Table 4 Representative reports of MMF studies for GvHD prophylaxis in HSCT

References	*N*	Age (range)	Regimen	Donor	Neutrophil engraftment (range)	Grade II–IV acute GvHD	Extensive chronic GvHD	NRM
CsA-based studies								
Jenke et al. [33]	15	32 (26–57)	Myeloablative	Related: 9 Unrelated: 6	11 days (8–27)	40 %	N.A.	27 %
Niederwieser et al. [77]	52	48 (6–65)	RIC	Unrelated	N.A.	63 %	30 %	29 % at 1 year
Maris et al. [45]	89	53	RIC	Unrelated	15 days (0–55)	52 %	37 % at 1 year	16 % at 1 year
Rodoriguez et al [86]	22	49 (18–66)	RIC	Unrelated	13 days (10–71)	63 %	45 %	32 %
Baron et al. [48]	21	54 (33–66)	RIC	Unrelated	7 days (0–20)	82 %	73 %	11 % at 1 year
Nash et al. [29]	46	49 (18–64)	Myeloablative	Related	15 days (10–20) in PII	62 %	72 %	37 %
Neumann et al. [61]	26	39 (22–57)	Myeloablative	Related	12 days	38 %	50 %	17 %
Giaccone et al. [38]	85	52 (18–70) in twice daily 52 (17–67) in thrice daily	RIC	Unrelated	N.A.	26 % in twice daily 32 % in thrice daily	N.A.	N.A.
Gupta et al. [46]	24	64.5 (60–71)	RIC	Related	13 days (7–27)	45 %	45 %	17 % at 2 years
Maris et al. [49]	103	54 (17–69.6)	RIC	Unrelated	7 days (0–44)	53 %	56 %	19 % at 2 years
Burnstein et al. [90]	110	51 (17–16)	RIC	Cord blood	12 days (0–32)	59 %	23 % at 1 year	26 % at 3 years
Baron et al. [50]	71	56 (17–75)	RIC	Unrelated	N.A.	77 %	45 %	29 % at 1 year
Perez–Simon et al. [87]	44	48 (17–60)	RIC	Unrelated	9 days	53 %	63 %	42 %
Pinana et al. [79]	52	57 (18–71)	RIC	Related	15 days (11–27)	38 %	39 %	25 %
FK506-based studies								
Osunkwo et al. [96]	34	7 (0.5–21)	Myeloablative: 21 RIC: 16	Cord blood: 22 Related: 15	16 days (3–79)	45.4 %	None developed extensive GvHD.	29.4 %
Haentzschel et al. [100]	29	53 (21–69)	Flu 120 mg/m^2 + BU 13.8 mg/kg	Related: 7 Unrelated: 22	13 days (10–30)	57 %	19 %	31 %
Sabry et al. [80]	131	54 (20–66)	RIC	Related	10 days (2–27)	19.7 %	76.1 % at 2 year	15.5 % at 7 years
Mizumoto et al. [78]	21	55 (24–66)	RIC	Unrelated	19 days (13–35)	33 %	55 %	19 %
Bhatia et al. [36]	38	8 (0.33–16)	Myeloablative: 17 RIC: 21	Related: 18 Unrelated: 20 (including cord blood)	N.A.	54.4 %	33.7 %	N.A.
Wakahashi et al. [89]	36	43 (33–66) in $AUC_{0-24\ h}$ <30 50 (20–66) in $AUC_{0-24\ h}$ >30	Myeloablative: 23 RIC: 13	Unrelated: 15 Cord blood: 21	11 days in BMT 20–21 days in CBT	46.7 % in $AUC_{0-24\ h}$ <30 15.8 % in $AUC_{0-24\ h}$ >30	30.8 % in $AUC_{0-24\ h}$ < 30 0 % in $AUC_{0-24\ h}$ > 30	30.6 %

Table 4 continued

References	*N*	Age (range)	Regimen	Donor	Neutrophil engraftment (range)	Grade II–IV acute GvHD	Extensive chronic GvHD	NRM
Zohren et al. [88]	50	51 (25–67)	RIC	Unrelated	8 days (0–30)	54 %	21 %	26 %
Kanda et al. [91]	27	33 (20–58)	Flu 160 mg/m^2 + TBI 1,350 cGy	Dual cord blood	24 days (13–45)	37 %	31 % (95 % CI, 15–49 %)	28 % at 2 yars
Uchida et al. [92]	29	62 (52–70)	Varies	Cord blood	19 days (13–32)	63 %	7 % at 2 years	28 % at 1 year

N.A. not applicable, *RIC* reduced intensity conditioning, *CsA* cyclosporine, *FK506* tacrolimus, *MTX* methotrexate, *MMF* mycophenolate mofetil, *CI* confidence interval, *NRM* non-relapse mortality, *GvHD* graft-versus-host disease, *HSCT* hematopoietic stem cell transplantation, *Flu* fludarabine, *TBI* total body irradiation, *BU* busulfan, *AUC* area under the curve

Prophylactic use for unrelated donors (except for umbilical cord blood)

Representative reports of MMF prophylaxis for unrelated donors are listed in Table 4 [38, 45, 47, 49, 77, 78, 86–88]. A large-scale study of eighty-nine patients transplanted under RIC using either oral or intravenous MMF and CsA showed 93 % engraftment. The rates of grade II, III, and IV acute GvHD were 42, 8, and 2 %, respectively [45]. We also showed 15 cases of myeloablative or RIC HSCT from unrelated donors using oral MMF and FK506. All, except one, were engrafted, and grade II–IV acute GvHD occurred in 6 patients (42.8 %) [89]. These results indicate that prophylactic use of MMF plus CI seems not to interfere with primary engraftment and prevent acute GvHD, as well as MTX plus CI, in HSCT from unrelated donors.

Prophylactic use for CBT

MMF is usually a feasible option for GvHD prophylaxis in CBT, wherein primary graft failure due to limited stem cell numbers is a concern. Large prospective study consisting of 110 adult umbilical cord blood transplantations demonstrated that the combination of MMF and CsA for GvHD prophylaxis facilitated engraftment (neutrophil engraftment was achieved in 92 % at a median of 12 days) and suppressed grade II to IV acute and chronic GvHD (59 and 29 %, respectively)[90]. In dual CBT, FK506 and MMF (1,000 mg twice daily) were given to 27 patients until at least 60 days after myeloablative conditioning [fludarabine (160 mg/m^2) plus TBI 1.350 cGy]. Neutrophil engraftment was achieved in 80 % with a median of 24 days. Grade II to IV acute GvHD occurred in 37 %. Cumulative incidence of total parental nutrition usage was up to 56 %. This reflected the less gastrointestinal mucosal damage caused by MMF than MTX even under myeloablative conditioning [91]. A Japanese group also reported 29 elderly (median age 62) RIC-CBT patients who received FK506 and MMF for acute GvHD prophylaxis. The patients were compared with matched-pair historical controls who received FK506 alone [92]. Primary engraftment until day 60 was significantly higher (90 %) in the FK506 plus MMF group than the control group (69 %). Cumulative incidence of grade II to IV acute GvHD was 63 %. Interestingly, severe pre-engraftment immune reaction (PIR), which was a factor that negatively affected overall survival [93–95], was significantly lower (16 %) in the FK506 plus MMF group than the control group (52 %). Consequently, NRM in the FK506 plus MMF group within 30 days was significantly lower compared to the control group (0 vs. 21 %). Our experiences with 21 adult myeloablative CBT patients, who received FK506 and MMF, also showed

