Table 1
(*continued*)

Characteristics	Leukemia Cohort	Lymphoma Cohort	All Patients
CSA + MTX ± other (except FK506, MMF)	461 (16)	173 (12)	634 (15)
CSA ± other (except FK506, MTX, MMF)	147 (9)	108 (8)	355 (9)
Other GVHD prophylaxis	91 (3)	22 (2)	113 (3)
Missing	15 (1)	16 (1)	31 (1)
Subsequent transplant or DLI	594 (21)	497 (35)	1091 (26)
Prior autologous transplant	245 (9)	536 (37)	781 (18)
Median follow-up of survivors, mo (range)	72 (2-188)	72 (1-169)	72 (1-188)

AML indicates acute myeloid leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myeloid leukemia; Cy, cyclophosphamide; Flud, fludarabine; Bu, busulfan; Mel, melphalan; FK506, tacrolimus; MMF, mycophenolate mofetil; MTX, methotrexate; CSA, cyclosporine; DLI, donor lymphocyte infusion.

* Disease risk classification: Early indicates AML/ALL in first complete remission, CML in first chronic phase, MDS refractory anemia, MDS refractory anemia with ringed sideroblasts, unspecified MDS with <5% marrow blasts; intermediate, AML/ALL in second or greater complete remission, CML in second or greater chronic phase, CML in accelerated phase; advanced, AML/ALL in primary induction failure or relapse, CML in blast phase, MDS refractory anemia with excess blasts, MDS refractory anemia with excess blasts in transformation, unspecified MDS with ≥5% marrow blasts, chronic myelomonocytic leukemia.

did not develop a second malignancy. Cox proportional hazards regression analyses were used to evaluate risk factors for cancers after RIC/NMC [29]. Risk factors considered included age at HCT, gender, Karnofsky performance score at transplant, diagnosis/disease status, time from diagnosis to HCT, TBI dose, donor/graft source, history of prior autologous transplant, GVHD prophylaxis regimen, year of HCT, and occurrence of acute (grades II to IV) or chronic GVHD.

The risk of cancer in patients receiving RIC/NMC regimens was compared with that of the general population using methods described in previous CIBMTR studies [1,5,6]. Briefly, for each transplant recipient, the number of person-years at risk was calculated from the date of transplantation until date of last contact, death, or diagnosis of new cancer, whichever occurred first. Incidence rates for all invasive cancers in the general population were obtained from selected registries in the United States, England and Wales, Europe, and Asia [28,30]. Age-, gender-, race- (for United States), calendar year–, and region-specific incidence rates for all invasive solid cancers combined and for cancers of specific anatomical sites were applied to the appropriate person-years at risk to compute the expected numbers of cancers. Observed-to-expected ratios, also called standardized incidence ratios (SIRs), were calculated, and the exact Poisson distribution was used to calculate 95% confidence intervals (CIs) [31].

For our second objective, we compared the risks of developing second solid cancers in recipients of RIC/NMC and MAC regimens. We limited this analysis to recipients who were ages 40 to 60 years at the time of transplantation to compare cancer risks between the 2 conditioning regimens in a relatively homogenous subgroup of patients. Furthermore, this age range represented the largest group of RIC/NMC recipients. Cox proportional hazards regression analyses were performed to evaluate the association of conditioning regimen intensity with solid cancer risk [29]. In addition to the main effect of conditioning intensity, variables considered were the same as the ones included in the RIC/NMC risk factor analyses (see above). We also compared the risk of cancer in this subgroup of patients with that of the general population using the methods described above. Given the relatively shorter follow-up for RIC/NMC recipients compared with MAC recipients, this analysis was restricted to 10 years of follow-up.

All *P* values are 2-sided. All analyses were carried out using SAS statistical software (*SAS Institute Inc., Cary, NC*).

RESULTS

Patient and Transplant Characteristics of RIC/NMC Recipients

A total of 4269 RIC/NMC recipients were included in our analysis (2833 with leukemia/MDS, 1436 with lymphoma) and represented 11,620 person-years of follow-up (Table 1). The median age of the cohort at the time of AHCT was 53 years. Most patients had received their transplant in the United States. Only 27% of patients received a TBI-based conditioning regimen. An unrelated donor was used in 68% of cases. The median follow-up for the whole cohort was 72 months (range, 1 to 188). There were some notable but expected differences in the leukemia/MDS and lymphoma cohorts. A greater proportion of lymphoma patients had received a prior autologous transplant (37% versus 9%) and their time from diagnosis to AHCT was longer (median 33 months versus 9 months). The cumulative incidence of acute grades II to IV GVHD at 100 days was 34% (95% CI, 32% to 36%) in patients with leukemia/MDS and 33% (95% CI, 30% to 36%) in patients with lymphoma. The 2-year cumulative incidence of chronic GVHD was 43% (95% CI, 41% to 45%) and 49% (95% CI, 46% to 51%), respectively. The follow-up completeness index for the entire cohort was 94% at 5 years and 83% at 10 years after transplantation.

Cumulative Incidence and Risk Factors for Second Solid Cancers in RIC/NMC Recipients

The cumulative incidence of second solid cancers for the entire cohort was .54 (95% CI, .34 to .79) at 1 year, 1.69 (95% CI, 1.32 to 2.12) at 5 years, and 3.35 (95% CI, 2.46 to 4.38) at 10 years after transplantation (Figure 1). Among patients with leukemia/MDS, the cumulative incidence probabilities at the 3 time points were .57 (95% CI, .32 to .88), 1.71 (95% CI, 1.26 to 2.24), and 3.61 (95% CI, 2.40 to 5.06), respectively. Among lymphoma patients, 1-, 5-, and 10-year cumulative incidences of second solid cancers were .49 (95% CI, .20 to .92), 1.65 (95% CI, 1.04 to 2.41), and 2.98 (95% CI, 1.78 to 4.47), respectively. In Cox regression analyses, age at AHCT was the only risk factor independently associated with solid cancers (hazard ratio [HR] 3.10 [95% CI, 1.89 to 5.07] for age >50 years versus ≤50 years, $P < .001$).

Cancer Risks of RIC/NMC Recipients Compared with the General Population

Table 2 depicts the SIRs (or observed/expected ratios) for second solid cancers in RIC/NMC recipients compared with age- and gender-matched general population control subjects. We observed no increase in the overall risk of second solid cancers in patients receiving RIC/NMC AHCT for leukemia/MDS (SIR .99, $P = 1.00$) or lymphoma (SIR .92, $P = .75$).

Increased risks compared with the general population were seen for some specific solid cancer sites. Among leukemia/MDS patients, these included cancers of the lip (SIR 14.28, $P = .02$), tonsil (SIR 8.66, $P = .05$), oropharynx (SIR 46.70, $P < .01$), bone (SIR 23.53, $P < .01$), soft tissue (SIR 12.92, $P < .01$), and vulva (SIR 18.55, $P = .01$). A reduced risk was noted for breast cancer (SIR .25, $P = .03$). Among patients who had received a transplant for lymphoma, increased risks were observed for cancers of the oropharynx (SIR 67.35, $P < .01$). The risks for melanoma of the skin were higher in control subjects than for patients with both leukemia (SIR 3.04, $P = .02$) and lymphoma (SIR 3.52, $P = .03$).

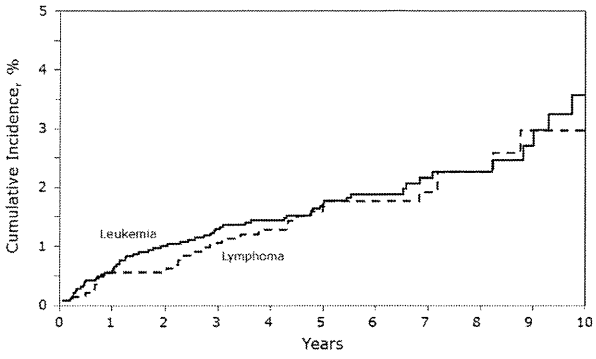

Figure 1. Cumulative incidence of second solid cancers (excluding nonmelanoma skin cancers) among patients receiving RIC/NMC regimens for leukemia/MDS and lymphoma.

Comparison of Second Solid Cancer Risks between RIC/NMC and MAC Regimens

Table 3 shows the characteristics of patients ages 40 to 60 years at AHCT who were included in the analysis comparing cancer risks between RIC/NMC and MAC recipients (leukemia/MDS: RIC/NMC = 1355 and MAC = 5728 patients; lymphoma: RIC/NMC = 783 and MAC = 700 patients). Among leukemia/MDS patients, 17,979 person-years of follow-up was available for MAC recipients versus 3544 person-years for RIC/NMC recipients. The follow-up available for lymphoma patients was 1914 person-years and 2552 person-years, respectively. The patients in the MAC cohort were younger than the RIC/NMC cohort. A greater proportion of RIC/NMC recipients had received AHCT more recently, received a regimen that did not contain TBI, and had peripheral blood stem cells as a graft source. They were also more likely to have received a prior autologous transplant. Among leukemia/MDS patients, the cumulative incidence of acute grades II to IV GVHD at 100 days was 42% (95% CI, 41% to 43%) and that of chronic GVHD at 2 years was 43% (95% CI, 42% to 45%). Among patients with lymphoma, the corresponding cumulative incidence estimates were 38% (95% CI, 35% to 40%) and 43% (95% CI, 40% to 45%), respectively.

The cumulative incidence of second cancers in the RIC/NMC and MAC cohorts is shown in Figure 2. Among patients with leukemia/MDS, the cumulative incidence of second solid cancers at 10 years post-transplantation was 3.03% (95% CI, 2.52% to 3.57%) among MAC recipients and 4.29% (95% CI, 2.43% to 6.65%) among RIC/NMC recipients ($P = .25$). The 10-year incidences of second solid cancers among lymphoma patients receiving MAC and RIC/NMC regimens were 3.95% (95% CI, 2.47% to 5.76%) and 3.05% (95% CI, 1.50% to 5.13%), respectively ($P = .48$).

In multivariable analyses for leukemia/MDS, after adjusting for patient, disease, and transplant variables, we observed no significant difference in the risks for second solid cancers after adjusting for patient and disease characteristics (HR .98 [95% CI, .64 to 1.45] for RIC/NMC versus MAC regimens, $P = .905$). After adjusting for other significant covariates, second solid cancer risks were lower in recipients of RIC/NMC regimens with lymphoma, although this difference was only marginally significant (HR .51 [95% CI, .26 to .99] for RIC/NMC versus MAC regimens, $P = .047$).

