ARTICLE

HLA discrepancy between graft and host rather than that graft and first donor impact the second transplant outcome

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ABSTRACT

Second allogeneic hematopoietic stem cell transplantation is a curative treatment option for patients with hematologic malignancies. However, it is unclear whether HLA discrepancy between graft and first donor has an impact on the outcome of second transplantation. We retrospectively analyzed 646 patients receiving second transplantation after an initial HLA mismatched transplantation. With regard to graft-*versus*-host, the one-allele mismatch (1 mismatch) group (SHR, 1.88; 95%CI: 0.79-4.45; *P*=0.163) and more than one-allele mismatch group (≥ 2 mismatch) (SHR, 1.84; 95%CI, 0.75–4.51; *P*=0.182) had higher risks of grade III–IV acute graft-*versus*-host disease (GvHD) compared to the HLA-matched (0 mismatch) group. In contrast, no difference in risk of acute GvHD was found among the 0, 1, and ≥ 2 mismatch group with respect to graft-*versus*-first donor. With regard to graft-*versus*-host, the ≥ 2 mismatch group showed a significantly higher risk of treatment-related mortality (SHR, 1.90; 95%CI, 1.04–3.50; *P*=0.038) compared to the 0 mismatch group, while the risk of relapse was slightly lower in the ≥ 2 mismatch group (SHR, 068; 95%CI, 0.44–1.06; *P*=0.086). In contrast, with regard to graft-*versus*-first donor, there were no significant differences in treatment-related mortality or relapse among the three groups. These findings suggested that HLA discrepancy between graft and host induces transplant-related immunological responses in second transplantation leading to an increase in treatment-related mortality, in contrast, the biological effects of HLA discrepancy between graft and first donor on outcome may be negligible.

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Introduction

For patients with malignant hematologic diseases who relapse after allogeneic hematopoietic stem cell transplantation (HSCT), a second HSCT is thought to be a curative option. It is believed that use of a second donor may confer increased therapeutic potency by inducing a more potent graft-*versus*-leukemia (GvL) effect; however, there are no data to support this assumption.[1-9] In early studies, a second HSCT after a first HLA-matched transplantation was associated with similar risks of relapse and acute graft-*versus*-host disease (GvHD) using a different HLA-matched donor.[3] There was no significant difference in survival between the transplantations from the original donor and another donor.

Over the years, the use of HLA-mismatched (MM) transplantation for hematologic diseases has increased, including haploidentical HSCT and cord blood transplantation (CBT). Following HLA-MM transplantation, a second donor is selected due to HLA discrepancy between the graft and the host. Physicians pay little attention to HLA discrepancy between the graft and the first donor, although the impact of this discrepancy on the outcome of second HSCT is unclear. Recipient non-hematopoietic gastrointestinal cells can express MHC class II, which is critical for inducing experimental acute GvHD in cases of minor histocompatibility antigen (mHAg) MM.[10,11] In contrast, hematopoietic antigen presenting cells (APCs), especially dendritic cells, induce MHC class I-dependent acute GvHD in mHAg MM cases.[12] Furthermore, in the MHC MM setting, hematopoietic APCs play an important role in the induction of both MHC class I- and II-dependent acute GvHD.[13-16] As hematopoietic APCs are of first donor origin, HLA discrepancy between the graft and the first donor may be related to transplant-related immunological responses of the second HSCT.

To elucidate the biological effects of HLA discrepancy between the graft and the first donor that impact the outcome of the second HSCT, we compared the effects of HLA-MM between the graft and the first donor to those between the graft and the host in 646 patients receiving a second HSCT after an initial HLA-MM transplantation.

Methods

Study population

Patients who were at least 16 years of age with acute myelogenous leukemia (AML), acute lymphoblastic leukemia (ALL), myelodysplastic syndrome (MDS), chronic myelogenous leukemia (CML), malignant lymphoma (ML), or other malignant hematologic disease, and who received a second HSCT after an initial HLA-MM transplantation, were included in this study. Furthermore, patients must have received first and second allogeneic HSCTs between 1994 and 2016, with full HLA-A, -B, and -DRB1 allele data. Hematopoietic stem cell transplantation recipient clinical data were collected by the Japan Society for Hematopoietic Cell Transplantation (JSHCT) and the Japanese Data Center for Hematopoietic Cell Transplantation (JDCHCT) using the Transplant Registry Unified Management Program (TRUMP).17-19 We excluded individuals who: 1) first received HLA-matched HSCT; 2) received a second HSCT within 30 days after the first HSCT, in a planned manner or due to rejection/engraftment failure; 3) died within 30 days and lacked data on survival status and survival date; 4) lacked accurate allele data; or 5) received more than two HSCTs. The final study population consisted of 646 patients. The study was approved by the Data Management Committee of TRUMP and the Institutional Review Board of Okayama University.

Study end points

The outcomes assessed included acute GvHD, chronic GvHD, neutrophil engraftment, transplant-related mortality (TRM), relapse, and overall survival (OS). Acute and chronic GvHD were diagnosed and graded using the standard criteria.[20,21] Neutrophil engraftment was considered to have occurred when the absolute neutrophil count was ≥ $0.5x10^9$ cells/L for 3 consecutive days. Death from any cause was the event of interest in determining OS. TRM was defined as death during remission.

