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Notes

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Regular Article

TRANSPLANTATION

Biological significance of HLA locus matching in unrelated donor bone marrow transplantation

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Key Points

- Significant HLA locus mismatches responsible for transplant-related events were determined in 7898 unrelated marrow donor transplants.
- This information provides a rationale for use of an algorithm for unrelated donor selection.

We hypothesized that the compatibility of each HLA loci between donor and patient induced divergent transplant-related immunologic responses, which attributed to the individualized manifestation of clinical outcomes. Here, we analyzed 7898 Japanese pairs transplanted with T-cell–replete marrow from an unrelated donor with complete HLA allele typing data. Multivariable competing risk regression analyses were conducted to evaluate the relative risk (RR) of clinical outcomes after transplantation. A significant RR of HLA allele mismatch compared with match was seen with HLA-A, -B, -C, and -DPB1 for grade III-IV acute graft-versus-host disease (GVHD), and HLA-C for chronic GVHD. Of note, only HLA-C and HLA-DPB1 mismatch reduced leukemia relapse, and this graft-versus-leukemia effect of HLA-DPB1 was independent of chronic GVHD. HLA-DRB1 and HLA-DQB1 double (DRB1_DQB1) mismatch was revealed to be a significant RR for acute GVHD and mortality, whereas single mismatch was not. Thus, the number of HLA-A, -B, -C, -DPB1, and DRB1_DQB1 mismatches showed a clear-cut risk difference for acute GVHD, whereas the number of mismatches for HLA-A, -B, -C, and DRB1_DQB1 showed the same for mortality. In conclusion, we determined the biological response to HLA locus mismatch in transplant-related immunologic events, and provide a rationale for use of a personalized algorithm for unrelated donor selection. (*Blood.* 2015;125(7):1189-1197)

Introduction

Allogeneic hematopoietic stem cell transplantation from unrelated donors (UR-HSCT) has been established as a mode of curative therapy for hematologic malignancies and other hematologic or immunologic disorders when an HLA-identical sibling donor is unavailable. Identification of the HLA locus matching at the allele level responsible for immunologic events related to HSCT is important in optimizing HLA matching and minimizing graft-versus-host disease (GVHD) and engraftment failure, as well as in enhancing the graft-versus-leukemia (GVL) effect.[1-3]

In the late 1990s, the Japan Marrow Donor Program (JMDP) demonstrated for the first time the effect of matching of HLA class I alleles on acute GVHD and the importance of HLA-A and -B allele matching for survival.[2] Analysis of a large cohort in the United States also indicated that HLA allele mismatching is a significant risk factor for severe acute GVHD and mortality.[3] Subsequent extensive analysis of the JMDP, US National Marrow Donor Program (NMDP), European registries, and the International Histocompatibility Workshop Group (IHWG) revealed considerable evidence that HLA allele

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compatibility,[4-11] HLA haplotype,[12,13] and HLA epitope[14-16] are significantly associated with clinical outcomes.

We hypothesized that the compatibility of the respective HLA loci between donor and patient accounts for the divergence in transplant-related immunologic responses, and that this effect influences the individualized manifestation of clinical outcomes overall.

Here, to elucidate the biological effects of HLA locus matching on clinical outcomes, we selected pairs transplanted with T-cell–replete marrow for whom precise data for the complete HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 alleles were obtained by retyping.

Methods

Study population

Unrelated donor transplant pairs (7898) from the JMDP database met the following criteria and were included in the analysis: (1) transplantation pairs retyped for HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 alleles; (2) T-cell–replete marrow without in vivo use of anti-thymocyte globulin or anti-T-cell monoclonal antibody for GVHD prophylaxis; (3) first transplantation; (4) Japanese ethnicity; and (5) survival for >7 days after transplantation. All pairs were transplanted between January 1993 and December 2010. A total of 12 502 pairs were facilitated through the JMDP during this period. The present 7898 study pairs with retyped HLA data consisted of 74.7% of the 10 575 pairs who matched selection criteria 2 to 5. No significant difference in clinical factors was seen between the HLA retyped and nonretyped pairs (data not shown). Patient diagnosis is listed in Table 1. Standard-risk leukemia was defined as chronic myeloid leukemia (CML) in the first chronic phase or acute lymphoblastic leukemia (ALL) and acute myeloblastic leukemia (AML) in the first complete remission (CR) at the time of transplantation, and diagnosed in 2508 patients, whereas high-risk leukemia was defined as transplantation at a more advanced stage than in standard-risk leukemia, and was diagnosed in 2772 patients. Sex matching between donor and patient was female (donor) to male (patient) in 1494 pairs, male to male in 3253, female to female in 1442, and male to female in 1709. For GVHD prophylaxis, no patient had in vivo use of anti-thymocyte globulin or a monoclonal antibody such as CAMPATH-1H. Tacrolimus-based regimens were used in 4779 patients, in combination with methotrexate in 4529; cyclosporine-based regimens were used in 3078, in combination with methotrexate in 2993; and other regimens were used in 41. The conditioning regimen was classified as myeloablative if it included total body irradiation (TBI) ≥8 Gy, oral busulfan (Bu) ≥9 mg/kg, IV Bu ≥7.2 mg/kg, or melphalan >140 mg/m^2; otherwise, it was classified as a reduced-intensity regimen. Transplantation conditioning was done with a myeloablative regimen in 6653 patients and with a reduced-intensity regimen in 1245 patients. Patient and donor characteristics and HLA matching in the GVH direction in total pairs are shown in Table 1, and by HLA locus matching in supplemental Table 1 (see supplemental Data available on the *Blood* Web site).

A final clinical survey of patients was completed by September 2012 using the Transplant Registry Unified Management Program.[17] Informed consent was obtained from patients and donors in accordance with the Declaration of Helsinki, and approval for the study was obtained from the Institutional Review Board of Aichi Cancer Center and the JMDP.

Outcome definition

Mortality was defined as time from transplantation to death from any cause. Clinical grading of acute GVHD was performed according to established criteria.[18,19] Chronic GVHD was defined as limited or extensive chronic GVHD according to the Seattle criteria.[20] Neutrophil engraftment was defined as more than 500 cells per cubic millimeter in peripheral blood at 3 consecutive measurements. Relapse was evaluated in patients with AML, ALL, or CML.

Table 1. Patient and donor characteristics

Characteristics	Value
HLA locus matching match/mismatch, no. (%)	
HLA-A	7048 (89)/850 (11)
HLA-B	7475 (95)/423 (5)
HLA-C	5565 (70)/2333 (30)
HLA-DRB1	5878 (74)/2020 (26)
HLA-DQB1	5681 (72)/2217 (28)
HLA-DPB1	2604 (33)/5294 (67)
Patient age, y	
Median (range)	35 (0-77)
Donor age, y	
Median (range)	34 (20-56)
Disease, no. (%)	
Acute lymphoblastic leukemia	1861 (24)
Acute myeloblastic leukemia	2609 (33)
Chronic myeloid leukemia	983 (12)
Myelodysplastic syndrome	841 (11)
Other leukemia	312 (4)
Lymphoid malignancy	542 (7)
Aplastic anemia	489 (6)
Multiple myeloma	33 (<1)
Others	228 (3)
GVHD prophylaxis, no. (%)	
Cyclosporine based	3078 (39)
Tacrolimus based	4779 (61)
Others	41 (<1)
Leukemia risk, no. (%)	
Standard	2508 (32)
High	2772 (35)
N/A	2618 (33)
Conditioning, no. (%)	
Myeloablative	6653 (84)
Reduced intensity	1245 (16)
Sex matching (donor to patient), no. (%)	
Female to male	1494 (19)
Male to male	3253 (41)
Female to female	1442 (18)
Male to female	1709 (22)
Transplanted year period, no. (%)	
1993-2000	2311 (29)
2001-2005	3084 (39)
2006-2010	2503 (32)

Patient and donor characteristics by HLA locus matching are shown in supplemental Table 1.

N/A, not applicable.

HLA typing and matching

All donor-patient pairs were retrospectively genotyped between 2009 and 2011 for all HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 alleles at the field 1 and field 2 level of the 2010 World Health Organization Nomenclature for factors of the HLA system.[21] The polymerase chain reaction–sequence specific oligonucleotide method was used for all samples, and the polymerase chain reaction–sequencing based typing method was used to confirm rare alleles and new alleles. HLA alleles were identified with >99.9% accuracy among Japanese. HLA alleles and their number are shown in supplemental Table 2, which also shows HLA loci and their level at confirmatory typing before transplantation.

HLA locus mismatch among the donor-recipient pairs was scored when the recipient's HLA alleles or antigens were not shared by the donor in the GVH direction for acute GVHD, chronic GVHD, leukemia relapse and survival analysis, and in the HVG direction for neutrophil engraftment. HLA allele match rate in the GVH direction by HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 was 89.2%, 94.6%, 70.5%, 74.4%, 71.9%, and 33.0%, respectively, whereas serological HLA antigen match rate in the GVH direction by HLA-A, -B, -C, and -DR was 99.7%, 99.5%, 72.3%, and 91.8%, respectively.