Table 4 demonstrates the results of the analysis of the risks of second solid cancers among MAC and RIC/NMC recipients ages 40 to 60 years compared with general population control subjects. In contrast to MAC regimens, the RIC/NMC regimens are relatively newer, and patient follow-up is comparatively shorter; hence, we restricted these analysis to 10 years post-transplant. Patients with leukemia/MDS and lymphoma who had received MAC regimens had a significantly higher risks of solid cancers compared with the age-, gender-, and region-matched general population (SIR 1.46, $P < .01$ and SIR 2.35, $P < .01$, respectively). However,

Table 2
Standardized Incidence (Observed/Expected) Ratios for Second Solid Cancers (Excluding Nonmelanoma Skin Cancers) among RIC/NMC Recipients Compared with the General Population

Cancer Site	Leukemia/MDS Cohort				Lymphoma Cohort			
	Cases	SIR	95% CI	*P*	Cases	SIR	95% CI	*P*
Lip	2	14.28	1.73-51.57	.02	0	.00	—	—
Tongue	1	2.48	.06-13.80	.66	1	4.17	.11-23.25	.42
Mouth	1	2.89	.07-16.08	.59	0	.00	—	—
Tonsil	2	8.66	1.05-31.27	.05	0	.00	—	—
Oropharynx	3	46.70	9.63-136.47	<.01	2	67.35	8.16-243.30	<.01
Esophagus	1	1.41	.04-7.87	1.00	0	.00	—	—
Small intestine	1	4.84	.12-26.97	.37	0	.00	—	—
Colon	1	.26	.01-1.42	.19	2	1.09	.13-3.92	1.00
Rectum	1	.48	.01-2.66	.76	1	.98	.03-5.47	1.00
Liver	2	2.70	.33-9.76	.34	2	5.96	.72-21.54	.09
Pancreas	2	1.60	.19-5.79	.71	1	1.70	.04-9.46	.89
Larynx	1	1.47	.04-8.20	.99	0	.00	—	—
Lung	8	.91	.39-1.79	.96	4	.96	.26-2.44	1.00
Bone	2	23.53	2.85-85.01	<.01	0	.00	—	—
Skin melanoma	7	3.04	1.22-6.27	.02	5	3.52	1.14-8.22	.03
Soft tissue	4	12.92	3.52-33.08	<.01	0	.00	—	—
Breast	2	.25	.03-.91	.03	3	.82	.17-2.39	1.00
Vulva	2	18.55	2.25-67.02	.01	0	.00	—	—
Cervix uteri	1	2.14	.05-11.93	.75	0	.00	—	—
Corpus uteri	1	.63	.02-3.52	1.00	1	1.44	.04-8.05	.99
Prostate	8	.66	.29-1.31	.30	5	.80	.26-1.86	.80
Testis	1	5.38	.14-29.99	.34	0	.00	—	—
Kidney	4	2.52	.69-6.45	.15	0	.00	—	—
Bladder	1	.37	.01-2.05	.49	0	.00	—	—
Thyroid	1	1.37	.04-7.64	1.00	1	2.22	.06-12.39	.72
All sites	58	.99	.75-1.28	1.00	27	.92	.61-1.34	.75

Table 3
Characteristics of Patients Ages 40 to 60 Years Included in the Analysis Comparing Risks of Second Solid Cancers among RIC/NMC and MAC Regimens

Characteristics	Leukemia/MDS			Lymphoma		
	MAC Regimens	RIC/NMC Regimens	*P*	MAC Regimens	RIC/NMC Regimens	*P*
Number of patients	5728	1355		700	783	
Age at transplant, yr			<.001			<.001
40-49	3581 (62)	407 (30)		423 (60)	345 (44)	
50-60	2147 (38)	948 (70)		277 (40)	438 (56)	
Male gender	3071 (54)	732 (54)	.79	467 (67)	490 (63)	.10
Karnofsky score before transplant ≥ 80	4844 (85)	1092 (81)	.001	588 (84)	640 (82)	.02
Disease risk before transplant			.02			
Early	2782 (49)	597 (44)		—	—	
Intermediate	1114 (19)	274 (20)		—	—	
Advanced	1788 (31)	470 (35)		—	—	
Unknown	44 (1)	14 (1)		—	—	
Year of transplant			<.001			<.001
1995-1998	2098 (37)	66 (5)		222 (32)	40 (5)	
1999-2002	1689 (29)	376 (28)		254 (36)	259 (33)	
2003-2006	1941 (34)	913 (67)		224 (32)	484 (62)	
Median interval from diagnosis to transplant, mo (range)	8 (<1-338)	9 (<1-302)	.02	20 (2-540)	36 (4-413)	<.001
Conditioning regimen			<.001			<.001
Bu + Cy ± other	2104 (37)	0		157 (22)	0	
TBI + Cy ± other	2782 (49)	0		425 (61)	0	
TBI + Flud ± other (no Cy)	0	279 (21)		0	139 (18)	
Bu + Flud ± other	0	334 (25)		0	93 (12)	
Mel + Flud ± other	0	308 (23)		0	163 (21)	
Cy + Flud ± other	0	111 (8)		0	147 (19)	
Other	842 (15)	323 (24)		118 (17)	241 (30)	
TBI dose, cGy			<.001			<.001
No TBI	2612 (46)	960 (71)		233 (33)	589 (75)	
≤400	19 (<1)	314 (23)		0	172 (22)	
401-800	154 (3)	80 (6)		30 (5)	22 (3)	
801-1200	1705 (30)	0		329 (47)	0	
>1200	1235 (22)	0		108 (15)	0	
TBI dose missing	3 (<1)	1 (<1)		0	0	
Donor*			<.001			<.001
HLA-identical sibling	2318 (40)	421 (31)		415 (59)	271 (35)	
Unrelated	3410 (60)	934 (69)		285 (41)	512 (65)	
Graft type			<.001			<.001
Bone marrow	3189 (56)	275 (20)		287 (41)	177 (23)	
Peripheral blood stem cells	2539 (44)	1080 (80)		413 (59)	606 (77)	
GVHD prophylaxis			<.001			<.001
Ex vivo T cell depletion ± other	497 (9)	37 (2)		98 (14)	17 (2)	
FK506 + MMF ± other	229 (4)	221 (16)		41 (6)	139 (18)	
FK506 + MTX ± other (except MMF)	1367 (24)	270 (20)		161 (23)	230 (29)	
FK506 ± others (except MTX, MMF)	194 (4)	96 (7)		43 (7)	41 (5)	
CSA + MMF ± other (except FK506)	81 (1)	352 (26)		10 (1)	179 (23)	
CSA + MTX ± other (except FK506, MMF)	2908 (51)	226 (17)		262 (37)	97 (12)	
CSA ± other (except FK506, MTX, MMF)	266 (5)	114 (8)		52 (8)	59 (8)	
Other GVHD prophylaxis	131 (2)	32 (2)		26 (4)	11 (2)	
Missing	52 (1)	7 (1)		7 (1)	10 (1)	
Subsequent transplant or DLI	541 (9)	285 (21)	<.001	65 (9)	242 (31)	<.001
Prior autologous transplant	117 (2)	130 (10)	<.001	62 (9)	241 (31)	<.001
Median follow-up of survivors, mo (range)	88 (2-204)	71 (2-163)		94 (3-189)	73 (3-168)	

* HLA-mismatched related donors were excluded from this analysis.

compared with their general population peers, solid cancer risks were not higher for patients receiving RIC/NMC regimens (leukemia/MDS: SIR 1.20, $P = .34$; lymphoma: SIR .92, $P = .85$).

DISCUSSION

Our study is the most comprehensive analysis of second solid cancers after RIC/NMC regimens to date. We present important information to assist clinicians when counseling patients about screening and prevention of second solid cancers. We observed a continued increase in the cumulative incidence of second solid cancers over time. Despite these findings, the overall risk of solid cancers was comparable with general population control subjects of the same age and gender as the RIC/NMC recipients. In MAC recipients, second solid cancer risks do not start increasing until 5 to 10 years post-transplantation and later [5,6,32,33]. Given their relatively recent advent in clinical practice, there is a need to continue research and conduct studies with even longer follow-up in RIC/NMC recipients to better understand and realize the true risk of second solid cancers.

In the subset of patients 40 to 60 years of age, we observed no significant difference in the cumulative incidence of solid cancers in multivariable analyses among RIC/NMC and MAC recipients with leukemia/MDS. The difference was only marginally significant among patients with lymphoma. When comparing the risks of solid cancers among recipients of both regimens with the risks expected in the general population of the same age and gender, only MAC recipients had higher risks than the general population. Because RIC/NMC regimens are a relatively new addition to clinical practice, the follow-up of RIC/NMC recipients was

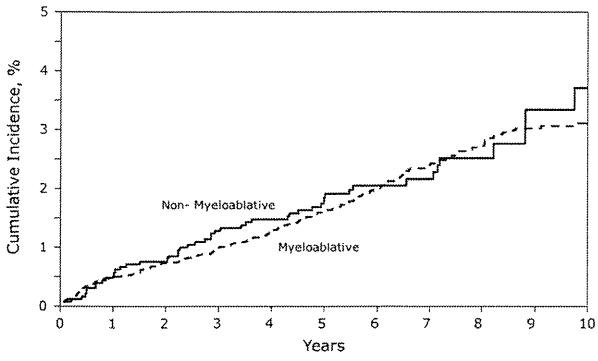

Figure 2. Cumulative incidence of second solid cancers (excluding non-melanoma skin cancers) among patients ages 40 to 60 years for leukemia/MDS and lymphoma treated with RIC/NMC and myeloablative conditioning, respectively.

substantially shorter than MAC recipients. Furthermore, the number of RIC/NMC recipients and second cancer events were much smaller than the MAC cohort. Hence, our study does not provide any definitive conclusions about risks of second solid cancers among RIC/NMC recipients relative to patients receiving MAC regimens. Because it can take more than a decade post-transplantation before second solid cancers begin to manifest, this comparative analysis between MAC and RIC/NMC recipients needs to be repeated as longer follow-up for the latter becomes available. At the same time, albeit the limitation of shorter follow-up for RIC/NMC recipients, the comparable risks for solid cancers between the 2 regimens may have other explanations. RIC/NMC recipients may have similar exposures to risk factors that increase solid cancer risks, such as pretransplant therapies (or even more exposure in RIC/NMC recipients, including autologous transplantation), acute and chronic GVHD, and the use and duration of post-AHCT immune suppression.

