been investigated. Several studies have reported that elevated *v*WF levels are positively associated with risk for coronary heart disease (CHD) [27,28]. Keightley et al. reported that the T allele (A allele in that study) of the *VWF* promoter variant rs7965413 had significantly lower *v*WF:Ag levels than the C allele (G allele in that study) [29] in a population of healthy individuals. In a case-control study, van der Meer et al. reported that a *VWF* promoter variant that was different from rs7965413 was also associated with risk for CHD [30]. However, because other studies could not detect an association with CHD [31], it is unclear if *VWF* promoter variants contribute significantly to CVD risk. We found that risk of MetS decreased as the number of T alleles of rs7965413 increased. This result may indicate a causal relationship between rs7965413 and CVD that is mediated via *v*WF:Ag and MetS.

As shown in Table 4, we identified a novel SNP × CP interaction between rs7965413 and platelet count significantly associated with MetS. This association of the SNP × CP interaction with MetS remained nominally significant in multiple logistic regression analysis after adjustment for the number of MetS components and significant after adjustment for the number of MetS components excluding obesity. This result indicates that this SNP × CP interaction is an independent risk marker for MetS. As described above, rs7965413 contributes to *v*WF:Ag levels, and *v*WF promotes platelet adhesion. The statistical interaction term between rs7965413 and platelet count is expected to reflect the biological interaction between *v*WF and PLT. This result reveals new information regarding platelet count as a risk marker for MetS. Moreover, for rs7965413, frequencies of the T allele across different ethnicities were as follows: African, 0.372; American, 0.445; Asian, 0.427; European, 0.621 in the 1000 Genomes Project Phase I version 3. This SNP was observed across different races, so the interaction may also be observed in other race/ethnic groups.

We found the association of rs7965413 with PLT count in both case and control groups. A GWAS recently showed that an SNP upstream of *VWF*, rs7342306, was associated with platelet count [32]. Although we did not genotype rs7342306, there was weak linkage disequilibrium (LD) between this SNP and rs7965413 in the 1000 Genomes Project Phase I version 3 ASN ($r^2 = 0.123$, $D' = 0.401$). Thus, the contribution of rs7965413 to platelet count is expected to be independent of the contribution of rs7342306 to platelet count. Our results indicate significant heterogeneity between case and control groups. The association of rs7965413 with platelet count might not be detected in the GWAS because of this heterogeneity.

It was also reported that variants in the *VWF* gene were associated with traits related to blood pressure, which was one of MetS components. Ruixing et al. reported that *VWF* rs1063856 (Thr789Ala) was significantly associated with hypertension in women [33]. Defago et al. reported that *VWF* rs2239153 was significantly associated with salt sensitivity [34]. These variants were weak LD with rs7965413 in the 1000 Genomes Project Phase I version 3 ASN ($r^2 = 0.0$, $D' = 0.013$ for rs1063856; $r^2 = 0.066$, $D' = 0.288$ for rs2239153). Thus, the contribution of rs7965413 to MetS is expected to be independent of the contribution of these variants to blood pressure.

As shown in Table 3, we found that four other SNPs were significantly associated with MetS: *LRP2* rs2544390, rs1800592 between *UCP1* and *TBC1D9*, *APOA5* rs662799, and rs1411766 between *MYO16* and *IRS2*. Of these SNPs, *APOA5* rs662799 was frequently reported to be associated with MetS and dyslipidemia, which was one of MetS components, based on several populations, including Japanese [35], Chinese [36], and Caucasian [37] populations. Our results are consistent with these reports. SNP rs1800592 between *UCP1* and *TBC1D9* is an A→G point mutation at the—3826 position in the 50 flanking region of the *UCP1* gene. The *UCP1* gene is a candidate gene for obesity and type 2 diabetes mellitus because the gene has been found to decrease mitochondrial membrane potential and increase thermogenesis [38]. Many association studies were conducted in various populations to elucidate the