Table 2. Effect of HLA locus matching on acute GVHD and chronic GVHD in a multivariable competing risk regression model

			Acute GVHD (Grade III-IV)†			Acute GVHD (Grade II-IV)†				Chronic GVHD‡		
HLA	Match or mismatch*	N	RR	95% CI	P	RR	95% CI	P	N	RR	95% CI	P
A	Match	7048	1.00		.001	1.00		.002	5892	1.00		.328
	Mismatch	850	1.29	1.10-1.51		1.18	1.06-1.32		636	1.06	0.94-1.21	
B	Match	7475	1.00		.001	1.00		.001	6217	1.00		.235
	Mismatch	423	1.42	1.16-1.73		1.28	1.11-1.48		311	1.10	0.94-1.30	
C	Match	5565	1.00		<.001	1.00		<.001	4716	1.00		<.001
	Mismatch	2333	1.63	1.45-1.83		1.27	1.17-1.37		1812	1.24	1.13-1.35	
DRB1	Match	5878	1.00		.022	1.00		<.001	4936	1.00		.262
	Mismatch	2020	1.21	1.03-1.43		1.24	1.11-1.39		1592	0.93	0.82-1.05	
DQB1	Match	5681	1.00		.336	1.00		.126	4758	1.00		.018
	Mismatch	2217	1.08	0.92-1.27		1.09	0.98-1.22		1770	1.15	1.03-1.30	
DPB1	Match	2604	1.00		.001	1.00		<.001	2223	1.00		.367
	Mismatch	5294	1.23	1.09-1.38		1.36	1.26-1.47		4305	1.04	0.96-1.12	

RR of respective HLA locus mismatches at the allele level was compared with HLA match adjusted with other HLA locus matching and clinical factors as listed in Table 1.
CI, confidence interval.
*GVH direction.
†Survived 7 or more days.
‡Survived 100 or more days.

Statistical analysis

Cumulative incidence of acute GVHD was assessed by a method described elsewhere.[22] Overall survival was calculated using the Kaplan-Meier method. Competing events were defined as death without acute GVHD for acute GVHD; death without chronic GVHD for chronic GVHD; death without neutrophil engraftment for neutrophil engraftment; and death without relapse for leukemia relapse. Multivariable competing risk regression analyses[23,24] were conducted to evaluate the impact of acute GVHD, chronic GVHD, leukemia relapse and neutrophil engraftment, and a Cox proportional regression model was used to evaluate the impact of mortality. The relative risk (RR) of HLA locus mismatch was compared with HLA locus match in the GVH direction for acute GVHD, chronic GVHD, leukemia relapse and mortality, and in the HVG direction for neutrophil engraftment. Confounders considered were sex (donor-recipient pair), patient age (linear), donor age (linear), disease, risk of leukemia relapse (standard and high), GVHD prophylaxis (cyclosporine-based regimen, tacrolimus-based regimen, and other regimen without cyclosporine and tacrolimus), preconditioning (myeloablative and reduced intensity), and period of transplant year (1992-2000, 2001-2005, 2006-2010). Transplanted cell number and ABO blood type matching were added as confounders in analyses of neutrophil engraftment. Missing data for confounder variables were treated as an unknown group. Acute GVHD, leukemia relapse, neutrophil engraftment, and survival were assessed in patients who survived >7 days, and chronic GVHD at 2 years was assessed in patients who survived 100 or more days after transplantation. Leukemia relapse at 5 years was assessed in patients who survived >7 days after transplantation for leukemia with AML, ALL, and CML. Risk of chronic GVHD on leukemia relapse was assessed by time-dependent covariate analysis in leukemia patients who survived 100 or more days after transplantation. Neutrophil engraftment at 100 days was assessed in all patients. A P value of <.01 was considered significant. All analyses were conducted using STATA version 12 (Stata Corp).

Results

Effect of HLA locus matching on acute GVHD and chronic GVHD

RR of HLA allele mismatch compared with HLA allele match for grade III-IV acute GVHD was highly significant for HLA-A, -B, -C, and -DPB1 (RR 1.29, $P = .001$; 1.42, $P = .001$; 1.63, $P < .001$; and 1.23, $P = .001$, respectively), but was not significant for HLA-DRB1 or -DQB1 (Table 2). RR of grade II-IV acute GVHD was highly significant for HLA-A, -B, -C, -DRB1, and -DPB1 (RR 1.18, $P = .002$; 1.28, $P = .001$; 1.27, $P < .001$; 1.24, $P < .001$; and 1.36, $P < .001$, respectively), but was not significant for HLA-DQB1 (Table 2).

RR of HLA allele mismatch compared with HLA allele match for chronic GVHD was significant for HLA-C (RR 1.24 $P < .001$), but not significant for HLA-A, -B, -DRB1, -DQB1, or -DPB1 (Table 2).

Effect of HLA locus matching on survival

RR of HLA allele mismatch compared with HLA allele match for mortality was highly significant in the HLA class I locus, namely HLA-A (1.29, $P < .001$), HLA-B (1.27, $P < .001$) and HLA-C (1.21, $P < .001$), but was not significant in the HLA class II locus, namely HLA-DRB1, -DQB1, and -DPB1 (Table 3).

Positive interaction of HLA-DRB1 mismatch and HLA-DQB1 mismatch in the risk of acute GVHD and survival

As HLA-DRB1 and HLA-DQB1 matching are closely linked in the HLA region and matching probability for HLA-DRB1 and HLA-DQB1 was 89%, stratified analysis of HLA-DRB1 matching and HLA-DQB1 matching was performed (Table 4). Pairs with HLA-DRB1 and HLA-DQB1 double (DRB1_DQB1) mismatch showed a significant risk of acute GVHD compared with pairs with both DRB1_DQB1 match (RR of grade III-IV, 1.32, $P < .001$; and RR of grade II-IV, 1.34, $P < .001$). HLA-DRB1 mismatch alone or HLA-DQB1 mismatch alone showed no significant difference in either grade III-IV or grade II-IV acute GVHD from DRB1_DQB1 match, respectively. Thus, DRB1_DQB1 mismatch induced a greater effect on acute GVHD than would be expected from the independent effect of either HLA-DRB1 or HLA-DQB1 mismatch alone.

As with acute GVHD, stratified analysis of both HLA locus matching showed that pairs with DRB1_DQB1 mismatch were at significantly higher risk of mortality than pairs with DRB1_ DQB1 match (RR 1.17, $P < .001$) (Table 4). In contrast, risk with HLA-DRB1 mismatch alone or HLA-DQB1 mismatch alone was not significantly different from that with DRB1_DQB1 match (RR 1.04, $P = .662$ and RR 1.04, $P = .532$, respectively).

The risk of double HLA locus mismatch combinations other than DRB1_DQB1 for grade III to IV acute GVHD and mortality were analyzed. As shown in supplemental Table 3, none of these double mismatch combinations revealed an epistatic effect of double HLA locus mismatch.

Table 3. Effect of HLA locus matching on leukemia relapse, engraftment, and mortality

		Leukemia relapse†				Engraftment‡				Mortality			
HLA	Match or mismatch*	N	RR	95% CI	P	N	RR	95% CI	P	N	RR	95% CI	P
A	Match	4847	1.00		.381	6898	1.00		.035	7048	1.00		<.001
	Mismatch	606	0.92	0.76-1.11		851	0.93	0.87-0.99		850	1.29	1.17-1.42	
B	Match	5163	1.00		.493	7320	1.00		.146	7475	1.00		<.001
	Mismatch	290	0.91	0.69-1.20		429	0.93	0.84-1.03		423	1.27	1.11-1.45	
C	Match	3865	1.00		<.001	5511	1.00		.049	5565	1.00		<.001
	Mismatch	1588	0.70	0.61-0.80		2238	0.95	0.90-1.00		2333	1.21	1.13-1.30	
DRB1	Match	4045	1.00		.468	5763	1.00		.212	5878	1.00		.125
	Mismatch	1408	0.93	0.76-1.14		1986	0.95	0.89-1.03		2020	1.09	0.98-1.21	
DQB1	Match	3924	1.00		.974	5583	1.00		.014	5681	1.00		.145
	Mismatch	1529	1.00	0.83-1.22		2166	0.91	0.85-0.98		2217	1.08	0.97-1.19	
DPB1	Match	1792	1.00		<.001	2531	1.00		.126	2604	1.00		.349
	Mismatch	3661	0.69	0.61-0.77		5218	0.97	0.92-1.01		5294	1.03	0.96-1.11	

Multivariable competing risk regression analyses were conducted to evaluate the impact of leukemia relapse and neutrophil engraftment, and a Cox proportional regression model was conducted for mortality. RR of respective HLA locus mismatches at the allele level was compared with HLA match adjusted with other HLA locus matching and the clinical factors listed in Table 1 for leukemia relapse and mortality. Transplanted cell number and ABO blood type matching were added for neutrophil engraftment.

*GVH direction for leukemia relapse and mortality; HVG direction for engraftment.

†At 5 years after transplantation.

‡Neutrophil recovery to successive >500 per microliter measurement at 3 time points in 100 days.

The same results were obtained using the same stratified analysis of HLA-DRB1 and -DQB1 with serological HLA-A, -B, and -DR match pairs (supplemental Table 4).

Effect of HLA locus matching on leukemia relapse

The occurrence of leukemia relapse within 5 years after transplantation was analyzed in patients with AML, ALL, and CML. RR of HLA allele mismatch compared with HLA allele match for leukemia relapse was low with high significance in HLA-C (RR 0.70, $P < .001$) and -DPB1 (RR 0.69, $P < .001$), but was not significant in HLA-A, -B, -DRB1, or -DQB1 (Table 3).

