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Appendix 1

The following centres participated in the bone marrow transplantations facilitated by the JMDP: Asahikawa Medical College Hospital, Asahikawa Red Cross Hospital, Sapporo Medical University Hospital, Sapporo Hokuyu Hospital, Hokkaido University Hospital, Asahikawa City Hospital, Hakodate City Hospital, Hirosaki University Hospital, Aomori Prefectural Central Hospital, Akita University Hospital, Iwate Medical University Hospital, Miyagi Cancer Centre, Tohoku University Hospital, Yamagata University Hospital, Fukushima Medical University Hospital, Ibaraki Children's Hospital, Tsukuba University Hospital, Tsuchiura Kyodo General Hospital, Jichi Medical School Hospital, Dokkyo Medical University Hospital, Saiseikai Maebashi Hospital, Gunma University Hospital, Saitama Medical University Hospital, Saitama Cancer Centre Hospital, Saitama Children's Medical Centre, Fukaya Red Cross Hospital, National Defense Medical College Hospital, Kameda General Hospital, Matsudo Municipal Hospital, Chiba Children's Hospital, Chiba Aoba Municipal Hospital, Chiba University Hospital, Jikei University Kashiwa Hospital, Keio University Hospital, Toranomon Hospital, National Cancer Centre Central Hospital, International Medical Centre of Japan, National Centre for Child Health and Development, Juntendo University Hospital, Showa University Hospital, Teikyo University Hospital, Tokyo Medical and Dental University Hospital, Tokyo Medical College Hospital, Jikei University Hospital, Tokyo Women's Medical University Hospital, Research Hospital of the Institute of Medical Science-the University of Tokyo, The University of Tokyo Hospital, Tokyo Metropolitan Komagome Hospital, Tokyo Metropolitan Kiyose Children's Hospital, Tokyo Metropolitan Hospital of Fuchu, Toho University Omori Medical Centre, National Hospital Organisation Tokyo Medical Centre, Nippon Medical School Hospital, Japanese Red Cross Medical Centre, Nihon University Itabashi Hospital, Yokohama City University Medical Centre, Yokohama City University Hospital, Kanagawa Cancer Centre, Kanagawa Children's Medical Centre, St. Marianna University School of Medicine Hospital, Tokai University Hospital, Niigata University Medical & Dental Hospital, Nagaoka Red Cross Hospital, Niigata Cancer Centre Hospital, University of Yamanashi Hospital, Saku Central Hospital, Shinshu University Hospital, Nagano Children's Hospital, Nagano Red Cross Hospital, Toyama Prefectural Central Hospital, Kanazawa Medical University Hospital, Kanazawa University Hospital, Ishikawa Prefectual Central Hospital, University of Fukui Hospital, Hamamatsu Medical Centre, Seirei Hamamatsu General Hospital, Shizuoka Children's Hospital, Shizuoka General Hospital, Shizuoka Red Cross Hospital, Hamamatsu University School of Medicine Hospital, Aichi Medical School Hospital, Aichi Cancer Centre Hospital, Anjo Kousei Hospital, Showa Hospital, National Hospital Organisation Nagoya Medical Centre, Fujita Health University Hospital, Nagoya City University Hospital, Nagoya University Hospital, Japanese Red Cross Nagoya First Hospital, Nagoya Daini Red Cross Hospital, Nagoya Ekisaikai Hospital, Meitetsu Hospital, Mie University Hospital, Yamada Red Cross Hospital, Suzuka Kaisei Hospital, Suzuka General Hospital, Shiga University of Medical Science Hospital, Kyoto Katsura Hospital, Kyoto City Hospital, Kyoto University Hospital, Kyoto First Red Cross Hospital, Kyoto Prefectural University of Medicine Hospital, Social Insurance Kyoto Hospital, Rinku General Medical Centre, Kansai Medical University Hospital, Kinki University Hospital, Matsushita Memorial Hospital, Osaka Medical College Hospital, Osaka City University Hospital, Osaka Red Cross Hospital, Osaka University Hospital, Osaka Medical Centre for Cancer and Cardiovascular Diseases, Osaka Medical Centre and Research Institute for Maternal and Child Health, Kobe City General Hospital, Kobe University Hospital, Hyogo College of Medicine Hospital, Hyogo Children's Hospital, Hyogo Medical Centre for Adults, Tenri Hospital, Nara Medical University Hospital, Wakayama Medical University Hospital, Tottori Prefectural Central Hospital, Tottori University Hospital, Shimane Prefectural Central Hospital, Okayama University Hospital, National Hospital Organisation Okayama Medical Centre, Hiroshima Red Cross Hospital and Atomic-Bomb Survivors Hospital, Hiroshima University Hospital, National Hospital Organisation Kure Medical Centre, Kurashiki Central Hospital, Yamaguchi University Hospital, Tokushima University Hospital, Kagawa University Hospital, Ehime Prefectural Central Hospital, Ehime University Hospital, Matsuyama Red Cross Hospital, Kochi Medical School Hospital, Kurume University Hospital, Kyushu University Hospital, Harasanshin General Hospital, Hamanomachi General Hospital, National Kyushu Cancer Centre, University of Occupational and Environmental Health Hospital, Kokura Memorial Hospital, St Mary's Hospital, Saga Prefectural Hospital, Nagasaki University Hospital, National Hospital Organisation Kumamoto Medical Centre, Oita Prefectural Hospital, Oita University Hospital, Miyazaki Prefectural Hospital, Imamura Hospital, and Kagoshima University Hospital.