Statistical analysis

Descriptive statistics were generated for patients' characteristics. Differences in characteristics between groups were evaluated by the χ^2 test and analysis of variance. The probability of OS was estimated according to the Kaplan-Meier method, and groups were compared using the log rank test. Subsequently, the probabilities of relapse, TRM, and acute and chronic GvHD were estimated on the basis of cumulative incidence curves.[22] Competing events were death without relapse for relapse, relapse for TRM, death without engraftment for engraftment, and death without GvHD for acute or chronic GvHD. The groups were compared using Gray's test.

To evaluate the impact of HLA discrepancy on transplant outcomes, we estimated the hazard ratios (HRs) or subhazard ratios (SHRs) and 95% confidence intervals (CIs) adjusted for potential confounders. The Cox proportional hazards model was used to evaluate the impact on OS, whereas multivariable competing-risks regression was used to evaluate the impact on the other end points. Several potential confounders considered in the multivariable analyses were provided in the *Online Supplementary Appendix*.

In all analyses, $P<0.05$ was considered statistically significant. All statistical analyses are performed with Stata (v.15,0; Stata Corp., College Station, TX, USA) and EZR software (Saitama Medical Center, Jichi Medical University, Japan).[23]

Results

Patients' and transplantation characteristics

A total of 646 patients who received a second HSCT after an initial HLA-MM transplantation were analyzed. Patients' and transplantation characteristics are presented in Table 1. With respect to the HLA discrepancy in the graft-*versus*-host direction (graft *vs.* host), HLA matching was categorized as follows: HLA -A, -B, -DRB1 match (0 MM, n=85), MM at one allele (1 MM, n=160), or mismatch at more than one allele (≥2 MM, n=401). With regard to HLA discrepancy in the graft-*versus*-first donor direction (graft *vs.* first donor), the second HSCT was categorized as follows: HLA -A, -B, -DRB1 match (0 MM, n=72), mismatch at one allele (1 MM, n=100), or mismatch at more than one allele (≥2 MM, n=474). In the graft-*versus*-host comparison, the ≥2 MM group received cord blood more frequently (0 MM, 20.0%; 1 MM, 21.3%; ≥2 MM, 60.2%, $P<0.001$), were more likely to use a reduced-intensity conditioning regimen (0 MM, 56.5%; 1 MM, 66.3%; ≥2 MM, 70.7%, $P=0.012$), and had a higher rate of *in vivo* T-cell depletion (0 MM, 10.6%; 1 MM, 18.1%; ≥2 MM, 25.4%, $P=0.003$). The interval between the first and second HSCT was shorter in this group (<12

Table 1. Patients' and donor characteristics.