Independence of GVL effect of HLA-DPB1 mismatch from chronic GVHD

As described in the previous paragraph, HLA-DPB1 mismatch induced the GVL effect, but did not induce chronic GVHD. Chronic GVHD also induced the GVL effect. Therefore, the GVL effect of HLA-DPB1 matching in relation to chronic GVHD was analyzed in 2129 leukemia patients with HLA-A, -B, -C, -DRB1, and -DQB1 allele complete match donors who survived 100 or more days after transplantation. Multivariate competing risk regression analysis, including HLA-DPB1 matching and chronic GVHD, were performed with chronic GVHD treated as a time-dependent covariate (Table 5). Both limited-type chronic GVHD and extensive-type chronic GVHD were associated with a significantly lower leukemia relapse risk than no chronic GVHD. Furthermore, 1 and 2 DPB1 allele mismatch was associated with a significantly lower leukemia relapse risk than HLA-DPB1 match. Interaction analysis between HLA-DPB1 matching and chronic GVHD was not significant (RR 1.26, 95% CI 0.85-1.88, $P = .255$), indicating the lack of any effect modification between HLA-DPB1 matching and chronic GVHD.

When acute GVHD was added to this analysis, RR of grade III-IV acute GVHD and grade II-IV acute GVHD was 0.77 (95% CI 0.57-1.04, $P = .091$) and 0.82 (95% CI 0.68-0.99, $P = .038$), respectively. Thus, the effect of acute GVHD on leukemia relapse was not significant in patients who survived more than 100 days after transplantation.

Effect of HLA locus matching on neutrophil engraftment

Engraftment risk of neutrophils at 100 days after transplantation was assessed in all patients. Although RR of engraftment by HLA locus mismatch in the HVG direction showed the relatively lower risk range of 0.91 to 0.97 compared with HLA locus match in all 6 HLA loci, there was no significant HLA locus matching for neutrophil engraftment (Table 4).

Effect of multiple HLA locus mismatch on acute GVHD and survival

As the above HLA locus matching analysis indicated that multiple HLA locus mismatch was associated with a higher risk of adverse

Table 4. Stratified analysis of HLA-DRB1 and HLA-DQB1 matching on acute GVHD and survival

		Acute GVHD (Grade III-IV)†			Acute GVHD (Grade II-IV)†			Mortality†		
HLA matching*	N	RR	95% CI	P	RR	95% CI	P	RR	95% CI	P
DRB1 match and DQB1 match	5356	1.00			1.00			1.00		
DRB1 mismatch and DQB1 match	325	0.98	0.74-1.28	.866	1.19	1.00-1.42	.046	1.04	0.88-1.22	.662
DRB1 match and DQB1 mismatch	522	0.92	0.73-1.16	.482	1.05	0.91-1.21	.517	1.04	0.92-1.19	.532
DRB1 mismatch and DQB1 mismatch	1695	1.32	1.16-1.50	<.001	1.34	1.23-1.46	<.001	1.17	1.08-1.27	<.001

Multivariable competing risk regression analyses were conducted to evaluate the impact of acute GVHD and Cox proportional regression model for mortality. RR of the combination of HLA-DRB1 and/or -DQB1 mismatch was compared with HLA-DRB1 and -DQB1 match. Adjusted confounders were HLA-A, -B, -C, and -DPB1 locus matching and the clinical factors listed in Table 1.

*GVH direction.

†Survived 7 or more days.

Table 5. Effect of chronic GVHD and HLA-DPB1 matching on leukemia relapse

	N	RR	95% CI	P
HLA-DPB1				
Match*	804	1.00		
1-allele mismatch*	971	0.70	0.58-0.84	<.001
2-allele mismatch*	354	0.54	0.41-0.72	<.001
Chronic GVHD				
No	1232	1.00		
Limited type	345	0.56	0.42-0.74	<.001
Extensive type	552	0.46	0.36-0.58	<.001

Multivariate competing risk regression analysis including HLA-DPB1 matching and chronic GVHD was performed by treating chronic GVHD as a time-dependent covariate adjusted for the clinical confounders listed in Table 1.

*GVH direction.

clinical outcomes of acute GVHD and survival, we next explored the appropriate HLA mismatch locus combination which revealed the effect of the number of HLA mismatch loci for acute GVHD and survival. The number of HLA 1-allele mismatches was summed after exclusion of 2-allele mismatches in each HLA locus. The combination of HLA-DRB1 1-allele mismatch and HLA-DQB1 1-allele mismatch (DRB1_DQB1 mismatch) was adopted and treated as 1 HLA locus mismatch.

The cumulative incidence curve of grade III-IV acute GVHD by the number of HLA-A, -B, -C, -DPB1 locus mismatches and DRB1_DQB1 mismatch showed a clear-cut risk difference which discriminated 0, 1, 2, 3, and 4 HLA locus mismatches (Figure 1A). Specifically, compared with 0 mismatches (n = 1476), RRs for grade III-IV acute GVHD were 1.37 with 1 mismatch (n = 2549), 2.19 with 2 mismatches (n = 1377), 2.82 with 3 mismatches (n = 415), and 3.25 with 4 mismatches (n = 60) ($P < .001$).

To clarify the risk of a 2 HLA loci single-mismatch combination, each 2 mismatch combination was compared with the combination of HLA-A and -C mismatch for grade III-IV GVHD. As shown in supplemental Table 5, the risk of double mismatch combination pairs showed no significant differences, except DRB1_DQB1 mismatch and -DPB1 mismatch combination, albeit that the number of some of these combinations was too small for any precise evaluation of risk.

The most clear-cut risk difference discriminating 0, 1, 2, 3, and 4 HLA locus mismatches is seen in the Kaplan-Meier curve for survival by the number of HLA locus mismatches of HLA-A, -B, -C, and DRB1_DQB1 (Figure 1B). Compared with 0 mismatches (n = 4076), the RR for mortality was 1.28 with 1 mismatch (n = 2352), 1.57 with 2 mismatches (n = 850), and 1.73 with 3 mismatches (n = 130) ($P < .001$). To clarify the risk of a 2 HLA loci single-mismatch combination, each 2 mismatch combination was compared with the combination of HLA-A and -C mismatch for mortality. As shown in supplemental Table 5, there were no significant differences between each double mismatch combination.

When HLA-DRB1 mismatch and HLA-DQB1 mismatch were added separately to this analysis, the survival curves of 1, 2, 3, 4, and 5 mismatches showed less clear-cut differences (Figure 1C).

Significant clinical factors other than HLA matching which affected transplant-related clinical outcomes

Significant variables ($P < .01$) other than HLA locus matching for acute GVHD, chronic GVHD, leukemia relapse, neutrophil engraftment, and mortality are listed in Table 6. Patient age affected acute GVHD, chronic GVHD and mortality, and donor age affected chronic GVHD and mortality. Compared with ALL, CML showed a lower risk of chronic GVHD, leukemia relapse and mortality, and a higher risk of neutrophil engraftment. AML showed a lower risk of mortality, and aplastic anemia showed a lower risk of acute GVHD, chronic GVHD and mortality. A reduced conditioning regimen