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ORIGINAL ARTICLE

High-dose chemotherapy and autologous peripheral blood stem cell transfusion for adult and adolescent patients with small round cell sarcomas

K Yamada[1], M Takahashi[1,3], M Ogura[2,4], Y Kagami[2,5], H Taji[2], Y Kamiya[2], H Sugiura[1] and Y Morishima[2]

[1]*Department of Orthopaedics, Aichi Cancer Center Hospital, Nagoya, Japan and* [2]*Department of Hematology and Cell Therapy, Aichi Cancer Center Hospital, Nagoya, Japan*

The treatment of small-round-cell tumors (SRCT) in adult patients remains a challenge to clinicians. In the present study, we analyzed the feasibility and efficacy of high-dose chemotherapy (HDCT) followed by autologous peripheral blood stem-cell rescue as a consolidation therapy exclusively for patients with good disease control through a single regimen of induction chemotherapy and local therapy. Twenty-one patients (12 females, median age 22.0 years) were analyzed, including seven cases with rhabdomyosarcoma (RMS) and 14 cases with Ewing's family tumors (EFT). Overall, survival was 46% and failure-free survival (FFS) was 33% at 3 years. Patients with EFT had better FFS than those with RMS, with an estimated 3-year FFS of 50% ($P<0.01$). There was a single case of possible treatment-related death and two cases of secondary malignancies. This study cannot conclusively determine the beneficial effects of HDCT for improving treatment outcomes in adult SRCTs due to the small number of subjects. However, study findings suggest that a subgroup of patients with EFT may obtain prolonged survival benefits from this therapy.

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Keywords: sarcoma; high-dose chemotherapy; PBSCT; adult; adolescent

Introduction

Rhabdomyosarcoma (RMS) and Ewing's sarcoma family of tumors (EFTs), including Ewing's sarcoma of bone, extra-skeletal Ewing's sarcoma and peripheral primitive neuro-ectodermal tumor, belong to the group of neoplasms commonly referred to as 'small round-cell sarcomas.' These tumors share a common morphologic histology and a similar treatment strategy consisting of multi-drug chemotherapy in combination with surgery and/or radiotherapy.[1,2]

A series of recent studies have reported improvements in outcomes of patients with the use of risk-adapted, intensive multimodal therapy, although the group of patients at high risk for treatment failure still remains.[3–5] Given that both diseases are considered to be chemotherapy-sensitive, dose intensification of chemotherapy is presumed to be a key factor in improving outcomes for patients with high risk factors. In particular, high-dose chemotherapy (HDCT) with stem cell rescue has been used in an attempt to improve clinical outcomes in high-risk patients. However, the impact of HDCT with stem cell rescue on the treatment of high-risk RMS or EFTs has yet to be established, as previous data have demonstrated inconsistent benefits.[6–12]

It is of particular importance to identify subsets of high-risk patients for the purpose of risk stratification. Various studies have identified prognostic factors for patients with RMS or EFTs, including older age, presence of metastatic disease, tumor volume >100 ml or axial site involvement.[13–16] In particular, patient age is one of the strongest predictors of outcome both in EFTs and RMS.[17,18] However, the management of adult patients with EFTs or RMS has been developed mainly from experiences with pediatric patients because of the rarity of the disease in adults.[19,20] Poor outcomes in adult patients[21] may be attributed to the fact that an adult may not respond well to treatments designed for children.

On the basis of these considerations, the purpose of this clinical study was to investigate the effects of HDCT on overall survival (OS) and time to disease progression in adults and adolescent patients with EFTs and RMS. In this report, we present the results from a prospective pilot study of a treatment protocol consisting of induction chemotherapy, local treatment, followed by HDCT as a consolidation therapy.

Correspondence: Dr K Yamada, Department of Orthopaedics, Aichi Cancer Center Hospital, 1-1 Kanokoden, Chikusa, Nagoya 464-8681, Japan.
E-mail: kyamada@aichi-cc.jp
[3]Current address: Division of Orthopaedic Oncology, Shizuoka Cancer Center Hospital, Naga-izumi, Shizuoka, Japan.
[4]Current address: Department of Hematology, Nagoya Daini Red Cross Hospital, Nagoya, Japan.
[5]Current address: Department of Hematology, Meitetsu Hospital, Nagoya, Japan.
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Patients and methods

Eligibility criteria

Patients between 15 and 55 years of age with primary and high-risk RMS or EFTs, as previously defined, were considered eligible for the present study. The pathologic diagnosis was confirmed by conventional histopathological and immunohistochemical examinations. Molecular genetic studies were not routinely conducted to investigate specific chromosomal translocations. Routine radiological examinations before study enrollment included computerized tomography (CT) and/or magnetic resonance imaging (MRI) of the primary site, plain radiographs and CT scans of the chest and a 99 technetium-diphosphonate bone scan. A performance status of <2, along with adequate organ function, was required.

Adequate organ function was defined as an absolute neutrophil blood cell count $>1.2 \times 10^9$/l, a platelet count $>100 \times 10^9$/l, a bilirubin level <2.0 mg/dl, a serum creatinine level <2.0 mg/dl, creatinine clearance >60 ml/min and cardiac ejection fraction rate $>50\%$.

Signed informed consent for the treatment was obtained from all patients or their legal guardian in accordance with our institutional review board guidelines.

Treatment protocol

The study treatment protocol included induction chemotherapy, local treatment (surgery and/or radiotherapy), followed by additional induction chemotherapy and, finally, HDCT with autologous peripheral blood stem-cell (APBSC) rescue. Induction chemotherapy consisted of a combination of four chemotherapeutic agents administered every 3 weeks, in modification of the CESS-86 regimen[22] (Table 1).

