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Effects of HLA Allele and Killer Immunoglobulin-Like Receptor Ligand Matching on Clinical Outcome in Leukemia Patients Undergoing Transplantation With T-cell–Replete Marrow From an Unrelated Donor

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ABSTRACT

The responsible human leukocyte antigen (HLA) locus and the role of killer immunoglobulin-like receptor (KIR) ligand matching on transplantation outcome were simultaneously identified by multivariate analysis in 1790 patients with leukemia who underwent transplantation with T-cell–replete marrow from an unrelated donor (UR-BMT) through the Japan Marrow Donor Program. The graft-versus-leukemia (GVL) effect depended on leukemia cell type. HLA-C mismatch reduced the relapse rate in acute lymphoblastic leukemia (ALL) (hazard ratio [HR] = 0.47; $P = .003$), and HLA-DPB1 mismatch reduced it in chronic myeloid leukemia (CML) (HR = 0.35; $P < .001$). In contrast, KIR2DL ligand mismatch in the graft-versus-host (GVH) direction (KIR-L-MM-G) increased in ALL (HR = 2.55; $P = .017$). An increased rejection rate was observed in KIR2DL ligand mismatch in the host-versus-graft direction (HR = 4.39; $P = .012$). Acute GVH disease (GVHD) was increased not only in the mismatch of HLA-A, -B, -C, and -DPB1, but also in KIR-L-MM-G. As a whole, the mismatch of HLA-A, -B, and -DQB1 locus and KIR-L-MM-G resulted in increased mortality. In conclusion, not only the mismatch of HLA-C and -DPB1, but also KIR-L-MM-G affected leukemia relapse, which should be considered based on leukemia cell type. Furthermore, KIR-L-MM induced adverse effects on acute GVHD (aGVHD) and rejection, and brought no survival benefits to patients with T-cell–replete UR-BMT.

KEY WORDS

KIR ligand incompatibility • HLA • Leukemia • Unrelated bone marrow transplantation

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) from a human leukocyte antigen (HLA)-matched unrelated (UR) donor has been established as one mode of curative therapy for hematologic malignancies and other hematologic or immunologic disorders [1,2]. Extensive research on genetic factors such

as HLA has produced mounting evidence of the presence of HLA alleles that drastically affect HSCT outcome through T cells. Induction of the graft-versus-leukemia (GVL) effect to reduce relapse of leukemia is considered an advantage of allogeneic HSCT [3]. There have been several large-scale analyses of UR-HSCT. The Japan Marrow Donor Program (JMDP) demonstrated the effect of matching of HLA class I alleles (HLA-A, -B, and -C) on the development of severe acute graft-versus-host disease (aGVHD) and the importance of HLA-A and -B allele matching for better survival in T-cell–replete UR-HSCT [4,5]. The Fred Hutchinson Cancer Research Center and the US National Marrow Donor Program (NMDP) reported the importance of HLA class II matching in GVHD and survival [6,7]. Updated analysis of the NMDP indicated that HLA-A allele-level mismatching, HLA-B serologic mismatching, and HLA-DRB1 mismatching are significant risk factors for severe aGVHD, and that disparity in HLA class I (HLA-A, -B, or -C) and/or HLA-DRB1 increased the mortality [8]. Furthermore, the role of HLA-DPB1 matching has been elucidated for aGVHD [9-11] and leukemia relapse [12]. However, the aforementioned reports have produced considerable conflicting results.

It has become evident that natural killer (NK) cells and the subpopulation of T cells express NK cell receptors, and that the activity of NK cells is controlled by the recognition of HLA class I molecules on the target cells by NK cell inhibitory and activating receptors [13,14]. The genotype and haplotype of the killer immunoglobulin-like receptors (KIRs) have been identified, and ligand specificities of KIRs have been characterized. C1 specificity of the HLA-C epitope (Asp80) is the ligand of inhibitory KIR2DL2/3, C2 specificity (Lys80) is the ligand of inhibitory KIR2DL1, and HLA-Bw4 is the ligand of KIR3DL1. With allogeneic HSCT, the disparities of these receptors between donor and recipient are suspected to induce transplant-related immunologic events through activation of NK cells, and evidence of the clinical outcome of HSCT in relation to KIR disparities has been accumulated [15]. However, reports of KIR ligand matching in UR-HSCT have shown contradictory results [16]. Limited patient numbers, different diseases, and various GVHD prophylaxes make it difficult to draw definite conclusions from these studies.

In the present study, the effects of HLA locus and KIR ligand matching were simultaneously analyzed in leukemia patients receiving T-cell–replete marrow from unrelated donors through the JMDP after a myeloablative conditioning regimen, focusing in particular on the influence of leukemia cell type on the GVL effect.

PATIENTS AND METHODS

Patients

A total of 1790 consecutive leukemia patients who underwent transplantation with marrow from a serologically HLA-A, -B, and -DR antigen–matched donor in Japan between January 1993 and March 2000 through the JMDP were analyzed. No patients receiving T-cell–depleted marrow and/or antithymocyte globulin (ATG) as GVHD prophylaxis were eligible for this study. Partial HLA-A and -B alleles and complete HLA-DRB1 alleles were identified as confirmatory HLA typing during the coordination process, and HLA-A, -B, -C, -DQB1, and -DPB1 alleles were retrospectively reconfirmed or identified after transplantation. The final clinical survey of these patients was completed as of June 1, 2005. Informed consent was obtained from patient and donor according to the Declaration of Helsinki, and approval was obtained from the JMDP and the Institutional Review Board of the Aichi Cancer Center.

Characteristics of patients and donors are listed in Table 1. The patients' age ranged from 0 to 59 years (median, 27 years), and donors' age ranged from 20 to 51 years (median, 35 years). There were 577 patients with acute myeloblastic leukemia (AML), of whom 186 underwent transplantation while in first complete remission (CR), 191 who did so while in second or further CR, and 200 who did so while in non-CR; 617 patients with acute lymphoblastic leukemia (ALL), of whom 236 underwent transplantation while in first CR, 207 who did so while in second or further CR, and 174 who did so while in non-CR; and 596 patients with chronic myeloid leukemia (CML), of whom 417 were in the first chronic phase (CP), 34 were in the second or further CP, 90 were in the accelerated phase, and 55 were in the blastic phase. Standard risk for leukemia relapse was defined as the status of the first CR of AML and ALL and the first CP of CML at transplantation, whereas high risk was defined as a more advanced status than standard risk in AML, ALL, and CML.

HLA Typing of Patients and Donors

Alleles at the HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 loci were identified as described previously [4,5]. HLA 6 locus alleles were typed in 1773 pairs, and HLA 5 locus alleles except HLA-DPB1 were typed in 17 pairs. HLA genotypes of HLA-A, -B, -C, -DQB1, and -DPB1 alleles of patient and donor were reconfirmed by the Luminex microbead method (100 System; Luminex, Austin, TX) adjusted for the JMDP [17] and in part by the sequencing-based typing method in 2004 and 2005. As a result, all HLA alleles that were observed with $> 0.1\%$ frequency among Japanese were identified. The numbers of

Table 1. *Patient characteristics and matching status of HLA allele and KIR2DL ligand*

	Patient Number (%) M/MM*	Patient Age Median (years) M/MM*	Patient Sex Female (%) M/MM*	Donor Age Median (years) M/MM*	Donor Sex Female (%) M/MM*	Sex Match (%) M/MM*	Stage at Transplant High (%) M/MM*	GVHD Prophylaxis Cyclosporine (%) M/MM*	Total Body Irradiation (%) M/MM*
All leukemia (n = 1790)									
HLA-A	1484/306	27/26	39/37	34/33	38/40	57/55	52/57	73/73	83/72
HLA-B	1645/145	27/26	40/34	34/35	39/36	56/63	52/51	72/76	83/84
HLA-C	1256/534	27/26	39/41	34/33	38/40	56/58	52/55	74/70	83/82
HLA-DRB1	1434/356	27/26	40/38	34/34	38/41	57/57	51/60	74/66	83/82
HLA-DQB1	1391/399	27/26	40/38	34/33	38/41	57/57	52/56	74/67	83/83
HLA-DPB1	612/1163	26/27	42/39	34/34	39/39	60/56	50/55	75/71	81/84
KIR2DL-G†	1693/97	26/27	39/35	34/34	39/43	57/74	53/63	73/64	83/84
KIR2DL-R‡	1679/111	27/25	39/40	34/32	39/60	57/51	53/59	73/67	83/84
Acute myeloblastic leukemia (n = 577)									
HLA-A	486/91	28/27	44/44	33/33	38/39	58/55	67/71	72/60	81/89
HLA-B	537/40	27/31	45/33	33/35	39/30	56/73	67/83	71/68	83/80
HLA-C	405/172	28/28	43/45	33/34	39/37	56/61	66/73	74/63	82/83
HLA-DRB1	474/103	28/27	44/43	33/33	37/47	58/55	66/77	72/63	82/86
HLA-DQB1	469/108	27/29	45/40	33/33	38/43	57/56	67/72	72/64	83/81
HLA-DPB1	206/366	27/28	48/42	34/33	40/38	58/57	65/70	71/70	81/84
KIR2DL-G†	546/31	28/28	43/55	33/33	38/39	57/65	67/71	72/52	82/83
KIR2DL-R‡	546/31	28/28	43/55	33/35	38/39	59/32	68/68	71/58	82/83
Acute lymphoblastic leukemia (n = 617)									
HLA-A	515/102	20/19	41/40	34/32	42/42	55/50	60/69	73/74	91/88
HLA-B	567/50	19/20	41/42	33/36	42/38	54/60	61/70	72/80	91/86
HLA-C	437/180	19/19	41/41	34/32	41/42	54/57	61/63	73/72	91/89
HLA-DRB1	485/132	19/19	41/42	33/33	43/36	55/52	61/64	74/70	90/90
HLA-DQB1	467/150	19/20	41/41	34/33	42/41	55/51	61/63	75/68	90/92
HLA-DPB1	190/425	19/29	43/40	34/33	38/43	61/52	61/62	77/71	89/91
KIR2DL-G†	587/30	20/17	42/20	33/35	42/40	55/53	61/73	73/73	91/83
KIR2DL-R‡	577/40	19/19	39/40	34/30	42/43	54/53	61/73	73/70	90/93
Chronic myelocytic leukemia (n = 596)									
HLA-A	483/113	32/31	33/35	34/34	35/40	59/60	29/35	73/81	76/72
HLA-B	541/55	32/29	34/27	34/37	36/38	56/60	29/36	74/78	74/85
HLA-C	414/182	32/31	33/36	35/34	35/39	60/58	30/31	74/76	75/74
HLA-DRB1	475/121	32/33	34/31	34/36	35/40	58/63	27/41	77/64	76/70
HLA-DQB1	455/141	32/31	34/33	35/33	35/39	57/65	28/35	76/69	75/74
HLA-DPB1	216/372	31/33	35/33	34/35	38/34	60/59	28/31	76/73	73/76
KIR2DL-G†	560/36	32/32	34/31	35/32	35/50	59/53	29/44	75/67	71/83
KIR2DL-R‡	556/40	32/27	34/28	35/31	36/38	59/65	29/38	75/68	75/75