Table 5 Overview of pharmacokinetics studies in HSCT

Reference	*N*	Age (range)	Regimen	Additional prophylaxis	MMF dosing (mg/kg)	MPA AUC (mg h/L)	C_{ss} (mg/L)	MPA CL (L/h kg)	MPA C_{max} (mg/L)	MPA C_{trough} (mg/L)	MPA $T_{1/2}$ (h)
Kiehl et al. [97]	14	N.A.	Myeloablative	CsA	1–3 g daily	N.A.	N.A.	N.A.	1.64 (median)	0.47 (median)	N.A.
Jenke et al. [33]	15	32 (26–57)	Myeloablative	CsA	12.5–17 mg/kg every 12 h	15.6–59.3	N.A.	0.73–2.73	8.48–38.6	N.A.	1.51–2.45
Maris et al. [45]	19	N.A.	RIC	CsA	15 mg/kg twice daily	20.3 (median)	N.A.	N.A.	5.3 (median)	0.5 (median)	3.0 (median)
Nash et al. [29]	46	49 (18–64)	Myeloablative	CsA	15 mg/kg every 6.8 and 12 h	16.4–34.5	1.19–4.84	0.4–1.0	5.9–12.7	0.13–0.64	0.8–1.48
Giaccone et al. [38]	85	52 (18–70) in twice daily 52 (17–67) in thrice daily	RIC	CsA	15 mg/kg every 8, 12 h	5.8–46.1 in twice daily 8.5–64.8 in thrice daily	1.9 in twice daily 3.8 in thrice daily	N.A.	1.0–29.3	0.8 in twice daily 2.5 in thrice daily	3.4 in twice daily 2.7 in thrice daily
van Hest et al. [28]	15	32 (17–58)	RIC	CsA	750–2,000 mg twice daily	7.6–35	N.A.	N.A.	2.6–23	0–4.0	0.8–5.7
Haentzschel et al. [100]	29	53 (21–69)	Myeloablative	FK506	1,500–2,500 mg (i.v.) twice daily	35.1–43.1	N.A.	N.A.	16–25	N.A.	N.A.
Perez-Simon et al. [87]	8	N.A.	RIC	CsA	1 g every 12 h or 1 g every 8 h	$AUC_{0-24\ h}$: 106.46 (60.2–199.17)	N.A.	N.A.	N.A.	1.11 (0.4–2.6)	N.A.
Royer et al. [99]	15	51 (20–60)	RIC	CsA	750 mg tid if BW <70 kg 1 g tid if BW >70 kg	21.83 (8.96–49.99) at day 7	N.A.	N.A.	N.A.	N.A.	N.A.
Jacobson et al. [63]	30	55 (29–69) in 1 g every 8 h 53 (21–67) in 1.5 g every 12 h	RIC	CsA	1 g every 8 h or 1.5 g every 12 h	$AUC_{0-24\ h}$: 53.59 (22.68–101.99) in 1 g every 8 h 60.9 (35.89–127.24) in 1.5 g every 12 h	2.33 (0.95–4.25) in 1 g every 8 h 2.53 (1.46–5.24) in 1.5 g every 12 h	N.A.	8.09 (1.4–10.8) in 1 g every 8 h 11.74 (7.22–26.5) in 1.5 g every 12 h	0.65 (0.39–8.38) in 1 g every 8 h 0.58 (0.29–4.18) in 1.5 g every 12 h	N.A.
Bhatia et al. [36]	38	8 (0.33–16)	Myeloablative 17 RIC 21	FK506	900 mg/m^2/dose (i.v.) every 6 h	26.82–33.71	4.73–6.46	1.17–1.46	12.31–16.54	0.33–0.72	1.02–2.49
de Winter et al. [37]	38	43 (17–65)	N.A.	CsA	500–2,000 mg daily (median 1,000 mg)	N.A.	N.A.	45.6L/h	N.A.	N.A.	N.A.

Table 5 continued

Reference	*N*	Age (range)	Regimen	Additional prophylaxis	MMF dosing (mg/kg)	MPA AUC (mg h/L)	C_{ss} (mg/L)	MPA CL (L/h kg)	MPA C_{max} (mg/L)	MPA C_{trough} (mg/L)	MPA $T_{1/2}$ (h)
Wakahashi et al. [89]	36	43 (33–60) in AUC <30 50 (20–66) in AUC >30	Myeloablative 23 RIC 13	FK506	15–25 mg/kg every 12 h or 1,000 mg every 8 h	$AUC_{0-24\ h}$: 30.4 (median)	N.A.	N.A.	2.5 (median)	N.A.	N.A.

N.A. not applicable, *RIC* reduced intensity conditioning, *CsA* cyclosporine, *FK506* tacrolimus, *MPA* mycophenolic acid, *MMF* mycophenolate mofetil, *AUC* area under the curve, C_{ss} concentration steady state, C_{max} maximum MPA peak concentrations, *MPA CL* MPA clearance, C_{trough} concentration at trough, *MPA* $T_{1/2}$ half-life of MPA

85.7 % engraftment and only 20 % grade II to IV acute GvHD. In pediatric CBT study, the combination of FK506 and MMF was used for GvHD prophylaxis in 22 cases with either myeloablative or RIC regimen [96]. The median time to recovery was 23 days, and grade II to IV acute GvHD occurred in 33.3 % among the evaluated patients.

Collectively in CBT setting, the addition of MMF to CI for the prophylaxis of acute GvHD seems to be feasible in RIC, as well as myeloablative conditioning.

Dose-finding studies and TDM

Many pharmacokinetics studies on MMF had been reported for organ transplantation, especially in renal transplantation. However, limited pharmacokinetics studies for finding the optimal dosage in HSCT have been observed to date. Table 5 shows the summary of pharmacokinetics studies in HSCT. In general, there is a large inter-patient, as well as intra-patient, variation in plasma MPA levels of HSCT patient as seen in organ transplantation. However, the peak of MPA levels in HSCT is significantly lower than those in organ transplantation. Some pharmacokinetics studies on MMF used for acute and chronic GvHD treatment showed that concentration at trough (C_{trough}) of MPA was significantly greater in the treatment responder than the non-responder [97, 98]. However, correlations between the efficacy on the prevention of acute GvHD and the MPA concentration have not been elucidated. Our small retrospective cohort showed that in patients with adjusted MPA $AUC_{0-24\ h}$ over 30 mg h/L ($C_{ss} > 1.25$ mg/L), acute GvHD, as well as chronic GvHD, occurred significantly less, especially in HSCT from unrelated bone marrow donors. On the contrary, lower MPA levels were enough to control acute and chronic GvHD in CBT. Moreover, a higher MPA level in CBT posed a tendency of GvHD relapse possibly due to weakened graft-versus-leukemia/lymphoma (GVL) effect of cord blood [89]. This finding is encouraging for prospective dose-finding studies depending on each donor source. Recently, one small prospective study demonstrated that at day 7, patients with $AUC_{0-8\ h} \geq 22.5$ mg h/L (concentration at steady state (C_{ss}) ≥ 2.8 mg/L) displayed no grade II to IV acute GvHD [99]. As a target range after organ transplantation, it has been suggested to keep C_{ss} MPA between 2.5 and 5 mg/L. In Japan, where HLA homogeneity and less GvHD incidence are more common than in Western countries, lower C_{ss} might be enough to prevent severe acute GvHD. Although MMF of 45 mg/kg/day dose reached a relatively high median C_{ss} MPA, 2.73–3.2 mg/L, it did not significantly reduce the occurrence of acute GvHD compared to historical controls receiving MTX instead of MMF for GvHD prophylaxis [29]. Further pharmacokinetics studies

should be carried out for optimal MMF dose finding, as well as understanding the precise pharmacodynamics of MPA in HSCT and the prevention of GvHD.

Surrogate marker for the prediction of MPA AUC, C_{trough} or C_{max}?

With limited evidences, there is a need to monitor the concentrations of MPA AUC or C_{ss} for evaluating the efficacy in HSCT, as well as in organ transplantation. As a surrogate marker for MPA AUC, the C_{trough} or C_{max} was often monitored. Haetzcshel et al. [100] reported that a significant correlation was observed between C_{max} and AUC for MPA in 28 patients evaluable at all points. Our data also showed that the concentration at 2 h (C_{2h}) after MMF administration was well correlated with AUC of MPA [89]. These results were encouraging for the utilization of C_{max} as surrogate marker of the AUC of MPA.

Table 6 Adverse effects of MMF

Hematological toxicities
Leukocytopenia
Anemia
Thrombocytopenia
Gastrointestinal toxicities
Nausea
Vomiting
Abdominal pain
Diarrhea
Infectious toxicities
Viral infections
CMV
EBV
HSV
Fungal infections
Aspergillosis
Candidiasis
Others

MMF mycophenolate mofetil, *CMV* cytomegalovirus, *EBV* Epstein–Barr virus, *HSV* herpes simplex virus

Toxicities and adverse effects

The adverse effects of MMF are listed in Table 6. The most common toxicities are gastrointestinal toxicity, opportunistic infections, and pancytopenia. Most of the physicians' concern is the gastrointestinal toxicity, which is usually manifested as diarrhea. MMF can produce colitis with focal ulcerations, marked apoptosis, and intense acute and chronic inflammation [101]. Histological features of MMF-related colitis are remarkably similar to the ones associated with intestinal GvHD. We are able to distinguish MMF-related colitis from intestinal GvHD only when there is improvement or resolution of symptoms on the withdrawal or reduction of MMF. This may cause some problems when we estimate the efficacy of MMF for salvage therapy of acute interstitial GvHD. However, few previous reports on prophylactic use of MMF discontinuation due to diarrhea until day 30 after HSCT have been published. There are insufficient data on whether MMF can also produce lesions in the upper gut or not.