Table 4
Standardized Incidence (Observed/Expected) Ratios Comparing Risks of Second Solid Cancers (Excluding Nonmelanoma Skin Cancers) in MAC and RIC/NMC Recipients Ages 40 to 60 Years with that of the General Population

Time Period Post-AHCT	Regimen	n	Cases	SIR (95% CI)	P*
Leukemia/MDS cohort					
<1 yr	MAC	5676	24	1.53 (.98-2.28)	.06
	RIC/NMC	1348	8	1.36 (.58-2.67)	.48
1-4 yr	MAC	2734	56	1.31 (.99-1.70)	.06
	RIC/NMC	689	16	1.09 (.62-1.78)	.79
5-9 yr	MAC	1581	53	1.61 (1.21-2.11)	<.01
	RIC/NMC	333	8	1.32 (.57-2.60)	.53
Overall until 10 yr‡	MAC	5676	133	1.46 (1.22-1.73)	<.01
	RIC/NMC	1348	32	1.20 (.82-1.70)	.34
Lymphoma cohort					
<1 yr	MAC	680	4	2.46 (.67-6.30)	.17
	RIC/NMC	775	3	.98 (.20-2.85)	1.00
1-4 yr	MAC	274	13	2.95 (1.57-5.05)	<.01
	RIC/NMC	454	10	1.08 (.52-2.00)	.88
5-9 yr	MAC	161	6	1.59 (.58-3.47)	.36
	RIC/NMC	259	3	.58 (.12-1.70)	.49
Overall until 10 yr‡	MAC	680	23	2.35 (1.49-3.52)	<.01
	RIC/NMC	775	16	.92 (.52-1.49)	.85

* *P* value comparing SIR of solid cancers in transplant recipients to age-, gender-, and region-specific general population.

† Person-years of follow-up was 16,611 years for MAC recipients and 3506 years for RIC/NMC recipients.

‡ Person-years of follow-up was 1722 years for MAC recipients and 2497 years for RIC/NMC recipients.

Significantly elevated SIRs were observed for cancers of the oropharyngeal tract, bone and soft tissue, and melanoma of the skin. Higher risks than the general population have also been reported at these sites in MAC recipients [5-7]. Patients with leukemia/MDS had a lower risk of breast cancer compared with the general population. However, breast cancer was reported in only 2 patients, and this observation has to be interpreted with caution. Furthermore, this is in contrast to the high risks of breast cancer seen in women who receive TBI containing MAC regimens [8]. Breast cancer is the most common cancer in women, and its incidence increases with age. The risks of breast cancer after RIC/NMC AHCT may be lower than that of MAC given the lack of exposure to high doses of TBI. At the same time, as illustrated by previous publications, the incidence of secondary breast cancer starts to rise about 1 decade after AHCT. Therefore, more patients and longer follow-up are needed to clarify the risks of secondary breast cancer after RIC/NMC regimens.

The data from our study have to be interpreted while considering the general limitations of a retrospective analysis using registry data. Details of pretransplant chemotherapy and radiation therapy exposures were not available. Although several thousands of patients were included, some cancers are indeed rare, and larger studies are required to provide a better understanding of the incidence and risks of specific second cancers. Observation time was more than 10 years for some patients, but longer follow-up is still needed to fully characterize the complete risks of second cancers in RIC/NMC recipients, because the risk of second cancers after AHCT continues to increase over time [1-3,5,6,34,35]. The present analysis is not conclusive but adds to our understanding of second cancer risks after AHCT. Despite these limitations, our study is the largest and most comprehensive analysis to date of second solid cancer risks after RIC/NMC transplantation.

Our study demonstrates that the incidence of second solid cancers after RIC/NMC AHCT continues to increase with time. RIC/NMC recipients should receive screening for solid cancers in a manner that is similar to what is recommended for MAC recipients [9]. Clinicians taking care of long-term survivors after RIC/NMC AHCT should be aware of the increased risk of cancers of the lip, tonsil, oropharynx, bone, soft tissue, and vulva and skin melanoma. Future studies with larger number of patients who have been followed for a longer period of time are needed to better understand the incidence and risks for secondary solid cancer after transplantation using RIC/NMC regimens.

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Donor Lymphocyte Infusion for the Treatment of Relapsed Acute Myeloid Leukemia after Allogeneic Hematopoietic Stem Cell Transplantation: A Retrospective Analysis by the Adult Acute Myeloid Leukemia Working Group of the Japan Society for Hematopoietic Cell Transplantation

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A B S T R A C T

Because the efficacy of donor lymphocyte infusion (DLI) for acute myeloid leukemia (AML) relapse after allogeneic hematopoietic stem cell transplantation (HSCT) remains uncertain, especially in the Asian population, a nationwide registry study was retrospectively performed by the Adult AML Working Group of the Japan Society for Hematopoietic Cell Transplantation to identify the factors affecting the patient survival after DLI. Among 143 adult AML patients who received DLI for the treatment of first hematological relapse after HSCT, the overall survival rates at 1 year, 2 years, and 5 years were 32% ± 4%, 17% ± 3%, and 7% ± 3%, respectively. Complete remission (CR) at the time of DLI, which was obtained in 8% of the patients, was the strongest predictive factor for survival after DLI. Therefore, long-term survival after DLI was achieved almost exclusively in patients who successfully achieved a CR before DLI, indicating the limited efficacy of DLI in a minority of patients.

INTRODUCTION

Relapse remains a major obstacle to the survival of patients with acute myelogenous leukemia (AML) after allogeneic hematopoietic stem cell transplantation (HSCT), accounting for 20% to 50% of the primary causes of death [1,2]. Although the best way to manage AML relapse after allogeneic HSCT is unclear, donor lymphocyte infusion (DLI) is 1 of the most common interventions used for AML relapse, with the expectation of inducing a graft-versus-leukemia (GVL) effect [2-4]. However, treatment success for AML relapse is limited, with overall survival (OS) rates of 10% to 20% at 3 years in previous studies [2-8]. To predict the efficacy of DLI in advance may lead to the selection of different treatments, including second HSCT, for patients predicted to be unresponsive to DLI. Until now, large-scale studies to analyze the risk factors for the success of DLI have been scarce, especially in the Asian population. The aim of this

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study was to retrospectively identify the factors affecting the efficacy of DLI for adult patients with a first hematological relapse after allogeneic HSCT, using national registry-based data of the Transplant Registry Unified Management Program (TRUMP) in Japan.

PATIENTS AND METHODS

Data Collection

The data for 14,286 Japanese patients with AML who underwent HSCT were obtained from the TRUMP in Japan [9]. Data regarding white blood cell count at diagnosis, blast count and chimerism at relapse, and cell dose of DLI were not available for this cohort. Inclusion was based on the following criteria: first allogeneic, bone marrow (BM) or peripheral blood stem cell (PBSC) HSCT between 1991 and 2011, age ≥ 16 years at transplantation, and DLI recipients after the first hematological relapse after HSCT without precedence of a second transplantation. Patients with myelodysplastic syndrome, secondary AML from myelodysplastic syndrome, or a subsequent relapse of AML were excluded. Patients never in remission at transplantation were excluded. A total of 143 patients met the criteria for study inclusion. The study design was approved by the TRUMP data management committee of the Japan Society for Hematopoietic Cell Transplantation and the institutional review board of Kanazawa University Hospital, where this study was organized.

Definitions

DLI was defined as transfusion of unstimulated lymphocyte concentrates, collected from the original stem cell donor as buffy coat preparations. According to a previous study [3], the transfusion of unmanipulated mobilized PBSC concentrates was also defined as DLI, if no prophylactic immunosuppressive medication was given, whereas the infusion of donor PBSC or BM after conditioning the patient with prophylactic immunosuppression for graft-versus-host disease (GVHD) prevention was defined as a second HSCT. The physicians who performed transplantation at each center diagnosed and graded acute and chronic GVHD according to traditional criteria [10,11]. *Complete remission* (CR) was defined by normal values for the absolute neutrophil count (>1000/μL) and platelet count (>100,000/μL), independence from red cell transfusion, and absence of signs of leukemia without ongoing antileukemic therapy, based on the revised recommendations of the international working group [12]. The classification of conditioning regimens as to whether they were myeloablative or reduced-intensity was based on the report by the Center for International Blood and Marrow Transplant Research [13]. Cytogenetic subgroups were classified according to the Southwest Oncology Group/Eastern Cooperative Oncology Group criteria [14].

Endpoints

The primary study endpoint was to identify the factors affecting the OS after DLI.

Statistical Analysis

All statistical analyses were performed with the EZR software package (Saitama Medical Center, Jichi Medical University), a graphical user interface for R (The R Foundation for Statistical Computing, version 2.13.0) [15]. Variables included the recipient's age at time of transplantation, sex, pretransplantation cytomegalovirus serostatus, disease characteristics (French–American–British classification [FAB] and cytogenetics), donor characteristics (age, sex, *ABO* and *HLA* compatibility), transplantation characteristics (year of transplantation, disease status at transplantation, conditioning, source of stem cells, acute GVHD, and/or chronic GVHD before DLI), and relapse and DLI characteristics (interval from transplantation to relapse, interval from relapse to DLI, chemotherapy before DLI, disease status at DLI, and acute GVHD after DLI). The median was used as the cutoff point for continuous variables. The chi-square test and the Mann-Whitney U test were used to compare data between 2 groups. The probability of OS was calculated using the Kaplan-Meier method and compared using the log-rank test. The probabilities of acute and chronic GVHD were analyzed using a cumulative incidence analysis [16], while considering death without acute GVHD and death without chronic GVHD as respective competing risks. All factors found to be significant in the univariate analyses ($P \leq .10$) were included in multivariate Cox hazard models. For both the univariate and multivariate analyses, P values were 2-sided and the outcomes were considered to be significant for values of $P \leq .05$.

RESULTS

Patient Characteristics

A total 143 patients with AML who received DLI for treatment of a first hematological relapse after allogeneic HSCT were included in the study (Table 1). The median time interval from HSCT to relapse was 149 days (range, 28 to 2153) and from relapse to DLI was 37 days (range, 0 to 841). Only 8% of patients had obtained CR at the time of DLI. One single infusion of DLI was given to 76% of patients, and the remaining patients received 2 or more infusions.