Standard-first complete remission or first chronic phase; high more advanced stage than standard.
*M/MM match/mismatch in GVH direction for HLA matching.
†KIR2DL ligand mismatching in GVH direction.
‡KIR2DL ligand mismatching in HVG direction.

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identified alleles in this study were 25 in HLA-A, 43 in HLA-B, 20 in HLA-C, 33 in HLA-DRB1, 14 in HLA-DQB1, and 21 in HLA-DPB1.

Matching of HLA Allele and KIR2DL Ligand

For the analysis of GVHD and leukemia relapse, HLA allele mismatch among the donor–recipient pair was scored when the recipient's alleles were not shared by the donor (graft-versus-host [GVH] direction). For graft rejection, HLA allele mismatch among the donor–recipient pair was scored when the donor's alleles were not shared by the patient (host-versus-graft [HVG] direction). For survival, the mismatch was defined as that of either the GVH direction or the HVG direction.

KIR2DL ligand specificity of HLA-C antigen was determined according to the HLA-C allele. The epitope of HLA-Cw3 group (C1 specificity) consists of Asn80, and that of the HLA-Cw4 group (C2 specificity) consists of Lys80.

KIR ligand mismatch in the GVH direction (KIR-L-MM-G) was scored when the donor's KIR2DL epitope of HLA-C was not shared by the patient epitope. This mismatch occurred when KIR2DL2/3- or KIR2DL1-positive effector cells were activated without the expression of corresponding HLA-C ligand (C1 or C2, respectively) on the patient's target cells. KIR ligand mismatch in HVG direction (KIR-L-MM-R) was scored when the patient's KIR2DL epitope of HLA-C was not shared by the donor. This mismatch occurred when patient KIR2DL2/3- or KIR2DL1-positive effector cells were activated without the expression of corresponding HLA-C ligand (C1 or C2, respectively) on donor cells.

Matching Status of HLA Locus in Allele Level and KIR2DL Ligand

The matching status of HLA allele matching in the GVH direction in each HLA locus and KIR ligand matching in both directions are given in Table 1. The HLA-C epitope of KIR2DL was estimated from HLA-C allele type, with 92.4% of the HLA-C allele belonging to the Cw3 group (C1 specificity) and 7.6% belonging to the Cw4 group (C2 specificity). KIR2DL ligand match in both directions occurred in 1583 pairs (88.4%). KIR-L-MM-G, which occurred in the combination of KIR2DL ligand in patient–donor pairs, was found in 97 pairs (5.4%): C1/C1 and C1/C2 in 92 pairs, C2/C2 and C1/C2 in 4 pairs, and C1/C1 and C2/C2 in 1 pair. KIR-L-MM-R, which occurred in the combination of KIR2DL ligand in patient and donor pairs, was found in 111 pairs (6.2%): C1/C2 and C1/C1 in 105 pairs, C1/C2 and C2/C2 in 5 pairs, and C1/C1 and C2/C2 in 1 pair. Mismatches in both directions were found in only 1 pair. Because all pairs were a serologic HLA-B match in this study, the combination of KIR3DL1 and its ligand of Bw4 matched in all pairs.

Definition of Transplantation-Related Events

The occurrence of aGVHD was evaluated according to grading criteria in patients who survived more than 8 days after transplantation, and chronic GVHD (cGVHD) according to the criteria in patients who survived more than 100 days after transplantation as described previously [5]. Rejection was defined as when the peripheral granulocyte count became $< 500/\mu L$ with the finding of severe hypoplastic marrow in engrafted patients. Engraftment was defined as a peripheral granulocyte count of $> 500/\mu L$ for 3 successive days in patients surviving > 21 days after transplantation.

GVHD Prophylaxis

Among the 1790 patients transplanted with T-cell–replete marrow, 1302 received a cyclosporine-based regimen and 488 received a tacrolimus-based regimen for GVHD prophylaxis. Anti-thymocyte globuline (ATG) was not given for GVHD prophylaxis.

Preconditioning Regimen

All patients were preconditioned with a myeloablative regimen, with 1480 receiving total body irradiation (TBI)-containing regimens and 310 receiving non-TBI regimens.

Statistical Analysis

All of the analyses were conducted using STATA version 8.2 (STATA Corp, College Station, TX). Overall survival rate was assessed by the Kaplan-Meier product limit method [18]. Cumulative incidences of aGVHD, cGVHD, rejection, and leukemia relapse were assessed as described previously to eliminate the effect of competing risk [19,20]. The competing events regarding aGVHD, cGVHD, rejection, and relapse were defined as death without aGVHD, cGVHD, rejection, and relapse, respectively. For each endpoint, a log-rank test was applied to assess the impact of the factor of interest.

Cox proportional hazard models [21] were applied to assess the impact of HLA allele matching (mismatch vs match [hazard risk = 1.0] as a reference group) as well as KIR ligand matching (mismatch vs match in the GVH direction and mismatch vs match in the HVG direction) including the following confounders. The confounders considered were sex (donor–recipient pairs), patient age (older: linear), donor age (older: linear), type of disease (AML, CML, or ALL), risk of leukemia relapse (high vs standard),

Table 2. *Effects of HLA and KIR ligand matching for leukemia relapse*

	All Leukemia Cell Types			Acute Myeloblastic Leukemia			Acute Lymphoblastic Leukemia			Chronic Myeloid Leukemia		
	HR*	(95% CI)	P	HR	(95% CI)	P	HR	(95% CI)	P	HR	(95% CI)	P
HLA-A	1.19	(0.89-1.59)	.251	0.92	(0.54-1.58)	.761	1.18	(0.76-1.86)	.462	1.63	(0.89-2.97)	.114
HLA-B	1.01	(0.65-1.59)	.953	1.36	(0.65-2.88)	.416	0.98	(0.48-1.98)	.952	0.62	(0.22-1.76)	.367
HLA-C	0.71	(0.53-0.96)	.025	0.8	(0.49-1.30)	.366	0.47	(0.28-0.78)	.003	1.2	(0.62-2.29)	.591
HLA-DRB1	1.05	(0.73-1.53)	.789	0.78	(0.40-1.52)	.466	0.91	(0.51-1.61)	.737	1.25	(0.55-2.85)	.59
HLA-DQB1	1.10	(0.77-1.58)	.579	1.55	(0.82-2.95)	.178	1.11	(0.63-1.95)	.71	0.86	(0.39-1.93)	.72
HLA-DPB1	0.68	(0.55-0.85)	.001	0.76	(0.52-1.09)	.137	0.92	(0.65-1.28)	.604	0.35	(0.21-0.58)	<.001
KIR2DL-G†	1.55	(0.92-2.63)	.103	1.05	(0.37-3.02)	.926	2.55	(1.18-5.52)	.017	1.23	(0.38-3.94)	.727
KIR2DL-R‡	0.73	(0.40-1.34)	.313	0.53	(0.15-1.78)	.305	1.30	(0.53-3.19)	.569	0.5	(0.14-1.80)	.292

HLA matching in GVH direction.

*Hazard ratio of mismatch with match as a reference adjusted for patient age, donor age, sex-matching disease, GVHD prophylaxis, total body irradiation, transplanted cell dose, risk status, and other matching status of HLA and KIR ligand.

†KIR2DL ligand mismatching in GVH direction.

‡KIR2DL ligand mismatching in HVG direction.

GVHD prophylaxis (tacrolimus-based vs cyclosporine-based and ATG vs cyclosporine-based), numbers of transplanted cells (linear), and preconditioning (non-TBI vs TBI). The numbers of nucleated cells before manipulation of bone marrow were replaced with the numbers of transplanted cells.