APBSCs were mobilized with the use of granulocyte colony-stimulating factor (G-CSF) after cycles 3 through 6 of the induction chemotherapy. Before stem cell harvesting, bone marrow aspirates and biopsies were performed to exclude bone marrow involvement of the tumor cells or abnormal myelopoietic functions. Harvesting was performed only when histological or cytological analyses indicated no evidence of tumor cells. A CD34+ cell count >10 CD34+ cells per μl was used as the harvest criterion. Total cell counts of CD34+ cells $>2.0 \times 10^6$/kg were required to proceed to further HDCT.

Imaging of the primary tumor by MRI and/or CT scanning was conducted after cycles 2 and 4 to aid decisions regarding local therapy, and once again before HDCT. Definitions of radiological antitumor effect were as follows: complete response (CR), no detectable tumors; partial response (PR), $>50\%$ reduction in measurable tumors; stable disease (SD), $<50\%$ reduction in measurable tumors and no new lesions; and progressive disease (PD), tumor growth or new lesions.

Local therapy was scheduled to be given after four courses of the induction chemotherapy, except when the primary tumor had been excised before the first visit to our hospital, or when the tumor was considered unresectable. Local therapy was planned individually, taking into consideration various factors such as tumor site, tumor size, tumor resectability, patients' performance status and expected disabilities after resection. Complete surgical resection was the theoretical goal, whenever feasible. Radiotherapy was prescribed in cases where the tumor was either unresectable or incompletely resectable, or where the surgical margin in the excised specimen proved to be inadequate.

Given that the aim of this study was to investigate the effect of HDCT as a consolidation therapy, patients were scheduled to be re-assessed after the induction chemotherapy with regard to the chemotherapeutic effect. If the patients were in CR or PR after four courses of induction chemotherapy, and had undergone definitive local therapy, they were considered to have minimal tumor burden and eligible for HDCT. If the patients were in SD, tumor excision without macroscopic residue was mandatory. If the patients were in PD, they were excluded from the study.

During the course of this study, two myeloablative regimens were used (Table 2). APBSCs were re-infused on day 0, followed by daily administration of G-CSF for enhancement of myeloid reconstitution until the absolute neutrophil count remained above 1000/μl for 3 consecutive days.

Table 1 Induction regimen (VAIA)

	Day	*Daily dosage (mg/m²)*	*Total dosage (mg/m²)*
Vincristine	1	1.0	1.0
Actinomycin-D	1–3	0.4	1.2
Doxorubicin (adriamycin)	1–3	20	60
Ifosfamide	1–5	2000	10 000

Table 2 HDCT conditioning regimen

A: CEC		*mg/m²*	*B: MEC*		*mg/m²*
Day −5 to −3	Cyclophosphamide	2000	Day −4 to −1	Melphalan	130
	Etoposide	400		Etoposide	500
	Carboplatin	300		Carboplatin	500
	Dexamethazon	40			
	APBSC rescue			*APBSC rescue*	
Day 0	$>2.0 \times 10^6$ CD34+ cells/kg		Day 0	$>2.0 \times 10^6$ CD34+ cells/kg	

Abbreviations: APBSC = autologous peripheral blood stem cell; CEC = cyclophosphamide, etoposide and carboplatin; MEC = melphalan, etoposide and carboplatin.

Toxicities
Toxicities were scored in accordance with the NCI Common Terminology Criteria for Adverse Events (CTCAE).

Statistical methods
OS rate was defined as the time interval from the date of HDCT to death. Failure-free survival (FFS) rate was defined as the time interval from the date of HDCT to disease progression, relapse, second malignancy or death. Survival analyses were conducted using the Kaplan–Meier method.[23] Comparisons of survival rates were performed using the log rank test.

Results

Patient characteristics
Twenty-five patients were enrolled into this study since 1995. Of the 25 enrolled patients (10 males and 15 females) with a median age of 22.0 years (15–35 years), eight patients (32%) had RMS, nine patients (36%) had EFT of the bone, and eight patients (32%) had EFT of the soft tissue. Twelve patients (48%) had metastatic lesions at the first presentation (Table 3).

Post-induction response
All patients received four courses of induction chemotherapy. Two patients received alternative induction chemotherapy before the prescribed regimen (VAIA) because of their huge tumor size (Case 4) and presence of disseminated intravascular coagulation at the first presentation (Case 24).

Twenty-four patients were assessable after induction chemotherapy, excluding one patient who had undergone radical surgery as an initial treatment. The overall response rate was 75% (18 of 24 patients), with 17% achieving CR (four out of 24), 63% achieving PR (15 out of 24), 13% achieving SD (two out of 24) and 8% resulting PD (two out of 24).

Four out of the 25 enrolled patients (16%) did not receive HDCT because two of them declined after finishing induction chemotherapy and the other two had disease progression during the induction chemotherapy. The remaining 21 patients were considered eligible for the HDCT.

The average CD34+ cell count at PBSCT was 4.21×10^6 CD34+ cells/kg body weight (range:1.7–14.9). The average number of harvesting performed was 1.8 (range 1–3). Stem cell harvesting in patients who had bone and/or bone marrow metastases at the first presentation was performed after cycles 5 or 6 to minimize tumor cell contamination, although there were no patients whose bone marrow aspirates or biopsies indicated tumor cell involvement.

Outcome
The median follow-up was 41.0 months (range 10–105 months) for the 21 patients who received HDCT. The final outcome of these patients included 13 DOD (died of disease), seven CDF (continuous disease free) and one NED (no evidence of disease). There were no AWD (alive with disease) cases at the time of this study.

