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H. 知的財産権の出願・登録状況

1. 特許取得

＜研究分担者　田中　淳司＞

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3. その他

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Ⅱ．研究成果の刊行に関する一覧

刊行に関する一覧 （in press 含む）

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Ⅲ. 研究成果の刊行物・別刷

Biol Blood Marrow Transplant 20 (2014) 526–535

Changes in the Clinical Impact of High-Risk Human Leukocyte Antigen Allele Mismatch Combinations on the Outcome of Unrelated Bone Marrow Transplantation

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A B S T R A C T

Several high-risk HLA allele mismatch combinations (HR-MMs) for severe acute graft-versus-host disease (GVHD) have been identified by analyzing transplantation outcomes in Japanese unrelated hematopoietic stem cell transplant recipients. In this study, we analyzed the effects of HR-MMs in 3 transplantation time periods. We confirmed that the incidence of grade III to IV acute GVHD in the HR-MM group was significantly higher than that in the low-risk (LR) MM group (hazard ratio [HR], 2.74; $P < .0001$) in the early time period (1993 to 2001). However, the difference in the incidence of grade III to IV acute GVHD between the HR-MM and LR-MM groups was not statistically significant (HR, 1.06; $P = .85$ and HR, .40; $P = .21$, respectively) in the mid (2002 to 2007) and late (2008 to 2011) time periods. Similarly, survival in the HR-MM group was significantly inferior to that in the LR-MM group (HR, 1.46; $P = .019$) in the early time period, whereas the difference in survival between the 2 groups was not statistically significant in the mid and late time periods (HR, 1.06; $P = .75$ and HR, .82; $P = .58$, respectively). In conclusion, the adverse impact of HR-MM has become less significant over time. Unrelated transplantation with a single HR-MM could be a viable option in the absence of a matched unrelated donor or an unrelated donor with a single LR-MM.

INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) from an unrelated donor has been established as an effective treatment option for patients with hematological diseases who lack a human leukocyte antigen (HLA)–matched related donor. However, an HLA mismatch at the genetic level (allele mismatch) may be observed even in HSCT from a serologically HLA-matched donor (antigen match), and the presence of an allele mismatch adversely affects the incidence of severe acute graft-versus-host disease (GVHD) and survival [1-4]. We recently showed that the presence of single HLA allele mismatches at the HLA-A, -B, -C, or -DRB1 loci equivalently affect the outcome of HSCT, although a previous study from Japan reported that an HLA-A or -B allele mismatch impairs overall survival more strongly than an HLA-C or -DRB1 allele mismatch [4,5]. These findings suggest that the

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Table 1
Patient Characteristics

Characteristic	Match n = 2504			Low-Risk Mismatch n = 1057			High-Risk Mismatch n = 157		
	Early	Mid	Late	Early	Mid	Late	Early	Mid	Late
	802	814	888	412	351	294	64	71	22
Age (recipient)									
Median	32	38	43	31	38	43	33	39	41
Age (donor)									
Median	34	34	36	33	34	37	35	36	37
Sex (recipient)									
Female	292	305	378	162	165	123	27	27	9
Male	510	509	510	250	186	171	37	44	13
Sex (donor)									
Female	286	262	266	164	158	107	20	28	5
Male	512	548	622	247	190	187	43	43	17
N.A.	4	4	0	1	3	0	1	0	0
Sex mismatch									
Match	507	537	512	238	209	166	35	40	14
Male to female	148	158	244	85	72	72	17	15	6
Female to male	143	115	132	88	67	56	11	16	2
N.A.	4	4	0	1	3	0	1	0	0
ABO blood type									
Match	454	462	500	167	151	121	33	31	9
Minor mismatch	154	162	175	112	84	81	15	18	3
Major mismatch	125	114	142	82	67	61	9	18	4
Bidirectional mismatch	58	70	71	45	46	31	7	4	6
N.A.	11	6	0	6	3	0	0	0	0
Disease									
AML	269	415	495	134	168	170	15	29	12
ALL	229	229	249	116	96	76	11	23	8
CML	237	84	29	125	42	14	30	3	0
MDS	67	86	115	37	45	34	8	16	2
Disease risk									
Low	552	533	607	265	219	181	40	38	12
High	230	239	280	135	116	113	21	28	10
Others	20	42	1	12	16	0	3	5	0
Cell dose (cells/kg)									
Median	3.0	2.7	2.7	3.0	2.6	2.6	3.1	2.8	2.6
GVHD prophylaxis									
CSA-based	545	306	185	267	114	47	45	21	2
TAC-based	240	499	689	135	227	240	19	50	20
N.A.	17	9	14	10	10	7	0	0	0
Conditioning regimen									
TBI regimen	760	639	560	394	272	194	59	53	15
Non-TBI regimen	30	114	328	17	52	100	3	11	7
N.A.	12	61	0	1	27	0	2	7	0

N.A. indicates not available; AML, acute myeloblastic leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome; GVHD, graft-versus-host disease; CSA, cyclosporine; TAC, tacrolimus; TBI, total body irradiation.

clinical impact of an HLA mismatch may have changed over time periods.

Some investigators have tried to identify specific donor-recipient allele combinations that may be associated with a higher risk of severe acute GVHD [6,7]. Kawase et al. found 16 high-risk HLA allele mismatch combinations (HR-MMs) for severe acute GVHD [7]. They also showed that the number of HR-MMs was associated with severe GVHD and poor survival, whereas the presence of mismatch combinations other than HR-MMs (low-risk mismatch combinations, LR-MMs) did not affect the outcome of HSCT. However, their study included a variety of benign and malignant hematological diseases. In addition, they included donor-recipient pairs with more than 1 HLA mismatch. The impact of each specific mismatch combination was evaluated after adjusting for the number of HLA mismatches in other loci in a multivariate model, but the possible presence of HR-MMs in other loci or the interaction between HLA mismatch combinations could not be appropriately treated in their model. At that time, the study design was inevitable, because the number of each HLA mismatch combination was limited. However, several years have passed and the amount of unrelated HSCT data in the Transplant Registry Unified Management Program (TRUMP) has increased to more than 13,500 donor-recipient pairs. Therefore, in this study, we reanalyzed the impact of HR-MMs, excluding HSCT with multiple HLA mismatches in patients with relatively homogeneous background diseases. In addition, we evaluated the impact of HLA mismatch on transplantation outcomes considering the period effect, because the impact of HR-MM mismatch might have changed over time periods, as we previously reported in an analysis of single HLA allele mismatches at the HLA-A, -B, -C, and -DRB1 loci [5].