Multivariate analysis for clinical outcomes, including KIR ligand matching and HLA-C matching in all pairs (not restricted to HLA-C mismatch), made it possible to evaluate whether these factors are independent. The results of all pairs by multivariate analysis are presented in the Results section and in Tables 2, 3, and 4. HLA-C–mismatched pairs were selected for the analysis of cumulative incidence in KIR ligand matching.

RESULTS

Effects of HLA Locus Mismatch and KIR Ligand Mismatch on Leukemia Relapse

When all leukemia patients (AML, ALL, and CML) were analyzed together, HLA-C mismatch was found to be a factor reducing the relapse rate (HR = 0.71; P = .025) (Table 2). This GVL effect was remarkable in ALL patients (HR = 0.47; P = .003), especially in high risk (HR = 0.40; P = .004) but not in standard risk (HR = 0.85; P = .728). No such effect was observed in AML patients (HR = 0.80; P = .366) or CML patients (HR = 1.20; P = .591).

Cumulative incidence curves of relapse by leukemia cell type are shown in Figure 1. The relapse rate 5 years after transplantation was 16.7% (95% confidence interval [CI] = 11.6%-30.9%) for HLA-C mismatch and 29.8% (95% CI = 25.5%-34.3%) for HLA-C match in ALL patients (P = .012); 17.6% (95% CI = 12.2%-23.8%) and 25.9% (95% CI = 21.1%-30.9%), respectively, in AML patients (P = .342); and 11.7% (955 CI = 12.2%-23.8%) and 12.0% (95% CI = 9.0%-15.4%), respectively, in CML patients (P = .485).

HLA-DPB1 mismatch was shown to reduce the overall leukemia relapse rate (HR = 0.68; P = .001) (Table 2). This effect was significant in CML (HR =

Table 3. *Effects of HLA and KIR ligand matching for acute GVHD, chronic GVHD, and rejection in all leukemia cell types*

	Acute GVHD (Grade 2-4) (n = 1751)			Acute GVHD (Grade 3-4) (n = 1751)			Chronic GVHD (n = 1109)			Rejection (n = 1664)		
	HR*	95% CI	P	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
HLA-A	1.22	(1.02-1.46)	.034	1.44	(1.11-1.86)	.006	1.41	(1.08-1.85)	.013	0.72	(0.24-2.14)	.555
HLA-B	1.43	(1.28-1.82)	.003	1.40	(1.00-1.95)	.05	1.00	(0.65-1.52)	.991	1.16	(0.32-4.16)	.82
HLA-C	1.29	(1.08-1.55)	.006	1.39	(1.06-1.83)	.017	1.38	(1.07-1.78)	.014	1.87	(0.72-4.86)	.201
HLA-DRB1	1.15	(0.90-1.47)	.254	1.09	(0.77-1.54)	.644	0.91	(0.63-1.31)	.607	0.49	(0.10-2.33)	.366
HLA-DQB1	1.02	(0.81-1.29)	.871	1.13	(0.81-1.59)	.465	1.20	(0.85-1.69)	.288	0.62	(0.07-5.16)	.536
HLA-DPB1	1.39	(1.19-1.63)	<.001	1.26	(1.00-1.60)	.053	0.86	(0.70-1.05)	.138	1.08	(0.59-2.41)	.843
KIR2DL-G†	1.70	(1.28-2.26)	<.001	2.35	(1.62-3.40)	<.001	1.13	(0.68-1.87)	.64	0.62	(0.07-5.16)	.655
KIR2DL-R‡	1.04	(0.77-1.42)	.78	1.33	(0.88-2.02)	.18	0.88	(0.55-1.42)	.603	4.39	(1.38-13.96)	.012

HLA matching in GVH direction for acute GVHD and chronic GVHD, and HLA matching in HVG direction for rejection.

*Hazard ratio of mismatch with match as a reference adjusted for patient age, donor age, sex-matching disease, GVHD prophylaxis, total body irradiation, transplanted cell dose, risk status, and other matching status of HLA and KIR ligand.

†KIR2DL ligand mismatching in GVH direction.

‡KIR2DL ligand mismatching in HVG direction.

Table 4. *Effects of HLA and KIR ligand matching for mortality*

	All Leukemia Cell Types			Acute Myeloblastic Leukemia			Acute Lymphoblastic Leukemia			Chronic Myeloid Leukemia		
	HR*	95% CI	P	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
HLA-A	1.36	(1.16-1.59)	<.001	1	(0.75-1.34)	.978	1.46	(1.11-1.90)	.006	1.77	(1.35-2.33)	<.001
HLA-B	1.40	(1.13-1.73)	.002	1.43	(0.96-2.12)	.079	1.47	(1.03-2.09)	.036	1.18	(0.80-1.72)	.402
HLA-C	1.17	(0.99-1.37)	.067	1.18	(0.89-1.55)	.246	0.99	(0.74-1.31)	.928	1.42	(1.04-1.93)	.025
HLA-DRB1	0.92	(0.74-1.15)	.463	0.74	(0.50-1.10)	.136	1.04	(0.72-1.49)	.849	0.99	(0.65-1.50)	.951
HLA-DQB1	1.28	(1.04-1.58)	.018	1.29	(0.89-1.87)	.184	1.33	(0.93-1.90)	.108	1.18	(0.79-1.75)	.422
HLA-DPB1	1.06	(0.91-1.23)	.474	0.96	(0.75-1.24)	.772	1.33	(1.02-1.75)	.038	0.97	(0.74-1.27)	.827
KIR2DL-G†	1.80	(1.39-2.34)	<.001	1.93	(1.22-3.05)	.005	1.57	(0.96-2.56)	.069	2.23	(1.42-3.50)	<.001
KIR2DL-R‡	1.07	(0.81-1.41)	.612	1.08	(0.66-1.75)	.769	0.98	(0.59-1.61)	.934	1.07	(0.66-1.72)	.787

*Hazard ratio of mismatch with match as a reference adjusted for patient age, donor age, sex-matching disease, GVHD prophylaxis, total body irradiation, transplanted cell dose, risk status, and other matching status of HLA and KIR ligand.
†KIR2DL ligand mismatching in GVH direction.
‡KIR2DL ligand matching in HVG direction.

0.35; $P < .001$), and both high-risk and standard-risk CML had a significantly lower relapse rate of HLA-DPB1 mismatch (HR = 0.35; $P < .001$ and HR = 0.39; $P = .012$, respectively). No significant effect was observed in AML (HR = 0.76; $P = .137$) or ALL (HR = 0.92; $P = .604$).

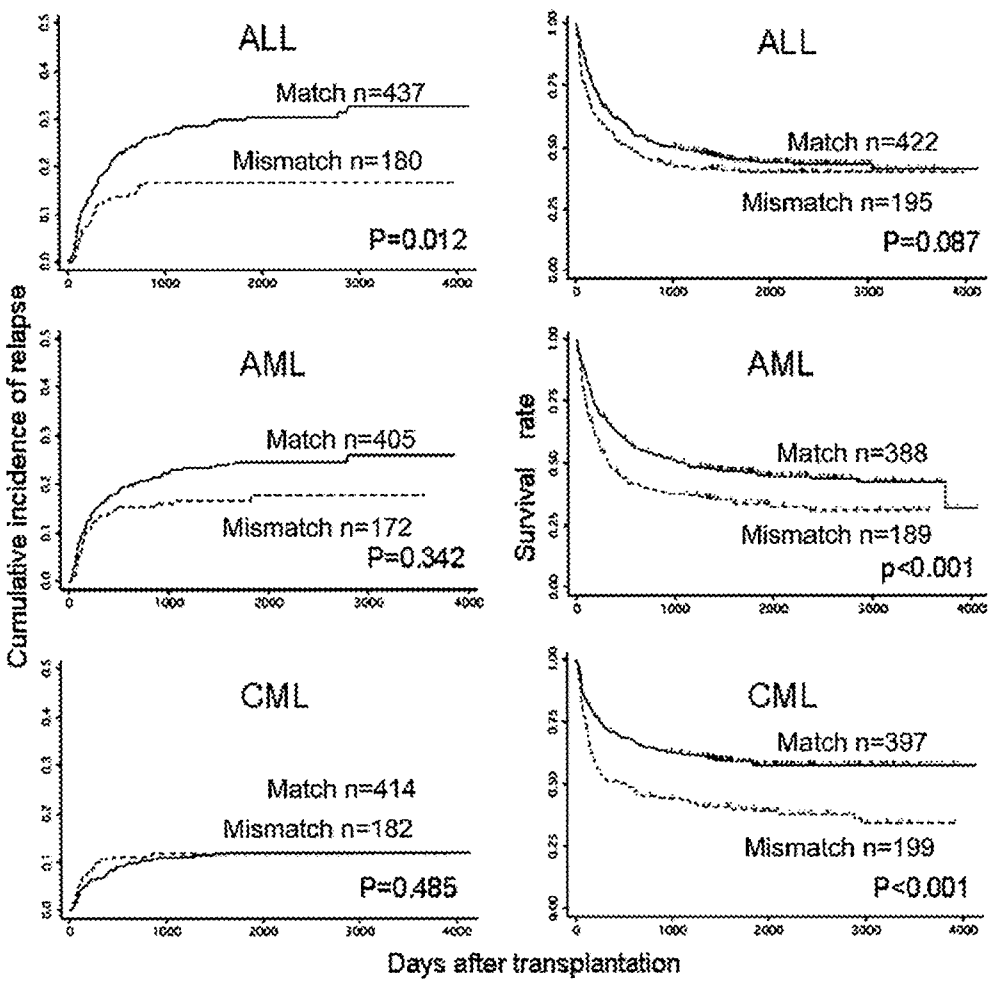

Figure 1. Cumulative incidence of relapse and survival by matching of HLA-C in patients with ALL, AML, and CML. All patients were analyzed. The direction of mismatching of HLA-C for relapse is GVH for relapse, and the direction for survival is GVH and/or HVG. The solid line represents match; the dotted line, mismatch.