Table 1
Characteristics of the Adult Patients who received DLI for the Treatment of Their First Hematological Relapse after HSCT for AML

No. of patients	143
Age at relapse, median (range), yr	49 (16-67)
Cytogenetics	
Good	20 (14)
Intermediate	81 (57)
Poor	42 (29)
Follow-up of survivors after DLI, median (range), d	459 (73-4377)
Interval from relapse to DLI, median (range), d	37 (0-841)
Extramedullary relapse	
No	131 (92)
Yes	12 (8)
Acute GVHD present at relapse	
No	69 (48)
Yes	71 (50)
Data missing	3 (2)
Chronic GVHD present at relapse	
No	95 (66)
Yes	28 (20)
Not evaluated or missing	20 (14)
Acute or chronic GVHD present at relapse	
No	62 (43)
Yes	79 (55)
Data missing	2 (1)
Chemotherapy before DLI	
No	21 (14)
Yes	55 (38)
Data missing	67 (47)
Status at DLI	
Active disease or aplasia	132 (92)
Complete remission	11 (8)
Transfusions, n	
1	109 (76)
2	22 (15)
≥ 3	12 (8)
Acute GVHD after DLI	
Yes	26 (18)
No	117 (82)
Cause of death	
Infection	11 (9)
Interstitial pneumonia	4 (3)
GVHD	2 (2)
Hemorrhage	6 (5)
Organ failure	11 (9)
Persistent or relapsed leukemia	86 (71)
Data missing	1 (1)

Data presented are n (%), unless otherwise indicated.

OS after DLI

In the 143 relapse patients who received DLI, the 1-year, 2-year, and 5-year OS rates from DLI were 32% ± 4%, 17% ± 3%, and 7% ± 3%, respectively. Among the 143 patients, 121 patients (85%) died after DLI, and the main cause of death was persistent or relapsed leukemia in 86 patients (71%), infections in 11 (9%), organ failure in 11 (9%), hemorrhage in 6 (5%), interstitial pneumonia in 4 (3%), and GVHD in 2 patients (2%). The median follow-up of the remaining 22 survivors after DLI was 459 days (range, 73 to 4377).

The factors significantly associated with a shorter OS after DLI based on the univariate analysis included male sex, sex match of the donor and recipient in contrast to a male donor for a female recipient, HLA mismatch of the donor and recipient, a related PBSC recipient at HSCT compared with a

Table 2
Results of Univariate Analysis of the Risk Factors for Survival after DLI

Characteristic	OS at One Year		OS at Two Years		*P* Value
	%	SE	%	SE	
Overall	32%	4%	17%	3%	
Patient age, yr					
<49	24%	5%	17%	5%	
≥49	21%	5%	9%	4%	.25
Patient sex					
Female	29%	6%	15%	5%	
Male	18%	4%	12%	4%	.02
Donor age, yr					
<37	25%	6%	15%	5%	
≥37	19%	5%	12%	5%	.47
Donor sex					
Male	24%	5%	14%	4%	
Female	20%	6%	12%	5%	.40
Sex matching					
Male donor to female recipient	35%	8%	21%	7%	
Female donor to male recipient	22%	8%	18%	8%	.07
Matched	16%	4%	9%	3%	.02
ABO matching					
Matched	22%	5%	13%	4%	
Major mismatched	36%	13%	NA	NA	.16
Minor mismatched	13%	7%	9%	6%	.76
Major-minor mismatched	22%	14%	NA	NA	.77
ABO major mismatching					
No	20%	4%	12%	3%	
Yes	30%	10%	NA	NA	.16
ABO minor mismatching					
No	24%	4%	16%	4%	
Yes	16%	6%	8%	5%	.71
HLA matching					
Matched	25%	4%	16%	4%	
Mismatched	16%	7%	NA	NA	.05
Type of HLA-matched donor					
Related	23%	5%	13%	4%	
Unrelated	29%	8%	25%	8%	.88
Source of stem cells					
Related BM	30%	8%	15%	6%	
Related PBSC	17%	5%	10%	4%	.03
Unrelated BM	24%	7%	21%	7%	.38
Status at transplantation					
CR1 or CR2	25%	5%	13%	4%	
Advanced	21%	5%	15%	5%	.69
Pretransplantation CMV serostatus					
CMV positive recipient	26%	4%	15%	4%	
CMV negative recipient	10%	7%	0%	NA	.30
Year of transplantation					
<2006	23%	6%	16%	5%	
≥2006	22%	5%	NA	NA	.47
Cytogenetic subgroup					
Good	33%	11%	27%	10%	
Intermediate	26%	5%	14%	4%	.36
Poor	10%	6%	NA	NA	.04
Conditioning for transplantation					
Myeloablative	22%	5%	16%	4%	
Reduced intensity	23%	6%	10%	5%	.78
Interval from transplantation to relapse, mo					
<5	15%	4%	7%	3%	
≥5	34%	7%	23%	6%	.001
Acute GVHD at time of relapse					
No	22%	5%	13%	4%	
Yes	23%	5%	15%	5%	.74
Chronic GVHD at time of relapse					
No	19%	4%	12%	4%	
Yes	33%	10%	19%	8%	.34
Acute or chronic GVHD at time of relapse					
No	22%	5%	12%	4%	
Yes	24%	5%	15%	4%	.68
Extramedullary relapse					
No	28%	7%	18%	6%	
Yes	17%	14%	NA	NA	.99
Interval from relapse to DLI, d					
≥37	32%	6%	19%	5%	
<37	12%	4%	9%	4%	.003
Chemotherapy before DLI					
No	29%	11%	NA	NA	
Yes	26%	7%	21%	6%	.41
Status at DLI					
CR	100%	NA	100%	NA	
Active disease or aplasia	17%	3%	8%	3%	.00001
Acute GVHD after DLI					
No	32%	8%	23%	7%	
Yes	26%	9%	NA	NA	.89
Second transplantation after relapse					
No	22%	4%	15%	4%	
Yes	25%	9%	8%	6%	.80

NA indicates not available; CMV, cytomegalovirus.

related BM recipient, poor cytogenetics compared with good cytogenetics, a shorter interval (<5 months) from HSCT to relapse, a shorter interval (<37 days) from relapse to DLI, active disease or aplasia at the time of DLI, and a single infusion of DLI (Table 2). Other factors, such as the patient and donor age, presence of GVHD at relapse, and the development of acute GVHD after DLI, did not significantly influence OS after DLI.

A total of 26 patients developed acute GVHD after DLI (Table 1), with grade I GVHD in 15 patients, grade II in 5, grade III in 5, and grade IV in 1 patient. Of the 26 patients, 17 (69%), 3 (12%), 2 (8%), and 4 (15%) patients experienced acute GVHD after 1, 2, 3, and 4 courses of DLI, respectively. Eight (31%) of the 26 patients achieved disease-free survival after DLI, with durations ranging from 82 to 2258 days (median, 362 days), whereas 14 (12%) of the 121 patients without acute GVHD experienced disease-free survival. It may be noted that 5 (33%) of the 15 patients who developed grade I acute GVHD after DLI survived without disease over 2 years, and that 2 of the 26 patients who developed GVHD subsequently developed chronic GVHD, and both patients survived long-term without disease. Three other patients developed chronic GVHD without experiencing acute GVHD after DLI, and 2 of these 3 patients survived without disease for over 2 years. These data might suggest the association of GVHD after DLI with a substantial GVL effect.

The impact of GVHD on OS after DLI was evaluated as a time-dependent variable. In a multivariate analysis, a shorter interval from HSCT to relapse (hazard ratio, 1.76; 95% confidence interval, 1.10 to 2.57; $P = .02$) and active disease or aplasia at time of DLI (hazard ratio, 9.98; 95% confidence interval, 2.27 to 43.9; $P = .002$) remained significantly associated with a shorter OS (Table 3). The number of DLI infusions was closely linked to the interval from relapse to DLI and was, therefore, eliminated from the multivariate model. Disease stage at DLI had a relatively greater impact on OS after DLI compared with the interval from HSCT to relapse. In addition, among the 11 patients who had obtained CR at the time of DLI, 10 patients showed a longer interval from HSCT to relapse.

Accordingly, 3 prognostic groups were categorized as follows: CR at DLI, regardless of the interval from HSCT to

Table 3
Results of Multivariate Analysis of Risk Factors for Survival after DLI

Prognostic Factor	*P* Value	Hazard Risk for OS	95% CI
Female versus male	.49	1.24	.68-2.25
Male donor to female recipient versus female donor to male recipient	.69	1.20	.50-2.87
Male donor to female recipient versus sex matched	.36	1.35	.71-2.57
HLA matched versus HLA mismatched	.19	1.39	.85-2.27
Good cytogenetics versus intermediate cytogenetics	.21	1.45	.81-2.59
Good cytogenetics versus poor cytogenetics	.09	1.76	.92-3.39
Interval from transplantation to relapse, ≥ 5 mo versus <5 mo	**.02**	**1.68**	**1.10-2.57**
Interval from relapse to DLI, ≥ 37 d versus <37 d	.35	1.23	.80-1.90
Disease stage at DLI (complete remission versus active disease or aplasia)	**.002**	**9.98**	**2.27-43.9**

The bold results show values with a $P \leq .05$.
CI indicates the confidence interval.

relapse (group 1; n = 11), a longer interval (≥5 months) from HSCT to relapse but not in CR at DLI (group 2; n = 51), and others (group 3; n = 81) (Table 4, Figure 1). Among the patients who received DLI while in CR (group 1), the 2-year OS was as high as 100%, which was significantly better than that observed in those with a longer interval from HSCT to relapse without CR at DLI (group 2; 12%, $P < .001$) and a shorter interval from HSCT to relapse without CR at DLI (group 3; 4%, $P < .001$). Of note, no significant differences in OS after DLI were noted between group 2 and group 3 ($P = .13$). Accordingly, CR at the time of DLI was the strongest factor with a significant impact on OS after DLI.

DISCUSSION

Despite advances in decreasing the nonrelapse mortality (NRM) after allogeneic HSCT [17], there has been little progress in reducing the incidence of relapse or in improving the subsequent outcome. The long-term survival rate after relapse for patients who underwent transplantation with AML was reported to be 5% [18,19], although salvage therapy, such as withdrawal of immunosuppression, chemotherapy, radiotherapy, a second HSCT, and DLI have been attempted. However, durable remission occasionally develops after DLI for AML relapse [2-8]. The current nationwide study confirmed that AML patients who successfully achieved CR after relapse may benefit from DLI. Although the 5-year OS from relapse was low at 7%, a subset of patients who achieved CR before DLI had a significantly better 5-year OS of 50%, supporting the use of this treatment strategy [2,3] when a CR is obtained by salvage treatment, such as withdrawal of immunosuppression and/or salvage chemotherapy, and immediate consolidation with DLI should be recommended to improve the chance for long-term survival after AML relapse.

Previous studies have identified several factors that are associated with a good prognosis after DLI, including achievement of hematological remission before DLI, a lower tumor burden at relapse, female sex, favorable cytogenetics, remission at the time of DLI, a longer duration of remission after HSCT, and the absence of acute GVHD after HSCT [2,3,5,20-22], the most important of which were the tumor burden at relapse and the duration of remission after HSCT. The present study supports the importance of disease control before DLI.