Infectious complications, including CMV reactivation, are also common and can be serious adverse effects. In HSCT patients within day 100 after the transplantation,

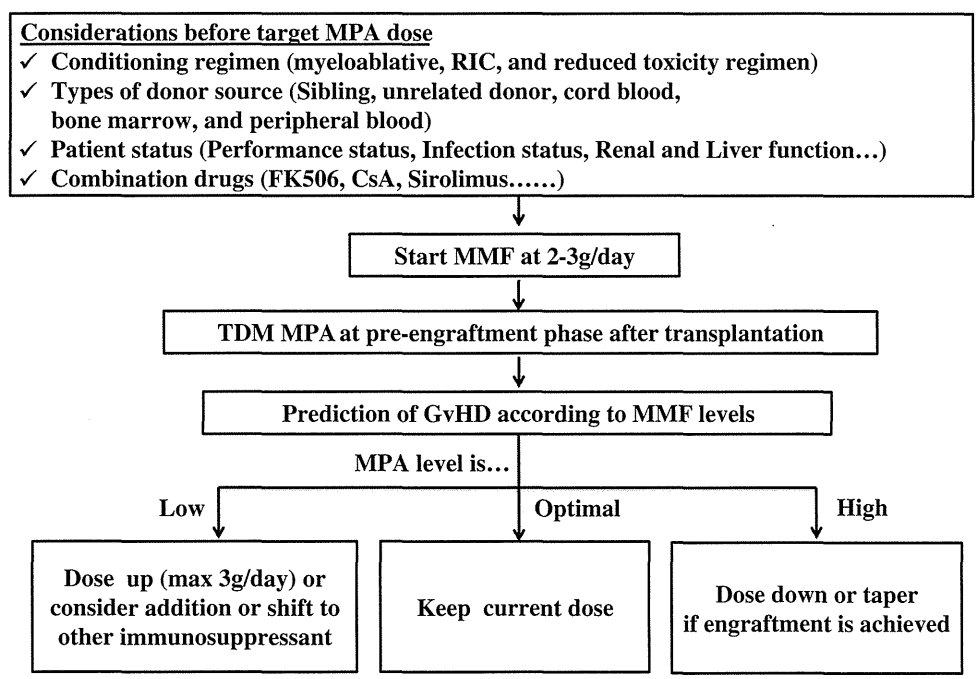

Fig. 2 New proposal algorithm for optimizing MMF dose. First, start MMF at 2–3 g/day. Second, monitor MPA concentration at pre-engraftment phase after transplantation. Third, adjust the MMF dosage according to the estimation of GvHD risk or switch to/add another immunosuppressant, such as steroids, if MPA concentration is too low

CMV infection, as well as antigenemia, are most likely to happen because of insufficient immune recovery after conditioning regimen and probably with acute GvHD. Prospective trial of 85 patients for HCST with RIC demonstrated that an elevated unbound C_{ss} was significantly associated with CMV reactivation [38]. A retrospective study of 21 consecutive patients with acute and extensive chronic GvHD showed the occurrence of 22 opportunistic or serious viral or bacterial infections in 10 patients [12]. Because MMF also prevents immune recovery, preemptive therapy should be required for CMV reactivation by monitoring CMV antigenemia, as well as empiric therapy for bacterial and fungal infections. Leukocytopenia, anemia, and thrombocytopenia are alarming when we worry about primary and secondary engraftment after HSCT.

A relationship between high MPA AUC values and drug-related adverse effects has been reported in some studies [102–105]. In a prospective, randomized, double-blind, multicenter, controlled study in 150 renal transplant patients, a dose-dependent increase in adverse effects was reported in the first 6 months post-transplant [106]. According to these data, it appears that an MPA $AUC_{0-12\ h}$ above 60 mg h/L may increase the risk of toxicity, although such high plasma MPA levels could be hardly achieved in HSCT as described above.

Conclusions and future directions

The relationship between MMF pharmacokinetics/pharmacodynamics and its effectiveness in HSCT is still obscure. However, MMF has been widely used for GvHD prophylaxis, as well as treatment of HSCT, in Europe and the USA.

One of the most important aspects of the pharmacokinetics of MMF is the wide intra-patient and inter-patient variations in the plasma MPA levels even under the same daily dose. On the other hand, similar to organ transplantation where higher MPA is correlated with lower rejection rate, higher MPA would correlate with the suppression of immune reactions, such as acute GvHD in HSCT. Then, we proposed a model of algorithm for the optimal dose finding using TDM of MMF (Fig. 2). For GvHD prophylaxis, MMF should be started at 2–3 g/day. In the earlier days after HSCT, such as at the pre-engraftment phase, the plasma MPA levels should be monitored (MPA AUC is preferred, but MPA $C_{2\ h}$ might be an alternative). If the MPA level is low, based on the prediction of upcoming GvHD as determined by individual risk factors such as conditioning, donor type and combination immunosuppressant, MMF dosage should be increased up to the maximum (3 g/day), or other immunosuppressants, such as steroids if the maximum dose had been administered, should be added/shifted to. For example, MPA $AUC_{0-24\ h}$ at day 9 or 16 should be >30 mg h/L for Japanese ordinary unrelated BMT. On the other hand, if MPA levels are high enough to prevent acute GvHD, MMF should be tapered as soon as the engraftment is achieved. In our study, we could predict that MPA $AUC_{0-24\ h}$ <30 mg h/L at day 9 or 16 would be usually enough for single unit Japanese CBT. The risk for relapse is higher at higher MPA levels. Thus, the MMF dose must be keep at the minimal requirement.

In conclusion, MMF is a safe and effective prophylaxis for the prevention of acute GvHD, as well as its treatment. MMF has been frequently used in RIC regimen and CBT. In a myeloablative setting, MMF has not been used by some clinicians due to limited clinical studies. To elucidate the advantage of the prophylactic use of MMF depending on the donor sources in the myeloablative regimen as well as RIC, larger prospective studies accompanying TDM are needed.

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Recipient *PTPN22* −1123 C/C Genotype Predicts Acute Graft-versus-Host Disease after HLA Fully Matched Unrelated Bone Marrow Transplantation for Hematologic Malignancies

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Article history:
Received 2 July 2012
Accepted 20 September 2012

Key Words:
Unrelated donor
Single nucleotide variation
Lymphoid specific phosphatase
rs2488457
Promoter gene variant

A B S T R A C T

PTPN22 is a critical negative regulator of T cell responses. Its promoter gene variant (rs2488457, −1123G>C) has been reported to be associated with autoimmune diseases. This study analyzed the impact of the *PTPN22* variant on transplantation outcomes in a cohort of 663 patients who underwent unrelated HLA-matched bone marrow transplantation (BMT) for hematologic malignancies through the Japan Marrow Donor Program. The recipient C/C genotype versus the recipient G/G genotype resulted in a lower incidence of grade II-IV acute graft-versus-host disease (hazard ratio [HR], 0.50; 95% confidence interval [CI], 0.29-0.85; $P = .01$), as well as a higher incidence of relapse (HR, 1.78; 95% CI, 1.10-2.90; $P = .02$), as demonstrated on multivariate analysis. In patients with high-risk disease, the recipient C/C genotype was associated with significantly worse overall survival rates than the recipient G/G genotype (HR, 1.60; 95% CI, 1.02-2.51; $P = .04$), whereas this effect was absent in patients with standard-risk disease. In addition, the donor G/C genotype was associated with a lower incidence of relapse (HR, 0.58; 95% CI, 0.40-0.85), which did not influence survival. Our findings suggest that *PTPN22* genotyping could be useful in predicting prognoses and creating therapeutic strategies for improving the final outcomes of allogeneic BMT.

INTRODUCTION

The *PTPN22* gene encodes lymphoid specific phosphatase (Lyp), expressed in T and B lymphocytes, monocytes, dendritic cells (DCs), neutrophils, natural killer cells and thymocytes [1]. *PTPN22* is an important negative regulator of T cell activation involved in the dephosphorylation and inactivation of TCR-associated kinases. A single nucleotide variant of the *PTPN22* promoter gene, rs2488457 (−1123G>C), is associated with susceptibility to autoimmune diseases, including type 1 diabetes and rheumatoid arthritis, in Caucasian and Asian populations [2-6].

The role of *PTPN22* in the immune response, as well as the association of the *PTPN22* variant with autoimmunity, prompted us to investigate the impact of donor and recipient −1123G>C variation in the *PTPN22* gene on the clinical outcomes of patients undergoing allogeneic bone marrow transplantation (BMT) using an HLA allele-matched unrelated donor through the Japan Marrow Donor Program (JMDP). Our data show that the recipient C/C genotype is associated with a significantly lower incidence of grade II-IV acute graft-versus-host disease (aGVHD) and a higher incidence of relapse, which predict worse survival outcomes for patients with high-risk disease.

PATIENTS AND METHODS

Patients

PTPN22 genotyping was performed on 663 patients with hematologic malignancies and their unrelated donors who underwent BMT through the JMDP with T cell–replete marrow from HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 allele-matched donors between January 1993 and December 2007. This cohort represents 7% (663 of 9229) of all recipients of unrelated BMT in Japan during the study period. All available data and samples for eligible patients and their donors were analyzed. None of the patients had a history of previous transplantation. The study cohort included Asian patients only. The final clinical survey of these patients was completed by November 1, 2008. Diagnoses included acute myelogenous leukemia (AML) in 215 patients (32%), acute lymphoblastic leukemia (ALL) in 164 patients (25%), chronic myelogenous leukemia (CML) in 118 patients (18%), myelodysplastic syndrome (MDS) in 89 patients (13%), malignant lymphoma (ML) in 73 patients (11%), and multiple myeloma in 4 patients (1%) (Tables 1 and 2). The median follow-up duration in the survivors was 2103 days (range, 124-5136 days); 183 recipients (28%) relapsed or progressed, and 322 (49%) died, 16 (2%) before engraftment. Recipients with AML or ALL in first complete remission, CML in any chronic phase, ML in any complete remission, or MDS were classified as having standard-risk disease. All others were classified as

Financial disclosure: See Acknowledgments on page 245.