METHODS

Patients

Patients aged at least 16 years with acute myeloblastic leukemia, acute lymphoblastic leukemia, myelodysplastic syndrome, or chronic myelogenous leukemia (CML) who underwent a first HSCT from a serologically HLA-A, -B, and -DR matched unrelated donors between 1993 and 2011, and who had full HLA-A, -B, -C, and -DRB1 allele data, were included in this study. Bone marrow was exclusively used as a stem cell source. Clinical data for

Table 2
Multivariate Analysis to Evaluate the Impact of Single HLA Allele Mismatches on the Incidence of Grade III to IV Acute GVHD Stratified according to the Transplantation Time Period

Year	Factor		Hazard Ratio	*P* Value
1993-2001				
	Donor age		1.02 (1.00-1.03)	.082
	Donor sex	Female	1.00	
		Male	1.65 (1.05-2.60)	.031
	Female to male transplantation	No	1.00	
		Yes	1.52 (.91-2.55)	.11
	Disease	AML	1.00	
		ALL	1.15 (.79-1.68)	.47
		CML	1.62 (1.11-2.36)	.012
		MDS	.65 (.32-1.35)	.25
	Disease risk	Low	1.00	
		High	1.30 (.93-1.83)	.13
		Others	.80 (.23-2.85)	.74
	GVHD prophylaxis	CSA-based	1.00	
		TAC-based	.83 (.61-1.14)	.25
	HLA	Low-risk mismatch	1.00	
		Match	.89 (.65-1.21)	.44
		High-risk mismatch	2.74 (1.73-4.32)	<.0001
2002-2007				
	Donor age		1.03 (1.01-1.05)	.0028
	Donor sex	Female	1.00	
		Male	1.50 (.96-2.33)	.076
	Female to male transplantation	No	1.00	
		Yes	1.53 (.89-2.64)	.13
	Disease	AML	1.00	
		ALL	1.36 (.95-1.96)	.094
		CML	1.27 (.74-2.20)	.38
		MDS	1.25 (.77-2.02)	.37
	Disease risk	Low	1.00	
		High	1.76 (1.25-2.48)	.0011
		Others	1.65 (.82-3.34)	.16
	GVHD prophylaxis	CSA-based	1.00	
		TAC-based	.86 (.63-1.19)	.37
	HLA	Low-risk mismatch	1.00	
		Match	.64 (.46-.89)	.008
		High-risk mismatch	1.06 (.58-1.93)	.85
2008-2011				
	Donor age		1.03 (1.01-1.06)	.0016
	Donor sex	Female	1.00	
		Male	1.28 (.78-2.12)	.33
	Female to male transplantation	No	1.00	
		Yes	.98 (.52-1.88)	.96
	Disease	AML	1.00	
		ALL	1.18 (.80-1.74)	.42
		CML	1.53 (.69-3.37)	.3
		MDS	.66 (.36-1.20)	.17
	Disease risk	Low	1.00	
		High	1.53 (1.08-2.17)	.018
		Others	NA (NA-NA)	NA
	GVHD prophylaxis	CSA-based	1.00	
		TAC-based	.82 (.55-1.24)	.34
	HLA	Low-risk mismatch	1.00	
		Match	.56 (.39-.80)	.0014
		High-risk mismatch	.40 (.10-1.64)	.21

AML indicates acute myeloblastic leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome; GVHD, graft-versus-host disease; CSA, cyclosporine; TAC, tacrolimus.

these patients were obtained from the TRUMP [8]. 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, such as antithymocyte globulin or alemtuzumab. Finally, 3718 patients were included in the main part of this study. As a post hoc analysis, 415 patients with 2 LR-MMs and 66 patients with 2 allele mismatches including at least 1 HR-MM were added to compare the impact of 1 HR-MM and 2 LR-MMs and to analyze the statistical interaction between HR-MM and the presence of an additional allele mismatch. The study was approved by the data management committee of TRUMP and by the institutional review board of Saitama Medical Center, 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 Japan Marrow Donor Program using frozen samples [7,9]. In this study, the following donor-recipient HLA-mismatch combinations were regarded as HR-MMs: A*02:06-A*02:01, A*02:06-A*02:07, A*26:02-A*26:01, A*26:03-A*26:01, B*15:01-B*15:07, C*03:03-C*15:02, C*03:04-C*08:01, C*04:01-C*03:03, C*08:01-C*03:03, C*14:02-C*03:04, C*15:02-C*03:04, C*15:02-C*14:02, DR*04:05-DR*04:03, and DR*14:03-DR*-DR1401, as we did not have enough data on HLA-DP and -DQ [7]. In HR-MM pairs, the donor and the recipient must have the HLA allele as shown above, and at the same time, these donor and recipient HLA alleles should not be shared by the recipient and the donor, respectively. For example, if the donor has HLA-A*02:06/02:06 and the recipient has HLA-A*02:01/02:06, this pair was not regarded as HR-MM pair, as the donor's HLA-A*02:06 was shared by the recipient. Other HLA mismatch pairs were regarded as LR-MM pairs. Only the HLA-C mismatch group included HLA mismatch at a serological (antigen) level.