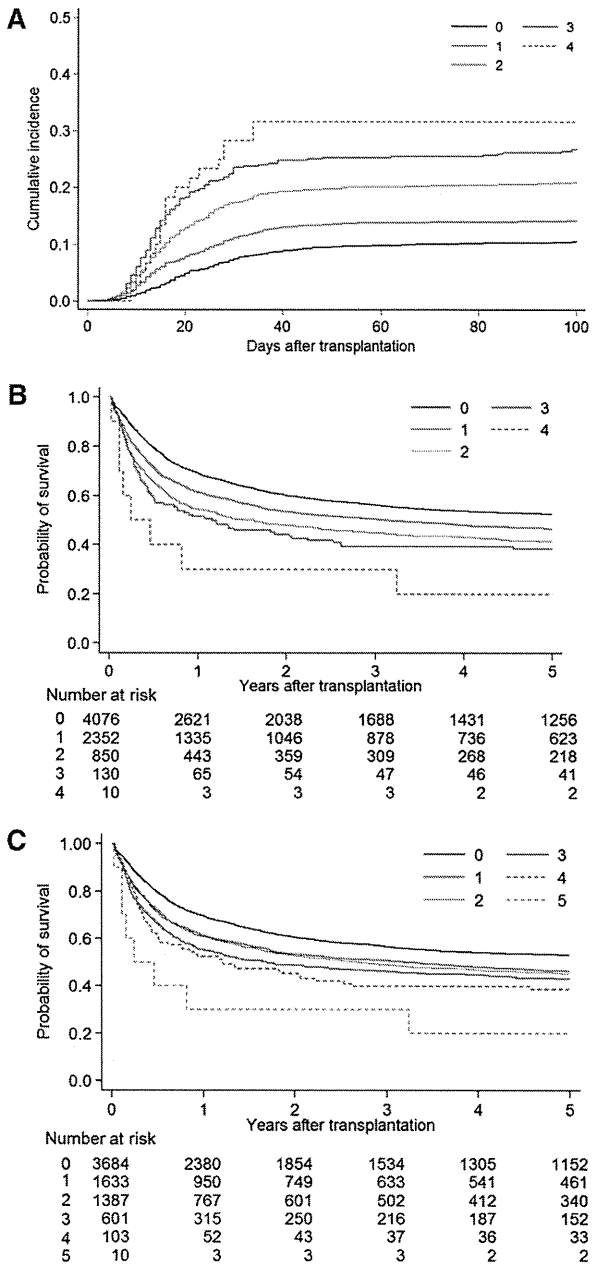

Figure 1. Acute GVHD and survival curve by the number of multiple HLA locus mismatches. The number of HLA 1-allele mismatches in the GVH direction, with exclusion of 2-allele mismatches, in each HLA locus was summed. (A) Cumulative incidence of grade III-IV acute GVHD by the mismatch number of HLA-A, -B, -C, -DRB1_DQB1, and -DPB1 at the allele level in the GVH direction. DRB1_DQB1: both HLA-DRB1 mismatch and HLA-DQB1 mismatch treated as 1 mismatch. 0: no mismatch (n = 1476); 1: 1 mismatch (n = 2549); 2: 2 mismatches (n = 1379); 3: 3 mismatches (n = 415); 4: 4 mismatches (n = 60). Cumulative incidence at 100 days was 0, 11% (95% CI, 9%-12%); 1, 14% (13%-16%); 2, 21% (19%-23%); 3, 27% (23%-31%); and 4, 32% (20%-44%). (B) Kaplan-Meier curve of survival by the mismatch number of HLA-A, -B, -C, and -DRB1_DQB1 at the allele level. Survival rate at 5 years was 0, 53% (95% CI, 51%-54%); 1, 46% (44%-49%); 2, 41% (38%-45%); 3, 38% (30%-47%); and 4, 20% (3%-47%). (C) Kaplan-Meier curve of survival by the mismatch number of HLA-A, -B, -C, -DRB1, and -DQB1 at the allele level.

Table 6. Significant factors other than HLA locus matching for clinical outcomes

Outcomes, Significant factor ($P < .01$)	N	RR	95% CI	P
Acute GVHD (grade III-IV)				
Patient age, year linear	7898	0.99	0.99-1.00	<.001
Disease				
ALL (Ref.)	1861	1.00		
Aplastic anemia	489	0.41	0.26-0.64	<.001
Conditioning				
Myeloablative (Ref.)	6653	1.00		
Reduced intensity	1245	1.26	1.07-1.50	.007
Sex matching				
Female to male (Ref.)	1494	1.00		
Female to female	1442	0.77	0.64-0.92	.005
Chronic GVHD				
Patient age, year linear	6528	1.01	1.00-1.01	<.001
Donor age, year linear	6528	1.00	1.00-1.00	<.001
Disease				
ALL (Ref.)	1568	1.00		
CML	813	1.28	1.13-1.46	<.001
Aplastic anemia	425	0.64	0.46-0.89	.008
Transplanted year				
1993-2000 (Ref.)	1865	1.00		
2006-2010	2117	0.74	0.65-0.83	<.001
Leukemia relapse				
Disease				
ALL (Ref.)	1861	1.00		
CML	983	0.49	0.39-0.60	<.001
Leukemia risk				
Standard (Ref.)	2508	1.00		
High	2772	2.62	2.31-2.98	<.001
Transplanted year				
1993-2000 (Ref.)	1815	1.00		
2001-2005	2079	1.34	1.14-1.56	<.001
2006-2010	1559	1.31	1.09-1.57	.004
Neutrophil engraftment				
Disease				
ALL (Ref.)	1831	1.00		
CML	959	0.90	0.84-0.97	.005
GVHD prophylaxis				
Cyclosporin based (Ref.)	2998	1.00		
Tacrolimus based	4716	1.12	1.07-1.18	<.001
Leukemia risk				
Standard (Ref.)	2486	1.00		
High	2703	0.81	0.77-0.85	<.001
Sex matching				
Female to male (Ref.)	1462	1.00		
Male to male	3182	1.10	1.03-1.16	.002
Male to female	1686	1.12	1.05-1.20	.001
ABO blood type matching				
Match (Ref.)	3455	1.00		
Major mismatch	1452	0.88	0.83-0.94	<.001
Transfused nuclear cell no./weight, kg, $\times 10^{E8}$				
<2.0 (Ref.)	1038	1.00		
2.0-4.0	4999	1.34	1.26-1.42	<.001
≤4.0	1068	1.42	1.31-1.55	<.001
Mortality				
Patient age, year linear	7898	1.02	1.02-1.02	<.001
Donor age, year linear	7898	1.01	1.01-1.02	<.001
Disease				
ALL (Ref.)	1861	1.00		
AML	2609	0.81	0.74-0.89	<.001
CML	983	0.72	0.63-0.81	<.001
MDS	841	0.50	0.40-0.64	<.001
Other leukemia	312	0.68	0.52-0.89	.005
Lymphoid malignancy	542	0.54	0.42-0.70	<.001
Aplastic anemia	489	0.30	0.23-0.40	<.001
Leukemia risk				
Standard (Ref.)	2508	1.00		
High	2772	2.19	2.01-2.39	<.001
Sex matching				
Female to male (Ref.)	1494	1.00		
Female to female	1442	0.81	0.72-0.90	<.001
Transplanted year				
1993-2000 (Ref.)	2311	1.00		
2001-2005	3084	0.81	0.74-0.89	<.001
2006-2010	2503	0.67	0.60-0.75	<.001

Multivariable competing risk regression analyses were conducted to evaluate the impact of acute GVHD, chronic GVHD, leukemia relapse and neutrophil engraftment, and a Cox proportional regression model for mortality. RR of respective factors was compared with the reference factor adjusted by HLA locus matching and clinical factors. Factors with significance ($P < .01$) were listed. RR of all variables is shown in supplemental Table 6.

Ref., reference factor.

showed a higher risk of acute GVHD (grade III-IV) compared with a myeloablative regimen. Tacrolimus-based GVHD prophylaxis showed a higher rate of neutrophil engraftment compared with cyclosporine-based GVHD prophylaxis, but no increase for acute GVHD and chronic GVHD. Sex matching conversely affected acute GVHD and neutrophil engraftment. ABO blood type matching and transplanted cell number affected neutrophil engraftment. The passage of time, reflecting an improvement in clinical selection for variables, was associated with a lower risk of mortality as a whole. RR of all variables for each factor are shown in supplemental Table 6.

Discussion

In this study, the accumulation of UR-HSCT clinical data and HLA retyping data through the JMDP allowed us to analyze biological immune responses of transplant-related events by HLA locus matching at the allele level. As data for some of the previously identified HLA alleles were no longer up to date, precise assessment of HLA matching required that we renew HLA allele types to meet the recent HLA nomenclature. We performed HLA allele typing for all HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1. In addition, to elucidate the biological immune responses, we strictly restricted pairs to non-T-cell–depleted bone marrow as stem cell source and to Japanese pairs as ethnic background.

Significant RRs of HLA allele mismatch compared with match were HLA-A, -B, -C and -DPB1 for grade III-IV acute GVHD; HLA-C for chronic GVHD; HLA-C and HLA-DPB1 for leukemia relapse; and HLA-A, -B, -C for mortality. Furthermore, stratified analysis of HLA-DRB1 and -DQB1 revealed that HLA-DRB1_DQB1 double mismatch was a significant RR for severe acute GVHD and mortality. These findings supersede previous JMDP studies[2,4,5] and provide a rationale for the development of an algorithm for unrelated donor selection.

HLA-A and/or -B locus mismatch induced significant severe acute GVHD but not the GVL effect, and resulted in a lower survival rate than in HLA match pairs. Since the first report from the JMDP showing the risk of HLA-A and/or -B for acute GVHD and survival, both the selection of HLA-A and/or -B mismatch donors and the impact of

this mismatch have dramatically decreased. In spite of this information bias, HLA-A and/or -B allele mismatch should be considered in donor selection and GVHD prophylaxis as a high-risk HLA locus of severe acute GVHD and mortality. The NMDP[6,7] and IHWG reports[10] also indicated the risk of HLA-A and/or -B mismatch.

HLA-C mismatch induces not only a high risk of acute GVHD but also a high risk of chronic GVHD and low risk of leukemia relapse. When an HLA-C mismatch donor is considered for the induction of GVL effect in general practice, the risk of acute GVHD and chronic GVHD should be kept in mind. This effect of HLA-C mismatch on leukemia relapse and survival confirms findings of previous JMDP[5,25] and NMDP reports.[6] In addition to T-cell recognition of the mismatched amino acid difference in HLA-C molecules,[14] NK-cell receptor KIR2DL ligand mismatch should also be considered, as described elsewhere.[5,26] The effect of KIR ligand mismatch remains controversial worldwide. Further analysis of HLA-C allele mismatch combination in conjunction with KIR receptor using JMDP pairs and comparison with non-JMDP pairs will help to elucidate the mechanism of HLA-C and KIR-related immunologic reaction and solve these discrepancies.

Our stratified analysis showed that the concurrent presence of HLA-DRB1 mismatch and HLA-DQB1 mismatch was associated with a high risk of severe acute GVHD and mortality, whereas the presence of HLA-DRB1 mismatch or HLA-DQB1 mismatch only did not induce a significantly higher risk of severe acute GVHD or survival. This epistasis of 2 HLA loci mismatch needs to be interpreted with care. In particular, the relatively small number of DRB1 alone mismatch pairs (n = 325) might have limited the statistical power. An additional consideration is that no other HLA 2 locus mismatch combination showed such an epistatic effect of DRB1 and DQB1 on the risk of severe acute GVHD and mortality (supplemental Table 3). Interaction of the HLA-DQB1 molecule with that of HLA-DR groups might evoke unique immune reactions related to allogeneic transplantation for severe acute GVHD. As reported by Fernández-Viña et al,[27] the effect of the low expression of HLA loci, not only of DP, DQ but also the DRB3/4/5 locus, needs to be explored.