association of rs1800592 with obesity phenotypes, diabetes mellitus, and lipid/lipoprotein-related disease, but the results have been controversial [39,40]. Our results indicate a significant association of this SNP with MetS. However, the associations of this SNP with obesity, diabetes, and lipids were not replicated in our data (results not shown). *LRP2* rs2544390 is located in the *LRP2* gene. It has been reported that the T allele of this SNP is associated with higher serum UA [41]. Our results indicate that the T allele was associated with risk of MetS development. However, the association of this SNP with UA was not replicated in our data (results not shown). SNP rs1411766 localizes to an intergenic region ~384 kb telomeric to *MYO16* and 120 kb centromeric to *IRS2*. This SNP was reported increase susceptible to diabetic nephropathy, as determined by a GWAS in European-American subjects with type 1 diabetes [42] and was also observed to be associated with susceptibility to diabetic nephropathy in a Japanese population with type 2 diabetes [43]. Obesity, hypertension, and other MetS components are expected to either cause or exacerbate the progression of nephropathy, independent of diabetes [44]. The rs1411766 was both a risk allele for diabetic nephrology as well as MetS development in our study. Although the function of rs1411766 has not been understood, this SNP may contribute to the development of MetS components excluding diabetes and result in the development of diabetic nephropathy.

Our study has some limitations. First, this is a case-control and exploratory study that does not establish a cause-and-effect relationship. Future studies are thus necessary to evaluate the predictive potential of SNP × CP interactions as risk markers in prospective cohorts. We assumed that the interaction is linear; that is, the per-allele effect of an SNP changes across the continuous spectrum of a CP. However, if the interaction effect is nonlinear or a threshold effect exists, in which case the association would only be present in one extreme of the CP distribution, this analysis is not suitable and other analytical methods should be applied.

In conclusion, our data demonstrate associations of five SNPs with MetS and of an interaction between SNP rs7965413 and platelet count for MetS. Our results reveal new insight into PLT count as a risk marker for MetS.

Supporting Information

S1 Table. The 99 genotyped SNPs.
(DOCX)

S2 Table. Multiplex PCR primers and PCR conditions for genotyping the 99 SNPs.
(XLSX)

S3 Table. Probes for genotyping the 99 SNPs.
(XLSX)

S4 Table. Multiple logistic regression analysis of the association between 98 SNPs and MetS in a screening analysis.
(XLSX)

S5 Table. Multiple logistic regression analysis including interactions between five SNPs and 15 clinical parameters in 2001 for MetS.
(XLSX)

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Author Contributions

Conceived and designed the experiments: MN Y. Yasuda Y. Yoshida RK TN MI YK MA NH TK HO MH S. Kato MY S. Maruyama S. Matsuo HH. Performed the experiments: Y. Yasuda Y. Yoshida TN S. Maruyama. Analyzed the data: MN YU S. Kawai RK MA HH. Wrote the paper: MN HH.

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ORIGINAL ARTICLE

Pharmacokinetics for once-daily modified release formulation of tacrolimus hydrate in unrelated hematopoietic stem cell transplantation

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Abstract A once-daily modified release formulation of oral tacrolimus (Tac QD) has been developed in response to the problem of nonadherence. However, there have been no data available about the efficacy of Tac QD conversion from intravenous Tac (Tac i.v.) in allogeneic hematopoietic stem cell transplantation (allo-SCT). We analyzed the pharmacokinetics (PK) of Tac QD in allo-SCT recipients. A total of 10 patients with hematological malignancies who received allo-SCT from unrelated donors were enrolled. Patients received Tac i.v. at 0.03 mg/kg a day before transplantation. Administration of Tac i.v. was converted to Tac QD at a 1:4 ratio when the patients had recovered from regimen-related gastrointestinal toxicity and could tolerate oral medication. After conversion, six out of 10 patients (60 %) showed a sustained decrease in Tac exposure and required dose adjustment. The conversion from Tac i.v. to Tac QD should be performed under close medical supervision. Area under the curve (AUC) and the trough of Tac QD showed a correlation, and the trough should be maintained above 7.5 ng/ml to provide an adequate AUC. Although four patients received bone marrow from an HLA DRB1 1 antigen-mismatched unrelated donor, no patients developed grade III–IV acute graft-versus-host disease (GVHD). The modification of Tac QD to maintain a whole-blood trough concentration above 7.5 ng/ml may be as effective as Tac BID.