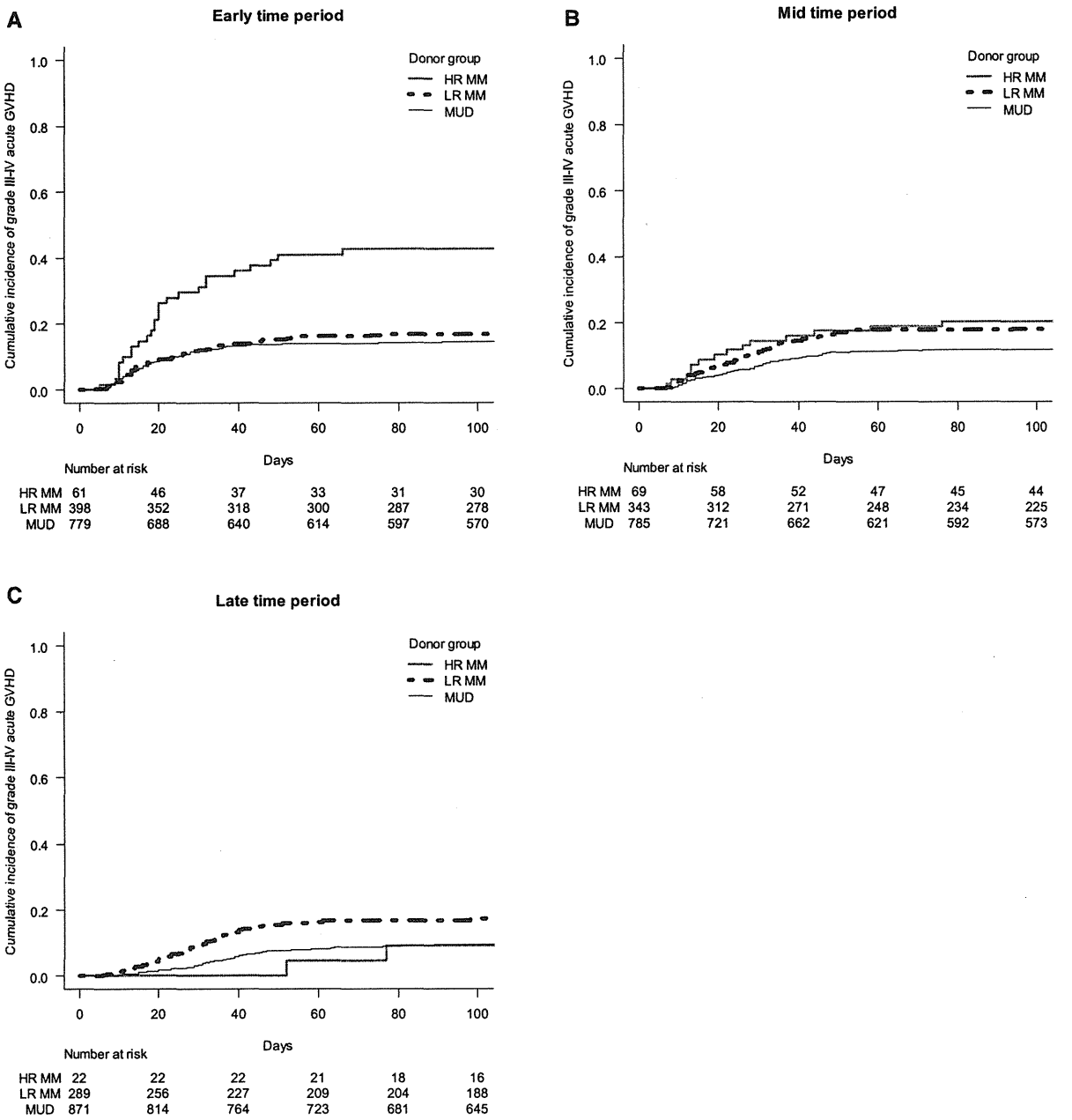

Figure 1. The cumulative incidence of grade III to IV acute GVHD grouped according to the HLA mismatch between the donor and recipient in the early (A), mid (B), and late time periods (C). HR-MM indicates high-risk mismatch; LR-MM, low-risk mismatch; MUD, matched unrelated donor.

Statistical Analyses

We divided the patients into 3 groups according to the time period when HSCT was performed to evaluate whether the impact of HR-MM changed over time periods: the early, mid, and late groups included HSCT performed from 1993 through 2001, 2002 through 2007, and 2008 through 2011, respectively. The break points among groups were determined to make the number of patients in each group equivalent (n = 1278, 1236, and 1204, respectively). To avoid making misleading conclusions by arbitrary grouping, we confirmed that there was a statistically significant interaction between the presence of HR-MMs and transplantation year as a continuous variable, both for overall survival ($P = .0098$) and the incidence of grade III to IV acute GVHD ($P < .001$). The following analyses were performed separately in each group. However, in post hoc analyses to evaluate the impact of HR-MMs at each locus and to compare 1 HR-MM and 2 LR-MMs, the mid and late groups were combined to increase the statistical power, after confirming that similar results were obtained in the 2 groups.

The primary endpoint was the incidence of grade III to IV acute GVHD. Overall survival was evaluated as a secondary endpoint. The chi-square test or Fisher exact test was used to compare categorical variables and Student *t*-test or an analysis of variance test was used for continuous variables to evaluate the homogeneity of background characteristics of the HR-MM, LR-MM, and HLA-matched (MUD) groups. *P* values were adjusted using the Bonferroni's method and Tukey's method for multiple comparisons between each pair. Overall survival was estimated according to the Kaplan-Meier method, and compared among groups with the log-rank test. The incidence of acute GVHD was calculated treating death without GVHD as a competing event, and it was compared using Gray's test [10].