One drawback is that the study was a retrospective registry analysis, limiting the risk factors that were available for analysis, including not only the blast count at relapse, but also the dose of mononuclear cells in the DLI grafts and the use of granulocyte colony–stimulating factor before harvesting the infused lymphocytes.

A second HSCT with or without DLI represents a good alternative treatment [3] because, at the current moment, the approaches expected to offer long-term survival for patients with AML who relapse after HSCT are confined to DLI and second HSCT. However, a second HSCT after myeloablative conditioning has historically been associated with poor survival, with higher NRM rates ranging from 25% to 45%. Recent approaches with a second HSCT after a reduced-intensity conditioning regimen minimized NRM rates to 0 to 30%, but this could be offset by the higher relapse rates after the second HSCT [1,23]. There have been few reports on whether DLI was superior to second HSCT, and a comparison of the efficacy of DLI and second HSCT for AML relapse is outside the scope of the present study. However, as shown Table 2, a second HSCT after DLI did not have a significant impact on the OS in patients with AML relapse.

Various modifications of DLI have been investigated, such as ex vivo activated DLI and earlier introduction of DLI [24-26]. A recent report [26] showed that preemptive DLI given when minimal residual disease (MRD) was detected effectively reverted MRD back to remission in all 16 treated patients with acute leukemia and offered long-term survival in 15 of the 16 patients without increasing the risk of GVHD development. Thus, early detection of potential disease progression by detecting MRD and subsequently performing DLI before overt relapse might be a better way to improve the success of HSCT for AML.

The major risk of DLI is the development of GVHD, which occurs in 40% to 80% of patients [20,27,28], placing patients at risk of significant morbidity and mortality. In the present study, the cumulative incidence of acute GVHD after DLI was as high as 82%. However, the development of acute GVHD after DLI did not significantly affect the long-term survival and it only caused 2% of the deaths. The majority of deaths resulted from original disease, which accounted for 79% of the deaths.

Table 4
Survival of Adult Patients Receiving DLI for Treatment of First Hematological Relapse after HSCT for AML (n = 143) Stratified according to Prognostic Group

Prognostic Group	n	OS at One Year		OS at Two Years		OS at Five Years		*P* Value
		%	SE	%	SE	%	SE	
Group 1: CR at DLI	11	100%	0%	100%	0%	50%	25%	
Group 2: Interval from transplantation to relapse, ≥5 mo, but not in CR at DLI	51	24%	6%	12%	4%	9%	5%	<.001
Group 3: Others	81	14%	4%	6%	3%	0%	0%	<.001

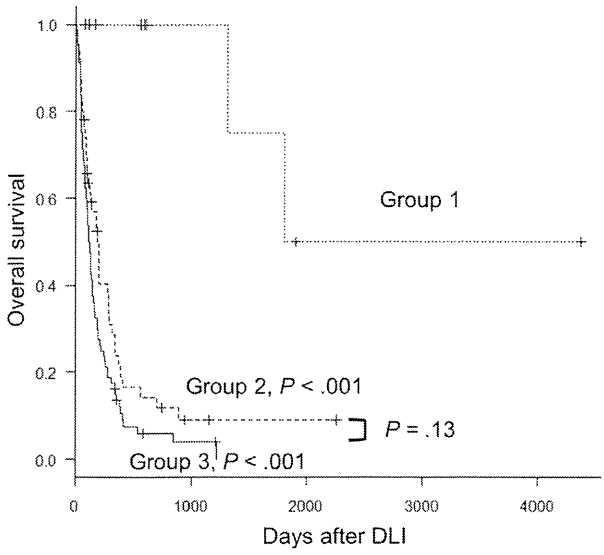

Figure 1. Survival after DLI according to the prognostic groups. Group 1 had a CR at DLI, regardless of the interval from HSCT to relapse (n = 11). Group 2 had a longer interval (≥5 months) from HSCT to relapse, but was not in CR at DLI (n = 51). Group 3 included the other patients (n = 81).

Despite the fact that there has been insufficient data about cases after DLI in the Asian population, several large-scale studies [2,3,6,7,27,29] that evaluated the efficacy of DLI for AML relapse after HSCT in non-Asian population have been reported. The OS rates from DLI in those studies ranged from 21% to 37%, 14% to 25%, 12% to 20%, and 10% to 15% at 1, 2, 3, and 5 years, respectively; comparable with the OS rates in the present study for the Asian population, which were 32%, 17%, 10%, and 7% at 1, 2, 3, and 5 years. The European Group for Blood and Marrow Transplantation Group [3] reported several factors that were associated with better OS, including remission at the time of DLI, as seen in the present study, bone marrow blasts less than 35% at relapse, female sex, and favorable cytogenetics. Therefore, there does not appear to be any major differences between the Asian and non-Asian populations in the context of the potent antileukemic effect of DLI for AML.

The nature of a retrospective, registry-based analysis implicates several limitations. There were missing data on the type of chemotherapy administered before DLI, no information about the cell doses and whether the DLI was a fresh infusion. Unfortunately, the present registry-based data do not include this information, and to collect such missing data is out of the scope of the present study. Therefore, further studies are warranted.

The present cohort does not include patients who received prophylactic immunosuppression, either after DLI or unmanipulated PBSC infusion, according to a previous report [3], to allow us to evaluate the pure GVL effect.

The results of this large retrospective study demonstrate that the efficacy of DLI is limited for the treatment of AML relapse after HSCT, and disease control at the time of DLI is critical for treatment success irrespective of operative chemotherapy before obtaining remission. However, the number of patients with CR was quite small (n = 11), and, therefore, conclusions should be considered with caution. New strategies to enhance and maintain the GVL effect of DLI while minimizing GVHD, which includes preemptive/prophylactic DLI before overt relapse, costimulation with cytokines or dendritic cells, and use of the leukemia-specific antibodies, such as gemtuzumab ozogamicin, should be considered.

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ORIGINAL ARTICLE

Impact of HLA allele mismatch on the clinical outcome in serologically matched related hematopoietic SCT

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In unrelated hematopoietic SCT (HSCT), HLA allele mismatch has been shown to have a significant role. To clarify the importance of HLA allele mismatch in the GVH direction in related HSCT, we retrospectively evaluated 2377 patients who received stem cells from an HLA serologically matched related donor in the GVH direction using the database of the Japan Society for Hematopoietic Cell Transplantation. The cumulative incidences of grade II–IV and grade III–IV acute GVHD in patients with an HLA allele-mismatched donor ($n = 133$, 5.6%) were significantly higher than those in patients with an HLA allele-matched donor. Multivariate analyses showed that the presence of HLA allele mismatch was associated with increased risks of grade II–IV and grade III–IV acute GVHD. In particular, HLA-B mismatch and multiple allele mismatches were associated with an increased risk of acute GVHD. The presence of HLA allele mismatch was associated with an inferior OS owing to an increased risk of non-relapse mortality (NRM). In conclusion, the presence of HLA allele mismatch in the GVH direction in related HSCT was associated with increased risks of GVHD and NRM, which led to an inferior OS. HLA allele typing is recommended in related HSCT.

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INTRODUCTION

Previous studies have shown that HLA allele mismatch significantly affects the clinical outcome after unrelated hematopoietic SCT (HSCT).[1,2] Several retrospective studies have demonstrated that the presence of HLA allele mismatch is associated with an increased risk of GVHD in unrelated HSCT.[3–5] Although the disparity of HLA molecules in HLA antigen mismatch is greater than that in HLA allele mismatch without HLA antigen mismatch, the impact of HLA mismatch on the clinical outcome was considered to be, for practical purposes, similar between antigen mismatch and allele mismatch, as reported previously.[4,6,7] Although the impact of an HLA mismatch at each locus varied among the studies, there is a consensus that an HLA mismatch at any locus, including A, B, C and DRB1, is in general associated with a poor clinical outcome.[2]

In related HSCT, the importance of HLA allele mismatch has not yet been well established, because an HLA antigen-matched sibling is in most cases an HLA allele fully matched donor. In Japan, HLA compatibility in related HSCT is usually assessed serologically or by low-resolution DNA typing at three loci, including HLA-A, -B and -DR. However, when the donor is not a sibling, such as a parent or child, the probability of HLA allele mismatch between the recipient and the donor is expected to be higher than that between siblings. Furthermore, there may also be an HLA allele mismatch with a sibling if we consider recombination and mutation. The presence of one HLA antigen mismatch has been reported to be associated with a poor overall clinical outcome in related HSCT.[8–10] Therefore, if the impact of allele mismatch is similar to that of antigen mismatch in related HSCT, as it is in unrelated HSCT, we could assume that the presence of HLA allele mismatch adversely affects the clinical outcome in related HSCT.

In this study, we assessed the impact of HLA allele mismatch on the clinical outcome in related HSCT using the database of the Japan Society for Hematopoietic Cell Transplantation (JSHCT), including patients without serological HLA mismatch in the GVH direction.

PATIENTS AND METHODS

Data collection

Data for all patients who received a first allogeneic HSCT from a serologically HLA-A, -B and -DR matched related donor in the GVH direction, irrespective of the number of mismatches in the HVG direction, between 1 January 2000 and 31 December 2011 were obtained from the Transplant Registry Unified Management Program, which includes data from the JSHCT.[11] We excluded patients who lacked data on survival status. Overall, 7089 patients satisfied the above criteria. In further analyses, we considered only 2377 patients (33.5%) for whom information

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on allele typing at the HLA-A, -B, and -DRB1 loci was available. The study was planned by the HLA working group of the JSHCT and was approved by the data management committees of TRUMP and by the institutional review board of Saitama Medical Centre, Jichi Medical University, Saitama, Japan.

Histocompatibility

Histocompatibility data for serological and genomic typing for the HLA-A, -B and -DR loci were obtained from reports obtained from the institution at which the transplantation was performed. To reflect current practice in Japan, HLA matching in related donors was assessed by serological data for HLA-A, -B, and -DR loci. When the recipient's antigens or alleles were not shared by the donor, this was considered an HLA mismatch in the GVH direction; when the donor's antigens or alleles were not shared by the recipient, this was considered a mismatch in the host-versus-graft (HVG) direction.