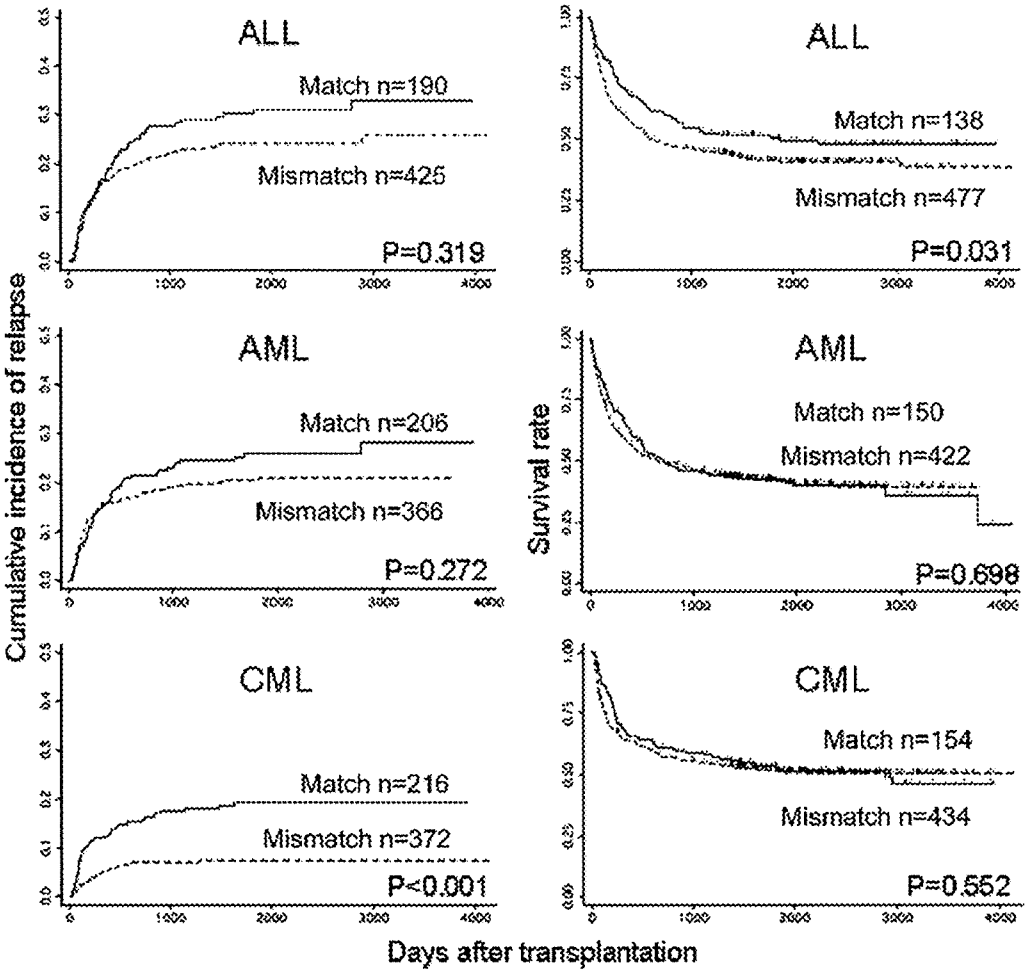

Figure 2. Cumulative incidence of relapse and survival by matching of HLA-DPB1 in patients with ALL, AML, and CML. All patients were analyzed. The direction of mismatching of HLA-DPB1 for relapse is GVH for relapse, and the direction for survival is GVH and/or HVG. Solid line, match; dotted line, mismatch.

As shown in Figure 2, the relapse rate 5 years after transplantation was 7.1% (95% CI = 5.0%-10.4%) for HLA-DPB1 mismatch and 19.3% (95% CI = 14.3%-24.9%) for HLA-DPB1 match in CML patients ($P < .001$); 20.4% (95% CI = 16.4%-24.8%) and 25.9% (95% CI = 19.9%-32.2%), respectively, in AML patients ($P = .272$); and 24.0% (95% CI = 19.9%-28.3%) and 30.2% (95% CI = 23.7%-37.0%), respectively, in ALL patients ($P = .319$).

Mismatch of HLA-A, -B, -DRB1, and -DQB1 was not a significant risk factor for leukemia relapse by multivariate analysis (Table 2).

Patients with KIR-L-MM-G had a higher relapse rate than those with KIR2DL ligand match in ALL (HR = 2.55; $P = .017$) (Table 2). This adverse effect on leukemia relapse was remarkable in high-risk ALL (HR = 3.03; $P = .013$), but not in standard-risk ALL (HR = 1.11; $P = .921$). In AML and CML, KIR-L-MM-G had no effect on leukemia relapse (HR = 1.05; $P = .926$ and HR = 1.23; $P = .727$, respectively).

Because KIR-L-MM occurs in HLA-C mismatch pairs, the cumulative incidence of leukemia relapse was analyzed in HLA-C mismatch patients in either direction by leukemia cell type (Figure 3). The relapse rate 5 years after transplantation was 31.0% (95% CI = 5.6%-47.9%) for KIR-L-MM-G and 16.3% (95% CI = 11.0%-22.4%) for match in ALL patients ($P = .026$); 11.1% (95% CI = 3.5%-23.6%) and 11.8% (95% CI = 7.4%-17.3%), respectively, in CML patients ($P = .634$); and 12.9% (95% CI = 4.1%-27.0%) and 16.3% (95% CI = 11.0%-22.6%), respectively, in AML patients ($P = .757$).

Significant clinical risk factors for leukemia relapse by multivariate analysis included status at transplantation (standard vs high, HR = 3.00; $P < .001$) and disease (HR = 0.75; $P < .001$) in all leukemia patients.

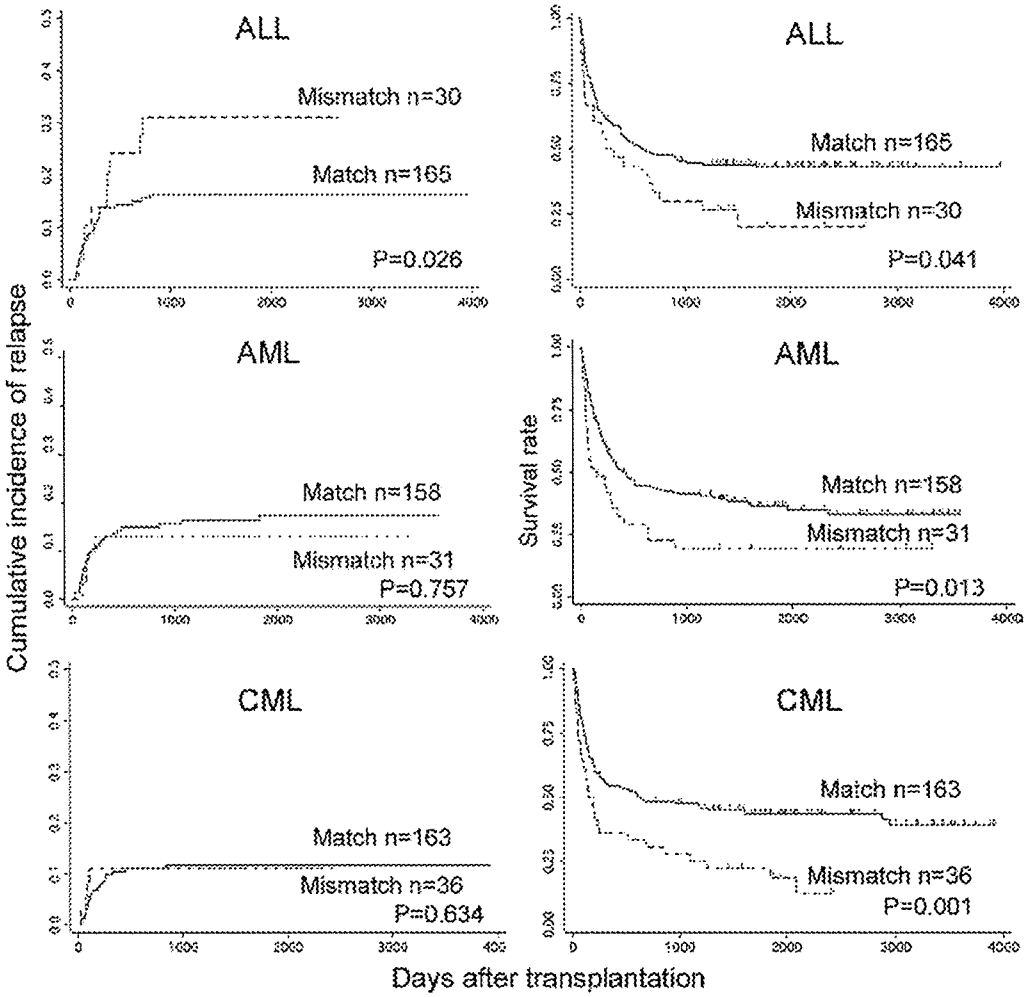

Figure 3. Cumulative incidence of relapse and survival by matching of KIR2DL ligand in the GVH direction in HLA-C–mismatched patients with ALL, AML, and CML. HLA-C–mismatched patients were selected for this analysis. The directions of HLA-C mismatching were GVH and/or HVG. The solid line represents KIR2DL ligand match in the GVH direction; the dotted line, KIR2DL mismatch in the GVH direction.