Table 3 Disease characteristics and outcome of the enrolled patients

Patients' number	*Age at diagnosis (years)*	*Gender*	*Histology*	*Primary tumor site*	*Initial metastatic disease*	*HDCT regimen*	*Status*
1	25	Female	RMS (alveolar)	Buttock	Lung	CEC	DOD
2	16	Male	EFT (bone)	Ilium	None	CEC	DOD
3	15	Female	EFT (bone)	Femur	None	CEC	CDF
4	23	Female	EFT (soft tissue)	Pelvic cavity	None	CEC	DOD
5	20	Male	EFT (soft tissue)	Retroperitneum	Bone	CEC	DOD
6	15	Female	RMS (embryonal)	Shoulder girdle	Bone, BM	CEC	DOD
7	20	Female	RMS (alveolar)	Forearm	BM	CEC	DOD
8	17	Female	RMS (alveolar)	Perineum	None	MEC	DOD
9	27	Female	EFT (bone)	Pubis	None	MEC	CDF
10	29	Female	EFT (soft tissue)	Iliopsoas muscle	None	N/A (PD)	DOD
11	17	Male	EFT (bone)	Femur	None	MEC	CDF
12	35	Male	EFT (bone)	Lumber vertebra	None	MEC	DOD
13	21	Male	RMS (pleomorphic)	Abdominal wall	Lung	MEC	NED
14	27	Female	EFT (bone)	Femur	None	MEC	CDF
15	15	Male	EFT (soft tissue)	Abdomem	Liver	MEC	CDF
16	27	Female	RMS (pleomorphic)	Lower leg	None	N/A (refused)	CDF
17	20	Male	EFT (soft tissue)	Pleura	Lung	MEC	DOD
18	22	Female	EFT (bone)	Femur	None	MEC	CDF
19	27	Female	EFT (soft tissue)	Retroperitoneum	None	N/A (PD)	DOD
20	22	Male	EFT (soft tissue)	Inguinal	None	N/A (refused)	CDF
21	22	Male	EFT (bone)	Rib	Pleura	MEC	DOD
22	25	Female	EFT (soft tissue)	Uterus	Peritoneum	MEC	DOD
23	32	Female	RMS (embryonal)	Foot sole	Bone, BM	MEC	DOD
24	29	Female	EFT (bone)	Pelvis	Bone, BM	MEC	CDF
25	18	Male	RMS (alveolar)	Perianal	LN, muscle, bone	MEC	DOD

Abbreviations: CDF = continuous disease free; DOD = died of disease; EFT = Ewing's family of tumors; LN = lymph node; N/A = not available; NED = no evidence of disease; PD = progressive disease; RMS = rhabdomyosarcoma.

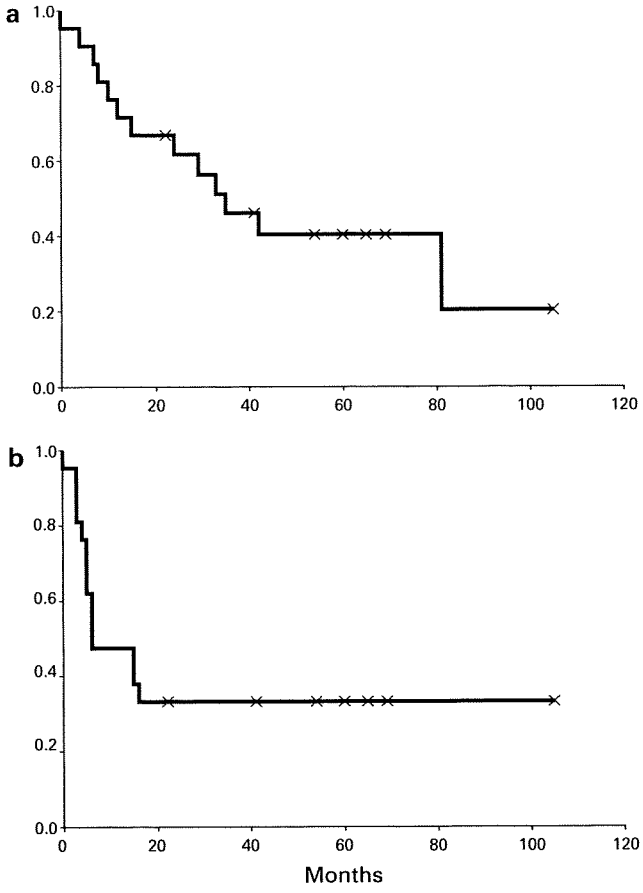

Figure 1 Kaplan–Meier plot of (**a**) overall and (**b**) FFS of 21 patients who underwent HDCT from the time of APBSC reinfusion. Tick marks indicate surviving patients.