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1083-8791/$ – see front matter © 2013 American Society for Blood and Marrow Transplantation.
http://dx.doi.org/10.1016/j.bbmt.2012.09.014

Table 1
Donor and Recipient Characteristics

Variable	Value
Number of cases	663
Recipient age, years, median (range)	34 (1-67)
Donor age, years, median (range)	34 (20-57)
Year of BMT, median (range)	2001 (1993-2007)
Recipient *PTPN22* genotype, n (%)	
G/G	228 (34)
G/C	331 (50)
C/C	104 (16)
Donor *PTPN22* genotype, n (%)	
G/G	219 (33)
G/C	324 (49)
C/C	120 (18)
Recipient sex, n (%)	
Male	395 (60)
Female	268 (40)
Donor sex, n (%)	
Male	420 (63)
Female	243 (37)
Donor/recipient sex match, n (%)	
Sex-matched	426 (64)
Female/male	106 (16)
Male/female	131 (20)

having high-risk disease. Myeloid malignancies included AML, CML, and MDS, and lymphoid malignancies included ALL, ML, and multiple myeloma. All patients received cyclosporine- or tacrolimus-based therapy for GVHD prophylaxis; none received anti–T cell therapy, such as antithymocyte globulin or ex vivo T cell depletion. All patients and donors provided written informed consent to participate in molecular studies of this nature at the time of transplantation, in accordance with the Declaration of Helsinki. This project was approved by the Institutional Review Board of Kanazawa University Graduate School of Medicine and the JMDP.

PTPN22 Genotyping

Genotyping of *PTPN22* was performed using the TaqMan-Allelic discrimination method as described previously [7]. The genotyping assay was conducted in 96-well PCR plates using specific TaqMan probes for the *PTPN22* gene single nucleotide polymorphism rs2488457 (catalog C_16027865_10) in a StepOne Plus real-time PCR system (Applied Biosystems, Foster City, CA).

Table 2
Pretransplant Characteristics

Variable	Value
Disease, n (%)	
AML	215 (32)
ALL	164 (25)
MDS	89 (13)
ML	73 (11)
CML	118 (18)
Multiple myeloma	4 (1)
Disease stage, n (%)	
Standard risk	406 (61)
High risk	257 (39)
ABO matching, n (%)	
Major or/and minor mismatch	255 (38)
Major mismatch	145 (22)
Minor mismatch	129 (19)
Bidirectional	19 (3)
Missing	9 (1)
Conditioning regimen, n (%)	
Myeloablative	583 (88)
Reduced intensity	80 (12)
With total body irradiation	525 (79)
Pretransplantation CMV serostatus, n (%)	
CMV-positive recipient	420 (72)
Missing	80 (12)
GVHD prophylaxis, n (%)	
With cyclosporine	376 (57)
With tacrolimus	285 (43)
Missing	2 (0)
TNC, $\times 10^8$/kg, median (range)	5.0 (0.1-316.8)

Data Management and Statistical Analysis

Data were collected by the JMDP using a standardized report form. Follow-up reports were submitted at 100 days and 1 year post-transplantation, and annually thereafter. Pretransplantation cytomegalovirus (CMV) serostatus was routinely tested in recipients only, not in donors. Engraftment was confirmed by an absolute neutrophil count of $>0.5 \times 10^9$/L for at least 3 consecutive days. Outcome classification, including GVHD, did not change over time.

After data collection, aGVHD and chronic GVHD (cGVHD) were diagnosed and graded based on classically defined criteria [8,9]; namely, aGVHD was defined as GVHD developing within the first 100 days post-transplantation, and cGVHD was defined as GVHD occurring after day 100. Data using the updated criteria for assessment of GVHD [10,11] were not available for our cohort. The overall survival (OS) rate was defined as the number of days from transplantation to death from any cause. Disease relapse was defined as the number of days from transplantation to disease relapse. Transplantation-related mortality (TRM) was defined as death without relapse. Any patients alive at the last follow-up date were censored. Data on infectious organisms, postmortem changes in causes of death, and supportive care, including prophylaxis for infections and therapy for GVHD given on an institutional basis, were not available for this cohort.

All statistical analyses were performed with the EZR software package (Saitama Medical Center, Jichi Medical University), a graphical user interface for R version 2.13.0 (R Foundation for Statistical Computing, Vienna, Austria) [12], as described previously [13]. The probability of OS was calculated using the Kaplan-Meier method and compared using the log-rank test. The probabilities of TRM, disease relapse, aGVHD, cGVHD, and engraftment were compared using the Gray test [14] and analyzed using cumulative incidence analysis [15], considering relapse, death without disease relapse, death without aGVHD, death without cGVHD, and death without engraftment as respective competing risks. Variables included recipient age at the time of BMT, sex, pretransplantation CMV serostatus, disease characteristics (ie, disease type, disease lineage, and disease risk at transplantation), donor characteristics (ie, age, sex, sex compatibility, and ABO compatibility), transplant characteristics (ie, conventional or reduced-intensity conditioning [16], total body irradiation–containing regimens, tacrolimus versus cyclosporine, and total nucleated cell count harvested per recipient weight), and year of transplantation. The median was used as the cutoff point for continuous variables. The χ^2 test and Mann-Whitney U test were used to compare data between 2 groups. The Hardy-Weinberg equilibrium for the *PTPN22* gene variant was determined using the Haploview program [17].

Multivariate Cox models were used to evaluate the hazard ratio (HR) associated with the *PTPN22* variation. Covariates found to be significant in the univariate analyses ($P \leq .10$) were used to adjust the HR. For both the univariate and multivariate analyses, P values were 2-sided, and $P \leq .05$ was considered to indicate statistical significance.

RESULTS

Frequencies of PTPN22 Genotypes

The rs2488457 single nucleotide polymorphism in the *PTPN22* gene was genotyped in 663 unrelated BMT donor–recipient pairs (Table 1). The genotype frequencies of G/G, G/C, and C/C were 34%, 50%, and 16% in recipients and 33%, 49%, and 18% in donors, respectively. These results are in accordance with the Hardy-Weinberg equilibrium ($P = .49$) and similar to HapMap data reported in the Japanese population [5]. Donor and recipient *PTPN22* genotype did not significantly influence the cumulative incidence of engraftment (data not shown).

Effects of Recipient PTPN22 Genotype on Transplantation Outcomes

Transplantation outcomes according to *PTPN22* genotype are summarized in Table 3. Recipient C/C genotype was significantly associated with a lower incidence of grade II-IV aGVHD (18%) compared with recipient G/G (33%; $P = .009$) and G/C (35%; $P = .02$) genotypes (Figure 1A), suggesting the homozygous recessive effects of the C allele. We randomly split the study cohort into 2 subcohorts to test the validity of these associations. Subcohort 1 included 116 (35%) recipient C/C, 164 (49%) recipient G/C, and 52 (16%) recipient G/G genotypes, and subcohort 2 comprised 116 (35%) recipient G/G, 167 (50%) recipient G/C, and 52 (16%) recipient C/C

Table 3
Univariate Analysis of Associations between *PTPN22* Variations and Clinical Outcomes after BMT

Variable	Number	5-Year OS, %	*P*	5-Year TRM, %	*P*	5-Year Relapse, %	*P*	Grade II-IV aGVHD, %	*P*	Grade III-IV aGVHD, %	*P*	Chronic GVHD, %	*P*
Recipient *PTPN22* genotype													
G/G	228	48		25		28		33		11		43	
G/C	331	50	.73	28	.67	27	.75	35	.69	15	.26	47	.36
C/C	104	48	.64	19	.43	40	.06	**18**	**.009**	6	.18	42	.79
Donor *PTPN22* genotype													
G/G	219	48		22		34		32		13		42	
G/C	324	48	.59	30	.08	**27**	**.04**	31	.73	11	.57	45	.42
C/C	120	53	.38	21	.62	29	.35	33	.85	14	.79	49	.24

Significant values ($P \leq .05$) are in bold.

genotypes, leading to an estimated statistical power of 57% to detect the difference between the recipient C/C genotype and recipient G/C or G/G genotype in both subcohort analyses. The association between recipient C/C genotype and a lower incidence of grade II-IV aGVHD remained positive in the analyses of subcohort 1 ($P = .04$) and subcohort 2 ($P = .03$) (Supplemental Figure 1).

In addition, the recipient C/C genotype was associated with a higher incidence of relapse (40%) compared with that seen in the recipient G/G (28%; $P = .06$) and G/C (27%; $P = .02$) genotypes (Figure 1B). This difference had no significant influence on OS or TRM, however.

In a comparison of the impact of the *PTPN22* genotype in recipients with standard-risk disease and those with high-risk disease to investigate the significant effect of recipient genotype on relapse rate, the effect of recipient genotype on the incidence of grade II-IV aGVHD appeared unchanged. In patients with high-risk disease, the incidence of grade II-IV aGVHD was 33% in those with the recipient G/G genotype, 38% in those with the G/C genotype, and 17% in those with the C/C genotype ($P = .10$). In patients with standard risk disease, these values were 33%, 34%, and 18% ($P = .09$), respectively. In patients with high-risk disease, the 5-year cumulative incidence of relapse associated with the recipient C/C genotype was as high as 50%, which was not significantly different from that in those with the recipient G/G (39%; $P = .28$) and G/C (35%; $P = .14$) genotypes; however, this likely contributed to a significantly lower 5-year OS rate associated with the recipient C/C genotype (20%) compared with the recipient G/C (37%; $P = .02$) and G/G genotypes (32%; $P = .05$) (Figure 2A). In patients with standard-risk disease, the 5-year cumulative incidence of relapse was 32% in those with the recipient C/C genotype, 22% in those with the G/G phenotype ($P = .23$), and 32% in those with the G/C genotype ($P = .17$), and there were no significant differences in OS rate (Figure 2B).