End points and statistical analyses

The primary end point was the cumulative incidence of acute GVHD. Secondary end points included the cumulative incidences of neutrophil engraftment and non-relapse mortality (NRM) and the probability of OS. The physicians who performed transplantation at each center diagnosed and graded acute GVHD according to the standard criteria.[12]

A descriptive statistical analysis was performed to assess the patients' characteristics. Medians and ranges are provided for continuous variables, and the percentages are shown for categorical variables. Patient's characteristics were compared by using the Chi-squared test or the Fisher's exact test for categorical variables. The probability of OS was calculated by the Kaplan–Meier method. A Cox proportional-hazards regression model was used to analyze OS. The cumulative incidences of NRM, GVHD and relapse were evaluated using the model of Fine and Grey[13] for univariate and multivariate analyses. In the competing risk models for GVHD, relapse and death before these events were defined as competing risks. In the competing risk models for NRM, relapse was defined as a competing risk. Factors that were associated with a two-sided *P*-value of < 0.10 in the univariate analysis were included in a multivariate analysis. We used a backward stepwise selection algorithm and retained only statistically significant variables in the final model. A two-sided *P*-value of < 0.05 was considered statistically significant. The variables evaluated in these analyses were as follows: sex mismatch (female to male vs others), patient's age at the time of HSCT (age $\geqslant 50$ years vs age < 50 years), disease risk (standard risk vs high risk), stem cell source (BM vs PBSC), relation to donor (sibling or others), ABO mismatch, use of *in vivo* T-cell depletion, performance status (0–1 vs 2–4), intensity of the conditioning regimen (myeloablative vs reduced intensity), GVHD prophylaxis (CYA based vs tacrolimus based), year of transplant ($\geqslant$2007 vs $<$ 2007) and HLA disparity as assessed by allele typing of HLA A, B and DRB1. Standard risk was defined as the first or second CR of acute leukemia, the first or second chronic phase of chronic myeloid leukemia, myelodysplastic syndrome refractory anemia or refractory cytopenia with multilineage dysplasia, malignant lymphoma in CR or PR or non-malignant disease. High risk was defined as some other status of malignancy. All statistical analyses were performed with EZR (Saitama Medical Centre, Jichi Medical University, Saitama, Japan; http://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmedEN.html), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria, version 2.13.0).[14]

RESULTS

Patient characteristics

The patient characteristics are summarized in Table 1. The median age was 40 years (range, 0–74). Compared with recipients with an HLA allele-matched donor (Match group, $n = 2244$), recipients with an HLA allele-mismatched donor (Mismatch group, $n = 133$) were more likely to have a poor performance status, to receive a transplantation from a non-sibling donor, to receive a transplantation at an earlier time period, to receive tacrolimus for GVHD prophylaxis and to receive an *in vivo* T-cell depletion (Table 1). More patients in the Mismatch group received a transplant from a donor with an HLA mismatch in the HVG direction. In the Match group, the number of antigen mismatches in the HVG direction

Table 1. Patient characteristics

Variable	*HLA allele match in the GVH direction* n = *2244*, n *(%)*	*HLA allele mismatch in the GVH direction* n = *133*, n *(%)*	P-value
Age at transplantation			
Median, years (range)	40 (0–74)	36 (0–69)	0.10
$\geqslant 50$	1491 (66.4%)	93 (69.9%)	0.46
< 50	753 (33.6%)	40 (30.1%)	
Sex combination of donors and recipients			
Female to male	608 (27.1%)	34 (25.6%)	0.60
Other combinations	1625 (72.4%)	98 (73.7%)	
Missing	11 (0.5%)	1 (0.8%)	
Performance status			
0–1	1967 (87.7%)	101 (75.9%)	< 0.001
2–4	232 (10.3%)	22 (16.5%)	
Missing	45 (2.0%)	10 (7.5%)	
Disease			
AML	813 (36.2%)	38 (28.6%)	0.35
ALL	468 (20.9%)	28 (21.1%)	
MDS	247 (11.0%)	12 (9.0%)	
CML	74 (3.3%)	7 (5.3%)	
Lymphoma	340 (15.2%)	26 (19.5%)	
Non-malignant disease	247 (11.0%)	17 (12.8%)	
Others	55 (2.5%)	5 (3.8%)	
Disease risk			
Standard	1325 (59.0%)	66 (49.6%)	0.083
High	906 (40.4%)	66 (49.6%)	
Missing	13 (0.6%)	1 (0.8%)	
Relation between donor and recipient			
Sibling	2048 (91.3%)	64 (48.1%)	< 0.001
Parent/child	185 (8.2%)	65 (48.9%)	
Others[a]	11 (0.5%)	4 (3.0%)	
Source of stem cells			
BM	1162 (51.8%)	65 (48.9%)	0.57
PBSC	1082 (48.2%)	68 (51.1%)	
HLA compatibility in the GVH direction[b]			
Matched	2244 (100%)	0 (0%)	< 0.001
One allele mismatch	0 (0%)	116 (87.2%)	
HLA-A		32	
HLA-B		18	
HLA-DRB1		66	
$\geqslant$ Two allele mismatch	0 (0%)	17 (12.8%)	
HLA compability in the HVG direction[b]			
Matched	2164 (96.4%)	75 (56.4%)	< 0.001
One antigen mismatch	46 (2.0%)	44 (33.1%)	
$\geqslant$ Two antigen mismatch	34 (1.5%)	14 (10.5%)	
Conditioning regimen			
Myeloablative	1426 (63.5%)	84 (63.2%)	0.92
Reduced intensity	761 (33.9%)	43 (32.3%)	
Missing	57 (2.5%)	6 (4.5%)	
GVHD prophylaxis			
CYA based	1891 (84.3%)	47 (35.3%)	< 0.001
Tacrolimus based	285 (12.7%)	79 (59.4%)	
Missing	68 (3.0%)	7 (5.3%)	
In vivo T-cell depletion			
Yes	154 (6.9%)	25 (18.8%)	< 0.001
No	2090 (93.1%)	108 (81.2%)	
Year of transplant			
2000–2006	522 (23.3%)	49 (36.8%)	< 0.001
2007–2011	1722 (76.7%)	84 (63.2%)	

Abbreviations: HVG = host-versus-graft; MDS = myelodysplastic syndrome. [a]Others included half-sibling ($n = 4$), aunt ($n = 3$), cousin ($n = 2$), nephew ($n = 1$) and grandchild in the Match group and half-sibling ($n = 1$), cousin ($n = 2$) and unknown ($n = 1$) in the Mismatch group. [b]HLA compatibility was defined according to the HLA-A, -B and -DR loci.

was 0 in 96.4%, 1 in 2.0%, 2 in 1.0% and 3 in 0.5%. In the Mismatch group, the number of antigen mismatches in the HVG direction was 0 in 56.3%, 1 in 33.1%, 2 in 7.5% and 3 in 3.0%. Information on HLA-C allele mismatch was available in only 1152 of 2377 (48.5%).

GVHD

The cumulative incidences of grade II–IV acute GVHD were 29.5% (95% confidence interval (CI) 27.6–31.4%) in the Match group and 40.6% (95% CI 32.2–48.8%) in the Mismatch group ($P=0.0018$, Figure 1a). A multivariate analysis showed that the presence of at least one allele mismatch was associated with an increased risk of grade II–IV acute GVHD (hazard ratio (HR) 1.77, 95% CI 1.31–2.38, $P=0.0002$, Table 2). An increase in the number of HLA mismatches was associated with a statistically significant increase in the risk of grade II–IV acute GVHD. The cumulative incidences of grade II–IV acute GVHD were 38.8% (95% CI 29.9–47.6%) and 52.9% (95% CI 26.5–73.8%) in patients with one allele mismatch and multiple allele mismatches, respectively ($P=0.0020$, Figure 1b). Compared with the Match group, both the one allele-mismatched and multiple allele-mismatched cohorts were associated with an increased risk of grade II–IV acute GVHD in multivariate analyses (one allele mismatch: HR 1.61, 95% CI 1.17–2.22, $P=0.0035$; multiple allele mismatches: HR 3.52, 95% CI 1.64–7.59, $P=0.0013$). We also assessed the impact of each locus excluding patients with multiple allele mismatches. The cumulative incidences of grade II–IV acute GVHD were 25.0% (95% CI 11.6–41.0%) in HLA-A mismatch, 50.0% (24.8–70.9%) in HLA-B mismatch and 42.4% (30.3–54.0%) in HLA-DRB1 mismatch (Figure 1c). In a multivariate analysis, the presence of HLA-B or -DRB1 mismatch was associated with an increased risk of grade II–IV acute GVHD (HLA-A: HR 0.86, 95% CI 0.40–1.84, $P=0.69$; HLA-B: HR 2.33, 95% CI 1.18–4.63, $P=0.015$; HLA-DRB1: HR 1.83, 95% CI 1.22–2.72, $P=0.0033$).

The cumulative incidences of grade III–IV acute GVHD were 9.5% (95% CI 8.3–10.8%) in the Match group and 21.8% (95% CI 15.2–29.2%) in the Mismatch group ($P<0.0001$, Figure 1d). A multivariate analysis showed that the presence of at least one allele mismatch was associated with an increased risk of grade III–IV acute GVHD (HR 2.39, 95% CI 1.60–3.58, $P<0.0001$, Table 2). Other factors that were associated with an increased risk of grade III–IV acute GVHD were use of PBSC and high disease risk. An increase in the number of HLA mismatches was associated with a significantly increased risk of grade III–IV acute GVHD. The cumulative incidences of grade III–IV acute GVHD were 19.8% (95% CI 13.1–27.6%) and 35.3% (95% CI 13.8–57.8%) in patients with one allele mismatch and multiple allele mismatches, respectively ($P<0.0001$, Figure 1e). Compared with the Match group, both the one allele mismatch and multiple allele mismatched cohorts were associated with an increased risk of grade III–IV acute GVHD in multivariate analyses (one allele