As also reported by Shaw et al,[8] the present study found that HLA-DPB1 mismatch induced acute GVHD and the GVL effect, but did not affect survival. HLA-DP antigen was originally typed using the in vitro–primed lymphocyte test. From this, HLA-DPB1 and its matching are known to play a distinct biological role in immunologic reactions. Indeed, the GVL effect in HLA-DPB1 mismatch combination in our previous analysis provided a rationale to explain the induction of the GVL effect and less acute GVHD.[25] In addition, our present results show for the first time that HLA-DPB1 mismatch and the occurrence of chronic GVHD affect the GVL effect independently of each other. The mechanism of the GVL effect induced by T-cell recognition of the HLA-DPB1 allele mismatch might differ from that induced by chronic GVHD. Potential candidates for the molecular implications of acute GVHD and the GVL effect include the high-risk HLA-DPB1 mismatch combinations for severe acute GVHD reported from the JMDP[14,25] and the effect of T-cell-epitope matching at HLA-DPB1 reported by Fleischhauer et al.[16]

When the impacts of the respective HLA locus matching described above are taken together, RR of mismatch of HLA class I loci is heightened, with a range of RR 1.29 to 1.63 for severe acute GVHD and RR 1.21 to 1.27 for mortality. For HLA class II loci, mismatch of double HLA-DRB1 and -DQB1 should be considered, with RR 1.32 for severe acute GVHD and 1.14 for mortality. Thus, appropriate combinations of HLA loci need to be selected according to the risk of each HLA locus and the interaction of HLA-DRB1 and -DQB1 for donor selection.

The number of multiple mismatches of HLA-A, -B, -C, -DRB1_DQB1 and -DPB1 showed good predictive value for the risk of severe acute GVHD. Furthermore, prediction of the risk of mortality after transplantation should consider the number of multiple mismatches of HLA-A, -B, -C, and -DRB1_DQB1 locus, and not of HLA-A, -B, -C, -DRB1, and -DQB1. This mismatch score is in agreement with reports from the NMDP[6,7,11] and Loiseau et al[28] showing that mismatch of HLA-DQB1 demonstrated an additive adverse effect in outcomes. Our analysis using the present data set is consistent with findings from a recent report[29] which showed a significant risk with single HLA-DRB1 mismatch using the Japanese HSCT dataset in leukemia patients with HLA-A, -B, -C and -DRB1 allele data.

Our analysis also provides further information for personalized unrelated donor selection. In cases where the transplant team is particularly concerned about the prevention of severe acute GVHD, leukemia relapse or early mortality, the specific HLA locus mismatches and number of mismatched locus should be considered with regard to the patient's disease, disease status, and clinical condition. The benefit of HLA-C mismatch and HLA-DPB1 mismatch for a specific GVL effect in leukemia patients is noted.

A number of other important factors will also impact clinical outcomes and change the magnitude of the HLA barrier. In the present study, clinical risk factors other than HLA matching are shown in Table 6. The magnitude of risks for HLA locus mismatch is compatible with that for clinical factors as a whole.

Candidates range widely, from ethnicity of the donor and patient[30] to HLA haplotype[12,13] and other genetic polymorphisms both inside and outside the HLA region.[31-33] Clinical risk factors in the present study agree with those reported previously, including procedures for GVHD prophylaxis, intensity of the conditioning regimen,[34] disease,[35,36] leukemia relapse risk, and stem cell source.[37] It will be interesting to determine whether these candidates shift the HLA barrier quantitatively and maintain the same divergent effect of each HLA locus, or qualitatively alter the HLA locus-specific barrier. As unrelated peripheral blood stem cell transplantation was not facilitated by the JMDP during the period of this study, we were unable to analyze the data for unrelated PBSCT. PBSCT might heighten the threshold of the HLA barrier, as reported by the NMDP.[37] Analysis for unrelated cord blood transplantation compared with unrelated donor transplantation[38,39] might shed light on the latter possibility and help elucidate the altered immune mechanisms which cause transplant-related events.

Our homogeneous cohort was restricted to Japanese pairs, which allowed us to elucidate biological responses based on this particular genetic background. However, individual ethnic groups present distinct HLA allele and HLA haplotypes, and these differences in the ethnic background of patient and donor might impact transplant-related clinical outcomes.[40] Our findings need to be validated using unrelated donor transplantation data for other ethnic groups.

In conclusion, we clearly determined the HLA locus mismatches responsible for diverse transplant-related immunologic events. Furthermore, we provide a rationale for the development of an algorithm for unrelated donor selection.

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Authorship

Contribution: Y.M., K. Kashiwase, K. Matsuo, M.M., T.I., H. Saji, S.K., Y.K., and T.S. participated in the design of the study; K. Kashiwase, F.A., and T.Y. performed the histocompatibility analysis; M.O., N.D., T.E., Y.M., K. Miyamura, T.M., H. Sao, Y.A., and K. Kawa organized and collected the clinical data and samples for transplantation; Y.M., S.M., and K. Matsuo performed statistical data analysis; Y.M., S.M., and K. Kashiwase performed the analysis and wrote the paper; and all authors checked the final version of the paper.

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Clinical Transplantation

Mycophenolate mofetil use after unrelated hematopoietic stem cell transplantation for prophylaxis and treatment of graft-vs.-host disease in adult patients in Japan

Iida M, Fukuda T, Uchida N, Murata M, Aotsuka N, Minagawa K, Oohashi K, Fukushima K, Kondo T, Eto T, Miyamoto T, Morishima Y, Nagamura T, Atsuta Y, Suzuki R. Mycophenolate mofetil use after unrelated hematopoietic stem cell transplantation for prophylaxis and treatment of graft-vs.-host disease in adult patients in Japan.

Abstract: Our previous study of 301 patients who received hematopoietic stem cell transplantation (HSCT) from related donors demonstrated the efficacy of mycophenolate mofetil (MMF) for prophylaxis and treatment of graft-vs.-host disease (GVHD). In this study, we investigated the safety and efficacy of MMF in 716 adult patients who received unrelated HSCT. The incidences of Grade II–IV and III–IV acute GVHD in the prophylactic administration group were 38.3% and 14.3%, respectively. These rates were not statistically significant when evaluating the MMF dosage and graft source. The incidences of limited and extensive chronic GVHD were 16.6% and 11.1%, respectively. In the therapeutic administration group, 69.1% of the subjective symptoms for both acute and chronic GVHD improved. With respect to the adverse events, 75 infections and 50 cases of diarrhea were observed, and the frequency of these events increased with increasing MMF dose. The overall survival rate was 36.4% after a median follow-up period of three yr. This study shows that MMF is safe and effective for the prevention and treatment of GVHD in patients who have received HSCT from unrelated donors.

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Key words: graft-vs.-host disease – mycophenolate mofetil – unrelated allogeneic stem cell transplantation

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Allogeneic hematopoietic stem cell transplantation (HSCT) is an effective therapy for a variety of hematological disorders. However, one of the limitations of allogeneic HSCT is donor availability; only 30% of patients can undergo transplantation with stem cells from an HLA-matched related donor (1–3). An HLA-matched unrelated donor (MUD) or umbilical cord blood (UCB) is an alternative for a patient lacking a related donor (4–7); however, unrelated HSCT is associated with a higher risk of graft-vs.-host disease (GVHD), which is a major complication of this procedure.

Mycophenolate mofetil (MMF) is an inhibitor of inosine monophosphate dehydrogenase, which impairs the proliferation of activated lymphocytes; MMF has been used as an immunosuppressant in HSCT (8–15). We previously surveyed MMF usage in more than 300 adult patients who received related HSCT in Japan and found that MMF is safe and effective for the prevention and treatment of GVHD (16). In this study, we conducted another survey of MMF use after HSCT from a MUD or a UCB donor in Japan.

Patients and methods

Study design

The basic study design was the same as in our previous study (16). The data on MMF use after allogeneic HSCT from unrelated donors were retrospectively collected using the questionnaire that we used for related donors. The items in the questionnaire included the purpose of treatment (prevention of GVHD or treatment of acute/chronic GVHD), the MMF dosage and dosing period, the presence or absence of subjective symptoms of GVHD, the GVHD grade and stage (before and after treatment), whether there was a decrease or increase in concomitant immunosuppressants, the drug effectiveness, adverse events (AEs), and the outcomes of HSCT. The basic information for each transplantation was extracted from the Transplant Registry Unified Management Program (TRUMP) system, which is a registry of patient outcomes in Japan (17). The number of HLA mismatches was defined as the numbers of mismatched HLA-A, HLA–B, and HLA-DRBI based on low resolution typing in the TRUMP dataset. Several demographic data were not available because they were not entered into the TRUMP system. The effects of MMF, including the rating of subjective symptoms (none, disappearance, improvement, no change or ingravescence) and the use of steroids (none, discontinuation, dose reduction, no change or dose increment), were assessed by the attending physicians at each hospital based on international standards (18). AEs were evaluated using the National Cancer Institute – Common Terminology Criteria for Adverse Events (NCI-CTCAE, ver. 3). This study was approved by the ethics committees of the Japan Society of Hematopoietic Cell Transplantation and the Nagoya University School of Medicine.