After adjusting for clinical factors in the multivariate model, recipient C/C genotype remained statistically significant compared with the recipient G/G genotype with respect to the development of grade II-IV aGVHD (HR, 0.50; 95% confidence interval [CI], 0.29-0.85; $P = .01$; Table 4) and relapse (HR, 1.78; 95% CI, 1.10-2.90; $P = .02$; Table 5). Although analysis of the entire cohort revealed no considerable effects of the *PTPN22* genotype on OS rates (Table 5), compared with recipient G/G genotype. recipient C/C genotype was associated with significantly lower OS in patients with high-risk disease (HR, 1.60; 95% CI, 1.02-2.51; $P = .04$; Table 6) and with a significantly higher incidence of relapse in patients with standard-risk disease (HR, 2.02; 95% CI, 1.02-4.00; $P = .04$). No effects of recipient C/C genotype on OS rates were seen in patients with standard-risk disease.

The increased risk of relapse associated with recipient C/C genotype could be outweighed by the decreased risk of grade II-IV aGVHD, given that the absence of grade II-IV aGVHD was closely linked to the higher incidence of relapse (31% versus 19% at 5 years; $P = .01$) in the landmark analysis completed at day 60, in agreement with a previous report [18]. Consequently, we analyzed the impact of recipient *PTPN22* genotype on relapse according to the development of grade II-IV aGVHD. The landmark time for aGVHD analysis was chosen as day 60 post-BMT, as in a previous study [18], because more than 90% of patients who develop grade II-IV aGVHD do so within 60 days after transplantation [19]. In patients who developed grade II-IV aGVHD before day 60, the cumulative incidence of relapse was higher in those with the recipient C/C genotype (47% at 5 years) compared with

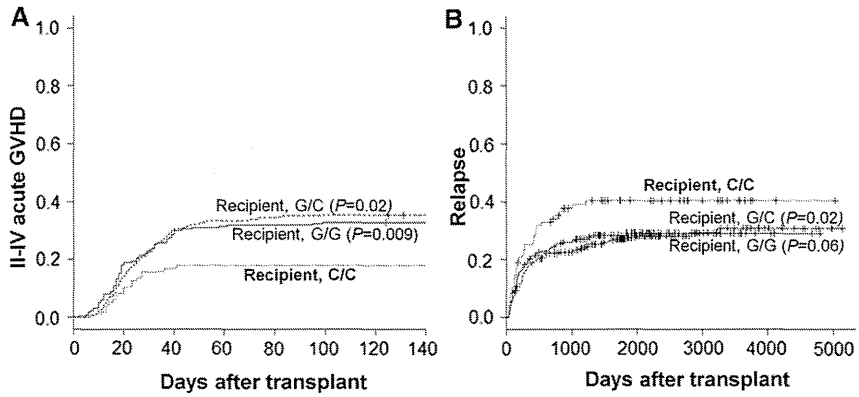

Figure 1. Estimated cumulative incidence curves of grade II-IV aGVHD (A) and relapse (B) according to recipient *PTPN22* genotype. Solid lines represent the recipient G/G genotype; dashed lines, the recipient G/C genotype; and dotted lines, the recipient C/C genotype.

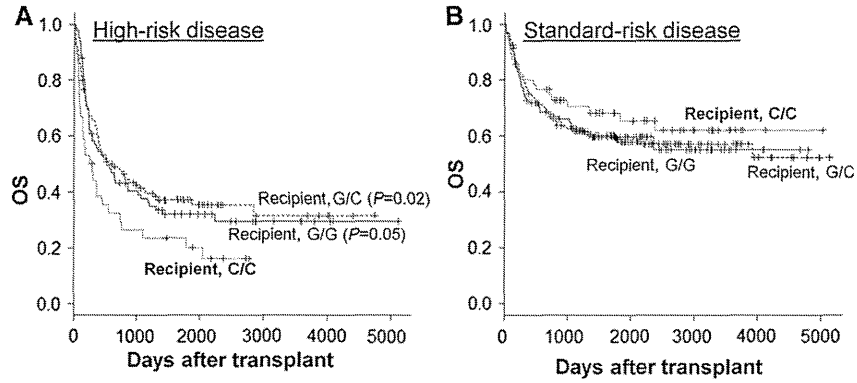

Figure 2. Kaplan-Meier analysis of OS after BMT according to the recipient *PTPN22* genotype in patients with high-risk disease (A) and those with standard-risk disease (B). Solid lines represent the recipient G/G genotype; dashed lines, the recipient G/C genotype; and dotted lines, the recipient C/C genotype.

those with the G/G (22%; $P = .04$) or G/C (20%; $P = .03$) genotype. The increased incidence of relapse associated with the recipient C/C genotype was confirmed on multivariate analysis, with an HR for relapse for the recipient C/C genotype versus G/G genotype as high as 4.5 (95% CI, 1.56-12.78; $P = .005$). In patients who survived more than 60 days without developing grade II-IV aGVHD, the 5-year cumulative incidence of relapse was higher in those with the recipient C/C genotype (39%) than in those with the recipient G/G (30%; $P = .22$), G/C (28%; $P = .24$), and G/G or G/C genotypes (30%; $P = .21$). After adjustment of covariates using the multivariate model, the increased incidence of relapse associated with the recipient C/C genotype was close to being significant compared with recipient G/G (HR, 1.79; 95% CI, 0.98-3.26; $P = .06$) and G/G or G/C (HR, 1.64; 95% CI, 0.99-2.71; $P = .06$) genotypes. Accordingly, the effects of recipient C/C genotype in increasing the incidence of relapse are considered independently significant irrespective of the development of grade II-IV aGVHD.

Effects of Donor PTPN22 Genotype on Transplantation Outcomes

Compared with donor G/G genotype, donor G/C genotype was correlated with a significantly lower incidence of relapse (27% versus 34%; $P = .04$) and with a trend toward increased TRM (30% versus 22%; $P = .08$). The effects of the lower relapse rate associated with the donor G/C genotype were also evident in the multivariate analysis (HR, 0.58; 95% CI, 0.40-0.85; $P = .005$; Table 5). The effects of donor G/C genotype on relapse and TRM had no significant impact on OS; this also held true in the analysis performed according to disease risk (data not shown).

DISCUSSION

In our study cohort, the recipient C/C genotype at the rs2488457 (−1123G>C) variant of the *PTPN22* promoter gene was associated with a lower incidence of grade II-IV aGVHD and a higher incidence of relapse after unrelated HLA-matched BMT performed through the JMDP. The recipient C/C genotype negatively affected OS in patients with high-risk disease, but not in those with standard-risk disease. In addition, the donor G/C genotype predicted a lower incidence of relapse, but had no significant impact on OS irrespective of disease risk.

Previous studies have identified 4 variations in the *PTPN22* gene associated with susceptibility to autoimmune diseases. The +1858C>T variation (rs2476601) is in near-perfect disequilibrium with rs6679677 [20] and is closely linked to the −1123G>C variation (rs2488457) analyzed in the present study [2,5,21-23]. The +1858C>T variation was first identified as associated with type 1 diabetes using a candidate gene approach [24]. Subsequent studies have confirmed this finding, as well as the variation's association with other autoimmune diseases, including Crohn's disease, ulcerative colitis, rheumatoid arthritis, Graves disease, autoimmune thyroid disease, vitiligo, alopecia, systemic lupus erythematosus, and acute allograft rejection [25]. The +1858C>T variation is not polymorphic in the Asian population [5]; instead, the −1123G>C variation is associated with type 1 diabetes and rheumatoid arthritis [2]. In addition, the −1123G>C variation is more closely associated with type 1 diabetes than the +1858C>T variation in the European population [5]. The remaining variation, +788G>A (Lyp-R263Q, rs33996649), is associated with ulcerative colitis, rheumatoid arthritis, and systemic lupus erythematosus [26].

Table 4
Multivariate Analysis of the Association between *PTPN22* Variations and GVHD after BMT

Variable	Grade II-IV aGVHD			Grade III-IV aGVHD			cGVHD		
	Adjusted HR	95% CI	*P*	Adjusted HR	95% CI	*P*	Adjusted HR	95% CI	*P*
Recipient *PTPN22* genotype, G/C (331) versus G/G (n = 228)	1.64	0.79-1.44	.68	1.32	0.80-2.18	.28	1.08	0.81-1.44	.59
Recipient *PTPN22* genotype, C/C (104) versus G/G (n = 228)	**0.50**	**0.29-0.85**	**.01**	0.54	0.22-1.34	.18	0.89	0.58-1.34	.59
Donor *PTPN22* genotype, G/C (324) versus G/G (n = 219)	0.95	0.70-1.30	.76	0.81	0.48-1.36	.42	1.13	0.84-1.53	.42
Donor *PTPN22* genotype, C/C (120) versus G/G (n = 219)	1.08	0.72-1.61	.72	1.10	0.59-2.07	.76	1.33	0.93-1.90	.11
Recipient age ≥34 years							**1.31**	**1.00-1.72**	**.05**
Total body irradiation–containing conditioning regimen							**1.44**	**1.01-2.06**	**.05**
High-risk disease							**0.75**	**0.56-0.99**	**.05**
Year of BMT 2001 or later				0.69	0.42-1.11	**.12**			

Covariates identified as significant in the univariate analyses ($P \leq .10$) were used to adjust the HR for the *PTPN22* genotype. Significant results ($P \leq .05$) are in bold.