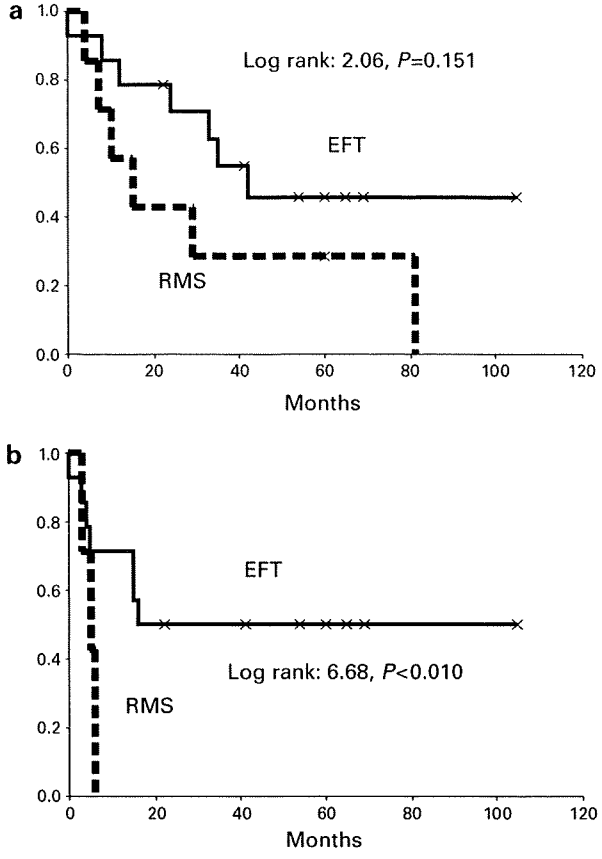

Figure 2 Kaplan–Meier plot of (**a**) overall and (**b**) FFS of EFT ($n = 14$) and RMS ($n = 7$) patients who underwent HDCT from the time of APBSC reinfusion. Tick marks indicate surviving patients.

The median OS and FFS from the date of PBSC reinfusion was 41.2 months (range 0–105) and 6.0 months (range 0–105), respectively. Figure 1 shows the Kaplan–Meier survival curves for OS (Figure 1a) and FFS (Figure 1b). The survival curves indicate that none of the patients relapsed beyond 16 months after HDCT.

Figure 2 shows the Kaplan–Meier survival curves for OS (Figure 2a) and FFS (Figure 2b) in patients with EFT and RMS. FFS for patients with EFT was significantly better compared with those with RMS. However, OS was not significantly different between the two groups of patients. Figure 3a and b show that survival estimates in patients with (M1) and without (M0) metastatic disease at the time of enrollment was significantly different with regard to FFS, but not for OS. Survival estimates comparing HDCT regimens and local treatment modalities were also performed, although there were no significant differences in either OS or FFS.

Toxicity

All patients who received HDCT experienced CTCAE grade 4 leukopenia, neutropenia and thrombocytopenia, which subsequently improved with stem cell re-infusion. One patient (Case 2) presented rapidly progressing left ventricular systolic dysfunction and pneumonitis on day 7, which resulted in a fatal cardiac arrest on day 8. As the patient's family did not consent to an autopsy, the definitive cause of the sudden cardiac arrest remains unknown. Nonetheless, it is suspected that cyclophosphamide (CPA)-induced or viral myocarditis was the possible cause.

Treatment-related second malignancy was observed in two patients (Cases 6 and 15). One case (Case 6: myelodysplastic syndrome (MDS)) occurred 34 months after the initial diagnosis of shoulder-girdle RMS with extensive bone/bone marrow metastases. The patient was treated with chemotherapy, followed by allograft bone marrow transplantation, which successfully resulted in complete remission of the MDS. The patient, however, died 51 months after the occurrence of the secondary MDS from graft-versus-host disease-induced pulmonary fibrosis. The second case (Case 15: AML) was diagnosed 23 months after the initial diagnosis of pleural EFT. This patient also received chemotherapy followed by allograft bone marrow transplantation. Given that AML recurred shortly after BM transplantation, the patient died 16 months after the occurrence of the secondary AML.

Discussion

To date, only a small number of studies have examined the clinical significance of HDCT exclusively in adult patients

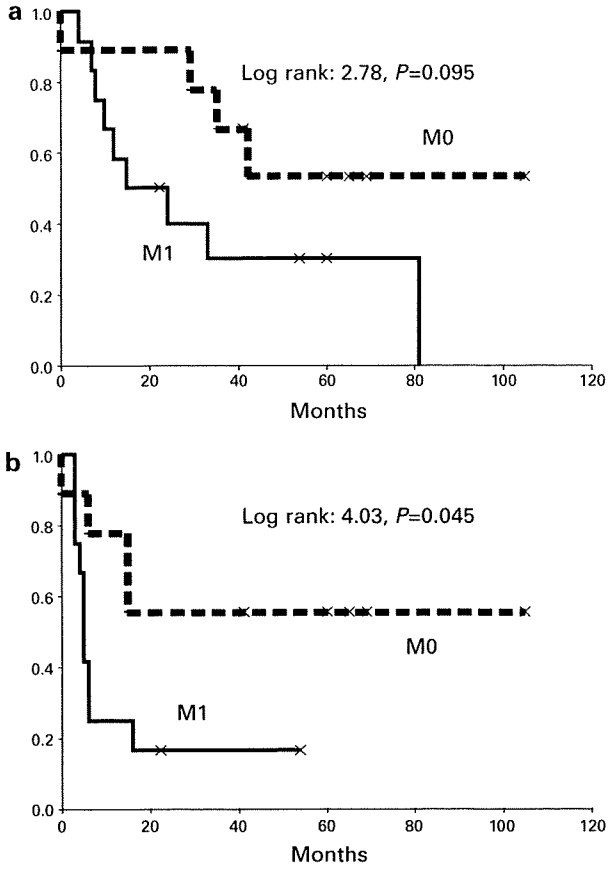

Figure 3 Kaplan–Meier plot of (**a**) overall and (**b**) FFS of patients with and without initial metastatic diseases who underwent HDCT from the time of APBSC reinfusion. Tick marks indicate surviving patients. M0: patients without metastatic diseases ($n=9$). M1: patients with metastatic diseases ($n=12$).

with RMS or EFT.[24,25] Older age is known to be a significantly negative prognostic factor for both diseases, as previous studies have revealed.[15–18] The present study was conducted at a single institution using a single-arm condition and, as such, we were unable to draw any conclusive results. However, this study demonstrated that a HDCT approach is feasible in subsets of adult patients with RMS or EFT. It should be noted that the median age (22.0 years) of the patients was considerably higher than in previously reported studies.