	HLA mismatch for graft-*versus*-host						*P*	HLA mismatch for graft-*versus*-first donor						*P*
	Match (N=85)		1 allele mismatch (N=160)		≥2 allele mismatch (N=401)			Match (N=72)		1 allele mismatch (N=100)		≥2 allele mismatch (N=474)		
	N	(%)	N	(%)	N	(%)		N	(%)	N	(%)	N	(%)	
Recipient age, years														
Median (range)	43 (18-73)		42 (16-69)		42 (16-70)		0.138	42 (18-69)		44 (16-73)		42 (16-71)		0.240
HLA mismatch														
A allele	0	0.0	24	15.0	276	67.3		0	0.0	23	23.0	339	71.5	
B allele	0	0.0	35	21.9	350	85.4		0	0.0	26	26.0	429	90.5	
DR allele	0	0.0	101	63.1	340	82.9		0	0.0	51	51.0	427	90.1	
Recipient gender (%)														
Male	47	55.3	96	60.0	229	55.9	0.740	47	65.3	59	59	266	56.1	0.326
Female	38	44.7	64	40.0	172	42.0		25	34.7	41	41	208	43.9	
Donor/recipient gender														
Match	45	52.9	79	49.4	168	41.0	0.183	32	44.4	49	49.0	211	44.5	0.852
Male to Female	23	27.1	30	18.8	78	19.0		17	23.6	19	19.0	95	20.0	
Female to Male	15	17.6	37	23.1	106	25.9		20	27.8	21	21.0	117	24.7	
Unknown	2	2.4	14	8.8	49	12.0		3	4.2	11	11.0	51	10.8	
Diagnosis														
Acute myeloid leukemia	45	52.9	89	55.6	254	62.0	0.049	38	52.8	56	56.0	294	62.0	0.556
Acute lymphoblastic leukemia	15	17.6	30	18.8	81	19.8		12	16.7	20	20.0	94	19.8	
Chronic myeloid leukemia	6	7.1	4	2.5	9	2.2		4	5.6	4	4.0	11	2.3	
Myelodysplastic syndrome	6	7.1	15	9.4	20	4.9		6	8.3	8	8.0	27	5.7	
Malignant lymphoma	10	11.8	11	6.9	22	5.4		6	8.3	7	7.0	30	6.3	
Others	3	3.5	11	6.9	15	3.7		6	8.3	5	5.0	18	3.8	
Disease risk at transplant														
Standard risk	31	36.5	51	31.9	110	26.8	0.200	24	33.3	34	34.0	134	28.3	0.406
High risk	54	63.5	109	68.1	291	71.0		48	66.7	66	66.0	340	71.7	
Stem cell source														
Bone marrow	37	43.5	52	32.5	78	19.0	<0.001	66	91.7	85	85.0	16	3.4	0.001
Peripheral blood	18	21.2	14	8.8	149	36.3		13	18.1	26	26.0	142	30.0	
Cord blood	17	20.0	34	21.3	247	60.2		6	8.3	49	49.0	243	51.3	
Conditioning regimen														
Myeloablative	37	43.5	54	33.8	111	27.1	0.012	26	36.1	44	44.0	132	27.8	0.004
Reduced intensity	48	56.5	106	66.3	290	70.7		46	63.9	56	56.0	342	72.2	
GvHD prophylaxis														
Cyclosporine based	22	25.9	27	16.9	74	18.0	0.228	17	23.6	25	25.0	81	17.1	0.088
Tacrolimus based	63	74.1	131	81.9	322	78.5		52	72.2	74	74.0	390	82.3	
Others	0	0.0	2	1.3	5	1.2		3	4.2	1	1.0	3	0.6	
In vivo T-cell depletion														
Yes	9	10.6	29	18.1	104	25.4	0.003	6	8.3	13	13.0	123	25.9	<0.001
No	76	89.4	131	81.9	297	72.4		66	91.7	87	87.0	351	74.1	
Year of transplant														
1994-2010	30	35.3	55	34.4	118	28.8	0.372	27	37.5	41	41.0	135	28.5	0.025
2011-2016	55	64.7	105	65.6	283	69.0		45	62.5	59	59.0	339	71.5	
Interval between first and second SCT														
<12 months	29	34.1	71	44.4	206	50.2	0.008	23	31.9	38	38.0	245	51.7	0.029
≥12-23 months	23	27.1	45	28.1	106	25.9		19	26.4	28	28.0	127	26.8	
≥24 months	27	31.8	37	23.1	67	16.3		18	25.0	27	27.0	86	18.1	
Missing	6	7.1	7	4.4	22	5.4		12	16.7	7	7.0	16	3.4	
Interval between first SCT and first relapse														
<12 months	34	40.0	67	41.9	175	42.7	0.793	29	40.3	35	35.0	212	44.7	0.217
≥2-12 months	32	37.6	57	35.6	145	35.4		23	31.9	42	42.0	166	35.0	
≥12 months	8	9.4	18	11.3	32	7.8		10	13.9	7	7.0	41	8.6	
Missing	11	12.9	18	11.3	52	12.7		10	13.9	16	16.0	55	11.6	

N: number; GvHD: graft-*versus*-host disease; SCT: stem cell transplantation.

Table 2. Effect of HLA allele mismatch on acute graft-*versus*-host disease (GvHD), chronic GvHD and engraftment in multivariate analyses.

	HLA mismatch for graft-*versus*-host			HLA mismatch for graft-*versus*-first donor		
	Match (N=85)	1 allele mismatch (N=160)	≥2 allele mismatch (N=401)	Match (N=72)	1 allele mismatch (N=100)	≥2 allele mismatch (N=474)
Grades III to IV acute GvHD						
SHR[1] (95%CI)	1 (ref)	1.88 (0.79-4.45, *P*=0.163)	1.84 (0.75-4.51, *P*=0.182)	1 (ref)	0.84 (0.35-2.02, *P*=0.669)	0.91 (0.43-1.93, *P*=0.800)
Chronic GvHD						
SHR[1] (95%CI)	1 (ref)	1.45 (0.84-2.50, *P*=0.181)	1.20 (0.60-2.38, *P*=0.605)	1 (ref)	0.98 (0.55-1.76, *P*=0.956)	0.91 (0.54-1.51, *P*=0.702)
Neutrophil engraftment						
SHR[1] (95%CI)	1 (ref)	0.81 (0.62-1.06, *P*=0.126)	0.77 (0.56-1.05, *P*=0.097)	1 (ref)	1.06 (0.75-1.48, *P*=0.753)	1.23 (0.92-1.66, *P*=0.167)

[1]Adjusted for recipient age at transplant (continuous), recipient gender, gender mismatch (match, male to female, female to male, unknown), diagnosis (acute myeloid leukemia, acute lymphoblastic leukemia, chronic myeloid leukemia, myelodysplastic syndrome, malignant lymphoma or others), disease risk at transplant (standard or high), stem cell source (bone marrow, peripheral blood, cord blood), conditioning regimen (myeloablative or reduced intensity), graft-*versus*-host disease (GvHD) prophylaxis (cyclosporine based, tacrolimus based, others), *in vivo* T-cell depletion (Yes, No), year of transplant (1994-2010, 2011-2016), interval between first and second stem cell transplantation (SCT) (<12 months, ≥12-23 months, ≥24 months, missing) and interval between first SCT and relapse (<2 months, ≥2-12 months, ≥12 months, missing). SHR: subdistribution hazard ratios.

months: 0 MM, 34.1%; 1 MM, 44.4%; ≥ 2 MM, 50.2%, *P*=0.008). With regard to graft-*versus*-first donor comparison, the ≥2 MM group showed a similar trend to that for the graft-*versus*-host group comparison. The ≥2 MM group required more cord blood (*P*=0.001), used a reduced-intensity conditioning regimen (*P*=0.004), and had greater *in vivo* T-cell depletion (*P*<0.001) and a shorter interval between the first and second HSCT (*P*=0.029).