Table 5
Multivariate Analysis of the Association between *PTPN22* Variations and Prognostic Outcomes after Transplantation

Variable	OS			TRM			Relapse		
	Adjusted HR	95% CI	*P*	Adjusted HR	95% CI	*P*	Adjusted HR	95% CI	*P*
Recipient *PTPN22* genotype, G/C (331) versus G/G (n = 228)	0.94	0.71-1.25	.69	0.84	0.55-1.28	.84	1.08	0.73-1.64	.71
Recipient *PTPN22* genotype, C/C (104) versus G/G (n = 228)	1.03	0.68-1.56	.87	0.67	0.33-1.35	.27	**1.78**	**1.10-2.90**	**.02**
Donor *PTPN22* genotype, G/C (324) versus G/G (n = 219)	0.91	0.68-1.21	.51	1.24	0.78-1.97	.37	**0.58**	**0.40-0.85**	**.005**
Donor *PTPN22* genotype, C/C (120) versus G/G (n = 219)	0.78	0.53-1.15	.21	1.08	0.60-1.97	.79	0.64	0.40-1.04	.07
Minor ABO incompatibility				**1.74**	**1.10-2.77**	**.002**			
Recipient age ≥34 years	**1.61**	**1.23-2.10**	**.001**	**2.21**	**1.45-3.37**	**<.001**			
CMV-positive recipient				**2.15**	**1.13-4.08**	**.002**	1.49	0.95-2.34	.08
Conventional conditioning regimen				1.33	0.64-2.78	.45			
Total body irradiation–containing conditioning regimen				0.95	0.60-1.52	.84			
High-risk disease	2.08	**1.60-2.69**	**<.001**	**1.75**	**1.14-2.70**	**.01**	**1.76**	**1.22-2.53**	**.003**
Female donor/male recipient							0.67	0.40-1.11	.12
TNC ≥ 5.0×10^8/kg				0.92	0.63-1.36	.69			
Year of BMT 2001 or later	0.98	0.74-1.31	.90						

Covariates identified as significant in the univariate analyses ($P \leq .10$) were used to adjust the HR for the *PTPN22* genotype. Significant results ($P \leq .05$) are in bold.

Recent genome-wide association studies and meta-analyses have validated the association of these variations with type 1 diabetes, inflammatory bowel disease, Graves disease, rheumatoid arthritis, and systemic lupus erythematosus [27].

Experimental evidence has demonstrated that +1858C>T (Lyp-R620W, rs2476601) and +788G>A (Lyp-R263Q, rs33996649) are functional [28,29]. Lyp-Trp620 (+1858T) is associated mainly with an increased risk of autoimmune diseases and impaired constitutive biding of Lyp with c-Src tyrosine kinase (Csk) [30]. The inability of Lyp-Trp620 to bind Csk results in a less efficient inhibition of TCR signaling, because Lyp and Csk concertedly down-regulate TCR signaling [28]. Previous studies in cell lines and primary human cells have shown conflicting results, however [28]. Lyp-Trp620–positive primary human T cells were found to produce less IL-2 on TCR signaling, and Lyp-Trp620 more potently reduced TCR signaling in a dose-dependent manner, suggesting a gain-of-function mutation [30]. Conversely, the

Table 6
Impact of Recipient *PTPN22* Genotype on OS and TRM According to Disease Risk in the Multivariate Analysis

Variable	OS			TRM			Relapse		
	Adjusted HR	95% CI	*P*	Adjusted HR	95% CI	*P*	Adjusted HR	95% CI	*P*
Patients with high-risk disease									
Recipient *PTPN22* genotype, G/C (128) versus G/G (n = 89)	0.95	0.57-1.35	.78	0.89	0.47-1.69	.73	1.05	0.60-1.84	.87
Recipient *PTPN22* genotype, C/C (40) versus G/G (n = 89)	**1.60**	**1.02-2.51**	**.04**	0.92	0.36-2.34	.85	1.51	0.75-3.05	.25
Donor *PTPN22* genotype, G/C (186) versus G/G (n = 142)	0.90	0.63-1.28	.55	1.29	0.60-2.80	.51	**0.53**	**0.31-0.91**	**.02**
Donor *PTPN22* genotype, C/C (69) versus G/G (n = 142)	0.81	0.51-1.29	.37	1.54	0.64-3.75	.34	0.56	0.29-1.11	.10
Minor ABO incompatibility				**2.32**	**1.14-4.73**	**.02**			
Recipient age ≥34 years	**1.76**	**1.28-2.43**	**.001**	**2.43**	**1.28-4.59**	**.006**			
CMV-positive recipient				1.33	0.58-3.06	.50	1.26	0.66-2.41	.49
Conventional conditioning regimen				1.33	0.35-5.14	.68			
Total body irradiation–containing conditioning regimen				1.80	0.53-6.15	.35			
Female donor/male recipient							0.85	0.41-1.78	.67
TNC ≥ 5.0×10^8/kg				1.11	0.61-2.03	.74			
Year of BMT 2001 or later	0.93	0.67-1.29	.67						
Patients with standard-risk disease									
Recipient *PTPN22* genotype, G/C (199) versus G/G (n = 138)	0.96	0.67-1.37	.81	0.78	0.46-1.34	.37	1.12	0.63-2.00	.70
Recipient *PTPN22* genotype, C/C (60) versus G/G (n = 138)	0.84	0.49-1.43	.52	0.51	0.18-1.41	.19	**2.02**	**1.02-4.00**	**.04**
Donor *PTPN22* genotype, G/C (186) versus G/G (n = 142)	1.17	0.82-1.69	.39	1.23	0.67-2.24	.51	0.65	0.39-1.10	.11
Donor *PTPN22* genotype, C/C (69) versus G/G (n = 142)	0.83	0.50-1.38	.48	0.81	0.35-1.86	.62	0.74	0.38-1.45	.39
Minor ABO incompatibility				1.39	0.72-2.71	.33			
Recipient age ≥34 years	**1.68**	**1.20-2.36**	**.003**	**2.04**	**1.16-3.59**	**.01**			
CMV-positive recipient				**3.45**	**1.19-9.96**	**.02**	1.74	0.90-3.39	.10
Conventional conditioning regimen				1.10	0.46-2.64	.83			
Total body irradiation–containing conditioning regimen				0.79	0.45-1.36	.39			
Female donor/male recipient							0.51	0.25-1.07	.08
TNC ≥ 5.0×10^8/kg				0.84	0.50-1.39	.50			
Year of BMT 2001 or later	1.24	0.88-1.74	.23						

Covariates identified as significant in the univariate analyses ($P \leq .10$) were used to adjust the HR for the *PTPN22* genotype. Significant values ($P \leq .05$) are in bold.

Lyp-Gln263 mutation, which is associated with a reduced risk of autoimmune diseases, reportedly results in loss of function [29].

The mechanisms through which the recipient −1123C allele of the *PTPN22* gene affects the incidence of aGVHD and disease relapse remain unclear. Previous reports of the number of regulatory T cells (Tregs) increasing inversely with the level of *PTPN22* in the thymus [31] and of thymus-derived Tregs operating to prevent aGVHD and promote disease relapse [32] suggest the hypothesis that in transplant recipients, the *PTPN22* −1123G>C variant influences the production of Tregs from the thymus. This hypothesis may be supported by the fact that the *PTPN22* gene has a functional variant, +1858C>T, that is closely linked to the −1123G>C variant [2,5,21-23], and that the minor +1858T allele functionally inhibits TCR signaling more potently than the major +1858C allele [30]. Hyporesponsive TCR signaling might lead to increased Treg production by the thymus, given that decreased TCR signaling can promote the development of intrathymic Tregs [33]. Thus, an increased number of Tregs in relation to the recipient −1123C/C genotype might prevent aGVHD at the expense of decreased graft-versus-tumor effects. These hypotheses must be considered speculative, however, given the lack of functional data on the −1123G>C variant. Elucidating the role of the *PTPN22* −1123G>C variant in Treg production will provide useful information in this regard.

A second possible mechanism includes the involvement of host DCs, which are critical for the initiation of aGVHD [34]. This possibility may be supported by a recent report indicating that the *PTPN22*+1858C>T variant plays key roles in antigen receptor signaling of DCs [28].

Why the *PTPN22* −1123G>C genotype displays different behaviors in the donor and recipient genotypes is obscure. Of note, the donor heterozygous −1123G/C genotype was associated with a reduced incidence of relapse, which could be attributed to increased graft-versus-tumor effects owing to donor G/C genotype. The effects of the heterozygous −1123G/C genotype on autoimmunity may be related to the association between this genotype and increased risk of developing autoimmune diseases, including type 1 diabetes and rheumatoid arthritis, in Asian populations [2,3,5]. However the present study showed no gene dose responses, and whether this phenomenon reflects a molecular heterosis is unclear [3,5,35].

The lack of considerable survival advantage in relation to donor *PTPN22* genotype may suggest that the beneficial effects of *PTPN22* genotyping are limited. However, determination of the recipient *PTPN22* genotype before transplantation might provide a recipient harboring the *PTPN22* G/C or G/G genotype an opportunity to avoid the risk of aGVHD by favoring a bone marrow or cord blood HLA-matched graft over a peripheral blood stem cell (PBSC) or HLA-mismatched graft. Conversely, a PBSC or HLA-mismatched graft, along with minimal aGVHD prophylaxis, could be acceptable for a recipient harboring the *PTPN22* C/C genotype. In addition, a recipient with the −1123G/G or G/C genotype may require a bone marrow or cord blood graft to avoid aGVHD. This may apply especially to recipients with a benign disease, such as severe aplastic anemia or primary immunodeficiency, in whom relapse does not matter.