This study was designed to examine patients with either RMS or EFT, given that treatment strategies for high-risk subsets of both diseases are very similar. The results indicate that RMS patients had very poor outcomes, with no failure-free cases at observation, whereas EFT patients had favorable outcomes, with an FFS rate of 50%. Our findings are consistent with those of Bertuzzi *et al.*,[12] who also investigated the effects of HDCT in adults. Both study findings suggest that, compared with EFT patients, high-risk RMS patients should not be exposed to intensive treatment using HDCT and PBSC rescue.

Most studies using APBSC as hematopoietic rescue included a mobilization phase with a regimen distinct from the induction phase. In the present study, a sufficient number of APBSCs were harvested after induction chemotherapy with G-CSF mobilization. Two out of 20 patients received additional mobilization chemotherapy consisting of a single agent. Harvesting APBSCs in the induction phase resulted in fewer chemotherapeutic agents and shorter treatment duration, both of which benefit patients.

Treatment intensification involves considerable risks.[26–28] In the present study, one possible case of treatment-related fatal adverse effect and two cases of secondary malignancy (one MDS and one AML) were observed. Treatment-related deaths in three out of 21 patients (14%) strongly suggest that such intensive treatment must be restricted to patients with a high risk of mortality.

The role of HDCT supported by stem cell rescue as a consolidation therapy for high-risk EFT and RMS patients has yet to be determined conclusively. Numerous reports have described disappointing survival outcomes in patients with EFT and RMS regarding the efficacy of HDCT.[7–13] However, it is difficult to deny completely the role of HDCT in patients with primary high-risk or recurrent diseases and abandon the application of this therapy, especially, as there are only a few new drugs that have been proven effective for refractory conditions. It has also been noted repeatedly that small studies conducted within a single institution cannot provide any definitive conclusions as to whether HDCT should be retained as a component of therapy for patients with high-risk EFT and RMS. A multicenter, prospective, randomized trial must be conducted to answer these difficult questions, although it should be difficult to carry out such a study due to the rare nature of these diseases. Oberlin *et al.*[29] recently reported on a nation-wide and multicenter scale that consolidation HDCT is feasible and may provide benefits for some EFT patients with refractory conditions.

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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

Yasuo Morishima,[1] Toshio Yabe,[2] Keitaro Matsuo,[3] Koichi Kashiwase,[2] Hidetoshi Inoko,[4] Hiroh Saji,[5] Ken Yamamoto,[6] Etsuko Maruya,[5] Yoshiki Akatsuka,[7] Makoto Onizuka,[4] Hisashi Sakamaki,[8] Hiroshi Sao,[9] Seishi Ogawa,[10] Shunichi Kato,[11] Takeo Juji,[12] Takehiko Sasazuki,[13] Yoshihisa Kodera,[14] for the Japan Marrow Donor Program

[1]Aichi Cancer Center Hospital, Department of Hematology and Cell Therapy, Nagoya, Japan; [2]Japanese Red Cross Tokyo Metropolitan Blood Center, Tokyo, Japan; [3]Aichi Cancer Center Research Institute, Division of Epidemiology and Prevention, Nagoya, Japan; [4]Tokai University School of Medicine, Division of Molecular Science, Isehara, Japan; [5]HLA Laboratory, NPO, Kyoto, Japan; [6]Kyushu University, Medical Institute of Bioregulation, Department of Molecular Genetics, Fukuoka, Japan; [7]Aichi Cancer Center Research Institute, Division of Immunology, Nagoya, Japan; [8]Tokyo Metropolitan Komagome Hospital, Department of Hematology, Tokyo, Japan; [9]Meitetsu Hospital, Department of Hematology, Nagoya, Japan; [10]Tokyo University Hospital, Tokyo, Japan; [11]Tokai University School of Medicine, Department of Cell Transplantation and Regenerate Medicine, Isehara, Japan; [12]Japanese Red Cross Central Blood Institute, Tokyo, Japan; [13]Japan International Medical Center of Japan, Tokyo, Japan; [14]Japanese Red Cross Nagoya First Hospital, Nagoya, Japan

Correspondence and reprint requests: Yasuo Morishima, MD, Department of Hematology and Cell Therapy, Aichi Cancer Center Hospital, 1-1 Kanokoden, Chikusa-ku, Nagoya 464-8681, Japan (e-mail: ymorisim@aichi-cc.jp).

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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; $P = .604$).