Effects of HLA Locus Mismatch and KIR Ligand Mismatch on Rejection

Rejection rates in patients who engrafted marrow and survived more than 21 days were analyzed. KIR-L-MM-R was found to be a significantly higher risk factor for rejection compared with match (HR = 4.39; P = .012), and no HLA mismatch was considered significant by multivariate analysis (Table 3). Older donor age was a significant clinical risk factor for rejection (HR = 1.08; P = .002); other clinical factors were not significant.

The cumulative incidence of graft rejection was 5.7% (95% CI = 2.3%-11.3%) in KIR-L-MM-R (n = 106) and 1.8% (95% CI = 0.8%-3.3%) in match (n = 447) (P = .019) 1 year after transplantation in HLA-C–mismatched patients in either direction. Engraftment rate was not influenced by HLA and KIR ligand matching (data not shown).

Effects of HLA Locus Mismatch and KIR Ligand Mismatch on Acute GVHD

HLA allele mismatch of each HLA-A, -B, and -C locus was found to be an independent risk factor for grade 3-4 aGVHD and grade 2-4 aGVHD, and the mismatch of each HLA-DRB1 and -DQB1 locus was not a significant risk factor. HLA-DPB1 mismatch was a significant risk factor for grade 2-4 aGVHD and a marginal risk factor for grade 3-4 aGVHD (Table 3). When analyzed by leukemia cell type, AML showed no significant HLA mismatch locus for aGVHD (data not shown).

KIR-L-MM-G was associated with a significantly higher risk of grade 2-4 aGVHD (HR = 1.70; $P < .001$) and grade 3-4 aGVHD (HR = 2.35; $P < .001$) compared with KIR ligand match (Table 3). By leukemia cell type, the HR of KIR-L-MM-G in grade 3-4 aGVHD was 2.76 for AML ($P = .005$), 1.75 for ALL ($P = .111$), and 2.79 for CML ($P < .001$).

In HLA-C mismatch patients, the incidence of 40.3% in KIR-L-MM-G (95% CI = 29.3%-50.9%) was significantly higher than the 25.8% in match (95% CI = 21.9%-30.0%) ($P = .011$) for grade 3-4 aGVHD.

Significant clinical risk factors for grade 3-4 GVHD by multivariate analysis were GVHD prophylaxis (tacrolimus vs cyclosporine, HR = 0.72; $P = .016$), patient age (HR = 0.99; $P = .019$), donor age (HR = 1.02; $P = .001$), and disease (HR = 1.28; $P = .001$) in all leukemia patients.

Effects of HLA Locus Mismatch and KIR Ligand Mismatch on Chronic GVHD

The occurrence of cGVHD was analyzed in patients who survived more than 100 days after transplantation. HLA-A mismatch and HLA-C mismatch were found to be significant factors (HR = 1.41; $P = .013$ and HR = 1.38; $P = .014$, respectively). KIR-L-MM-G was not significant (HR = 1.13; $P = .640$) (Table 3).

In HLA-C mismatch patients, the cumulative incidence of cGVHD 3 years after transplantation was 43.2% in KIR-L-MM-G (95% CI = 27.2%-58.3%) and 40.4% in KIR2DL ligand match (95% CI = 35.4%-46.1%) ($P = .727$). Significant clinical risk factors for cGVHD by multivariate analysis were patient age (HR = 1.01; $P = .0004$), disease (HR = 1.23; $P = .003$), and TBI (HR = 1.54; $P = .004$).

Effects of HLA Allele Mismatch and KIR Ligand Mismatch on Survival

In all leukemia patients, HLA allele mismatch of each HLA-A, -B, and -DQB1 locus was found to be an independent risk factor for mortality after transplantation, and the mismatch of HLA-C was of marginal risk. HLA mismatch in each HLA-DRB1 and -DPB1 locus was not a significant factor. By leukemia cell type, mismatch of HLA-A, -B, and -DPB1 was a significant risk factor in ALL, and mismatch of HLA-A and -C was a significant risk factor in CML (Table 4).

Survival 5 years after transplantation was 39.8% in HLA-C mismatch (95% CI = 32.8%-46.7%) and 44.5% in HLA-C match (95% CI = 39.6%-49.3%) in ALL ($P = .088$); 33.7% (95% CI = 26.9%-40.6%) and 46.3% (95% CI = 41.2%-51.2%), respectively, in AML ($P < .001$); and 39.7% (95% CI = 32.8%-46.5%) and 58.3% (95% CI = 53.2%-63.1%), respectively, in CML ($P < .001$) (Figure 1).

Survival 5 years after transplantation was 40.9% in HLA-DPB1 mismatch (95% CI = 36.3%-45.4%) and 50.3% in HLA-DPB1 match (95% CI = 41.5%-58.4%) in ALL ($P = .031$); 41.8% (95% CI = 37.0%-46.6%) and 42.6% (95% CI = 34.5%-50.4%), respectively, in AML ($P = .698$); and 51.4% (95% CI = 46.5%-56.1%) and 53.4% (95% CI = 45.1%-61.0%), respectively, in CML ($P = .522$) (Figure 2).

KIR-L-MM-G was also found to be a significant risk factor for mortality (HR = 1.80; $P < .001$). Particularly in AML and CML patients, KIR-L-MM-G had a significantly higher adverse effect than match (HR = 1.93; $P = .005$ and HR = 2.23; $P < .001$, respectively); its effect was moderate in ALL patients (HR = 1.57; $P = .069$) (Table 4).

In HLA-C mismatch patients in either direction, the survival rate 5 years after transplantation was 20.0% for KIR-L-MM-G (95% CI = 6.9%-38.0%) and 43.0% in match (95% CI = 35.3%-50.5%) in ALL ($P = .041$); 19.4% (95% CI = 7.9%-34.6%) and 36.5% (95% CI = 28.8%-44.2%), respectively, in AML ($P = .013$); and 22.2 (95% CI = 10.5%-36.7%) and 43.6% (95% CI = 35.8%-51.1%), respectively, in CML ($P = .001$) (Figure 3).

Significant clinical factors for mortality by multivariate analysis were patient age (HR = 1.02; $P < .001$), donor age (HR = 1.01; $P = .037$), disease (HR = 0.88; $P = .006$), and the status at transplantation (high vs standard, HR = 2.14; $P < .001$).

DISCUSSION

In the present study, we attempted to elucidate how disparities of HLA and KIR affect leukemia relapse and the other transplantation-related immunologic events and to explore how these findings can be applied to induce a GVL effect and improve patient survival in the unrelated setting. Simultaneous analysis of HLA and KIR ligand matching by multivariate analysis made it possible to clarify the role of these antigens in UR-HSCT.

To the best of our knowledge, this is the first report to elucidate the HLA locus responsible for the GVL effect by leukemia cell type in T-cell–replete UR-HSCT. The sequentially registered 577 AML, 617 ALL, and 596 CML patients sufficed to analyze the effects of HLA and KIR ligand matching in the 3 major leukemia cell types.

HLA-C mismatch reduced the relapse rate overall, as reported previously [4]. The GVL effect of HLA-C mismatch depended on the leukemia cell type. ALL patients with HLA-C mismatch showed a significantly lower leukemia relapse risk than those with match, whereas AML and CML patients did not. Furthermore, CML patients with HLA-DPB1 mismatch

showed a significantly lower leukemia relapse rate than those with match, whereas AML and ALL patients did not. Although the reason why the HLA locus responsible for the GVL effect differs with leukemia cell type remains unknown, the different expression of HLA antigens, such as HLA-C, HLA-DPB1, or co-stimulatory molecules on leukemia cells, might modify the immune response of effector cells to leukemia cells. The finding of HLA-DPB1 is in line with a previous report in CML and ALL patients treated with T cell–depleted UR-HSCT [12].

In contrast, an impact of HLA-A and -B allele mismatch on leukemia relapse was not observed. Because HLA-A and/or -B allele mismatch induces severe aGVHD, no GVL effect of HLA-A and /or -B allele mismatch might imply that the target antigenic peptide recognized by effector T cells responsible for aGVHD is not expressed on leukemia cells.

Unexpectedly, KIR-L-MM-G increased the leukemia relapse rate overall. A significantly increased relapse rate in the mismatched group was observed in ALL, but not in AML and CML. Simultaneous multivariate analysis of HLA-C mismatch and KIR-L-MM-G revealed that contrary reactions of these mismatches occurred independently. Although the mechanism involved in this detrimental effect of KIR-L-MM-G on leukemia relapse is not known, the activation of KIR-positive NK cells or T cells might cause immune dysfunction, which abrogates the GVL effect.