A previous study investigated the impact of the *PTPN22* +1858C>T variant on transplantation outcomes in a cohort of European patients who underwent hematopoietic stem cell transplantation for hematologic malignancies [36]. Although a relatively small number of patients were included in that analysis, the authors found that the donor +1858C/C genotype was consistently linked with severe bacterial infections [36]. Another study [37] showed that recipient–donor pairs carrying 2 or more PTPN22 −1858T alleles were at increased risk for grade III-IV aGVHD, but not for grade II-IV aGVHD. Although determining whether such associations are also present in Japanese patients is not possible, because the +1858C>T variant is not polymorphic in Asian populations [2,3,5], these results might support involvement of the *PTPN22* gene in the pathophysiology of aGVHD, as suggested in the present study.

In conclusion, our data suggest that the specific *PTPN22* variant affects prognosis after unrelated donor BMT. Thus, *PTPN22* genotyping in transplant donors and recipients can be a useful tool for evaluating pretransplantation risk and, in combination with other known risk factors, can form the basis for tailoring individual treatment strategies. Nonetheless, care should be taken when drawing conclusions from our data; experimental evidence is needed to verify the effects of *PTPN22* variations. Moreover, the present study did not include adjustment for multiple testing, because the analyses were conducted in an exploratory context, and thus the interpretation of analyses in the subgroups should be taken into account. Finally, transplantation outcomes, including aGVHD and relapse are multifactorial, and single polymorphisms in one cytokine gene are unlikely to determine the majority of outcomes. Further studies are needed to ascertain whether the findings of this study can be extended to other stem cell sources or to HLA-mismatched transplantation, and to validate these data in other ethnic groups.

ACKNOWLEDGMENTS

We thank Professor Yoshinobu Kanda, Jichi Medical University, for assistance with the EZR software program. We also thank all of the Japan Marrow Donor Program transplant teams who provided valuable assistance in caring for the patients and donors investigated in this study.

Financial disclosure: This study was supported by grants from the Ministry of Health, Labor, and Welfare of Japan (Research on Allergic Disease and Immunology in Health and Labor Science grant H23-010); the Ministry of Education, Culture, Sports, and Technology of Japan; and the Hokkoku Gan Kikin Fund. The funding agencies played no role in study design, data collection and analysis, decision to publish, or manuscript preparation. The authors have no conflicts of interest to disclose.

Authorship statement: J. Luis Espinoza and Akiyoshi Takami designed research and wrote the manuscript. J. Luis Espinoza performed experiments. Akiyoshi Takami analyzed data and performed statistical analysis. All authors contributed to data and sample collection and reviewed the manuscript.

SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.bbmt.2012.09.014.

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Leukemia (2013) **27**, 286–294

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ORIGINAL ARTICLE

Unrelated cord blood transplantation vs related transplantation with HLA 1-antigen mismatch in the graft-versus-host direction

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Little information is available regarding whether an unrelated cord blood (UCB) unit or a related donor with a 1-antigen mismatch at the HLA-A, HLA-B or HLA-DR locus in the graft-versus-host direction (RD/1AG-MM-GVH) should be selected as an alternative donor for patients without an HLA-matched related/unrelated donor. Therefore, we conducted a retrospective study using national registry data on patients with leukemia or myelodysplastic syndrome who received transplantation using a single UCB ($n = 2288$) unit or an RD/1AG-MM-GVH ($n = 525$). We found that the survival rate in the UCB group was comparable to that in the RD/1AG-MM-GVH group, although the RD/1AG-MM-GVH group with an HLA-B mismatch showed significantly higher overall and non-relapse mortality. Neutrophil and platelet engraftment were significantly faster, whereas the incidence of acute or chronic graft-versus-host disease (GVHD) was significantly higher in the RD/1AG-MM-GVH group. The incidence of acute or chronic GVHD in the RD/1AG-MM-GVH group with *in vivo* T-cell depletion was comparable to that in the UCB group, which translated into a trend toward better overall survival, regardless of the presence of an HLA-B mismatch. In conclusion, UCB and RD/1AG-MM-GVH are comparable for use as an alternative donor, except for RD/1AG-MM-GVH involving an HLA-B mismatch.

Leukemia (2013) **27,** 286–294; doi:10.1038/leu.2012.203

Keywords: cord blood transplantation; related transplantation; HLA mismatch; alternative donor

INTRODUCTION

For patients who lack an HLA-identical sibling, an HLA-matched unrelated donor (MUD) is considered to be the preferred alternative donor in allogeneic hematopoietic cell transplantation (HCT).[1–5] However, it is difficult to find an MUD for patients with rare HLA haplotypes. Furthermore, it takes at least a few months from the start of an unrelated donor search to actually receive a graft. Therefore, there is a large demand for an alternative source to an HLA-identical sibling or MUD, particularly for patients who have a rare haplotype or who need immediate transplantation.

Unrelated cord blood (UCB) has emerged as a promising alternative source for pediatric and adult patients.[6–17] In UCB transplantation, up to two antigen/allele mismatches between a recipient and cord blood unit are acceptable without an increased risk of acute graft-versus-host disease (GVHD). The clinical outcome in UCB transplantation is improving, and is almost comparable to that in HLA 8/8 allele MUD transplantation, although a high risk of graft failure and early treatment-related complications are still major issues.[15–17]

Another alternative source is an HLA-mismatched related donor, particularly when a related donor with a 1-antigen mismatch at the HLA-A, HLA-B, or HLA-DR locus in the graft-versus-host (GVH) direction (RD/1AG-MM-GVH) is available. HCT from an RD/1AG-MM-GVH results in a higher but acceptable incidence of acute GVHD.[18–20] In previous studies, HLA mismatches in the host-versus-graft (HVG) direction were associated with a higher incidence of graft failure and lower overall survival (OS).[18,19,21] However, the risk of graft failure might have been improved by the use of conditioning regimens that strongly suppress the recipient's immune system.[22] Therefore, in current clinical practice in Japan, stem cell transplantation from an RD/1AG-MM-GVH is being performed while accepting multiple antigen mismatches in the HVG direction without specific *ex vivo* stem cell manipulation.[18,19,23] We have recently reported that OS in transplantation from an RD/1AG-MM-GVH involving an HLA-B antigen mismatch was inferior, whereas that from an RD/1AG-MM-GVH involving an HLA-A or -DR antigen mismatch was comparable to that from an 8/8-MUD in standard-risk diseases.[23]

Unlike transplantation from an MUD, transplantation using a UCB unit or an RD/1AG-MM-GVH can be performed immediately when necessary. However, little information is available regarding the priority in selecting these alternative donors. Therefore, we conducted a retrospective study using national registry data on 2813 patients with leukemia or myelodysplastic syndrome (MDS)

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Received 15 June 2012; revised 5 July 2012; accepted 11 July 2012; accepted article preview online 18 July 2012; advance online publication, 10 August 2012

who received transplantation using a single UCB or an RD/1AG-MM-GVH.

MATERIALS AND METHODS

Data collection

Data for patients (age: ⩾16 years) with acute myeloid leukemia, acute lymphoblastic leukemia, MDS and chronic myelogenous leukemia who received a first HCT using a single HLA 0–2 antigen-mismatched UCB unit or an RD/1AG-MM-GVH between 1 January 1998 and 31 December 2009 were obtained from the Transplant Registry Unified Management Program (TRUMP),[24] which includes data from the Japan Cord Blood Bank Network (JCBBN) and the Japan Society for Hematopoietic Cell Transplantation (JSHCT). Our analysis included 2306 patients who received a single UCB graft (UCB group) and 541 patients who received a graft from an RD/1AG-MM-GVH (RD/1AG-MM-GVH group). As of January 2012, double UCB grafts for HCT are not available in Japan. The following patients were excluded: 26 patients who lacked data on survival status, survival date, sex of recipient, or GVHD prophylaxis and 8 patients who received stem cells that had been manipulated by *ex vivo* T-cell depletion or CD34 selection. Overall, 2288 patients who received a UCB unit and 525 who received a graft from an RD/1AG-MM-GVH fulfilled the criteria. The study was approved by the data management committees of TRUMP and by the institutional review boards of Japanese Red Cross Nagoya First Hospital and Saitama Medical Center, Jichi Medical University, where this study was organized.

Histocompatibility

Histocompatibility data for the HLA-A, HLA-B and HLA-DR loci were obtained from reports from the institution where the transplantation was performed or from cord blood banks. To reflect current practice in Japan, HLA matching in UCB or RD/1AG-MM-GVH transplantation was assessed by serological data for HLA-A, HLA-B, and HLA-DR loci. An HLA mismatch in the GVH direction was defined as when the recipient's antigens or alleles were not shared by the donor, whereas a mismatch in the HVG direction was defined as when the donor's antigens or alleles were not shared by the recipient.