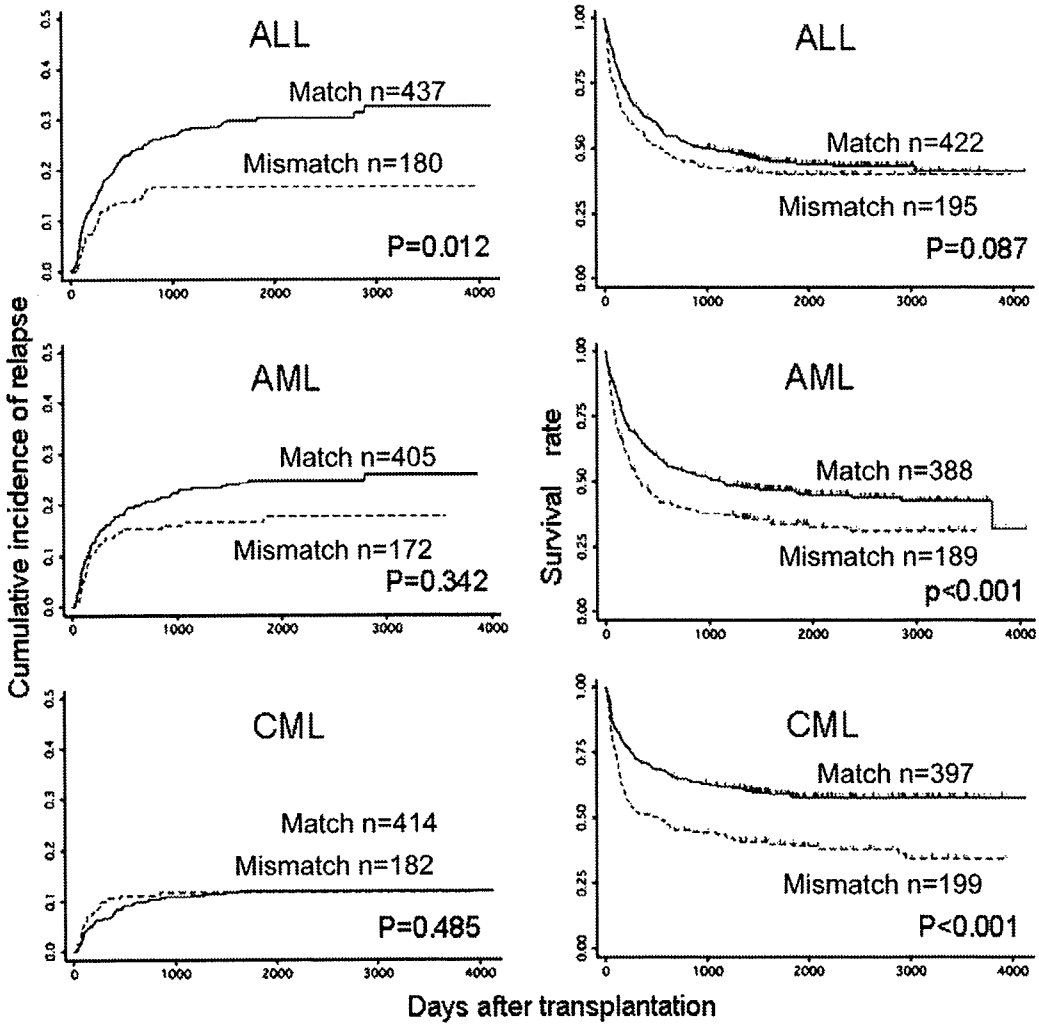

Figure 1. Cumulative incidence of relapse and survival by matching of HLA-C in patients with ALL, AML, and CML. All patients were analyzed. The direction of mismatching of HLA-C for relapse is GVH for relapse, and the direction for survival is GVH and/or HVG. The solid line represents match; the dotted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