Acute graft-*versus*-host disease, chronic graft-*versus*-host disease, and engraftment

With regard to the graft-*versus*-host results, the unadjusted cumulative incidence rates of grade III-IV acute GvHD at 100 days post transplantation were 9.5% (95%CI: 4.4-17.0%) in the 0 MM group, 13.8% (95%CI: 9.0-19.7%) in the 1 MM group, and 11.0% (95%CI: 8.2-14.3%) in the ≥2 MM group (Figure 1). In multivariate analysis, the 1 MM group (SHR, 1.88; 95%CI: 0.79-4.45; *P*=0.163) and ≥2 MM group (SHR, 1.84; 95%CI: 0.75-4.51; *P*=0.182) tended to have higher risk of grade III-IV acute GvHD compared to the 0 MM group, although the results were not statistically significant (Table 2). With regard to affected organ, the risk of skin, gut and liver acute GvHD increased among the 1 MM group and ≥2MM group compared to the 0 MM group (Table 3). There was no statistically significant difference in risk of chronic GvHD among the groups in multivariate analysis. The cumulative incidence rate of neutrophil engraftment at day 50 was 94.0% (95%CI: 85.6-97.6%) in the 0 MM group, 96.9% (95%CI: 92.3-98.7%) in the 1 MM group, and 91.0% (95%CI: 87.7-93.4%) in the ≥2 MM group. In multivariate analysis, the ≥2 MM group tended to show delayed engraftment compared to the 0 MM group (SHR, 0.77; 95%CI: 0.56-1.05; *P*=0.097).

With regard to the graft-*versus*-first donor results, there were no significant differences in the risk of grade III-IV acute GvHD, chronic GvHD, or neutrophil engraftment among the groups in multivariate analysis (Table 2).

Next, the association of each HLA allele MM with GvHD was evaluated (*Online Supplementary Table S1*). With regard to graft-*versus*-host, B allele MM was associated with an increased risk of grade III-IV acute GvHD in multivariate analysis (SHR, 2.87; 95%CI: 1.42-5.79; *P*=0.003), and DR allele MM was associated with delayed neutrophil engraftment (SHR, 0.80; 95%CI: 0.67-0.95, *P*=0.011); no such associations were found for the other MM types. With regard to the graft-*versus*-first donor results, no HLA allele MM showed an association with grade III-IV acute GvHD, chronic GvHD, or neutrophil engraftment in multivariate analysis.

Transplant-related mortality, relapse, and overall survival

With regard to the graft-*versus*-host results, the unadjusted cumulative incidence rates of TRM and relapse at 5 years post transplantation were 19.8% (95%CI: 11.8-29.2%) and 55.6% (95%CI: 43.9-65.7%) in the 0 MM group, 32.5% (95%CI:25.2-39.9%) and 45.2% (95%CI: 37.3-52.8%) in the 1 MM group, and 34.7% (95%CI: 30.0-39.4%) and 46.8% (95%CI: 41.8-51.7%) in the ≥2 MM group, respectively (Figures 2 and 3). Multivariate analysis indicated that the risk of TRM was marginally higher in the 1 MM group (SHR, 1.67; 95%CI: 0.94-2.98; *P*=0.081), and significantly higher in the ≥2 MM group (SHR, 1.90; 95%CI: 1.04-3.50; *P*=0.038), *versus* the 0 MM group. In contrast, the risk of relapse was slightly lower in both the 1 MM group (SHR, 073; 95%CI: 0.50-1.07; *P*=0.110) and the ≥2 MM group (SHR, 068; 95%CI: 0.44-1.06; *P*=0.086). Consequently, no significant differences in OS were found among the three groups in multivariate analyses (Table 4). Analysis of each HLA allele MM revealed that only HLA DR allele MM was significantly associated with a lower risk of relapse (SHR, 0.75; 95%CI: 0.58-0.95; *P*=0.018) and a higher risk of TRM (SHR, 1.44; 95%CI: 1.03-2.00; *P*=0.033) (*Online Supplementary Table S2*). The main causes of TRM differed among the three groups. The rates of interstitial pneumonia, TMA, and especially acute GvHD