End points

The primary end point of the study was to compare OS rates between the UCB and RD/1AG-MM-GVH groups. Other end points were the cumulative incidences of neutrophil and platelet engraftment, acute and chronic GVHD, relapse, and non-relapse mortality (NRM). Neutrophil recovery was considered to have occurred when the absolute neutrophil count exceeded 0.5×10^9/l for 3 consecutive days following transplantation. Platelet recovery was considered to have occurred when the absolute platelet count exceeded 50×10^9/l without platelet transfusion. The physicians who performed transplantation at each center diagnosed and graded acute and chronic GVHD according to the traditional criteria.[25,26] The incidence of chronic GVHD was evaluated in patients who survived for at least 100 days.

Statistical analysis

Descriptive statistics were used to summarize variables related to the patient characteristics. Comparisons between groups were performed with the χ^2-test or extended Fisher's exact test as appropriate for categorical variables and the Mann–Whitney *U*-test for continuous variables. The probability of OS was estimated according to the Kaplan–Meier method, and the groups were compared with the log-rank test. The adjusted probability of OS was estimated according to the Cox proportional-hazards model, with other significant variables considered in the final multivariate model. The probabilities of neutrophil and platelet engraftment, acute and chronic GVHD, NRM, and relapse were estimated on the basis of cumulative incidence methods, and the groups were compared with the Gray test;[27,28] competing events were death without engraftment for neutrophil and platelet engraftment, death or relapse without GVHD for acute and chronic GVHD, death without relapse for relapse, and relapse for NRM. The Cox proportional-hazards model was used to evaluate variables that may affect OS, whereas the Fine and Gray proportional-hazards model was used to evaluate variables that may affect engraftment, GVHD, NRM and relapse.[29] We classified the conditioning regimen as myeloablative if either total body irradiation >8 Gy, oral busulfan ⩾9 mg/kg, intravenous busulfan ⩾7.2 mg/kg, or melphalan >140 mg/m^2 was used in the conditioning regimen, and otherwise classified it as reduced intensity, based on the report by the Center for International Blood and Marrow Transplant Research.[30] For patients for whom the doses of agents used in the conditioning regimen were not available, we used the information on conditioning intensity (myeloablative or reduced intensity) reported by the treating clinicians. Acute leukemia in the first or second remission, chronic myelogenous leukemia in the first or second chronic phase or accelerated phase, and MDS with refractory anemia or refractory anemia with ringed sideroblasts were defined as standard-risk diseases, and other conditions were defined as high-risk diseases. The following variables were considered when comparing the UCB and RD/1AG-MM-GVH groups: the recipient's age group (⩽50 years or >50 years at transplantation), sex of recipient, disease (acute myeloid leukemia, acute lymphoblastic leukemia, chronic myelogenous leukemia or MDS), disease status before transplantation (standard- or high-risk), type of conditioning regimen (myeloablative or reduced intensity), type of GVHD prophylaxis (calcineurin inhibitor and methotrexate, calcineurin inhibitor only, or other), year of transplantation (1998–2004, 2005–2009), and the time from diagnosis to transplantation (<6 months or ⩾6 months). In the analysis within the RD/1AG-MM-GVH group, the use of *in vivo* T cell depletion (no vs yes), stem cell source (peripheral blood (PB) stem cells vs bone marrow (BM)), and the number of HLA mismatches in the HVG direction (0–1 vs 2–3) were also considered. Factors without a variable of main interest were selected in a stepwise manner from the model with a variable retention criterion of $P<0.05$. We then added a variable of main interest to the final model. All tests were two-sided, and $P<0.05$ was considered to indicate statistical significance. All statistical analyses were performed with Stata version 12 (Stata Corp., College Station, TX, USA) and EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan).[31] EZR is a graphical user interface for R (The R Foundation for Statistical Computing, version 2.13.0, Vienna, Austria). More precisely, it is a modified version of R commander (version 1.6–3) that was designed to add statistical functions that are frequently used in biostatistics.

RESULTS

Characteristics of patients and transplants

Table 1 shows the patient and transplant characteristics. Recipients of an RD/1AG-MM-GVH were younger than recipients of a UCB unit. Approximately half of the recipients in the RD/1AG-MM-GVH group received PB. The number of HLA mismatches in the GVH direction between a UCB unit and recipient was 0 in 10%, 1 in 33% and 2 in 57%. In the RD/1AG-MM-GVH group, the number of antigen mismatches in the HVG direction was 0 in 12%, 1 in 68%, 2 in 18% and 3 in 3%. Most of the recipients of an RD/1AG-MM-GVH received a calcineurin inhibitor with methotrexate for GVHD prophylaxis, whereas 25% of UCB recipients received only calcineurin inhibitor. *In vivo* T-cell depletion including antithymocyte globulin (ATG) or alemtuzumab was used in 10% of the RD/1AG-MM-GVH group, but in only 1% of the UCB group. Alemtuzumab was used in only one patient, who received transplantation from an RD/1AG-MM-GVH. Information regarding the dose and type of ATG was missing in two-third of the patients who received ATG. Available data showed that the median dose of thymoglobulin was 2.5 (range 2.5–9.0, $n=9$) and 2.5 (range 1.25–5.0, $n=10$) mg/kg and the median dose of ATG-Fresenius was 8.0 (range 5.0–10.0, $n=3$) and 8.0 (range 5.0–10.0, $n=7$) mg/kg, in the UCB and RD/1AG-MM-GVH groups, respectively. Two-third of UCB transplantations were performed between 2005 and 2009. The median duration of follow-up for survivors was 2 and 4 years in the UCB and RD/1AG-MM-GVH groups, respectively.

Neutrophil and platelet engraftment

The incidence of neutrophil engraftment at day 50 in the RD/1AG-MM-GVH group was higher than that in the UCB group (UCB group, 73%, 95% confidence interval (CI), 71–75%; RD/1AG-MM-GVH group, 93%, 95% CI, 91–95%; Gray test, $P<0.001$; Figure 1a). The incidence of platelet engraftment at day 150 in the

Table 1. Patient characteristics

Variable	*UCB (n = 2288)*	*RD/1AG-MM-GVH (n = 525)*	P
Age at transplant, median (range)	49 (16–82)	43 (16–74)	<0.001
Recipient sex			
Female	1004 (44%)	239 (46%)	0.494
Male	1284 (56%)	286 (54%)	
Disease			
Acute myelogenous leukemia	1365 (60%)	269 (51%)	0.003
Acute lymphoblastic leukemia	498 (22%)	137 (26%)	
Chronic myelogenous leukemia	124 (5%)	42 (8%)	
Myelodysplastic syndrome	301 (13%)	77 (15%)	
Duration from diagnosis to transplant			
Median time (range), months	7.9 (0.2–768.5)	7.6 (0–251.7)	0.233
Disease risk			
Standard	959 (42%)	249 (47%)	0.050
High	1217 (53%)	257 (49%)	
Unknown	112 (5%)	19 (4%)	
Source of stem cells			
Bone marrow	—	251 (48%)	—
Peripheral blood	—	274 (52%)	
Cord blood	2288 (100%)	—	
HLA compatibility in the graft-versus-host direction			
Matched	225 (10%)	—	<0.001
One-antigen mismatch	753 (33%)	525 (100%)	
Two-antigen mismatch	1310 (57%)	—	
HLA compatibility in the host-versus-graft direction			
Matched	233 (10%)	62 (12%)	<0.001
One-antigen mismatch	716 (31%)	355 (68%)	
Two-antigen mismatch	1339 (59%)	94 (18%)	
Three-antigen mismatch	—	14 (3%)	
Conditioning regimen			
Myeloablative	1390 (61%)	253 (48%)	<0.001
CY + TBI ±	1062	164	
Other TBI regimen	130	20	
BU + CY ±	88	45	
Other non-TBI regimen	110	24	
Reduced intensity	894 (39%)	162 (31%)	
FLU ± TBI ±	840	138	
Other regimen	54	24	
Unclassifiable	4 (0.2%)	110 (21%)	
GVHD prophylaxis			
CSA/TAC + MTX	1410 (62%)	448 (85%)	<0.001
CSA/TAC + MMF	246 (11%)	12 (2%)	
CSA/TAC + Steroid	28 (1%)	13 (2%)	
CSA/TAC only	571 (25%)	45 (9%)	
Unknown	33 (1%)	7 (1%)	
Use of in vivo *T-cell depletion*			
No	2258 (99%)	472 (90%)	<0.001
Yes	30 (1%)	53 (10%)	
Year at transplant			
1998–2004	760 (33%)	260 (50%)	<0.001
2005–2009	1528 (67%)	265 (50%)	
Follow-up of survivors			
Median time (range), years	2.1 (0.0–10.0)	4.0 (0.1–12.2)	<0.001

Abbreviations: BU, busulfan; CSA, cyclosporine; CY, cyclophosphamide; FLU, fludarabine; MMF, mycophenolate mofetil; MTX, methotrexate; TAC, tacrolimus; TBI, total body irradiation; UCB, unrelated cord blood.

RD/1AG-MM-GVH group was also higher than that in the UCB group (UCB group, 53%, 95% CI, 51–55%; RD/1AG-MM-GVH group, 70%, 95% CI, 66–74%; Gray test, $P<0.001$; Figure 1b). The use of RD/1AG-MM-GVH was significantly associated with a higher incidence of neutrophil and platelet engraftment in the multivariate analysis (neutrophil engraftment, hazard ratio (HR), 3.46,