The impact of HR-MMs was evaluated using multivariate models: the Cox proportional hazards model was used for overall survival and Fine and Gray's proportional hazards model was used for acute GVHD [11]. The LR-MM group was regarded as the reference group. 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

Table 3
Multivariate Analysis to Evaluate the Impact of Single High-Risk Allele Mismatches on Overall Survival Stratified According to the Transplantation Time Period

Year	Factor		Hazard Ratio	P Value
1993-2001				
	Age		1.02 (1.01-1.03)	<.0001
	Sex	Female	1.00	
		Male	1.06 (.90-1.23)	.51
	Disease	AML	1.00	
		ALL	1.20 (.99-1.45)	.065
		CML	.89 (.72-1.10)	.29
		MDS	.61 (.45-.83)	.0015
	Disease risk	Low	1.00	
		High	2.72 (2.30-3.23)	<.0001
		Others	2.03 (1.27-3.23)	.0029
	ABO major mismatch	Absent	1.00	
		Present	1.25 (1.06-1.47)	.0092
	GVHD prophylaxis	CSA-based	1.00	
		TAC-based	.85 (.72-1.00)	.049
	HLA	Low-risk mismatch	1.00	
		Match	.86 (.73-1.01)	.063
		High-risk mismatch	1.46 (1.06-2.01)	.019
2002-2007				
	Age		1.01 (1.00-1.02)	.0025
	Sex	Female	1.00	
		Male	1.20 (1.02-1.41)	.0027
	Disease	AML	1.00	
		ALL	1.16 (.96-1.39)	.13
		CML	.84 (.62-1.12)	.23
		MDS	.56 (.43-.73)	<.0001
	Disease risk	Low	1.00	
		High	2.87 (2.41-3.40)	<.0001
		Others	2.23 (1.58-3.15)	<.0001
	ABO major mismatch	Absent	1.00	
		Present	.97 (.81-1.16)	.77
	GVHD prophylaxis	CSA-based	1.00	
		TAC-based	.97 (.83-1.15)	.76
	HLA	Low-risk mismatch	1.00	
		Match	.83 (.69-.98)	.032
		High-risk mismatch	1.06 (.75-1.48)	.75
2008-2011				
	Age		1.02 (1.01-1.03)	<.0001
	Sex	Female	1.00	
		Male	1.08 (.89-1.31)	.42
	Disease	AML	1.00	
		ALL	.97 (.76-1.25)	.83
		CML	.97 (.57-1.64)	.9
		MDS	.65 (.48-.87)	.004
	Disease risk	Low	1.00	
		High	2.73 (2.23-3.35)	<.0001
		Others	NA (NA-NA)	NA
	ABO major mismatch	Absent	1.00	
		Present	1.14 (.92-1.41)	.22
	GVHD prophylaxis	CSA-based	1.00	
		TAC-based	.95 (.75-1.21)	.69
	HLA	Low-risk mismatch	1.00	
		Match	.86 (.69-1.06)	.15
		High-risk mismatch	.82 (.42-1.62)	.58

AML indicates acute myeloblastic leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome; GVHD, graft-versus-host disease; CSA, cyclosporine; TAC, tacrolimus.

total body irradiation in the conditioning regimen, cell dose in the bone marrow graft, the use of cyclosporine or tacrolimus as GVHD prophylaxis, background disease, and disease risk. Acute leukemia in first or second remission, CML in first or second chronic phase, CML in accelerated phase, and myelodysplastic syndrome of refractory anemia or refractory anemia 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 fashion from the model to exclude factors with a P value of .05 or higher. Finally, HLA mismatch was added to the model. Different multivariate models were compared using the likelihood ratio test. The quantity of interest was the deviance difference between the 2 models, under the null hypothesis that 2 models fit the data equally well and the deviance difference has an approximate chi-square distribution with degrees of freedom equal to the difference in the number of independent variables between the compared models.

All P values were 2 sided and P values of .05 or less were considered statistically significant. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University) [12], which is a graphical user interface for R (The R Foundation for Statistical Computing). More precisely, it is a modified version of R commander that was designed to add statistical functions frequently used in biostatistics.

RESULTS

Patients

The patient characteristics are summarized in Table 1. HR-MMs were observed in 64 of 1278, 71 of 1236, and 22 of 1204 donor-recipient pairs in the early, mid, and late time periods, respectively. On the other hand, 412, 351, and 294 pairs had LR-MMs, respectively. With regard to the