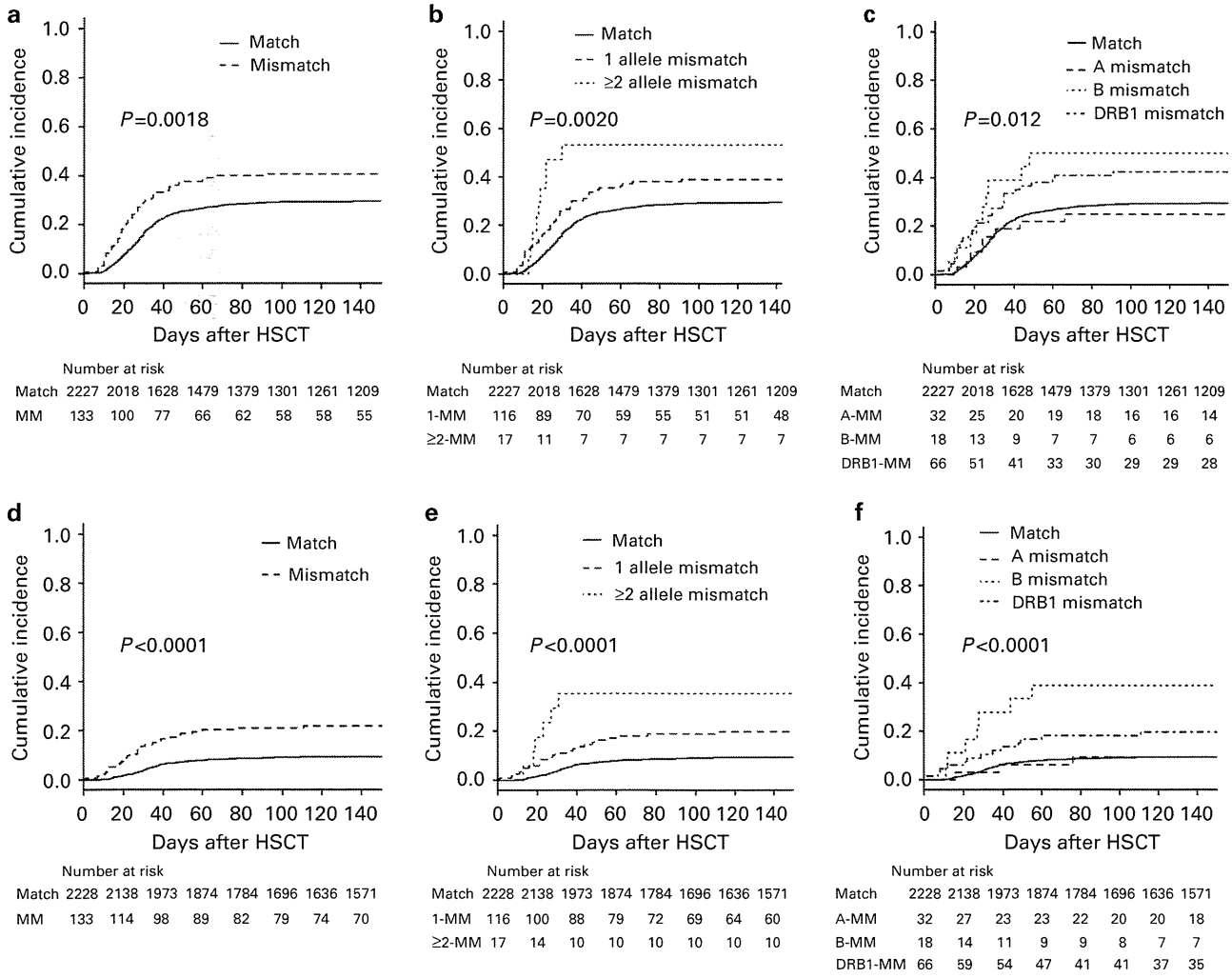

Figure 1. Cumulative incidence of acute GVHD. Cumulative incidences of grade II–IV (**a**–**c**) and grade III–IV (**d**–**f**) acute GVHD grouped according to (**a**, **d**) allele mismatch, (**b**, **e**) the number of allele mismatches and (**c**, **f**) locus of allele mismatches.

Table 2. Multivariate analysis

Outcomes and significant factors	*HR*	*95% CI*	*P-value*
Grade II–IV acute GVHD			
Use of *in vivo* TCD (vs no *in vivo* TCD)	0.58	0.39–0.85	0.0059
Age ⩾ 50 years (vs age < 50 years)	1.19	1.01–1.41	0.039
Reduced intensity (vs myeloablative)	0.78	0.66–0.92	0.0041
PBSC (vs BM)	1.32	1.13–1.53	0.0005
Allele mismatch in the GVH direction	1.77	1.31–2.38	0.0002
Grade III–IV acute GVHD			
PBSC (vs BM)	1.85	1.41–2.44	< 0.0001
Disease risk, high (vs standard)	1.59	1.22–2.08	0.0001
Allele mismatch in the GVH direction	2.39	1.60–3.58	< 0.0001
NRM			
Age ⩾ 50 years (vs age < 50 years)	1.93	1.52–2.46	< 0.0001
PBSC (vs BM)	1.52	1.19–1.94	< 0.0001
Disease risk, high (vs standard)	1.57	1.23–2.00	0.0003
Allele mismatch in the GVH direction	1.57	1.01–2.43	0.043
OS			
Age ⩾ 50 years (vs age < 50 years)	1.45	1.27–1.66	< 0.0001
Use of *in vivo* TCD (vs no *in vivo* TCD)	0.50	0.35–0.73	0.0003
Performance status, 2–4 (vs 0–1)	2.36	1.99–2.79	< 0.0001
PBSC (vs BM)	1.41	1.23–1.61	< 0.0001
Disease risk, high (vs standard)	2.08	1.81–2.38	< 0.0001
Allele mismatch in the GVH direction	1.43	1.11–1.85	0.0058

Abbreviations: CI = confidence interval; HR = hazard ratio; NRM = non-relapse mortality; TCD = T-cell depletion.

mismatch: HR 2.12, 95% CI 1.36–3.30, $P < 0.0001$; multiple allele mismatches: HR 4.73, 95% CI 1.88–11.87, $P < 0.0001$). We also assessed the impact of each locus, excluding patients with multiple allele mismatches. The cumulative incidences of grade III–IV acute GVHD were 9.4% (95% CI 2.3–22.6%) in HLA-A mismatch, 38.9% (16.7–60.8%) in HLA-B mismatch and 19.7% (11.1–30.2%) in HLA-DRB1 mismatch (Figure 1f). In a multivariate analysis, the presence of HLA-B mismatch or HLA-DRB1 mismatch was associated with an increased risk of grade III–IV acute GVHD (HLA-A: HR 0.89, 95% CI 0.29–2.68, $P = 0.830$; HLA-B: HR 4.74, 95% CI 2.00–11.28, $P < 0.0001$; HLA-DRB1: HR 2.16, 95% CI 1.22–3.85, $P = 0.0009$).

To exclude the possibility that HLA antigen mismatch in the HVG direction may affect the incidence of acute GVHD, we performed a subgroup analysis that included patients without HLA antigen mismatch in the HVG direction. In this subgroup analysis, the cumulative incidences of grade II–IV and grade III–IV acute GVHD in the Mismatch group were significantly higher than those in the Match group (grade II–IV 41.3% vs 29.5%, $P = 0.010$; grade III–IV 24.0% vs 9.6%, $P < 0.0001$). In multivariate analyses, the presence of an HLA allele mismatch in the GVH direction was still associated with increased risks of grade II–IV and grade III–IV acute GVHD (HR 1.75, 95% CI 1.30–2.35, $P = 0.0002$; HR 2.39, 95% CI 1.60–3.58, $P < 0.0001$, respectively).

Graft failure

The cumulative incidence of neutrophil engraftment at 60 days was 96.3% (95% CI 95.4–97.0%) in the Match group and 90.4% (95% CI 83.6–94.5%) in the Mismatch group ($P = 0.0044$). Although the presence of HLA antigen mismatch in the HVG direction was associated with an increased risk of graft failure in a multivariate analysis (HR of engraftment 0.79, 95% CI 0.65–0.95, $P = 0.013$), the presence of at least one allele mismatch in the GVH direction was not associated with an increased risk of graft failure.

NRM and relapse

The cumulative incidences of NRM at 2 years were 13.7% (95% CI 12.3–15.3%) in the Match group and 19.2% (95% CI 12.8–26.6%) in the Mismatch group ($P = 0.022$, Figure 2a). A multivariate analysis showed that the presence of at least one allele mismatch was associated with an increased risk of NRM (HR 1.64, 95% CI 1.11–2.41, $P = 0.012$, Table 2). The cohort with a one allele mismatch was associated with an increased risk of NRM, compared with the allele-matched cohort, in a multivariate analysis (one allele mismatch HR 1.83, 95% CI 1.18–2.84, $P = 0.0073$; multiple allele mismatch HR 0.93, 95% CI 0.22–3.94, $P = 0.92$). We also assessed the impact of each locus excluding patients with multiple allele mismatches. The cumulative incidences of 2-year NRM were 29.3% (95% CI 14.2–46.2%) in HLA-A mismatch, 23.5% (6.9–45.8%) in HLA-B mismatch and 15.1% (7.3–25.5%) in HLA-DRB1 mismatch (Figure 2b). In a multivariate analysis, the presence of an HLA-A mismatch was associated with an increased risk of NRM (HLA-A: HR 2.73, 95% CI 1.34–5.54, $P = 0.0056$; HLA-B: HR 2.08, 95% CI 0.74–5.88, $P = 0.17$; HLA-DRB1: HR 1.31, 95% CI 0.69–2.50, $P = 0.41$).

The cumulative incidences of relapse at 2 years were 32.7% (95% CI 30.7–34.7%) in the Match group and 30.1% (95% CI 22.3–38.3%) in the Mismatch group ($P = 0.54$, Figure 2c). The presence of allele mismatch did not affect the incidence of relapse. The cumulative incidences of relapse at 2 years were 22.9% (95% CI 9.7–39.3%) in HLA-A mismatch, 24.2% (6.9–47.0%) in HLA-B mismatch and 35.4% (23.6–47.4%) in HLA-DRB1 mismatch (Figure 2d). There was no statistically significant difference among the four groups.

OS

The probabilities of OS at 2 years after allogeneic HSCT were 61.7% in the Match group and 54.0% in the Mismatch group ($P = 0.0090$, Figure 2e). A multivariate analysis showed that the presence of at least one allele mismatch was associated with an inferior OS (HR 1.43, 95% CI 1.11–1.85, $P = 0.0058$, Table 2). Other factors that were associated with an increased risk of overall mortality were age (⩾50 years), poor performance status (2–4), use of PBSC and high disease risk. Compared with an allele match, the presence of a one allele mismatch was associated with an inferior OS in a multivariate analysis (one allele mismatch: HR 1.46, 95% CI 1.11–1.90, $P = 0.0059$; multiple allele mismatch: HR 1.25, 95% CI 0.59–2.66, $P = 0.56$). We also assessed the impact of each locus excluding patients with multiple allele mismatches. The probabilities of 2-year OS were 57.6% (95% CI 38.0–72.9%) in HLA-A mismatch, 55.0% (29.8–74.5%) in HLA-B mismatch and 51.0% (37.7–62.9%) in HLA-DRB1 mismatch (Figure 2f). In a multivariate analysis, patients with an HLA-A or HLA-DRB1 mismatch tended to have a worse OS (HLA-A: HR 1.51, 95% CI 0.93–2.45, $P = 0.094$; HLA-B: HR 1.49, 95% CI 0.77–2.87, $P = 0.24$; HLA-DRB1: HR 1.43, 95% CI 1.00–2.03, $P = 0.050$).