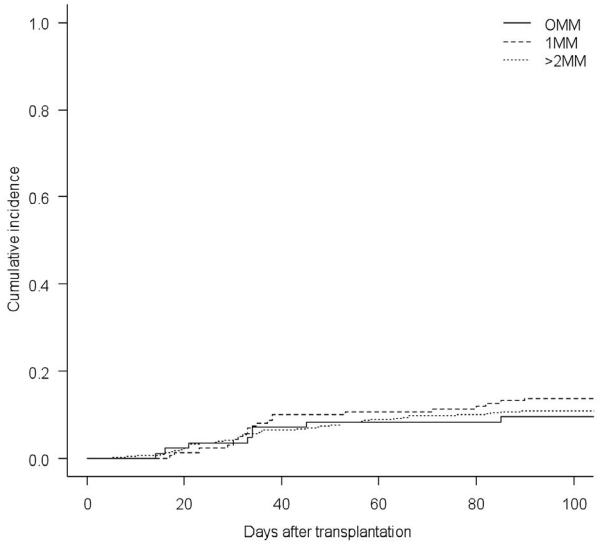

Figure 1. The unadjusted cumulative incidence of grades III to IV acute graft-*versus*-host disease (GvHD) by HLA mismatch (MM) for graft. With regard to the graft-*versus*-host results, the unadjusted cumulative incidence rates of grade III-IV acute GvHD were 9.5% (95%CI: 4.4-17.0%) in the 0 MM group, 13.8% (95%CI: 9.0%–19.7%) in the 1 MM group, and 11.0% (95%CI: 8.2%–14.3%) in the ≥ 2 MM group.

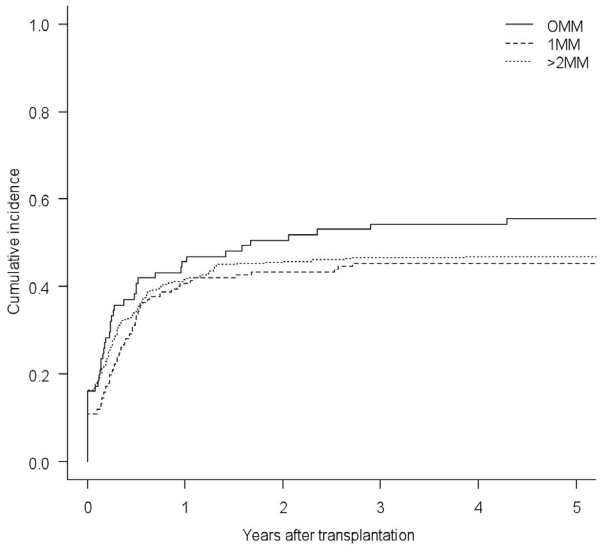

Figure 2. The unadjusted cumulative incidence of relapse by HLA mismatch (MM) for graft-*versus*-host. With regard to the graft-*versus*-host results, the unadjusted cumulative incidence rates of relapse were 55.6% (95%CI: 43.9-65.7%) in the 0 MM group, 45.2% (95%CI: 37.3-52.8%) in the 1 MM group, and 46.8% (95%CI: 41.8-51.7%) in the ≥ 2 MM group.

(0 MM, 0.0%; 1 MM, 11.8%; ≥2 MM 10.9%) were increased in the 1 MM group and the ≥2 MM group (*Online Supplementary Table S3*). With regard to the graft-*versus*-first donor outcomes, there were no significant differences in TRM, relapse, or OS among the three groups (Table 4). In addition, no allele MM was associated with relapse, TRM, or OS in the analysis of each HLA allele MM (*Online Supplementary Table S2*).

Analyses by stem cell sources

Finally, we performed analyses according to stem cell source (*Online Supplementary Tables S4 and 5*). We did not observe any obvious statistically heterogeneity among stem cell sources. However, the small sample size for some categories partially precluded evaluation of significance.

Discussion

There have been several studies on the role of donor change in the outcome of second HSCT; however, these studies were performed mainly in HLA-matched or 1 Ag-MM cases and focused on procedures in which a second HSCT from the same donor was performed.[1-9] In this study, we evaluated the role of HLA discrepancy between the graft and host and between the graft and the first donor on the outcome of second HSCT after HLA-MM initial HSCT. On evaluating 646 recipients of a second HSCT, it was found that graft-host HLA-match was associated with a reduced rate of TRM compared to HLA-MM, while HLA discrepancy between the graft and the first donor had no impact on the outcome of second HSCT.

In the largest retrospective analysis performed to date (n=1285 patients) to compare the incidence of GvHD in the same cohort, the incidence rate of grade II-IV acute GvHD in first HSCT was 26% *versus* 46% in second HSCT.[24] In our study, the incidence of grade II-IV and