The GVL effect of donor-derived KIR-positive NK cells transplanted purified $CD34^+$ stem cells with HLA haploidentical donor was reported in AML patients, but not in ALL patients [22]. In T-cell–replete UR-HSCT, published reports show contradictory effects of KIR ligand mismatch on leukemia relapse. A GVL effect in myeloid malignancies [23-25], a higher leukemia relapse rate [26], and no significant effect [27-29] all have been reported. The use of ATG for GVHD prophylaxis might be a key to understanding these diverse results. Our analysis of T-cell–replete UR-BMT with no use of ATG provided reliable evidence for the adverse effect of KIR-L-MM-G on relapse of ALL relapse. No effect on relapse of AML or CML was reported in a recent large-scale study of myeloid malignancy from the Center for International Blood and Marrow Transplant Research, the European Blood and Marrow Transplant Registry, and the Dutch Registry [30]. Whether KIR ligand match affects leukemia relapse adversely or beneficially is a critical issue for clinical transplantation and immunotherapy using NK cells, and further large-scale comparative studies considering GVHD prophylaxis are warranted.

A higher rejection rate (HR = 4.39; $P = .012$) was found for KIR-L-MM-R; that is, in this mismatch combination, patient KIR2DL-positive effector cells lacking donor KIR ligand are reconstituted and activated after transplantation, which induces the rejection of engrafted donor-derived hematopoietic stem cells. "Hybrid resistance" has been extensively analyzed in mice to induce graft rejection by NK cells [31]. The same mechanism of rejection induced by NK cells might be considered in humans, although 88% of KIR ligand mismatch pairs and 86% of match pairs were given cyclophosphamide as a preconditioning. The effects of HLA class I mismatch for graft rejection were reported [5,32,33]; our data suggest that the effect of HLA-C mismatch were mainly because of KIR2DL ligand mismatch in the HVG direction, and may not result from the HLA-C allele mismatch itself. Our findings are in agreement with a report showing the effect of rejection but not engraftment by KIR2DL ligand mismatch in UR-HSCT [29].

Since the first JMDP report [4], HLA-class I mismatch has been known to significantly increase aGVHD, whereas HLA-DRB1 mismatch has only a marginal effect on aGVHD. The present study has confirmed those earlier findings. We could add the new data on HLA-DPB1 matching showing that HLA-DPB1 mismatch induces moderate aGVHD. Our finding of the effect of HLA-DPB1 on aGVHD concurs with other reports [9-11], although there we found no difference in aGVHD between 2 allele mismatches and 1 allele mismatch of HLA-DPB1.

The international collaborative study is expected to reconcile discrepancies of allele matching in ethnically diverse transplantation populations. Furthermore, the identification of nonpermissive HLA allele mismatch and amino acid substitution responsible for aGVHD, leukemia relapse, and survival might explain these discrepancies in diverse ethnic populations.

Interestingly, KIR-L-MM-G had a higher HR of severe aGVHD than did match. Because these values were adjusted by HLA allele matching and clinical factors, this finding demonstrates that KIR-L-MM-G is a factor independent of HLA allele matching. In fact, among HLA-C mismatch patients, KIR-L-MM-G was associated with a higher rate of grade 3-4 aGVHD than match. In KIR-L-MM-G, the donor-derived KIR2DL2/3- or KIR2DL1-positive effector cells are suspected to react with patient cells that lack the corresponding KIR2DL epitope of HLA-C. These effector cells induce aGVHD through several possible mechanisms. First, NK cells derived from donor graft might directly attack the patient target cells. This is unlikely, however, because in vivo infusion of alloreactive NK cells were found to not cause aGVHD [34], and NK cells were seen to play mainly a protective role for GVHD in a murine experimental model [35]. Alternatively, activated NK cells might

affect donor-derived T cells that induce aGVHD. Third, KIR2DL-positive T cells might induce aGVHD directly. The presence of KIR2DL-positive T cells was reconstituted after UR-HSCT [36].

Conflicting findings have been reported in terms of the effect of KIR-L-MM-G on aGVHD in T-cell–replete UR-HSCT. Some studies have found a trend toward less aGVHD [23], whereas others have reported an increased risk of aGVHD [27,29]. The variety of GVHD prophylaxis, HLA matching, and other clinical factors, and limited patient numbers in each study makes it difficult to determine the role of KIR ligand matching in clinical outcomes. The use of ATG and/or the T-cell depletion method for GVHD prophylaxis will be a key strategy in resolving the discrepancy regarding aGVHD in UR-HSCT [35,37] and in HLA haplotype–identical related HSCT with T-cell depletion [38]. That is, T cell and NK-cell reconstitution after transplantation might affect immunologic events induced by the interaction of KIR and HLA-C epitopes. In addition, genotyping of KIR genes, especially for activating KIR such as KIR2DS, is required to understand the mechanism of KIR involved in aGVHD and the GVL effect [39]. The East Asian population, including Japanese, is known to have several characteristic HLA types. Similarly, the frequencies of both the KIR ligand epitope and the KIR genotype are distinctive in the Japanese population. For example, a higher frequency of C1 epitope and dominance of the KIR "A" haplotype were reported [40]. Those features might contribute considerably to our results. The combination of KIR2DL1 and C2 epitope has been reported to show higher affinity and a stronger inhibitory signal compared with the combination of KIR2DL2/3 and C1 epitope [14].

HLA-A and HLA-C mismatch have been identified as significant independent factors inducing cGVHD, underscoring our previous finding of the importance of HLA class I matching. No influence of KIR-L-MM-G on cGVHD (in contrast to aGVHD) indicates that the KIR-related immunologic reaction has no relation to cGVHD.

There is another model regarding the KIR ligand effect in HSCT, the so-called "missing KIR ligand theory." Hsu et al reported this effect on survival and relapse of AML and myelodysplastic syndrome in T-cell–depleted HLA-matched related HSCT [41] and on relapse in AML, ALL, and CML in UR-HSCT in non-JMDP populations [42]. Lack of either KIR2DL ligand in a patient should activate the corresponding donor NK cells and induce the GVL effect.

In the analysis of KIR matching including HLA mismatch pairs, the mismatch pairs in the "missing KIR ligand theory" with either C1C1 or C2C2 patient epitope were divided into match and mismatch in the "KIR ligand matching theory" by donor epitope. When the donor has either C1C1 or C2C2, the KIR ligand matching theory indicates match, and when the donor has C1C2, the theory indicates mismatch. In this combination, donors with C1C2 (n = 92) had a significantly higher rate of severe aGVHD (44.4%) than donors with either C1C1 or C2C2 (19.2%) (n = 1413; $P < .001$). Therefore, we considered the "ligand matching model" to be applied in this JMDP study.

Finally, because survival after transplantation is influenced not only by leukemia relapse, but also by transplantation-related mortality resulting from aGVHD, cGVHD, fatal infections, or graft failure, the effect of HLA matching and KIR ligand matching should be discussed in light of these events.

The present study has more precisely elucidated the impact of HLA matching on leukemia patient survival. The mismatch of HLA-A and -B alleles resulted in significantly higher mortality. HLA-C and HLA-DQB1 mismatch emerged as a risk factor for poorer survival for the first time in the JMDP study. Increased survival in ALL with HLA-C mismatch cannot be linked to the compensation from a lower leukemia relapse rate. HLA-DPB1 mismatch did not significantly affect overall mortality despite the increase in moderately aGVHD. These observations of HLA-C and -DQB1 mismatch in the JMDP are in line with those of other recent reports. The NMDP reported an adverse effect of HLA-C mismatch [8], and another study reported that not only HLA-C mismatch in early-stage CML, but also HLA-DQB1 mismatched CML patients with multiple mismatch posed increased risk for mortality [43].

It should be noted that KIR-L-MM-G resulted in higher mortality in UR-HSCT with T-cell–replete marrow regardless of leukemia cell type. KIR-L-MM-G might induce an immunodeficient state that would result in a higher risk for opportunistic infections [44,45]. Thus, infectious complications by cytomegalovirus and the like should be explored in relation to KIR.

We estimate that about 30% of patients in the Japanese population have HLA-C mismatch donors, of whom 15.0% are KIR-L-MM in the GVH direction, 20.8% are KIR-L-MM in the HVG direction, and 35.6% are KIR-L-MM in either direction, when HLA-A, -B, and -DRB1 genotyping is used as the donor confirmatory typing. Because both KIR2DL ligand matching and/or HLA matching itself affect aGVHD, cGVHD, rejection, ALL relapse, and survival, as described earlier, HLA-C typing is essential in selecting a suitable donor to reduce the risk of aGVHD and improve survival in practice.

In conclusion, our analysis has produced important findings for transplantation immunology and the selection of donors in UR-HSCT. First, HLA-C and HLA-DPB1 mismatches are expected to induce a ben-

eficial GVL effect, which should be considered in terms of the leukemia cell type of individual patients. Second, KIR-L-MM should be avoided, because it induces only adverse effects on transplantation outcome and provides no benefits for patients undergoing T-cell–replete UR-HSCT.