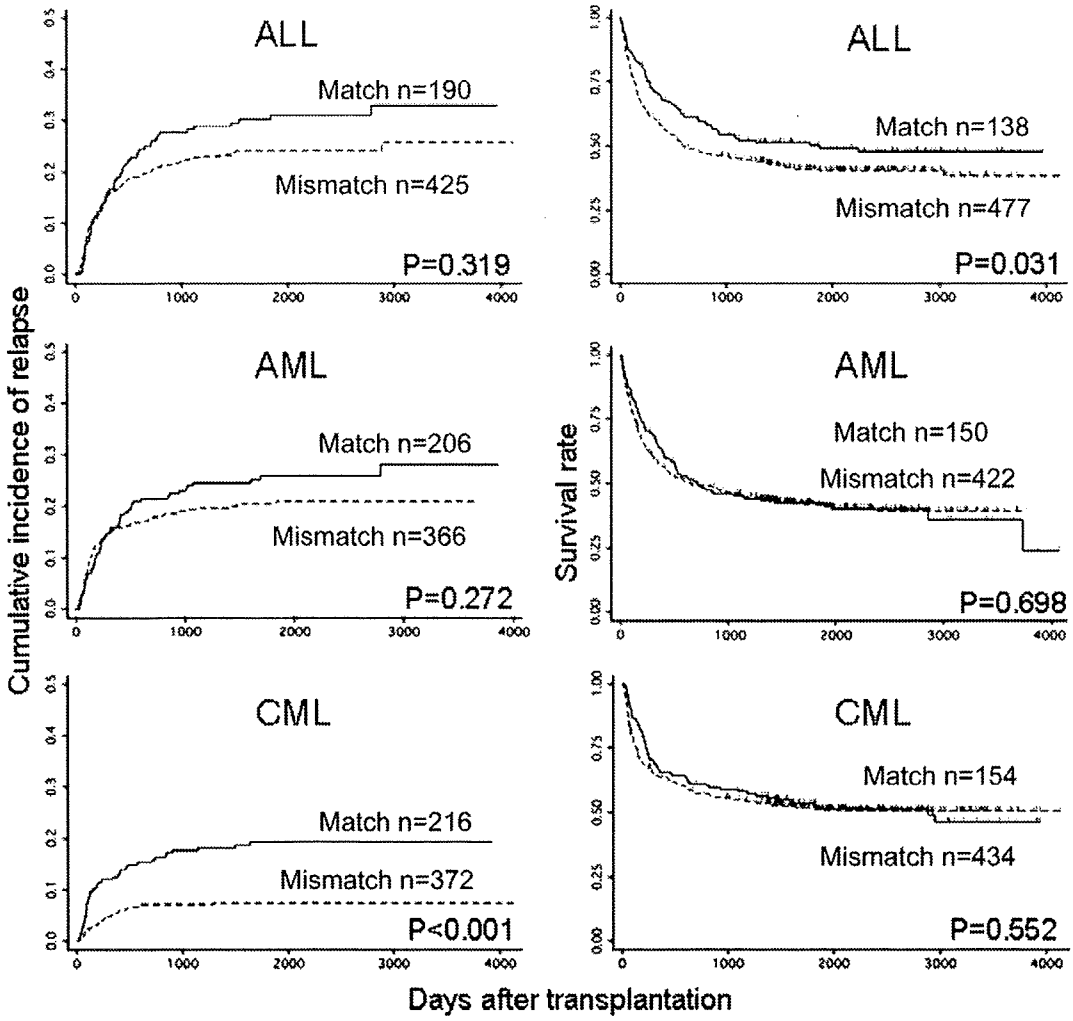

Figure 2. Cumulative incidence of relapse and survival by matching of HLA-DPB1 in patients with ALL, AML, and CML. All patients were analyzed. The direction of mismatching of HLA-DPB1 for relapse is GVH for relapse, and the direction for survival is GVH and/or HVG. Solid line, match; dotted line, mismatch.

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.

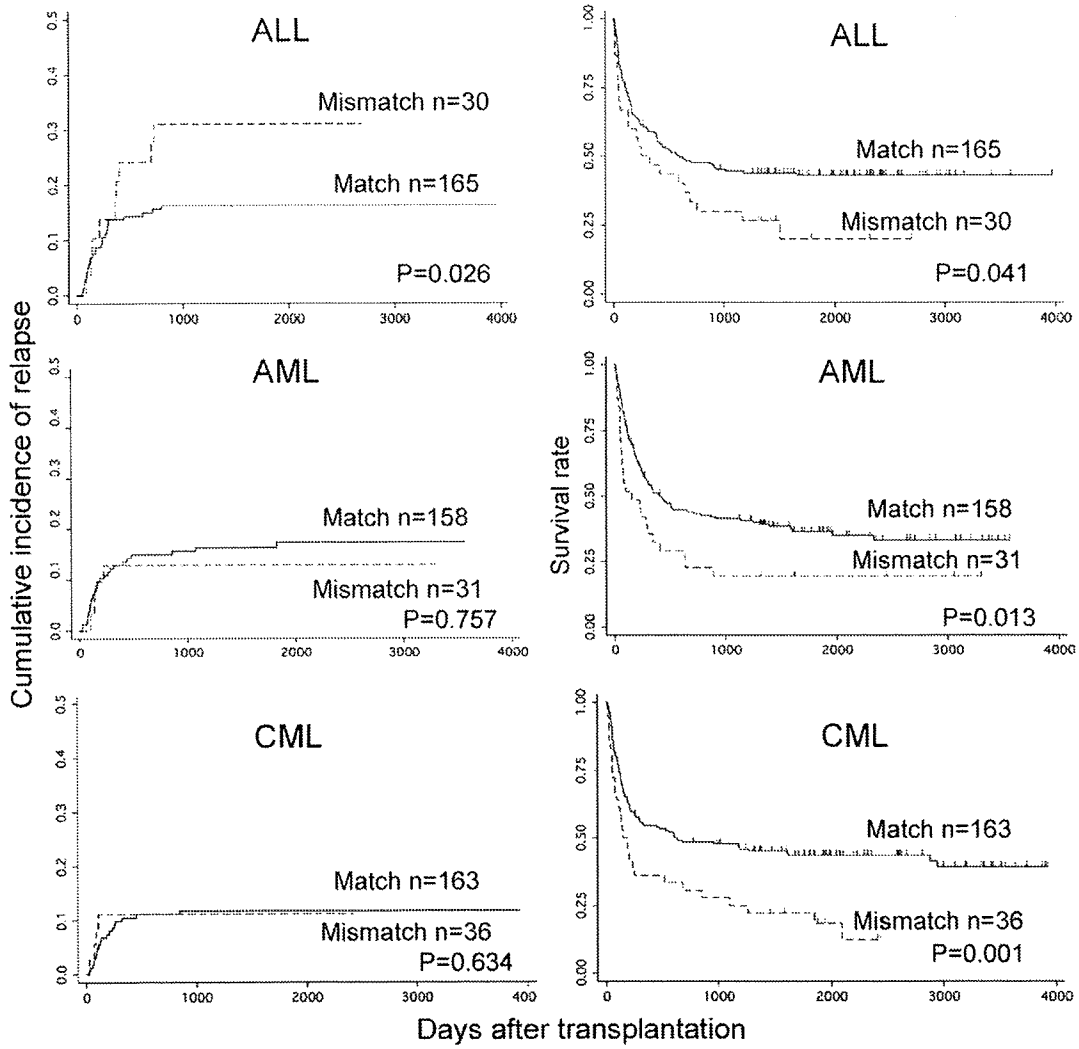

Figure 3. Cumulative incidence of relapse and survival by matching of KIR2DL ligand in the GVH direction in HLA-C–mismatched patients with ALL, AML, and CML. HLA-C–mismatched patients were selected for this analysis. The directions of HLA-C mismatching were GVH and/or HVG. The solid line represents KIR2DL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