to -3), oral busulfan at 4 mg/kg/day for 2 days (days -5 to -4), and TBI at 4 Gy on day -1. The other patients received a myeloablative conditioning regimen (CY-TBI) that consisted of cyclophosphamide at 60 mg/kg/day for 2 days (days -3 to -2) and TBI at 4 Gy/day for 3 days (days -7 to -5). Alemtuzumab was added to this regimen for 6 days (days -8 to -3).

Dose of alemtuzumab

These studies consisted of two stages. In the first stage, the dose of alemtuzumab was started at 0.2 mg/kg/day, which was used in our previous study [10], and then we planned to change the dose to between 0.16 mg/kg/day and 0.25 mg/kg/day according to the conventional 3 + 3 cohort design. The starting dose of alemtuzumab in the second stage was defined by the results in the first-stage patients, and thereafter, the dose was changed according to the continual reassessment method [13].

To prevent acute infusion-related reactions to alemtuzumab, patients were pretreated with 1 mg/kg of methyl-prednisolone. Alemtuzumab was infused over 4 hr. On the first day of alemtuzumab infusion, 3 mg of alemtuzumab was infused over 2 hr and, after confirming that no severe infusion-related toxicities were observed, we infused the remaining alemtuzumab over the next 2 hr.

Other transplantation procedures

With regard to the stem cell source, bone marrow was exclusively used in the AA study, whereas peripheral blood was used in the MM study. Prophylaxis against GVHD was performed with cyclosporine (CSA) and short-term methotrexate. CSA was started on day -1 at a dose of 3 mg/kg/day by continuous infusion and the dose was adjusted to maintain a blood concentration between 250 and 350 ng/mL. CSA was changed to an oral form when it could be tolerated by the patient. Methotrexate was administered at 10 mg/m^2 on Day 1 and 7 mg/m^2 on days 3 and 6 in the AA study, whereas it was administered at 15 mg/m^2 on Day 1 and 10 mg/m^2 on days 3, 6, and 11 in the MM study. For patients without GVHD, we started to taper CSA from Day 100 by 5% per week in the AA study, whereas we started to taper CSA from Day 30 by 10% per week and discontinued CSA on Day 100 in the MM study.

Prophylaxis against bacterial, fungal and *Pneumocystis jiroveci* infection consisted of fluoroquinolones, azoles, and sulfamethoxazole/trimethoprim. As prophylaxis against herpes simplex virus infection and varicella zoster infection, acyclovir was given from days -7 to 35, followed by long-term low-dose administration [14]. Preemptive therapy with ganciclovir against cytomegalovirus (CMV) was performed by weekly monitoring of an antigenemia assay [15]. EB virus (EBV) reactivation was monitored by polymerase chain reaction (PCR) assay on days 90 and 180 and at optional occasions at the discretion of attending physicians. Patients who developed Grade II–IV acute GVHD were treated with 1 mg/kg of methyl-prednisolone.

Chimerism and immune recovery

Host/donor cell chimerism after transplantation was analyzed by sex-chromosome FISH or the short tandem repeat method using peripheral blood CD3+ cells [16]. Immune reconstitution was evaluated by the quantification of CD3+/CD4+, CD3+/CD8+, CD3-/CD19+, and CD3-/CD56+ cells by flowcytometry. For patients who had HLA-A*0201 or HLA-A*2402, the number of cytotoxic T-cells that were specific for CMV was measured using tetramers loaded with CMV pp65 antigen peptides [17]. T-cell receptor rearrangement excision circles (TRECs) were quantified in purified CD4+ and CD8+ T-cells by real-time quantitative PCR (RQ-PCR) with the 5'-nuclease (TaqMan) assay and an ABI7900 system (Life Technologies, Foster City, CA) as described elsewhere [18]. The mean copy numbers of TRECs in 10^5 CD4+ and CD8+ T-cells from 10 healthy adults were 1440 ± 880 and 1920 ± 1300, respectively [19]. The overall complexity of the T-cell receptor repertoire within a Vβ subfamily was determined by counting the number of discrete peaks and determining their relative sizes on the electropherogram by a complexity scoring system as described elsewhere [19–21]. The mean complexity scores of CD4+ and CD8+ T-cells from 10 healthy adults were 116.1 ± 2.36 and 109.49 ± 6.77, respectively [19].

Pharmacokinetics of alemtuzumab and detection of anti-alemtuzumab antibody

Serum samples were collected before each dose and at various time points afterward and stored frozen at −80°C until analysis. Serum alemtuzumab concentrations were measured using flowcytometry as described elsewhere [22]. The presence of anti-alemtuzumab antibodies was analyzed using YID13.9, a monoclonal anti-idiotype-specific antibody for alemtuzumab, as a standard [22].

Statistical considerations

Treatment was considered successful if all three of the following criteria were met: achievement of neutrophil engraftment, patients alive at Day 60 after HSCT, and the absence of Grade II–IV and Grade III–IV acute GVHD in the AA study and the MM study, respectively. Overall survival was calculated using the Kaplan-Meier method. The serial changes in the alemtuzumab concentrations or the number of lymphocytes were analyzed using a repeated-measures analysis of variance after logarithmic transformation by discarding the patients with missing values. All *P* values were two-sided and *P* values less than 0.05 were considered to indicate statistical significance.

Results

Patients

In the AA study, six patients were enrolled in the first-stage study. The first three patients who received alemtuzumab at 0.20 mg/kg/day for 6 days (CAM20) achieved treatment success and the dose of alemtuzumab was decreased to 0.16 mg/kg/day for 6 days (CAM16). Thereafter, the next three patients in the first stage and all 12 patients in the second stage received alemtuzumab at this decreased dose. Similarly, in the MM study, the first three patients in the first stage received CAM20, whereas the next three patients in the first stage and all 11 patients in the second stage received CAM16. In both studies, no dose modification was required in the second stage according to the continual reassessment method protocol.

In the following analyses, only the 23 patients who received CAM16 were evaluated as predefined in the protocol, unless otherwise specified. The donor was an HLA-matched related donor, a matched unrelated donor, and a one-allele-mismatched unrelated donor in three, four, and five patients, respectively, in the AA study. In the MM study, there was a two- and three-antigen mismatch in the graft-versus-host direction in seven and four patients, respectively.

Engraftment, chimerism, and GVHD

All patients, including 12 in the AA study and 11 in the MM study, achieved engraftment (Table I). However, one patient in the AA study developed secondary graft failure and died on Day 69 after HSCT.