Keywords Once-daily tacrolimus · Pharmacokinetics · Allogeneic hematopoietic stem cell transplantation · GVHD

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Introduction

In solid organ transplantation, lifelong immunosuppression is required to preserve graft function. Medication nonadherence is a major risk factor for graft failure. Oral tacrolimus (Tac) was first developed as a twice-daily formulation (Tac BID) and has been widely used in solid organ and allogeneic hematopoietic stem cell transplantation (allo-SCT), but long-term adherence remains a concern. It has been reported that morning dosing is associated with significantly higher adherence than evening dosing in kidney transplant patients [1]. In response to this potential adherence problem, a once-daily modified release formulation of oral tacrolimus (Tac QD) has been developed with a morning dosing regimen that maximizes the potential for adherence [2]. Tac QD possibly contributes to reduce physical and mental stress for patients who need to take several different medications. In de novo kidney, liver, and heart transplantation, phase II studies have demonstrated that patients can be converted from Tac BID to Tac QD on a one-to-one total daily-dose basis [3], and the efficacy and safety of Tac QD were maintained in long-term graft survival [4]. Randomized phase III studies have reported

that Tac QD was well tolerated with similar efficacy and safety profiles to Tac BID in kidney and liver transplantation [5, 6]. However, several investigators have recently reported a sustained decrease in Tac exposure after conversion from Tac BID to Tac QD, and it is necessary to use a higher dose of Tac QD than Tac BID to achieve similar trough levels [7–10]. Therefore, the switch from Tac BID to Tac QD should be performed under close medical supervision.

In allo-SCT, prevention and treatment of graft-versus-host disease (GVHD) are critical factors in transplant-related morbidity and mortality. Tac plus methotrexate has been used as one of the standard regimens for GVHD prophylaxis after allo-SCT from unrelated donors [11, 12]. However, there have been no data available about the efficacy and safety of Tac QD conversion from intravenous Tac (Tac i.v.) in allo-SCT recipients. In this study, we analyzed the pharmacokinetics (PK) of Tac QD. The aim of this study was to evaluate the PK profile of Tac QD in allo-SCT patients. This is the first report of a PK study of Tac QD in patients who received allo-SCT from unrelated donors for the prevention of GVHD.

Methods

This is a prospective clinical trial involving investigation of the PK of Tac QD in allo-SCT patients. Patients who were 15–65 years of age were eligible to participate in this study. Patients were excluded if their serum creatinine was over 2 mg/dl, performance status by the ECOG criteria was over 2, and serology was positive for human immunodeficiency virus, or if they had uncontrolled infections. This study was approved by the Institutional Review Board at Jikei University and was registered at www.umin.ac.jp/ctr/ as UMIN000002441. Each patient provided written informed consent in accordance with the Declaration of Helsinki.

Study design

The patients were treated with various conditioning regimens (Table 1) and received unrelated bone marrow or umbilical cord blood transplantation. Micafungin was administered at a dose of 50 mg/day intravenously as antifungal prophylaxis until granulocyte counts exceeded 0.5×10^9/l. All patients received Tac i.v. at 0.03 mg/kg by continuous infusion beginning a day before transplantation with short-term methotrexate, 10 mg/m^2 on day 1, and 7 mg/m^2 on days 3, 6, and 11 in unrelated bone marrow, or 15 mg/m^2 on day 1 and 10 mg/m^2 on days 3 and 6 in umbilical cord blood. The dose of Tac i.v. was adjusted to maintain the whole-blood Tac concentration between 10 and 20 ng/ml. Administration of Tac i.v. was converted to Tac QD at a 1:4 ratio when the patients had recovered from regimen-related gastrointestinal toxicity and could tolerate oral medication. Tac QD exposure was adjusted to maintain a whole-blood trough concentration of 8–12 ng/ml. A dose reduction of Tac QD was recommended if serum creatinine increased more than 1.5 times from the baseline or if other serious toxicities associated with the agent appeared.

PK sampling and analysis

When the patients did not require a dose adjustment after conversion to Tac QD, blood samples were collected 0, 1, 2, 3, 6, 12, and 24 h after exposure. These blood samples were frozen at −20 °C within 2 h after collection and stored until shipment to the SRL laboratory in Tokyo, Japan, for analysis. The plasma concentration of Tac was determined by the ACMIA method. Area under the curve (AUC) was determined by the linear trapezoidal rule using a noncompartmental approach.