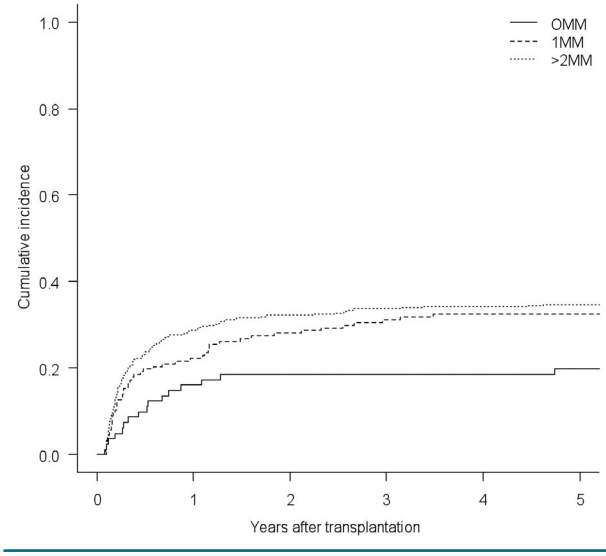

Figure 3. The unadjusted cumulative incidence of transplant-related mortality (TRM) by HLA mismatch (MM) for graft-*versus*-host. With regard to the graft-*versus*-host results, the unadjusted cumulative incidence rates of treatment-related mortality TRM were 19.8% (95%CI: 11.8-29.2%) in the 0 MM group, 32.5% (95%CI: 25.2-39.9%) in the 1 MM group, and 34.7% (95%CI: 30.0-39.4%) in the ≥ 2 MM group.

grade III-IV acute GvHD for first HSCT was 36.4% and 9.0% *versus* 34.2% and 11.2%, respectively, for second HSCT. Due to the higher rate of GvHD for second HSCT, prevention of acute GvHD represents an important, and as yet unmet, medical need.

Experimental murine studies reported that hematopoietic APCs play an important role in the induction of acute GvHD in an MHC MM setting.[13-16]

In the present study, HLA-MM between the graft and first donor was not associated with an increased risk of acute GvHD in HSCT recipients having hematopoietic

Table 3. Effect of HLA allele mismatch on Grades III to IV acute graft-*versus*-host disease (GvHD) by affected organ.

	HLA mismatch for graft-*versus*-host		
	Match (N=85)	1 allele mismatch (N=160)	≥2 allele mismatch (N=401)
Skin GvHD			
SHR1 (95%CI)	1 (ref)	2.49 (0.87-7.13, *P*=0.088)	2.94 (0.94-9.19, *P*=0.063)
Gut GvHD			
SHR1 (95%CI)	1 (ref)	3.33 (0.90-12.3, *P*=0.072)	3.14 (0.82-12.0, *P*=0.094)
Liver GvHD			
SHR1 (95%CI)	1 (ref)	2.16 (0.53-8.85, *P*=0.283)	3.24 (0.73-14.4, *P*=0.122)

[1]Adjusted for recipient age at transplant (continuous), recipient gender, gender mismatch (match, male to female, female to male, unknown), diagnosis (acute myeloid leukemia, acute lymphoblastic leukemia, chronic myeloid leukemia, myelodysplastic syndrome, malignant lymphoma or others), disease risk at transplant (standard or high), stem cell source (bone marrow, peripheral blood, cord blood), conditioning regimen (myeloablative or reduced intensity), graft-*versus*-host disease (GvHD) prophylaxis (cyclosporine based, tacrolimus based, others), *in vivo* T-cell depletion (Yes, No), year of transplant (1994-2010, 2011-2016), interval between first and second stem cell transplantation (SCT) (<12 months, ≥12-23 months, ≥24 months, missing) and interval between first SCT and relapse (<2 months, ≥2-12 months, ≥12 months, missing). SHR: subdistribution hazard ratios.

Table 4. Effect of HLA allele mismatch on transplant-related mortality, relapse and overall survival in multivariate analyses.

	HLA mismatch for graft-*versus*-host			HLA mismatch for graft-*versus*-first donor		
	Match (N=85)	1 allele mismatch (N=160)	≥2 allele mismatch (N=401)	Match (N=72)	1 allele mismatch N=100)	≥2 allele mismatch (N=474)
Transplant-related mortality						
SHR[1] (95%CI)	1 (ref)	1.67 (0.94-2.98, *P*=0.081)	**1.90** (1.04-3.50, *P*=0.038)	1 (ref)	0.89 (0.52-1.52, *P*=0.665)	0.67 (0.42-1.07, *P*=0.095)
Relapse						
SHR[1] (95%CI)	1 (ref)	0.73 (0.50-1.07, *P*=0.110)	0.68 (0.44-1.06, *P*=0.086)	1 (ref)	1.18 (0.72-1.95, *P*=0.516)	1.41 (0.89-2.22, *P*=0.143)
Overall survival						
HR[1] (95%CI)	1 (ref)	1.00 (0.72-1.41, *P*=0.952)	1.21 (0.84-1.73, *P*=0.313)	1 (ref)	0.84 (0.57-1.21, *P*=0.347)	0.85 (0.61-1.17, *P*=0.313)

*Bold denotes statistical significance. [1]Adjusted for recipient age at transplant (continuous), recipient gender, gender mismatch (match, male to female, female to male, unknown), diagnosis (acute myeloid leukemia, acute lymphoblastic leukemia, chronic myeloid leukemia, myelodysplastic syndrome, malignant lymphoma or others), disease risk at transplant (standard or high), stem cell source (bone marrow, peripheral blood, cord blood), conditioning regimen (myeloablative or reduced intensity), graft-*versus*-host disease (GvHD) prophylaxis (cyclosporine based, tacrolimus based, others), *in vivo* T-cell depletion (Yes, No), year of transplant (1994-2010, 2011-2016), interval between first and second stem cell transplantation (SCT) (<12 months, ≥12-23 months, ≥24 months, missing) and interval between first SCT and relapse (<2 months, ≥2-12 months, ≥12 months, missing). SHR: subdistribution hazard ratios; HR: hazard ratios.