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Allogeneic Bone Marrow Transplantation from Unrelated Human T-Cell Leukemia Virus-I–negative Donors for Adult T-Cell Leukemia/Lymphoma: Retrospective Analysis of Data from the Japan Marrow Donor Program

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ABSTRACT

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) from an HLA-matched related donor has been suggested to improve the poor prognosis of adult T-cell leukemia/lymphoma (ATLL). However, the infusion of HTLV-I–infected cells from HTLV-I–positive related donors could lead to the development of donor-derived ATLL under immunosuppressive conditions. Although most ATLL patients lack a suitable HLA-matched related donor and require an HTLV-I–negative unrelated donor, little information is currently available regarding the outcome of unrelated bone marrow transplantation (UBMT) for ATLL. To evaluate the role of UBMT in treating ATLL, we retrospectively analyzed data from 33 patients with ATLL treated by UBMT through the Japan Marrow Donor Program (JMDP). Overall survival (OS), progression-free survival, and cumulative incidence of disease progression and progression-free mortality at 1 year after UBMT were 49.5%, 49.2%, 18.6%, and 32.3%, respectively. Multivariate analysis identified recipient age as an independent prognostic factor for OS (P = .044). Patients age ≥50 years who showed nonremission at transplantation tended to have higher rates of treatment-related mortality. Our observations suggest that UBMT could represent a feasible treatment option for ATLL patients and warrant further investigation based on these risk factors.

KEY WORDS

Adult T-cell leukemia/lymphoma • Allogeneic hematopoietic stem cell transplantation • Unrelated donor • Graft-versus-adult T-cell leukemia/lymphoma

INTRODUCTION

Adult T-cell leukemia/lymphoma (ATLL) is a peripheral T-cell neoplasm caused by human T-cell leukemia virus type I (HTLV-I) [1,2]. ATLL is generally classified into 4 clinical subtypes based on clinical and laboratory features: acute, chronic, smoldering, and lymphoma type. Clinically, acute- and lymphoma-type ATLL show an aggressive course, with tumor

burden, severe hypercalcemia, multiorgan failure, and poor performance status. ATLL has an extremely poor prognosis, with a median survival of about 6 months for the acute type and about 10 months for the lymphoma type; these patients are usually highly immunocompromised and develop various opportunistic infections. [3] Furthermore, their tumor cells are usually resistant to conventional chemotherapies, because overexpression of multidrug-resistance genes leads to intrinsic drug resistance. [4,5] Intensified chemotherapy [6,7] and autologous stem cell transplantation [8] likewise have failed to improve the prognosis. Thus, alternative treatment strategies for ATLL are needed.

Some cases of successful treatment with allogeneic stem cell transplantation (allo-HSCT) from an HLA-matched related donor have been reported, and a graft-versus-ATLL (GvATLL) effect has been implicated for improving treatments outcomes in transplant patients undergoing transplantation for ATLL. [9–11] However, more than 2/3 of patients with ATLL lack HLA-matched related donors. Furthermore, approximately 2/3 of the siblings of patients with ATLL are HTLV-I carriers [12], and allo-HSCT from an HTLV-I–positive donor may carry a risk of promoting the development of ATLL through the addition of a new HTLV-I load on the immunocompromised host. [13,14] Although most ATLL patients lack a suitable HLA-matched related donor and require an unrelated donor to benefit from allo-HSCT, few reports are available concerning the results of unrelated donor bone marrow transplantation (UBMT) for ATLL [9,11,15–18], and the number of patients in these few reports has been too small on which to base any solid conclusions. Therefore, to clarify the feasibility and efficacy of UBMT from an HTLV-I-negative donor for ATLL, we retrospectively analyzed registered data and clinical outcomes of UBMT for ATLL through the Japan Marrow Donor Program (JMDP).

PATIENTS AND METHODS

Patients and Transplantation Procedure

The subjects of this retrospective study consisted of 33 patients with ATLL (acute type, n = 20; lymphoma type, n = 7; not described, n = 6) who received UBMT from a donor mediated and recruited through the JMDP between September 1999 and January 2004. The clinical indications for UBMT were determined by each individual institution. The median time from diagnosis of ATLL to UBMT was 8 months (range, 5–28 months). At the time of transplantation, 13 patients were in complete remission (CR), 2 patients were in partial remission (PR), and 14 patients were in nonremission (NR); disease status at the time of transplantation was not described in 4 patients. CR status was reported in detail for 13 patients, with 11 patients in first CR (CR1) and 2 patients in second CR (CR2) (Table 1). All unrelated donors were HTLV-I antibody–negative. Serologic typing for HLA-A, -B, and -DR was performed using a standard 2-stage complement-dependent test of microcytotoxicity. [19] Alleles at the HLA-A, -B, and -DRB1 loci were identified by high-resolution DNA typing as described previously. [20] Serologic typing revealed that 22 patients were matched at the HLA-A, -B, and -DR loci. Four patients were mismatched at 1 HLA-DR locus, and 1 patient was mismatched at 2 loci of HLA-A and -DR. DNA typing revealed that 13 patients were matched at HLA-A, -B and -DRB1 loci. Ten patients were mismatched at 1 locus; 9 patients were mismatched at the HLA-DRB1 locus, and the remaining patient was mismatched at 1 HLA-A locus. Another 4 patients were mismatched at 2 loci. HLA typing data were not described in 6 patients. Patient and donor characteristics are summarized in Table 2.

Table 1. *Patient characteristics*

Characteristic	Value
Median age at transplantation, years (range)	49 (range, 24–59)
Sex, n	
Male	18
Female	15
Performance status, n	
0–1	21
2–4	4
ND	8
Subtypes of ATLL, n	
Acute	20
Lymphoma	7
ND	6
Disease status at transplantation, n	
CR or PR	15
NR	14
ND	4
Duration from diagnosis to UBMT, n	
Within 1 year	21
Beyond 1 year	11
ND	1
Conditioning, n	(TBI-containing, 22; non-TBI-containing, 11)
CST	27
RIST	6
Cell dose, n	
$< 3.0 \times 10^8$/kg	16
$\geq 3.0 \times 10^8$/kg	14
ND	3
GVHD prophylaxis, n	
CsA + MTX	13
TCR + MTX	20

ND indicates not described; CR, complete remission; PR, partial remission; NR, nonremission; UBMT, unrelated bone marrow transplantation; TBI, total body irradiation; CST, conventional stem cell transplantation; RIST, reduced-intensity stem cell transplantation; GVHD, graft-versus-host disease; CsA, cyclosporine; MTX, methotrexate; TCR, tacrolimus.

Table 2. *Patient and donor characteristics*

Characteristic	Value
HLA-A, -B, and -DRB1 allele mismatches, n	
0	13
1	10
2	4
ND	6
Sex of donor/patient, n	
Male/male	13
Female/female	8
Female/male	5
Male/female	7
Extent of ABO match, n	
Match	19
Minor mismatch	4
Major mismatch	7
Major/minor	2
ND	1

ND indicates not described.

Transplantation was performed according to the protocol of each institution; therefore, conditioning regimens and prophylaxis against graft-versus-host disease (GVHD) differed among patients. Conditioning regimens were myeloablative in 27 patients; total body irradiation (TBI) was incorporated in 22 patients. Reduced-intensity conditioning regimens were used in 6 patients. GVHD prophylaxis included cyclosporine (n = 13) and tacrolimus (n = 20) combined with methotrexate. All recipients received bone marrow transplantation, which was not manipulated.

Assessment of Engraftment, GVHD, Survival, and Progression-Free Mortality

The day of sustained engraftment was defined as the first of 3 consecutive days with an absolute neutrophil count exceeding 0.5×10^9/L. Acute GVHD was diagnosed and graded according to the standard criteria described previously. [21,22] Chronic GVHD was evaluated according to standard criteria [23] in patients who survived more than 100 days after transplantation. Overall survival (OS) was defined as the duration (in days) from transplantation to death from any cause. Progression-free survival (PFS) was defined as days from transplantation to disease progression or death from any cause. Progression-free mortality was defined as death without disease progression.

Data Management and Statistical Considerations

Data were collected by the JMDP using a standardized report form. Follow-up reports were submitted at 100 days, 1 year, and every subsequent year after transplantation. The cumulative incidence of disease progression and progression-free mortality were evaluated using Gray's method, [24] considering each other risk as a competing risk. OS and PFS were estimated using the Kaplan-Meier method. Potential confounding factors considered in the analysis were age, sex, disease status, duration from diagnosis to transplantation, Eastern Cooperative Oncology Group (ECOG) performance status, [25] conditioning regimen, number of bone marrow cells transplanted, and presence of grade II–IV acute GVHD. Proportional hazard modeling was used to evaluate any influence of these factors on OS, treating development of acute GVHD as a time-dependent covariate. Factors associated with at least borderline significance ($P < .05$) in univariate analyses were subjected to multivariate analyses using backward-stepwise proportional hazards modeling. P values $P < .10$ were considered statistically significant.

RESULTS

Engraftment and GVHD

Transplantation outcomes are summarized in Table 3. The median number of cells transplanted was 2.44×10^8 nucleated cells/kg of recipient body weight (range, $0.58–3.58 \times 10^8$ nucleated cells/kg of recipient body weight). Five patients (15%) died within 20 days. Neutrophil engraftment was achieved in 28 patients. Late graft failure occurred in 1 of these 28 patients, although the patient showed engraftment on

Table 3. *Transplantation outcome*

	Value
Alive/dead, n	19/14
Median follow-up for survivors, days (range)	139 (87–600)
Cause of death	
Progression, n	2
Death without progression, n	9
Median days after transplantation (range)	32 (10–71)
Late graft failure, n	1
GVHD, n	1
Infection, n	3
TMA, n	2
VOD, n	1
Arrhythmia, n	1
Not described, n	3
Disease progression, n	5
Median days after transplantation (range)	122 (61–223)
Engraftment, n	
Engraftment	28
Death within 20 days	5
Late graft failure	1
Acute GVHD, n	
None	3
Grade I	8
Grade II	12
Grade III	3
Grade IV	2
Chronic GVHD, n	
None	14
Limited	1
Extensive	3

GVHD indicates graft-versus-host disease; TMA, thrombotic microangiopathy; VOD, venooculusive disease.