Statistics

Correlations between the two subgroups were examined using the chi-square test and Fisher's exact test. p-Values < 0.05 in two-sided tests were considered statistically significant. The data were

analyzed using STATA version 10 statistical software (STATA Corp, College Station, TX, USA).

Results

Patient background data

From 1999 to 2011, MMF was administered to 716 adult patients. The patient background data are summarized in Table 1. The patient ages at the time of transplantation ranged from 16 to 74 yr (median 51 yr), and the number of male patients was greater than the number of female patients (445 [62.2%] vs. 271 [37.8%], respectively). Unrelated peripheral blood stem cell (PBSC) transplantation had not commonly been conducted in Japan until 2011; therefore, a one-to-one ratio of bone marrow (BM) to cord blood (CB) was approximately achieved (340 vs. 359, respectively). With respect to the donor type, 289 patients (40.4%) received transplants from HLA-matched donors and 400 patients (55.9%) received transplants from HLA-mismatched donors. The HLA data were missing for 27 patients (3.8%). Of the HLA-mismatched donors, 153 (38.3%) were mismatched at one antigen, 242 (60.5%) at two antigens and five (1.2%) at three antigens. The rate of HLA-mismatched transplantation in the BM group was 20.9%, whereas the rate was 95.9% in the CB group. The distribution of diseases in this survey indicated that 95.1% of all of the diseases were hematological malignancies, including acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myelogenous leukemia (CML), myelodysplastic/myeloproliferative syndrome (MDS), malignant lymphoma (ML), and multiple myeloma (MM). The aim of MMF use was GVHD prevention in 440 patients, acute GVHD treatment in 230 patients and chronic GVHD treatment in 84 patients; several of the aims overlapped. In the prevention group (n = 440), CB accounted for 73.2%. The pre-conditioning regimen was myeloablative (MAST) in 290 patients and non-myeloablative (RIST) in 407 patients. In the MAST group, BM accounted for 74.1% of the transplantations, whereas CB accounted for 67.3% of the transplantations in the RIST group.

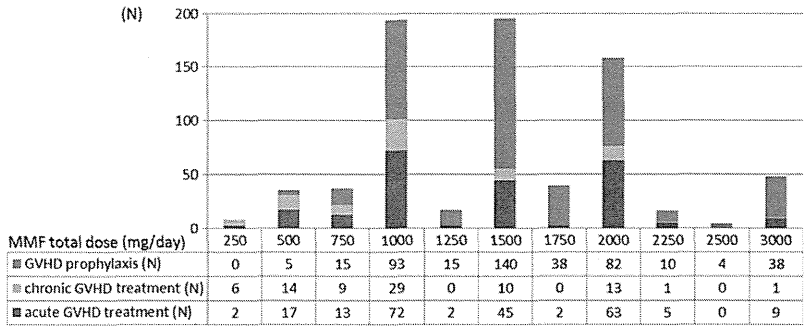
Table 1. Patient characteristics

Variables	Number
Patient number	716
Median age (range)	51 (16–74)
Male/female	445/271
Disease	
Acute myeloid leukemia	315
Acute lymphoblastic leukemia	102
Chronic myelogenous leukemia	28
Myelodysplastic/myeloproliferative syndrome	87
Malignant lymphoma	133
Multiple myeloma	16
Aplastic anemia	18
Other diseases	17
Purpose of mycophenolate mofetil[a]	
Graft-vs.-host disease (GVHD) prophylaxis	440
aGVHD treatment	230
cGVHD treatment	84
Graft source	
Bone marrow (BM)	351
Peripheral blood stem cell	3
Cord blood (CB)	362
Donor type[b]	
Matched (UBM/UPB/CB)	289 (272/3/14)
Mismatched (UBM/UPB/CB)	400 (72/0/328)
1 antigen mismatch (UBM/UPB/CB)	153 (68/0/85)
2 antigens mismatch (UBM/UPB/CB)	242 (4/0/238)
3 antigens mismatch (UBM/UPB/CB)	5 (0/0/5)
Conditioning regimen[b]	
Myeloablative	290
Non-myeloablative	407

[a]Some of these three were overlapped.
[b]There were some blanks in the donor type and conditioning regimen due to lack of patient entry to the Transplant Registry Unified Management Program system.

MMF administration

The daily MMF dosage varied from 250 to 3000 mg. According to the total dosage by purpose, the most common dosage in the prevention group was 1500 mg MMF per day (N = 140), whereas the most common dosage was 1000 mg/d in the acute and chronic GVHD treatment group (N = 72 and 29, respectively; Fig. 1). The number of doses per day ranged from one to eight. The most common dosages and frequencies of MMF administration were 500 mg two times per day, followed by 1000 mg two times per day and 750 mg two times per day, which were given to 156 patients (21.8%), 115 patients (16.0%), and 112 patients (15.6%), respectively. The dosing period varied from <11 d to more than 4000 d. The reasons for early termination of MMF therapy were four early deaths and three adverse events (Quincke's edema, vertigo, and poor oral intake). The average dosing periods in each group were 32, 60, and 221 d in the prophylactic, acute GVHD treatment, and chronic GVHD treatment groups, respectively. In the prophylactic group, most patients (429 patients, 97.5%) were given MMF concurrently with the following immunosuppressants: cyclosporine in 178 patients, tacrolimus in 152 patients, short-term methotrexate plus tacrolimus in 44 patients, and tacrolimus plus steroid in 31 patients. Only 11 patients (2.5%) received MMF alone.



MMF total dose (mg/day)	250	500	750	1000	1250	1500	1750	2000	2250	2500	3000
GVHD prophylaxis (N)	0	5	15	93	15	140	38	82	10	4	38
chronic GVHD treatment (N)	6	14	9	29	0	10	0	13	1	0	1
acute GVHD treatment (N)	2	17	13	72	2	45	2	63	5	0	9

Fig. 1. Mycophenolate mofetil (MMF) daily dose (mg/d) by purpose. MMF was given from 250 to 3000 mg/d. The most common dosages of MMF administration by purpose were 1500 and 1000 mg/d given for prophylaxis (N = 140) and treatment of acute (N = 72)/chronic (N = 29) graft-vs.-host disease, respectively.

Adverse events

All of the AEs that may have been caused by MMF administration are listed in Table 2. The most frequent AE was infection (75 cases, accounting for 30.7% of all cases). The four most common infections were sepsis (19 cases), pneumonia (17 cases), CMV infections (seven cases), and adenovirus infection (four cases). For human herpes virus type 6 (HHV-6) infection, one case of gastritis and four cases of encephalitis/encephalopathy were observed with CB transplantation. Diarrhea was the second most common AE (50 cases, 20.5%); however, excluding one case, the cases were graded as 1–3. In addition, gastrointestinal adverse events, such as nausea, vomiting, stomatitis, and constipation, were less serious. Regarding the therapeutic responses to AEs of grade 3 and 4, the recovery rates for infections and gastrointestinal system-related AEs were relatively favorable (79.8% and 89.2–100%, respectively), whereas the extent of recovery from hematological AEs (thrombocytopenia and neutropenia) were inferior (31.6% and 60.8%, respectively). Overall, 37 patients died of complications that were potentially associated with MMF use, and 73.0% (27) of these cases were attributed to infections (13 cases of pneumonia, five cases of sepsis, two cases of fungal infection, two cases of adeno virus infections, two cases of brain abscess, one case of CMV-related infection, one case of methicillin-resistant *Staphylococcus aureus*/multidrug-resistant *Pseudomonas aeruginosa* infection, and one case of intestinal bleeding due to CMV colitis).

Efficacy of MMF

Among the 440 patients who received MMF for GVHD prophylaxis, the incidence of grade II–IV

Table 2. Adverse events whose relationships to mycophenolate mofetil were not necessarily denied by the NCI-CTCAE (ver. 3) grade

	Grade 1		Grade 2		Grade 3		Grade 4		Grade 5[a]		Total	
Adverse events	N	%	N	%	N	%	N	%	N	%	N	%
Infection	0	0.0	7	1.0	31	4.3	10	1.4	27	3.8	75	10.5
Diarrhea	6	0.8	11	1.5	32	4.7	1	0.1	0	0.0	50	7.0
Neutropenia	0	0.0	4	0.6	10	1.4	9	1.3	0	0.0	23	3.2
Thrombocytopenia	0	0.0	4	0.6	8	1.1	11	1.5	0	0.0	23	3.2
Nausea	2	0.3	8	1.1	7	1.0	0	0.0	0	0.0	17	2.4
Gastrointestinal bleeding	1	0.1	1	0.1	4	0.6	1	0.1	0	0.1	7	1.0
Myelosuppression	0	0.0	5	0.7	2	0.3	1	0.1	0	0.0	8	1.1
Vomiting	2	0.3	3	0.4	1	0.1	0	0.0	0	0.0	6	0.8
Stomatitis	1	0.1	1	0.1	0	0.0	0	0.0	0	0.0	2	0.3
Constipation	0	0.0	1	0.1	0	0.0	0	0.0	0	0.0	1	0.1
Others[b]	4	0.6	6	0.8	8	1.1	4	0.6	10	1.4	32	4.5

[a]Details about Grade 5: pneumonia (13), sepsis (5), fungal infection (2), adenovirus infection (2), brain abscess (2), CMV infection (1), multiple organ failure (2), organ failure (lung) (2), gastrointestinal bleeding due to CMV enteritis, MRSA/MDRP infection, organ failure (central nervous system), EBV-related lymphoproliferative disease, hemophagocytic syndrome, ileus, thrombotic microangiopathy.
[b]Others: grade 1: hypogammaglobulinemia, Quincke's edema, renal tubular acidosis, poor oral intake; grade 2: renal damage, vertigo, heartburn, tongue fur, abdominal pain, drug eruption; grade 3: hypoalbuminemia (2), rhabdomyolysis, thrombotic microangiopathy (2), vertigo, pure red cell aplasia, ileus; grade 4: interstitial pneumonia (2), thrombotic microangiopathy, graft failure.