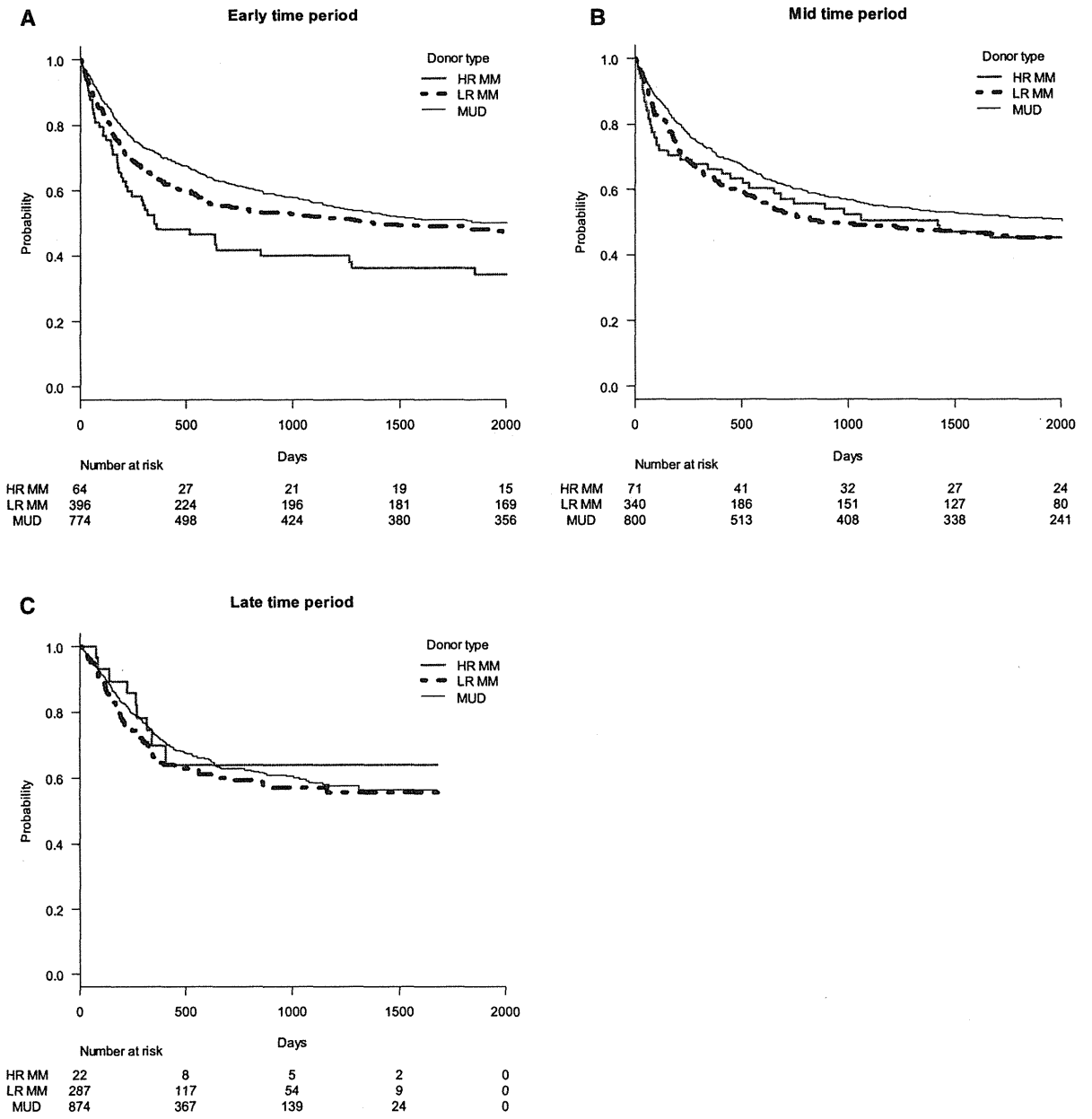

Figure 2. Overall survival grouped according to the HLA mismatch between the donor and recipient in the early (A), mid (B), and late time periods (C). The survival curves were adjusted for other significant factors by the mean of covariates method, in which average values of covariates are entered into the Cox proportional hazards model. HR-MM, high-risk mismatch; LR-MM, low-risk mismatch; MUD, matched unrelated donor.

differences among transplantation time periods, the numbers of LR-MMs and HR-MMs decreased in the late time periods, ie, after the introduction of routine typing for HLA-C and the publication of a paper about HR-MMs [7]. The proportion of HSCTs for CML also dramatically decreased over time periods (30.7%, 10.4%, and 3.6% in the early, mid, and late periods, respectively). With regard to the difference among HLA mismatch groups, the proportion of patients with high-risk underlying disease in the MUD group (29.9%) was significantly lower than those in the HR-MM (37.6%) and LR-MM groups (34.4%). In addition, the proportion of HSCTs for CML was significantly higher in the HR-MM group in the early time period (29.6%, 30.3%, and 46.9% in the MUD, LR-MM, and HR-MM groups, respectively).

Incidence of Grade III to IV Acute GVHD

To adjust the impact of HLA mismatch for possible confounding factors, we identified the following independently significant factors for the incidence of grade III to IV acute GVHD: donor age, donor sex, sex mismatch, disease, disease risk, and GVHD prophylaxis. After we adjusted for these factors, we confirmed that the incidence of grade III to IV acute GVHD in the HR-MM group was significantly higher than that in the LR-MM group (hazard ratio [HR], 2.74; 95% confidence interval [CI], 1.73 to 4.32; $P < .0001$) in the early time period, whereas the difference between the MUD and LR-MM groups was not significant (HR, .89; 95% CI, .65 to 1.21; $P = .44$) (Table 2, Figure 1). On the other hand, in the mid and late time periods, the difference in the incidence of