Table 1 Patient characteristics

	Age	Sex	Disease	Disease status at HSCT	Stem cell sources	Conditioning regimens
1	57	M	AML	CR2	HLA-matched unrelated	Flu/Bu 16
2	65	M	MDS	Overt	Umbilical cord blood	Flu/Mel 80/TBI 2
3	42	M	AML	CR1	HLA-matched unrelated	Flu/Bu 16
4	23	F	PTCL	PD	HLA-mismatched unrelated	ETOP/CY/TBI 10
5	42	M	AML	Non-CR	Umbilical cord blood	Flu/Bu 8/TBI 10
6	33	M	MDS	RAEB-1	HLA-mismatched unrelated	CY/TBI 12
7	45	M	ALL	CR1	HLA-mismatched unrelated	ETOP/CY/TBI 10
8	38	F	AML	CR2	HLA-mismatched unrelated	CY/TBI 12
9	63	M	AML	CR2	HLA-matched unrelated	Flu/Mel 140
10	57	F	AML	CR1	HLA-matched unrelated	Flu/Bu 16

M male; *F* female; *AML* acute myeloid leukemia; *MDS* myelodysplastic syndrome; *PTCL* peripheral T cell lymphoma, not otherwise specified; *Flu* fludarabine; *Bu* busulfan; *Mel* melphalan; *TBI* total body irradiation; *ETOP* etoposide; *CY* cyclophosphamide; *HSCT* hematopoietic stem cell transplantation; *RAEB-1* refractory anemia with excess blasts 1; *PD* progressive disease; *CR* complete remission; *HLA* major histocompatibility complex

Statistical analysis

The primary endpoint of this study was analysis of the pharmacokinetic behavior of Tac QD. The secondary endpoints were to assess the development of grade II–IV acute GVHD after the conversion. Acute GVHD was evaluated according to the standard criteria [13]. Neutrophil engraftment was defined as the first of three consecutive days with an absolute neutrophil count of greater than 0.5×10^9/l. Adverse events were graded according to the Common Terminology Criteria for Adverse Events Version 4.0. Spearman's rank correlation coefficient was calculated to assess the correlation between AUC and the trough of Tac QD. EZR was used for statistical analysis [14].

Results

Patient characteristics

This study included 10 consecutive patients with acute myeloid leukemia (AML) (n=6), acute lymphoblastic leukemia (ALL) (n=1), myelodysplastic syndrome (MDS) (n=2), and non-Hodgkin lymphoma (NHL) (n=1) who underwent allo-SCT from unrelated donors between October 2009 and May 2011. These patients' characteristics are summarized in Table 1. Median age was 45 (23–65)years. Six patients were classified into a standard-risk group (first complete remission: CR1=3, CR2=3) and four patients into an advanced-risk group (non-CR=3, refractory anemia with excess blast-1=1) at allo-SCT. Stem cell sources were bone marrow (BM) from an HLA-matched unrelated donor (n=4), BM from an HLA DRB1 1 antigen-mismatched unrelated donor (n=4), or umbilical cord blood (n=2).

Tac trough concentration after conversion from Tac i.v. to Tac QD

All patients received Tac i.v. at 0.03 mg/kg by continuous infusion beginning a day before transplantation. When the patients had recovered from regimen-related gastrointestinal toxicity and could tolerate oral medication, we converted Tac i.v. to Tac QD at a 1:4 ratio. After conversion, six out of 10 patients (60 %) showed a sustained decrease in Tac exposure and required dose adjustment (Fig. 1). It took a median of 11 (2–33)days to reach stable Tac exposure after the conversion. According to the conditioning regimens, five out of seven patients (71 %) who received total body irradiation (TBI≥ 10 Gy) or high-dose melphalan (≥80 mg/m^2) needed an increase in the dose of Tac QD, whereas one out of three patients (33 %) treated with busulfan without TBI and melphalan required dose escalation.