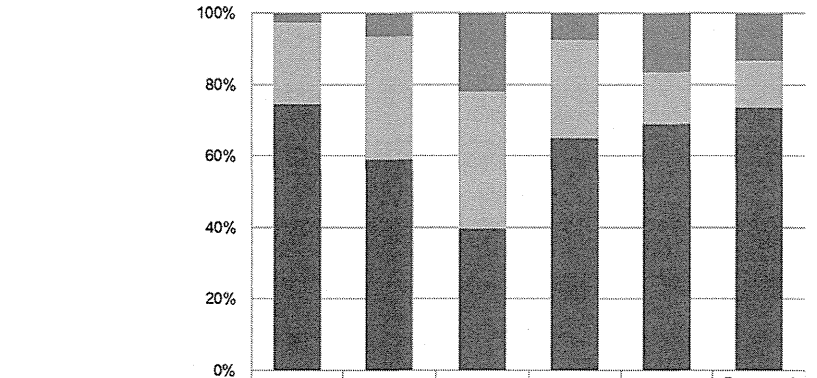
acute GVHD was 38.3% (164/428), and the incidence of grade III–IV was 14.3% (61/428). The incidence of grade II–IV acute GVHD decreased with increasing MMF dosage (~1000, ~2000, and 2001 mg/d~); however, the incidence of grade III–IV acute GVHD remained the same regardless of the dosage. Assessing the incidence of acute GVHD according to the graft source, the rates of grade II–IV and III–IV acute GVHD in the BM group were lower than that in the CB group (33.6% vs. 38.2% and 12.9% vs. 14.0%, respectively; Table 3). No significant differences were found in the incidence of grade II–IV/III–IV acute GVHD between HLA-matched and mismatched transplant patients, excluding grade III–IV gut GVHD (0/94 [0%] vs. 13/295 [4.4%], $p = 0.04$). With respect to the chronic GVHD incidence, 16.6% (60/361) and 11.1% (40/361) of patients experienced limited and extensive chronic GVHD. The MMF dose was not associated with the incidence of limited and extensive chronic GVHD. In the evaluation according to the graft source, 20.2% (21/104) of patients in the BM group and 14.5% (37/255) of patients in the CB group developed limited chronic GVHD and 15.4% (16/104) of patients in the BM group and 9.4% (24/255) of patients in the CB group developed extensive chronic GVHD (Table 3). There were no significant differences between the HLA-matched and mismatched groups in the development of chronic GVHD (25/93 [37.6%] vs. 61/249 [24.5%], $p = 0.31$). When we compared the incidence of acute and chronic GVHD with the disease and source, the incidence of grade II to IV acute GVHD in the BM and CB cases were 31% vs. 39% in AML, respectively, and 60% vs. 39% in ALL, respectively. For chronic GVHD, the incidences for BM and CB cases were 18% vs. 21% in AML and 53% vs. 18% in ALL, respectively.

Table 3. Incidence of graft-versus-host disease (GVHD) with prophylactic mycophenolate mofetil use

	a GVHD		c GVHD	
	II–IV (%)	III–IV (%)	Limited (%)	Extensive (%)
Total	38.3	14.3	16.6	11.1
Dosage (mg/d)				
~1000	38.5	16.0	13.3	7.8
1001~2000	39.7	13.5	15.0	11.9
2001~	30.8	15.4	31.1	13.3
Graft source				
Bone marrow	33.6	12.9	20.2	15.4
Cord blood	38.2	14.0	14.5	9.4
Peripheral blood	100.0	50.0	100.0	0
Conditioning regimen				
Myeloablative	38.3	13.1	25.8	11.2
Non-myeloablative	38.1	14.3	14.0	11.6

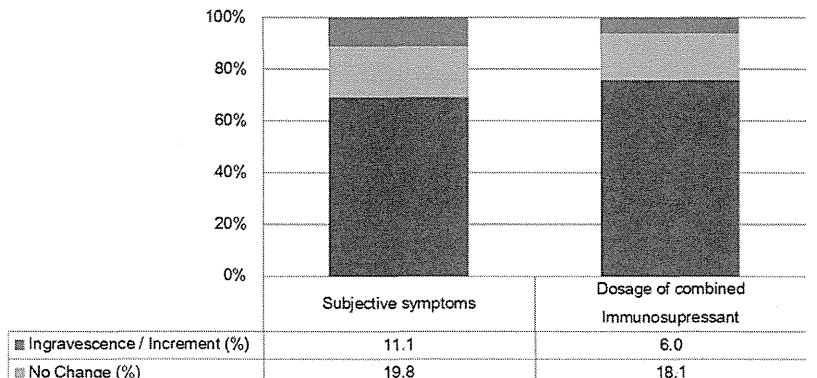
In the acute GVHD treatment group, the disappearance or improvement of subjective symptoms occurred in 69.1% of patients, and 73.7% of the patients in this group reduced or discontinued the combined immunosuppressants (Fig. 2). Especially in the HLA-matched group, the improvement rate in the subjective symptoms was significantly higher than that in the HLA-mismatched group (92/132 [69.7%] vs. 47/85 [55.3%], $p = 0.03$). The comparison of the effects of MMF according to the target organ indicated that MMF was more effective for skin GVHD than for gut and liver GVHD (143/192 [74.5%], 72/122 [59.0%] and 27/68 [39.7%], respectively; Fig. 2). In the chronic GVHD treatment group, 56/81 (69.1%) of the cases had improved subjective symptoms and 63/83 (75.9%) of the cases reduced or discontinued the dosage of combined immunosuppressants (Fig. 3). In addition, there were no significant differences between the HLA-matched and mismatched patients in each of these observed items ($p = 0.44$~0.77, data not shown). To assess the efficacy of MMF for GVHD treatment, we divided all of the patients into the following three subgroups according to the MMF dosage: <1000 mg/d, <2000 mg/d, and more than 2001 mg/d (Table 4). The efficacy rates for every acute and chronic GVHD survey item, including improvement in the grade and subjective symptoms and a reduction in the dose of combined immunosuppressants, were higher in the more than 2001 mg/d dosage group than in the <1000 mg/d and <2000 mg/d groups; however, there were no differences in the dose efficacy observed among the three dosage groups ($p = 0.13$–0.99 for acute GVHD items and $p = 0.56$–0.99 for chronic GVHD items).

We collected data from a large number of patients who underwent CB transplantation with prophylactic use of MMF (N = 322); 90.4% of these patients were HLA mismatched. The median daily dosage and dosing days in this group were 1500 mg and 32 d, respectively. One hundred and seventy-six (54.7%) and 114 (35.4%) patients were given MMF with cyclosporine and tacrolimus, respectively. The rates of grades II–IV and III–IV acute GVHD were 38.2% and 14.0%, respectively, and the rates of limited and extensive chronic GVHD were 14.5% and 9.4%, respectively (Table 3). Three out of five cases of HHV-6 encephalitis/encephalopathy in this group developed grade II–IV acute GVHD (2 grade III cases and 1 grade IV).



	Skin	Gut	Liver	Grade	Subjective symptoms	Dosage of combined Immuno supressant
Ingravescence / Increment (%)	2.6	6.6	22.1	7.4	16.4	13.2
No Change (%)	22.9	34.4	38.2	27.5	14.5	13.2
Disappearance· Improvement/ Reduction· Discontinuation (%)	74.5	59.0	39.7	65.1	69.1	73.7

Fig. 2. Response to acute graft-vs.-host disease (GVHD) in the treatment group. In the acute GVHD treatment group, the disappearance or the improvement of subjective symptoms occurred in 69.1% of patients and 73.7% of the patients in this group could reduce or discontinue the combined immunosuppressants.



	Subjective symptoms	Dosage of combined Immunosupressant
Ingravescence / Increment (%)	11.1	6.0
No Change (%)	19.8	18.1
Disappearance· Improvement/ Reduction· Discontinuation (%)	69.1	75.9

Fig. 3. Response to chronic graft-vs.-host disease (GVHD) in the treatment group. In the chronic GVHD treatment group, 56/81 (69.1%) of cases improved subjective symptoms and 63/83 (75.9%) of cases reduced or discontinued the dosage of combined immunosuppressants.

Transplantation outcomes

In the GVHD prevention group, engraftment was observed in 360 of 423 patients (85.1%). The engraftment rates according to the graft source were 94.0% and 77.3% for the BM and CB groups, respectively. Of the total 716 patients, 168 (23.5%) relapsed and 425 (59.4%) died after transplantation. The overall survival rates were 40.6% and 36.4% after a median follow-up of period of two and three yr, respectively. The main causes of death were disease recurrence, bacterial infection and acute or chronic GVHD. In the prophylactic group, disease recurrence was the most common cause of death (43%), which was followed by bacterial infection (11%) and acute GVHD (6%). Four out of five cases of HHV-6 encephalitis/encephalopathy died of other infections (3) and relapse (1) instead of acute GVHD.