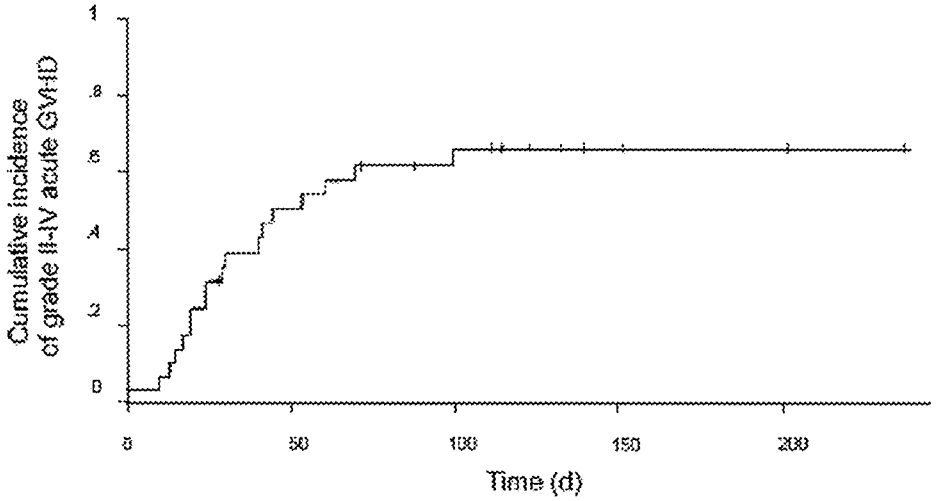

Figure 1. Cumulative incidence of grade II–IV acute GVHD in patients who achieved neutrophil engraftment.

day 14. Acute GVHD developed in 25 of the 28 patients who achieved engraftment (89%): grade I GVHD in 8 patients, grade II in 12 patients, grade III in 3 patients, and grade IV in 2 patients. The cumulative incidence of grade II–IV acute GVHD was 61% (Figure 1). Chronic GVHD developed in 4 of 18 patients, with limited disease in 1 patient and extensive disease in the other 3 patients.

Survival and disease progression

The 1-year OS and PFS were 49.5% (95% confidence interval [CI], 31.2%–78.5%) and 49.2% (95% CI, 33.6%–72.1%), respectively (Figure 2). Disease progression was observed in 5 patients, and the median number of days from transplantation to disease progression was 122 (range, 61–223 days). As of the last follow-up, 14 deaths had been reported. Primary cause of death was disease progression in 2 patients and was not described in 3 patients, but the other 9 deaths were not due to disease progression (see Table 3). Primary causes of transplantation-related death within 100 days after transplantation were late graft failure in 1 patient, GVHD in 1 patient, infection in 3 patients (with methicillin-resistant *Staphylococcus aureus*–positive sepsis in 1 patient and pulmonary infection in 2 patients), thrombotic microangiopathy (TMA) in 2 patients, veno-occlusive disease (VOD) in 1 patient, and arrhythmia in 1 patient.

Univariate and Multivariate Analyses for OS

Pretransplantation and posttransplant factors were calculated for OS (Table 4). In univariate analyses, OS was not significantly associated with sex, duration from diagnosis to transplantation, ECOG performance status, conditioning regimen, number of bone marrow cells transplanted, or presence of grade II–IV acute GVHD. On the other hand, patient age and disease status at transplantation were identified as significant independent risk factors. In multivariate analyses, only patient age at transplantation was identified as exerting a significant independent risk impact on OS (≥50 years vs <50 years; relative risk, 3.47; 95% CI, 1.03–11.6; $P = .044$). Disease status at transplantation exerted a marginally significant impact on OS (NR vs CR or PR; relative risk, 3.17; 95% CI, 0.96–10.5; $P = .059$) (Figure 3).

Influence of Pretransplantation Factors on Disease Progression and Progression-Free Mortality

The cumulative incidence of disease progression and progression-free mortality at 1 year were 18.6% and 32.3%, respectively (Figure 4). To clarify how age and disease status at transplantation affected OS, we evaluated the relationship between these factors and the incidence of progression-free mortality. The cumulative incidence of progression-free mortality was significantly higher in patients age ≥50 years at transplantation (50% vs 18%; $P = .048$; Figure 5A). NR at transplantation exerted a marginally significant effect on increased progression-free mortality (54% vs 20%; $P = .070$; Figure 5B).

DISCUSSION

This study analyzed the data and evaluated treatment outcomes for 33 patients with ATLL who received UBMT. Two important findings were identified regarding UBMT for ATLL. First, UBMT from HTLV-I–negative donors for ATLL represents a feasible treatment. Second, recipient age (≥50 years) and NR disease status at transplantation were independent risk factors for OS, and patients with ATLL displaying these risk factors tended to exhibit higher frequencies of treatment-related mortality.

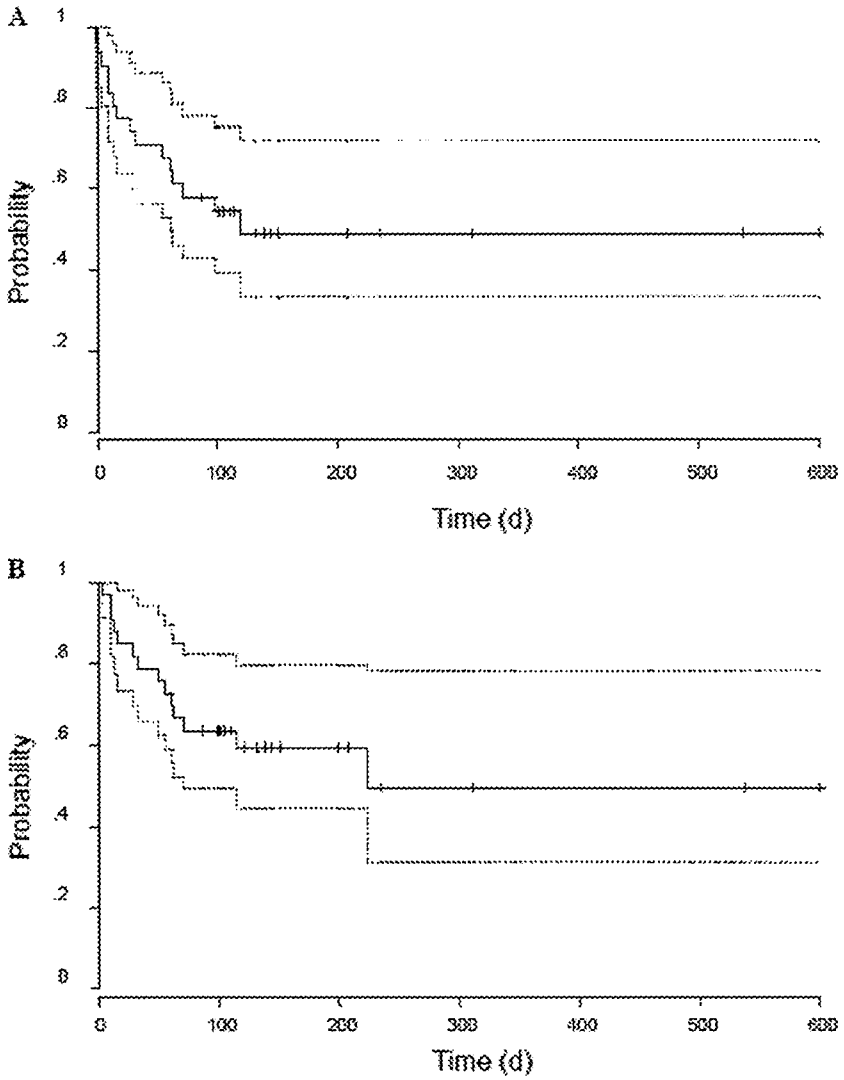

Figure 2. Probability of progression-free survival (A) and overall survival (B) after unrelated bone marrow transplantation for adult T-cell leukemia/lymphoma. Dashed lines represent 95% confidence intervals.

Table 4. *Prognosis factors in univariate and multivariate analyses*

	Univariate		Multivariate	
	Relative risk (95% CI)	*P*	Relative risk (95% CI)	*P*
Age ≥50 versus <50 years	4.03 (1.23–13.3)	.022	4.03 (1.23–13.3)	.022
Male versus female	0.97 (0.34–2.80)	.95		
PS 0–1 versus 2–4	0.44 (0.11–1.70)	.23		
NR versus CR or PR	3.37 (1.03–11.0)	.044		.059
UBMT within 1 year versus beyond 1 year	0.54 (0.15–2.00)	.35		
RIST versus CST	0.71 (0.19–2.59)	.60		
TBI versus non-TBI	1.35 (0.45–4.04)	.59		
Cell dose < 3.0×10^8/kg versus ≥ 3.0×10^8/kg	0.98 (0.31–3.05)	.97		
GVHD II–IV present versus absent	1.91 (0.50–7.26)	.34		

CI indicates confidence interval; PS, performance status; NR, nonremission; CR, complete remission; PR, partial remission; UBMT, unrelated bone marrow transplantation; RIST, reduced-intensity stem cell transplantation; CST, conventional stem cell transplantation; TBI, total body irradiation; GVHD, graft-versus-host disease.