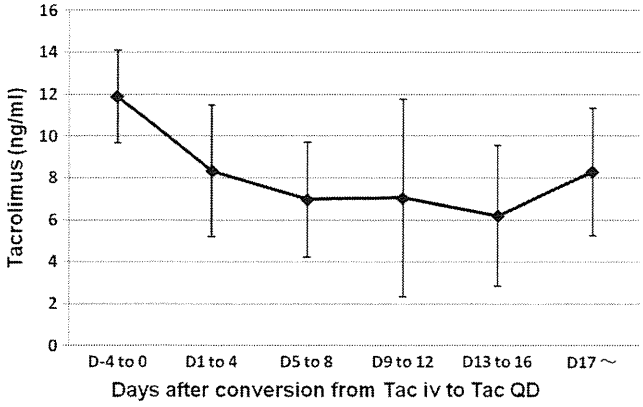

Fig. 1 The whole-blood trough of Tac tended to decrease after the conversion from Tac i.v. to Tac QD

PK analysis

In the PK analysis, the median C_0 was 9.8 ng/ml and C_{max} was 19.5 ng/ml at a T_{max} of 4.5 h post-dose (Fig. 2). The median area under the curve (AUC_{0-24}) was 246 ng·h/ml. There was a correlation between AUC and the trough of Tac QD (Fig. 3, ρ=0.8754, P=0.0009). To obtain AUC_{0-24} of more than 240 ng·h/ml, it is necessary to maintain the whole-blood trough at more than 7.5 ng/ml.

Development of acute GVHD and adverse effect of Tac QD

Engraftment was confirmed in all patients. One patient experienced a rapid decrease in the trough concentration of Tac from 9.8 to 3.6 ng/ml right after the conversion, and he developed grade II acute GVHD in the skin. No patients developed grade III–IV acute GVHD within the first 100 days after allo-SCT. The most common adverse event was grade 1–2 elevation of AST or ALT (10 patients), but all patients improved promptly without dose modification of Tac QD. Three patients developed nephrotoxicity (grade 1 in one patient, grade 2 in two patients), and one patient needed to reduce the dose of Tac because of a transient increase of serum creatinine up to 1.85 mg/dl. Within the first 100 days after allo-SCT, all patients were alive and disease free. The probability of overall survival at 3 years was 70 %. Three patients died because of relapse in two patients and cardiac failure in one patient.

Discussion

GVHD remains one of the main causes of treatment-related mortality after allo-SCT, so that prevention of GVHD is an important issue for the treatment to succeed. The combination of calcineurin inhibitor and short-term methotrexate has been one of the standard prophylaxis methods. Calcineurin inhibitors are usually started intravenously a day before

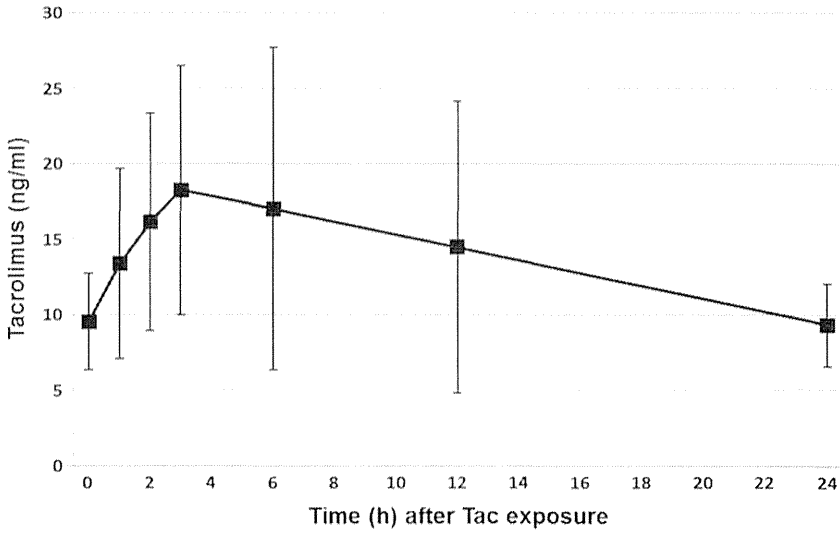

Fig. 2 Pharmacokinetic analysis of Tac QD

transplantation, and the agents are converted to an oral formulation when patients can tolerate oral medication. Twice-daily formulation of oral calcineurin inhibitors has been widely used in allo-SCT and solid organ transplantation. As lifelong immunosuppression is required to prevent graft dysfunction in solid organ transplantation, medication nonadherence is a matter of utmost concern. Tac QD has been developed to improve compliance in kidney and liver transplantation, and it has been well tolerated with similar efficacy to Tac BID [5, 6]. Although patients who have undergone allo-SCT are supposed to take oral Tac for a shorter period than solid transplant recipients, once-daily Tac may reduce physical and mental stress for patients who need to take several different medications. However, there have been no data available about the efficacy and safety of Tac QD conversion from Tac i.v. in the setting of allo-SCT. We speculated that the use of Tac QD instead of Tac BID for GVHD prophylaxis exhibits an equivalent effect.