In the acute GVHD treatment group, the causes of death were acute GVHD (22%), disease recurrence (18%) and bacterial infection (11%). In the chronic GVHD treatment group, the most common cause of death was bacterial infection (22%), which was followed by chronic GVHD and disease recurrence (16%, respectively).

Discussion

Over the past few years, several types of allogeneic HSCT have become available, including CB, non-myeloablative, and haplo-identical transplantation. As the number of new strategies increases, methods for controlling GVHD must be developed

Table 4. Response rate of acute and chronic graft-versus-host disease (GVHD) during therapeutic mycophenolate mofetil use by daily dosage

	Dosage (mg/d)					
	~1000		1001~2000		2001~	
	N	%	N	%	N	%
Acute GVHD						
Grade						
Improvement	66	63.5	71	63.4	12	85.7
No change/ ingravescence	38	36.5	41	36.6	2	14.3
Subjective symptoms						
Disappearance/ improvement	70	72.2	70	64.2	12	85.7
No change/ ingravescence	27	27.8	39	35.8	2	14.3
Dosage of combined immunosuppressant						
Reduction/ discontinuation	75	73.5	82	73.2	11	78.6
No change/ increment	27	26.5	30	26.8	3	21.4
Chronic GVHD						
Subjective symptoms						
Disappearance/ improvement	38	67.9	16	69.6	2	100.0
No change/ ingravescence	18	32.1	7	30.4	0	0.0
Dosage of combined immunosuppressant						
Reduction/ discontinuation	45	77.6	16	69.6	2	100.0
No change/ increment	13	22.4	7	30.4	0	0.0

because GVHD remains one of the greatest post-transplantation complications. MMF is one of the most effective drugs available and is used under various conditions for HSCT (8–10, 12–14, 19–23). As we previously reported, MMF is an important therapeutic agent for the treatment and prophylaxis of acute and chronic GVHD after related HSCT in Japan (16). In this study, we evaluated more than 700 patients who received MMF after unrelated HSCT in Japan. There were few differences in the method of MMF administration for unrelated and related HSCT. The most frequent daily dosage was 1000 mg (dosage/frequency of 500 mg twice a day) in both groups, which was followed by 750 mg (250 mg three times per day) and 2000 mg (1000 mg twice a day) in the related donor group and followed by 1500 mg (750 mg twice a day or 500 mg three times per day) and 2000 mg (1000 mg twice a day) in the unrelated donor group. The most common MMF dosing period was 10–30 d in the related group and 30–60 d in the unrelated group. The MMF dosages and dose regimens in Japan were relatively lower/shorter than those in other countries because of the low incidence of GVHD, the risk for infection due to excess immunosuppressive states, and the fact that MMF is not approved for the treatment and prevention of GVHD.

Murata et al. (24) revealed that the response rate of grade II–IV acute GVHD to systemic corticosteroid therapy in Japanese patients was very high (~64%), especially for CB transplantation. However, if systemic corticosteroid therapy is ineffective, Japanese patients cannot achieve a satisfactory survival rate, and the authors concluded that the establishment of second-line treatment for corticosteroid refractory acute GVHD is required for Japanese patient. Kanda et al. (25) reported that in Japan, the incidences of grade II to IV acute GVHD among unrelated HSCT patients were 41% in the unrelated BM group and 45% in the CB group with conventional GVHD prophylaxis, such as cyclosporine or tacrolimus. In the same report, the incidences of chronic GVHD at two yr were 34%, 40%, and 30% in the matched unrelated BM, mismatched-unrelated BM and CB groups, respectively. Atsuta et al. conducted a disease-specific comparison of Japanese unrelated BM and CB patients with acute leukemia (AML and ALL) using the same GVHD prophylaxis regimen as Kanda et al. The incidences of grade II to IV acute GVHD were lower for CB cases than for BM cases (32% vs. 35% in AML, 28% vs. 42% in ALL) and were the same as for chronic GVHD (8% vs. 20% in AML, 10% vs. 17% in ALL; 26). Considering the results in Table 3, the incidence of grade II to IV acute GVHD and chronic GVHD in this study seems to be better than with conventional prophylaxis; however, the results were too varied to directly compare the use of conventional prophylaxis and our results from MMF administration for each risk factor, such as the disease and source.

MMF has been increasingly used after HSCT worldwide. Among recent reports, Minagawa et al. (11) summarized more than 100 representative reports from studies of MMF use as GVHD prophylaxis in HSCT. Additionally, Wolff et al. (22) conducted a survey in 72 allo-HSCT centers in Germany, Austria, and Switzerland and reported that MMF was used more frequently as a first- and second-line treatment for acute GVHD. There have been an increasing number of reports on the effectiveness of MMF. Xhaard et al. (23) found that the overall response to second-line therapy with MMF for the treatment of steroid-resistant acute GVHD is greater than that with inolimomab

and etanercept. Rodriguez et al. and Basara et al. retrospectively studied the effectiveness of MMF for chronic GVHD treatment compared to historical controls (14, 20). Alousi et al. (19) conducted a randomized phase 2 trial using four drugs (MMF, etanercept, denileukin, and pentostatin plus corticosteroids) for acute GVHD treatment and reported that the use of MMF plus corticosteroids was significantly better than other agents according to the treatment response and long-term survival. Furthermore, Furlong et al. (21), in a prospective trial of refractory acute and chronic GVHD treatment, concluded that MMF is effective at treating GVHD. In contrast, Martin et al. (27) failed to show the efficacy of adding MMF to a prednisolone regimen for the first-line therapy of chronic GVHD in a randomized study. They concluded that adding MMF to immunosuppressive regimens has no effect on controlling chronic GVHD, and the risk of the overall mortality and malignancy recurrence in the MMF group may be higher than in the control group. Retrospective data can be very helpful; however, we should consider the flaws of retrospective data, including the diversity of the background and investigator's biases.

We found it interesting that many patients who received CB as their graft source also underwent prophylactic MMF treatment in Japan. Uchida et al. (28) reported 29 CB transplantation cases with MMF from Japan, and they concluded that the MMF and tacrolimus combination is well tolerated. Styczynski et al. (29) also reported on 29 CB transplantation cases (20 of which received MMF), and the incidences of acute and chronic GVHD were relatively low. Most previous studies have demonstrated that the incidence of acute and chronic GVHD after CB transplantation is ~40% for HLA-mismatched CB grafts (6, 30–33). The incidences of acute and chronic GVHD in our survey were as low as 40%, and the results of our large cohort encourage us to conclude that MMF compares favorably with other immunosuppressants.

HHV-6 reactivation and HHV-6 encephalitis occur more frequently in patients with CB transplantation (34), and Zerr et al. (35) reported that HHV-6 reactivation is associated with an increased risk of grade II–IV acute GVHD and non-relapse mortality. Five cases of HHV-6 encephalitis/encephalopathy were observed with CB transplantation in this study. Among these five cases, 60% developed grade III–IV acute GVHD and 80% died. Because the treatment for HHV-6 encephalitis/encephalopathy has not been established, determining the role of immunosuppressants such as MMF, as well as antibiotics, will become more important.

Regarding other adverse events, no cases of severe mucositis were observed in this study, which is in agreement with previous studies (13, 36). Additionally, severe nephrotoxicity, which is the most common type of toxicity due to cyclosporine and tacrolimus administration (37), did not occur.

Martin et al. (38) reviewed reports on the treatment of acute GVHD that were published from 1990 to 2011, and they summarized 11 first- and second-line systemic treatments, including MMF. They concluded that none of the comparative data demonstrated the superior efficacy of any particular agent and that patients should be treated according to various conditions, such as previous treatments, drug toxicity and drug interactions. Each physician should fully understand the advantages and disadvantages of MMF and immunosuppressants and determine which agent is the most appropriate for each patient/situation. Additionally, Martin et al. reported on the risks of viral infections such as CMV, Epstein-Barr virus and adenovirus caused by long-term immunosuppression. The data from our study on adverse events are in agreement with their findings. Physicians should be more cautious about considering the conditions of each patient when prescribing MMF.

This study has several limitations. One is the possibility of selection bias of the patients. Because this study is retrospective and based on data from questionnaire, a disproportionately refractory and higher risk population may have been included. The physicians who participated in this study may unconsciously have selected patients from a higher risk population, and they might have added MMF in a desperate attempt to control GVHD. Moreover, MMF was used more frequently in patients who participated in another clinical trial or was more often prescribed at the discretion of other physician. The third limitation is that TRUMP does not include information on MMF, and we were missing some data. Fourth, we could not analyze items that were not included in the questionnaire, such as whether MMF was given for first-line or salvage treatment and how many previous treatment regimens had been given to the patients. However, this survey of more than 700 cases of unrelated HSCT is the largest to date and thoroughly demonstrates the effectiveness and safety of MMF, and we found that MMF is effectively and safely used in a large number of HSCT patients in Japan. This is the first step to establishing the value of MMF in Japanese HSCT. We understand the importance of randomized studies,

as Martin et al. (27) emphasized in their report. Future well-designed, prospective phase 2 studies will be needed to confirm our results.

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Authors' contributions

MI, RS, and TF designed the study and wrote the paper. NU, MM, NA, KM, KO, KF, TK, TE, and TM submitted the data. MI, RS, and YA performed the statistical analysis. YM and TN reviewed and compiled the data as well as reviewed the results.

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