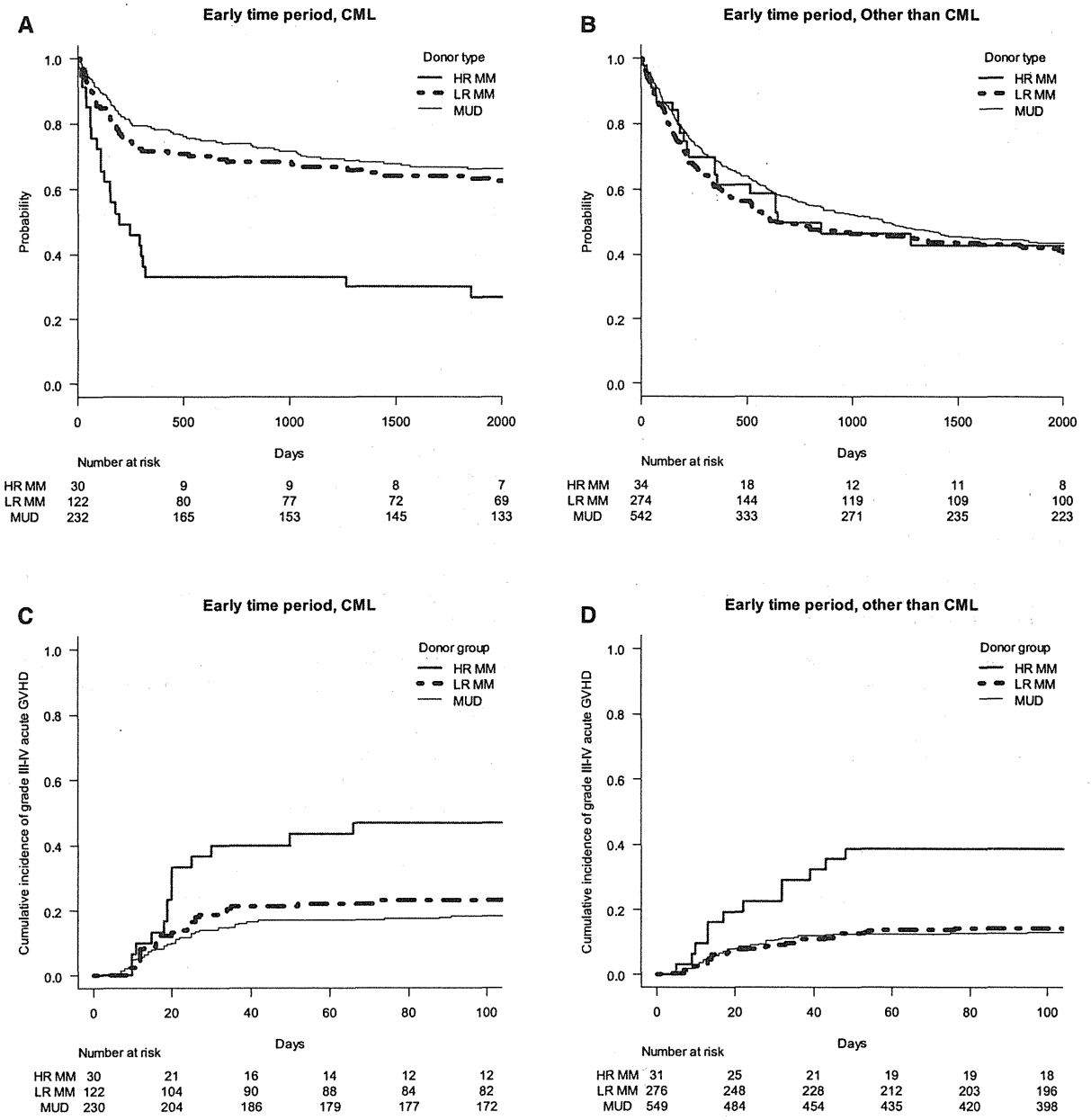

Figure 3. Adjusted overall survival (A,B) and the cumulative incidence of grade III to IV acute GVHD (C,D) grouped according to the underlying disease in the early time period. CML, chronic myelogenous leukemia; HR-MM, high-risk mismatch; LR-MM, low-risk mismatch; MUD, matched unrelated donor.

grade III to IV acute GVHD between the HR-MM and LR-MM groups was not statistically significant (HR, 1.06; 95% CI, .58 to 1.93; $P = .85$ and HR, .40; 95% CI; .10 to 1.64; $P = .21$, respectively). The presence of LR-MM significantly adversely affected the incidence of grade III to IV acute GVHD in the mid and late periods (HR, .64; 95% CI, .46 to .89; $P = .008$ and HR, .56; 95% CI, .39 to .80; $P = .0014$, respectively, for the MUD group).

Similarly, the presence of HR-MM significantly affected the incidence of grade II to IV acute GVHD compared with LR-MM only in the early time period (HR, 1.53; 95% CI, 1.05 to 2.24; $P = .028$), and not in the mid and late periods (HR, .92; 95% CI, .61 to 1.37; $P = .67$ and HR, .79; 95% CI, .40 to 1.58; $P = .51$, respectively).

Overall Survival

After adjusting for recipient age, recipient sex, presence of ABO-major mismatch, disease, disease risk, and GVHD prophylaxis, we again confirmed that survival in the HR-MM group was significantly inferior to that in the LR-MM group (HR, 1.46; 95% CI, 1.06 to 2.01; $P = .019$) in the early time period, whereas there was no significant difference between the MUD and LR-MM groups (HR, .86; 95% CI, .73 to 1.01; $P = .063$) (Table 3). On the other hand, the difference in survival between the HR-MM and LR-MM groups was not statistically significant in the mid and late time periods (HR, 1.06; 95% CI, .75 to 1.48; $P = .75$ and HR, .82; 95% CI, .42 to 1.62; $P = .58$, respectively). The difference in survival between the MUD and LR-MM groups was consistent among

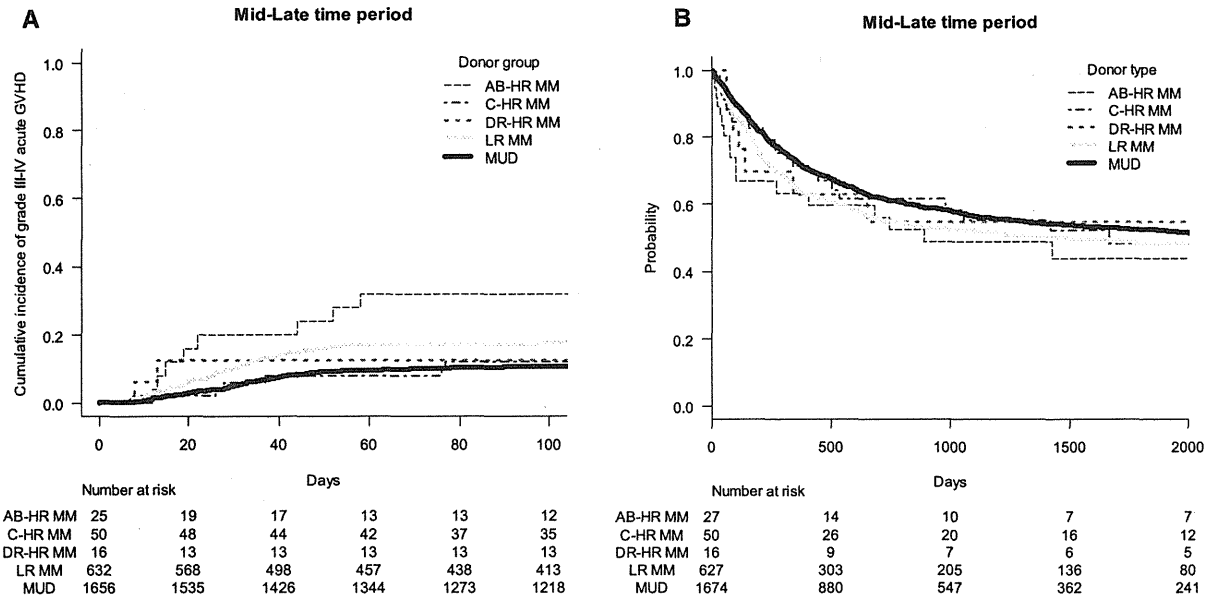

Figure 4. The cumulative incidence of grade III to IV acute GVHD (A) and adjusted overall survival (B) grouped according to the HLA mismatch loci between the donor and recipient in the mid or late time period. AB-HR MM, high-risk mismatch at the HLA-A or -B locus; C-HR MM, high-risk mismatch at the HLA-C locus; DR-HR MM, high-risk mismatch at the DRB1 locus; LR-MM, low-risk mismatch; MUD, matched unrelated donor.

the 3 time periods but statistically significant only in the mid period (HR, .83; 95% CI, .69 to .98; $P = .032$). Figure 2 shows the overall survival curves grouped according to the HLA-mismatch groups in each time period, adjusted for other significant factors by the mean of covariates method.