Recently, the necessity of using higher doses of Tac QD to achieve a therapeutic level compared with the Tac BID dose in a kidney transplant has been reported [7–10], despite initial reports showing the bioequivalence of Tac QD with Tac BID. These reports suggest that lower Tac exposure is observed after conversion from Tac BID to Tac QD because of decreased bioavailability. In this study, the patients were converted from Tac i.v. to Tac QD at a 1:4 ratio according to one of the standard approaches for the conversion from Tac i.v. to Tac BID [15, 16]. The 1:4 ratio resulted in a decrease of Tac exposure for six out of 10 patients (60 %) and required dose adjustment. According to the conditioning regimens, most of the patients who needed dose adjustment received TBI ≥10 Gy or high-dose melphalan. Mucosal injury of the intestine might reduce absorption of the agent. Importantly, one patient experienced a rapid decrease in the trough concentration of Tac right after the conversion and he developed grade II acute GVHD in the skin. Therefore, the conversion from

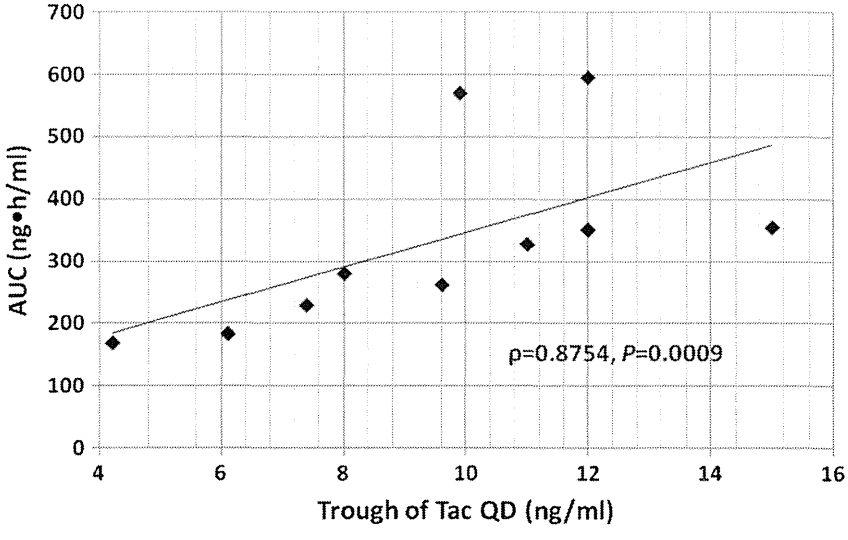

Fig. 3 There was a correlation between AUC and trough of Tac QD

Tac i.v. to Tac QD should be performed under close medical supervision. The patients who receive high-dose TBI or melphalan as a conditioning regimen in particular may have to increase the conversion ratio to more than 1:4. Mita et al. reported that the optimal initial dose ratio of Tac i.v. to Tac QD was 1:8 in the setting of liver transplantation [17].

PK analysis showed that there was a correlation between AUC and the trough of Tac QD (Fig. 3). We set a target AUC_{0-24} of Tac QD of more than 240 ng·h/ml because we had adjusted the dose of Tac i.v. by continuous infusion to maintain the whole-blood Tac concentration between 10 and 20 ng/ml, and preservation of Tac blood concentration 10 ng/ml for 24 h would obtain an AUC_{0-24} of 240 ng·h/ml. Patients required a whole-blood Tac QD trough of at least 7.5 ng/ml to achieve an AUC of more than 240 ng·h/ml (Fig. 3). Although four patients received bone marrow from an HLA DRB1 1 antigen-mismatched unrelated donor, no patients developed grade III–IV acute GVHD. Obtaining a Tac QD trough above 7.5 ng/ml may be a valid approach for the prevention of acute GVHD.