APCs originating from the first donor. The antigen-presenting function of the first-donor hematopoietic cells may be insufficiently strong to induce GvHD. An alternative explanation is that recipient hematopoietic APCs have a limited capacity to induce acute GvHD, possibly owing to their predisposition to induce donor T-cell death.[11] In contrast, HLA discrepancy between the graft and host may impact the risk of acute GvHD during the second transplant. In this study, HLA-MM between the graft and host showed increased risk of grade III-IV acute GvHD, although the results were not significant. In addition, B allele MM was significantly associated with an increased risk of grade III-IV acute GvHD in the analysis of each HLA allele mismatch [relative risk (RR) 2.87, 95%CI: 1.42-5.79; *P*=0.003]. Several experimental studies showed that non-hematopoietic gastrointestinal cells are able to express MHC class II and induce $CD4^+$ T-cell-dependent acute GvHD.[10,11] As the antigen-presenting function of epithelial cells is enhanced in the presence of an inflammatory environment, epithelial cells after the first HSCT could play a major role in inducing GvHD following second HSCT, although further studies are needed to validate this.

The length of remission after first HSCT and the disease status at second HSCT, are two main independent prognostic factors for predicting the outcome of a second HSCT.[2,3,5] Despite a significant increase in the proportion of patients of advanced age, having an advanced disease stage, and receiving alternative donor transplants, there has been a continual decrease in TRM, reflecting the impact of advances in supportive care and more widespread use of reduced-intensity conditioning regimens. However, the reduction in rate of TRM has been less obvious in patients following a second remission or refractory disease.[25] Due to more advanced disease and accumulating toxicity, second transplants are more problematic than first transplants, and often result in an increase in TRM and overall mortality rates. Attempted enhancement of the GvT effect by switching donor may be affected by the toxicity of the second HSCT. Reducing TRM remains one of the most significant challenges in second HSCT. Our analysis showed that HLA-MM between the graft and first donor had no influence on GvHD, relapse, TRM, or OS. In contrast, with regard to graft-*versus*-host, the risk of TRM was significantly higher in the ≥2 MM group *versus* the 0 MM group (RR, 1.90; 95%CI: 1.04-3.50; *P*=0.038). Analysis of each HLA allele MM revealed that the DR allele MM was significantly associated with a lower rate

of relapse *versus* the 0 MM group (RR, 0.75; 95%CI: 0.58-0.95; *P*=0.018), but this was offset by a higher rate of TRM (RR, 1.44; 95%CI: 1.03-2.00; *P*=0.033). Our data suggested that use of an HLA-MM donor may induce a more potent GvL effect, but also increases the allogeneic responses of the second HSCT and provokes an increase in TRM events. These effects tended to cancel each other out in respect to OS.

This is the first study to focus on patients after initial HLA-MM transplantation and identify risk factors for a poor second HSCT outcome. However, several limitations of the study should be mentioned. First, although this was a relatively large-scale study on second transplant, the sample size was still modest, and therefore further studies with larger sample sizes are required. Second, it used a retrospective design and included a heterogeneous patient group. Moreover, the strategies of the different treatment centers with respect to donor change are unknown, and any heterogeneity in transplantation procedure, year of transplant, and patients' characteristics may have biased the results, although we attempted to reduce bias by adjusting for these factors in multivariate analyses. Third, we did not adjust for multiple comparisons and therefore caution is required when interpreting the results, in particular those of the stratified analyses. In addition, HLA-C typing and high-resolution DNA typing were either rarely, or not routinely, performed on the donors. Finally, donor chimerism was not systematically analyzed and cell subset chimerism data were not available for most patients.

In conclusion, HLA-MM donor is an option after initial HLA-MM transplantation. However, TRM remains a challenge, particularly with a ≥2 MM donor regarding graft-*versus*-host. In this study, the biological effects of HLA discrepancy between the graft and the first donor on the outcome appeared negligible, and our findings shed light on the role of non-hematopoietic APCs on transplant-related immunological responses.