Disease-specific Effects of HR-MM in the Early Period

The number of patients with CML was significantly higher in the early period than in the mid and late periods. Therefore, we evaluated the disease-specific impact of HR-MM in the early period. As shown in Figures 3A and B, the presence of HR-MM had an adverse impact on overall survival only in patients with CML, although HR-MM showed a similar adverse impact on the incidence of grade III to IV acute GVHD regardless of the underlying disease (Figure 3C, D). Of the 24 CML patients who died after HSCT with HR-MM, 23 died without relapse of CML, and 10 of these patients died without grade III to IV acute GVHD.

Impact of HR-MM at Each Locus

To evaluate the impact of HR-MM at each locus in the mid and early periods, we combined the 2 periods together to

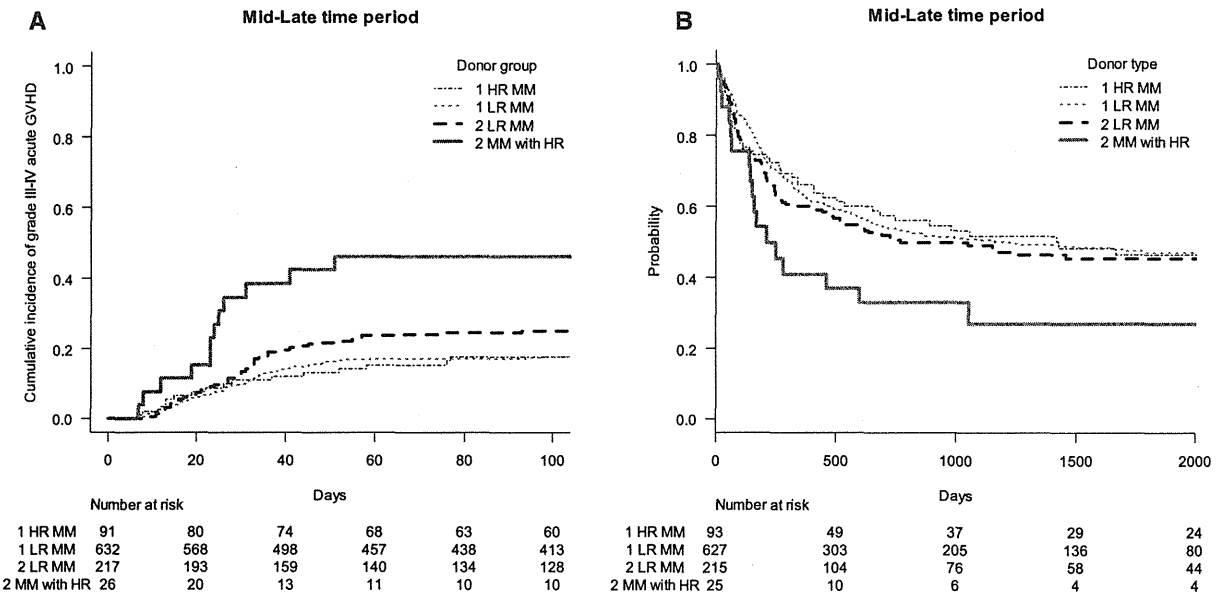

Figure 5. The cumulative incidence of grade III to IV acute GVHD (A) and adjusted overall survival (B) grouped according to the HLA mismatch between the donor and recipient in the mid or late time period. 1HR-MM, 1 high-risk mismatch; 1LR-MM, 1 low-risk mismatch; 2LR-MM, 2 low-risk mismatches; 2MM with HR, 2 allele mismatches including at least 1 HR-MM.

increase statistical power because the impact of HR-MM on acute GVHD and survival tended to be similar in these 2 time periods. The presence of HR-MMs at the HLA-A/B (HLA-A or -B), HLA-C, and HLA-DRB1 loci was not associated with significantly different survival compared with the LR-MM group (HR, 1.23; 95% CI, .76 to 1.98; $P = .41$; HR, .96; 95% CI, .65 to 1.44; $P = .86$; and HR, .95; 95% CI, .45 to 2.02; $P = .89$, respectively. Figure 4A). However, the incidence of grade III to IV acute GVHD was higher in patients who had HR-MM at the HLA-A/B locus than in those with LR-MM, although this difference was not statistically significant (HR, 1.78; 95% CI, .86 to 3.66; $P = .12$; HR, .63; 95% CI, .28 to 1.41; $P = .26$; and HR, .69; 95% CI, .15 to 3.12; $P = .63$ for HLA-A/B, HLA-C, and HLA-DRB1, respectively.) (Figure 4B).

Comparison of One HR-MM and Two LR-MMs

To evaluate whether a donor with 1 HR-MM or a donor with 2 LR-MMs should be preferred, we added patients with 2 LR-MMs and those with 2 allele mismatches including at least 1 HR-MM to the dataset, and we compared the outcome of HSCT from these donors with that of HSCT from a donor with 1 LR-MM as a reference in the combined mid and late periods.

The presence of 2 LR-MMs was associated with a significantly higher incidence of grade III to IV acute GVHD (HR, 1.44; 95% CI, 1.04 to 2.00; $P = .030$), but the impact of 1 HR-MM was not statistically significant (HR, .94; 95% CI, .56 to 1.59; $P = .83$) (Figure 5A). However, the impact of 2 LR-MMs was not associated with inferior survival. The HR for survival of 1 HR-MM and 2 LR-MMs were 1.05 (95% CI, .78 to 1.42; $P = .75$) and 1.12 (95% CI, .90 to 1.39; $P = .33$), respectively (Figure 5B).

On the other hand, the presence of 2 allele mismatches including at least 1 HR-MM was associated with an extremely poor outcome; HR, 3.61 (95% CI, 1.96 to 6.66; $P < .001$) for grade III to IV acute GVHD and HR, 2.02 (95% CI, 1.25 to 3.26; $P = .0040$) for overall survival. These results suggested that the impact of HR-MM may change according to the presence or absence of an additional allele mismatch. In fact, there was a statistically significant interaction between the presence of HR-MM and the presence of an additional allele mismatch ($P = .020$). The likelihood ratio test revealed that the prognostic value of Fine and Gray's proportional hazards model for acute GVHD was significantly improved by adding the interaction term to the model ($P = .024$).