In this study, engraftment was confirmed in all patients, and no patients died within 100 days after transplantation. Grade 1–2 renal and hepatic toxicities were observed, but they were mild and transient. The increased frequency of nephrotoxicity found in previous studies might have resulted from the higher doses of Tac or the higher ranges of blood concentration [18, 19]. One patient developed transient congestive heart failure on day 6 after allo-SCT. The symptom of heart failure immediately resolved after the treatment, and left ventricular ejection fraction recovered from 41 to 54 %, as measured by echocardiography. This patient could be discharged with no complications on day 55; however, he died of cardiac failure on day 220. Autopsy confirmed dilated cardiomyopathy. Although there have been several reports about Tac-associated reversible myocardial hypertrophy [20–22], only a few case reports of Tac-related dilated cardiomyopathy have been publicized in the setting of allo-SCT. However, we could not completely rule out an effect of Tac on this cardiac complication.

In conclusion, despite initial reports showing the bioequivalence of Tac QD with Tac BID, we found that 60 % of patients experienced a sustained decrease in Tac exposure. The conversion from Tac i.v. to Tac QD should be performed under close medical supervision. AUC and the trough of Tac QD showed a correlation in allo-SCT recipients. The whole-blood trough should be maintained around at least 7.5 ng/ml to provide an adequate level of AUC. The modification of Tac QD to maintain a whole-blood trough concentration above 7.5 ng/ml may be as effective as Tac BID.

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Conflict of interest The authors declare that they have no conflict of interest.

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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

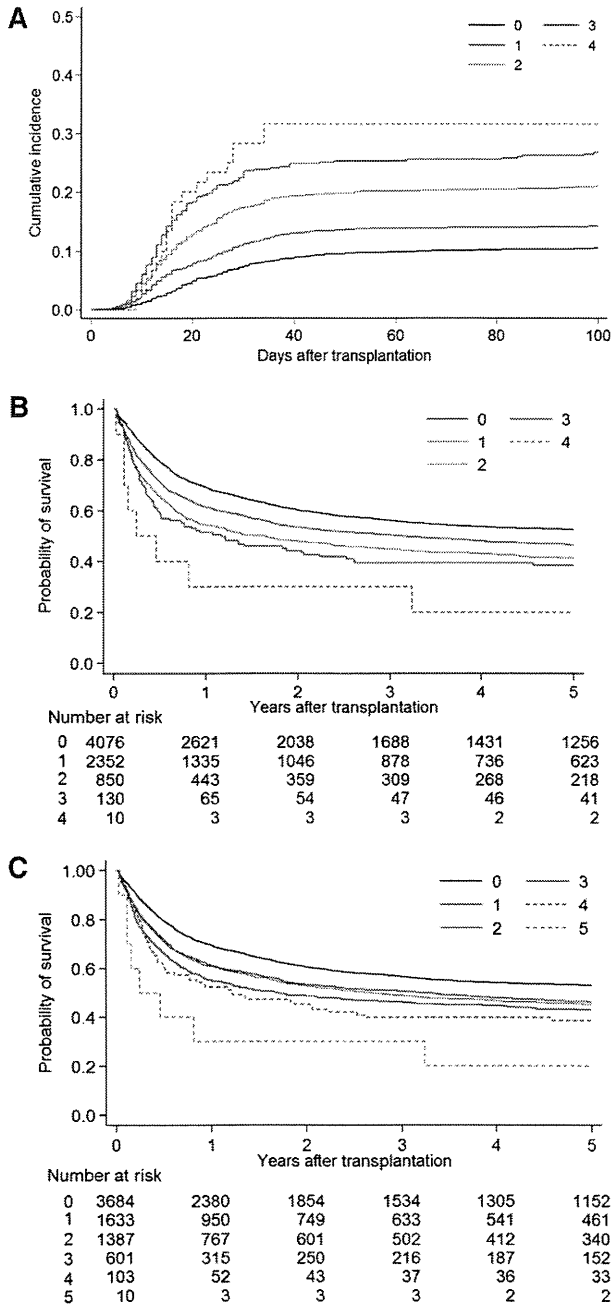

Figure 1. Acute GVHD and survival curve by the number of multiple HLA locus mismatches. The number of HLA 1-allele mismatches in the GVH direction, with exclusion of 2-allele mismatches, in each HLA locus was summed. (A) Cumulative incidence of grade III-IV acute GVHD by the mismatch number of HLA-A, -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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