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References

1. Bosi A, Laszlo D, Labopin M, et al. Second allogeneic bone marrow transplantation in acute leukemia: results of a survey by the European Cooperative Group for Blood and Marrow Transplantation. J Clin Oncol. 2001;19(16):3675-3684.
2. Shaw BE, Mufti GJ, Mackinnon S, et al. Outcome of second allogeneic transplants using reduced-intensity conditioning following relapse of haematological malignancy after an initial allogeneic transplant. Bone Marrow Transplant. 2008;42(12):783-789.
3. Eapen M, Giralt SA, Horowitz MM, et al. Second transplant for acute and chronic leukemia relapsing after first HLA-identical sibling transplant. Bone Marrow Transplant. 2004;34(8):721-727.
4. Christopeit M, Kuss O, Finke J, et al. Second allograft for hematologic relapse of acute leukemia after first allogeneic stem-cell transplantation from related and unrelated donors: the role of donor change. J Clin Oncol. 2013; 31(26):3259-3271.
5. Ruutu T, de Wreede LC, van Biezen A, et al. Second allogeneic transplantation for relapse of malignant disease: retrospective analysis of outcome and predictive factors by the EBMT. Bone Marrow Transplant. 2015; 50(12):1542-1550.
6. Vrhovac R, Labopin M, Ciceri F, et al. Second reduced intensity conditioning allogeneic transplant as a rescue strategy for acute leukaemia patients who relapse after an initial RIC allogeneic transplantation: analysis of risk factors and treatment outcomes. Bone Marrow Transplant. 2016;51(2):186-193.
7. Orti G, Sanz J, Bermudez A, et al. Outcome of Second Allogeneic hematopoietic cell transplantation after relapse of myeloid malignancies following allogeneic hematopoietic cell transplantation: a Retrospective Cohort on Behalf of the Grupo Espanol de Trasplante Hematopoyetico. Biol Blood Marrow Transplant. 2016;22(3):584-588.
8. Andreola G, Labopin M, Beelen D, et al. Long-term outcome and prognostic factors of second allogeneic hematopoietic stem cell transplant for acute leukemia in patients with a median follow-up of 10 years. Bone Marrow Transplant. 2015;50(12):1508-1512.
9. Horstmann K, Boumendil A, Finke J, et al. Second allo-SCT in patients with lymphoma relapse after a first allogeneic transplantation. A retrospective study of the EBMT Lymphoma Working Party. Bone Marrow Transplant. 2015;50(6):790-794.
10. Jones SC, Murphy GF, Friedman TM, Korngold R. Importance of minor histocompatibility antigen expression by non-hematopoietic tissues in a CD4+ T cell-mediated graft-versus-host disease model. J Clin Invest. 2003;112(12):1880-1886.
11. Koyama M, Kuns RD, Olver SD, et al. Recipient nonhematopoietic antigen-presenting cells are sufficient to induce lethal acute graft-versus-host disease. Nat Med. 2011; 18(1):135-142.
12. Shlomchik WD, Couzens MS, Tang CB, et al. Prevention of graft versus host disease by inactivation of host antigen-presenting cells. Science. 1999;285(5426):412-415.
13. Teshima T, Ordemann R, Reddy P, et al. Acute graft-versus-host disease does not require alloantigen expression on host epithelium. Nat Med. 2002;8(6):575-581.
14. Duffner UA, Maeda Y, Cooke KR, et al. Host dendritic cells alone are sufficient to initiate acute graft-versus-host disease. J Immunol. 2004;172(12):7393-7398.
15. Zhang Y, Louboutin JP, Zhu J, Rivera AJ, Emerson SG. Preterminal host dendritic cells in irradiated mice prime CD8+ T cell-mediated acute graft-versus-host disease. J Clin Invest. 2002;109(10):1335-1344.
16. Reddy P, Maeda Y, Liu C, Krijanovski OI, Korngold R, Ferrara JL. A crucial role for antigen-presenting cells and alloantigen expression in graft-versus-leukemia responses. Nat Med. 2005;11(11):1244-1249.
17. Atsuta Y, Suzuki R, Yoshimi A, et al. Unification of hematopoietic stem cell transplantation registries in Japan and establishment of the TRUMP System. Int J Hematol. 2007;86(3):269-274.
18. Atsuta Y. Introduction of Transplant Registry Unified Management Program 2 (TRUMP2): scripts for TRUMP data analyses, part I (variables other than HLA-related data). Int J Hematol. 2016;103(1):3-10.
19. Kanda J. Scripts for TRUMP data analyses. Part II (HLA-related data): statistical analyses specific for hematopoietic stem cell transplantation. Int J Hematol. 2016;103(1):11-19.
20. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. Bone Marrow Transplant. 1995;15(6):825-828.
21. Sullivan KM, Agura E, Anasetti C, et al. Chronic graft-versus-host disease and other late complications of bone marrow transplantation. Semin Hematol. 1991;28(3):250-259.
22. Gooley TA, Leisenring W, Crowley J, Storer BE. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. Stat Med. 1999; 18(6):695-706.
23. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant. 2013; 48(3):452-458.
24. Duncan CN, Majhail NS, Brazauskas R, et al. Long-term survival and late effects among one-year survivors of second allogeneic hematopoietic cell transplantation for relapsed acute leukemia and myelodysplastic syndromes. Biol Blood Marrow Transplant. 2015;21(1):151-158.
25. Bacigalupo A, Sormani MP, Lamparelli T, et al. Reducing transplant-related mortality after allogeneic hematopoietic stem cell transplantation. Haematologica. 2004; 89(10):1238-1247.