DISCUSSION

In this study, we reevaluated the clinical impact of HR-MMs in unrelated HSCT. We confirmed that the presence of HR-MMs was associated with a significantly higher incidence of grade III to IV acute GVHD and significantly inferior survival in the early transplantation time period. However, in the mid and late periods, ie, after 2002, there was no statistically significant difference in overall survival or the incidence of grade III to IV acute GVHD between patients with HR-MMs and those with LR-MMs. The methods used for the statistical analyses were somewhat different than those in a previous study, but this is not the major reason for the different results, as the significant impact of HR-MMs on survival and acute GVHD was reproduced in the early time period. Another possible explanation is a bias caused by the availability of information about HR-MMs. After the publication of a paper that reported the importance of HR-MM, physicians may have tended to intensify prophylaxis against GVHD in unrelated HSCT with HR-MMs, and, thereby, the impact of HR-MMs might have become less significant. However, this is not the case because the impact of HR-MMs was already not apparent in the mid time period, before the paper was published. We also considered that the difference in the underlying disease might have influenced the effect of HR-MMs. The proportion of patients with CML decreased from 30.7% in the early period to 10.4% and 3.6% in the mid and late periods, respectively. Therefore, we analyzed the impact of HR-MMs grouped according to the underlying disease in the early period. The effect of HR-MMs on survival was observed only in patients with CML (Figure 3A,B). However, HR-MMs had an adverse effect on the incidence of grade III to IV acute GVHD regardless of the underlying disease (Figure 3C,D). Therefore, the different effects of HR-MMs on the incidence of grade III to IV acute GVHD among the time periods could not be explained solely by the underlying diseases. We could not clarify the reason for this different effect, but the changes in the transplantation procedure, including prophylaxis against GVHD, might have reduced the clinical impact of HR-MM. In fact, the incidence of grade III to IV acute GVHD decreased from 42.6%, 16.8%, and 14.5% in the HR-MM, LR-MM, and MUD groups, respectively, in the early time period to 17.6%, 17.7%, and 10.6% in the mid or late period. Improved survival in patients who developed severe acute GVHD might also reduce the effect of HR-MMS on survival. The 1-year survival in patients who developed grade III to IV acute GVHD improved from 32.1% in the early period to 44.4% in the mid and late time periods. This change may have resulted from the progress in supportive care, including strategies against fungal or viral infections.

Another important finding is that the impact of HR-MM was significantly enhanced by the presence of an additional allele mismatch in the mid and late time periods. This fact may be explained by a hypothesis that the HR-MM biologically increases the graft-versus-host (GVH) reaction, but the recent improvement in GVHD prophylaxis has masked its effect, if HR-MM exists as a single allele mismatch, whereas the adverse impact of HR-MM is not suppressed even by recent methods of GVHD prophylaxis when an additional allele mismatch is present. Based on these findings, interaction terms should be incorporated into the statistical model when the impact of HR-MMs is analyzed in datasets that include HSCT with multiple allele mismatches.

A major limitation of this study is the small number of patients with HR-MMs, especially in the late time period. We cannot deny the possibility that an important effect of HR-MMs might be overlooked because of the poor statistical power. The lack of a significant difference in the incidence of grade III to IV acute GVHD between unrelated HSCT with HR-MMs at the HLA-A/B locus and HSCT with LR-MM should be interpreted with caution, because of the small number of patients. Furthermore, it was impossible to evaluate the effect of each mismatch combination, as the number of patients with each mismatch combination was most often fewer than 10. HR-MMs associated with at least a 20% incidence of grade III to IV acute GVHD in the mid and late periods included A*0206-A*0201 (4 of 14), A*0206-A*0207 (3 of 4), B*1501-B*1507 (1 of 1), C*0801-C*0303 (4 of 15), and C*1402-C*0304 (1 of 5), but the number of patients in each pair was too small to draw any definitive conclusions.

When we consider the impact of HR-MMs, especially at the HLA-C locus, we should also consider the effect of a killer immunoglobulin-like receptor ligand (KIR) mismatch [13,14]. Among the 50 patients with HR-MMs at the HLA-C locus in the mid and late periods, 20 had a KIR mismatch in the GVH direction, whereas 30 did not. The incidence of grade III to IV acute GVHD was 5% and 16.7%, respectively, but this

difference was not statistically significant ($P = .24$). The incidence of grade III to IV acute GVHD in the 21 patients who had LR-MMs and a KIR mismatch in the GVH direction was 15.0%. We could not conclude that a KIR mismatch had an impact in this study because of the small number of patients with a KIR mismatch in the GVH direction.

We should note that the results of the current study are applicable to patients who receive bone marrow graft after a myeloablative conditioning regimen. The impact of HR-MMs may change according to the stem cell source or the conditioning regimen. Therefore, further analyses are required to evaluate the impact of HR-MMs in peripheral blood stem cell transplantation and reduced-intensity conditioning transplantation.

In conclusion, this retrospective study revealed that the clinical impact of HR-MMs became less significant after 2002. Although HR-MMs may have a biological impact, their effect may be controlled by recent methods for GVHD prophylaxis when they exist as a single allele mismatch. It may still be prudent to avoid a donor with HR-MMs, especially at the HLA-A or -B locus, if a donor with the other mismatch combination is available. However, in the absence of MUD or an unrelated donor with a LR-MM, a donor with a single HR-MM could be a viable option for unrelated HSCT, and it is preferred over a donor with 2 LR-MMs. In addition, we should be aware that the clinical impact of risk factors may change over time periods, and therefore, we should repeatedly confirm the validity of risk factors.

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Authorship statement: Y.K. designed the study. Y.K. and J.K. analyzed the data. Y.A., S.F., Y.M., T.I., M.T., K.O., T.F., K.M., T.M., C.K., N.K., K.I., A.S., and S.M. gathered the data. Y.K. wrote the first draft of the paper and all other authors contributed to the final version.

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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.