DISCUSSION

In this study, we have demonstrated for the first time that HLA allele mismatch in the GVH direction in related HSCT was associated with increased risks of acute GVHD and NRM, which led to a poor OS. No previous study has assessed the impact of HLA allele mismatch in the related HSCT setting, as it is generally believed that HLA is completely matched in serologically HLA-matched related HSCT, especially in sibling donors if the parental HLA types are missing. Our result demonstrated that there is a possibility of HLA allele mismatch even in serologically matched related HSCT (5.6% in an HLA serologically matched donor/recipient combination). Our current result in related HSCT was consistent with the findings in unrelated HSCT, which suggests that serological HLA typing is insufficient to assess HLA

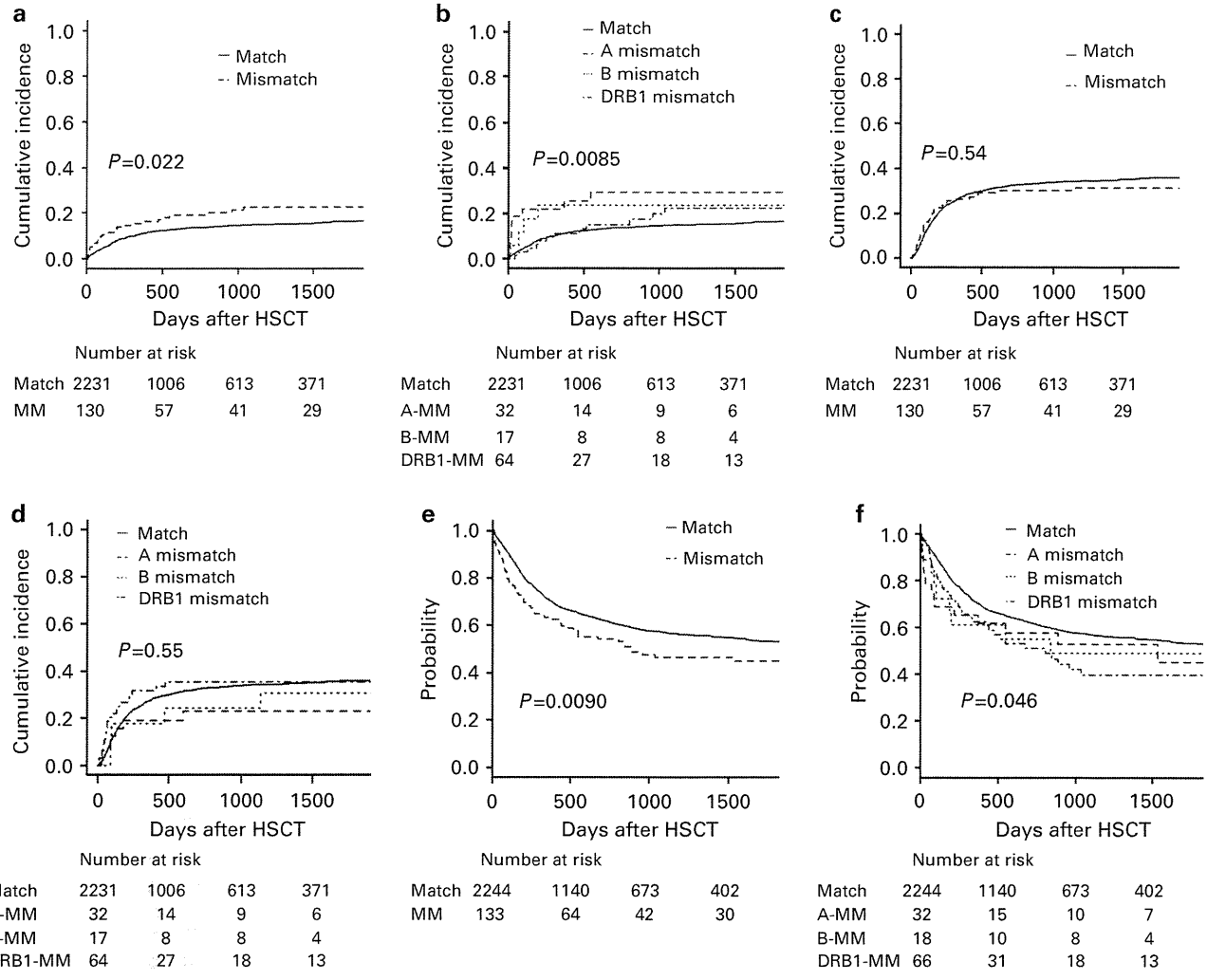

Figure 2. NRM, relapse and OS. Cumulative incidence of NRM grouped according to (**a**) allele mismatch and (**b**) locus of allele mismatch. Cumulative incidence of relapse grouped according to (**c**) allele mismatch and (**d**) locus of allele mismatch. The probability of OS grouped according to (**e**) allele mismatch and (**f**) locus of allele mismatches.

compatibility.[1,2] Therefore, HLA typing at high resolution (allele-level typing) should be done in all patients, including matched related transplants. The presence of HLA allele mismatch in the GVH direction should be taken into consideration when selecting a stem cell donor and determining the intensity of GVHD prophylaxis. In this study, the presence of HLA-B allele mismatch was associated with a significantly increased risk of severe acute GVHD. The significant impact of HLA-B antigen mismatch seemed to be similar to that in a previous report from Japan that assessed the impact of HLA-one antigen mismatch in related HSCT.[10] An important limitation here is the lack of HLA-C information in our current database. The frequency of an HLA-C mismatch in an HLA-B-mismatched group was shown to be substantially higher than those in the HLA-A and -DR antigen-mismatched groups.[15,16] In our database, information about the HLA-C allele was available in only 1152 cases (48.5%). Therefore, the impact of HLA-B and -C allele mismatch in related HSCT should be clarified in analyses using larger cohorts with complete HLA-C allele information.

One important issue in this study was the result that the use of PBSC was significantly associated with an increased risk of grade III–IV acute GVHD (HR 1.85, 95% CI 1.41–2.44, $P < 0.0001$, Table 2), which led to an increased risk of NRM and overall mortality. Therefore optimization of GVHD prophylaxis is particularly important in patients who receive PBSC to improve the clinical outcome.

A major limitation of this study is the small sample size in the Mismatch group, which is largely due to the fact that we included patients for whom data on the HLA allele were available. Because of the limited number of cases with HLA allele mismatch, it was difficult to assess the effect of the type of GVHD prophylaxis, such as the use of T-cell depletion, on the incidence of acute GVHD. Although the use of T-cell depletion seems to reduce the risk of GVHD, this association was not statistically significant (data not shown). This may have been due to the limited number of cases with T-cell depletion in this cohort.

In conclusion, our findings suggest that the presence of an HLA allele mismatch in serologically matched related HSCT was associated with increased risks of acute GVHD and NRM, which led to a poor OS. Therefore, HLA typing at high resolution (allele-level typing) should be done in all patients, including matched related transplants. The optimal GVHD prophylaxis in patients who receive stem cells from an HLA allele-mismatched related donor should be explored prospectively.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Salvage Allogeneic Stem Cell Transplantation in Patients with Pediatric Myelodysplastic Syndrome and Myeloproliferative Neoplasms

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Background. Allogeneic hematopoietic stem cell transplantation (HSCT) is the only curable approach for myelodysplastic syndrome (MDS) and myeloproliferative neoplasms (MPN); however, the event-free survival rate of patients with pediatric MDS and MPN is still only approximately 60%. Although salvage HSCT is the only curative approach for patients with the failure of previous HSCT, its safety and efficacy have yet to be determined. ***Procedures.*** We retrospectively analyzed 51 pediatric MDS or MPN who received salvage HSCT for relapse or graft failure following HSCT using registry data of the Japan Society for Hematopoietic Cell Transplantation. The indications used for salvage HSCT were relapse in 22 patients and graft failure in 29 patients. ***Results.*** The overall survival (OS) rate for salvage HSCT in relapsed patients was 49.0 ± 10.8% at 3 years. The cumulative incidence of relapse following salvage HSCT was 29.8 ± 10.7% at 3 years, whereas the incidence of non-relapse mortality (NRM) was 28.6 ± 10.2%. No significant differences were observed in the OS after salvage HSCT between disease types. Twenty-four of 29 patients who received salvage HSCT for graft failure achieved engraftment, resulting in an engraftment probability of 81.5 ± 8.0% on day 100. The OS rate after salvage HSCT for graft failure was 56.8 ± 9.6% at 3 years. ***Conclusions.*** Second HSCT should be considered as a valuable option for the patients with relapse and graft failure in patients with pediatric MDS or MPN after HSCT, but high NRM is an important issue that needs to be addressed. Pediatr Blood Cancer 2014;61:1860–1866. © 2014 Wiley Periodicals, Inc.

Key words: allogeneic transplantation; children; graft failure; myelodysplastic syndrome; relapse

INTRODUCTION

Myelodysplastic syndrome (MDS) and myeloproliferative neoplasms (MPN) are a clonal disorder of hematopoietic stem cells, and allogeneic hematopoietic stem cell transplantation (HSCT) is the only curative approach for pediatric MDS and MPN. Although recent studies have reported improved outcomes, the event-free survival (EFS) rate is still approximately 60% [1–9].

The main cause of failure after allogeneic HSCT for malignant diseases is generally accepted to be relapsing; however, previous studies demonstrated that transplantation-related mortality was as frequent an event as relapse in patients with pediatric MDS/MPN [2]. Thus, optimizing the conditioning regimen, including a reduction in the conditioning intensity, is required to improve the outcome of HSCT in patients with pediatric MDS and MPN, as shown by recent studies on adult MDS [10,11]. However, excessive reductions in the conditioning intensity may potentially cause an increase in the incidence of relapse or probability of graft failure. Although salvage HSCT is required for both these events, the number of patients investigated has been limited owing to rarity of this situation [5,12]. Furthermore, the efficacy and safety of salvage allogeneic HSCT have yet to be determined in detail.

We performed a retrospective analysis of 51 patients who received a second course of allogeneic HSCT in an attempt to salvage events after the first allogeneic HSCT in the present study to obtain fundamental information for establishing a standard therapeutic strategy for patients with pediatric MDS and MPN.

METHODS

Patients and Transplantations

This study was approved by the Institutional Ethics Committee of Saitama Children's Medical Center. Of 550 pediatric MDS/MPN patients in registry data of the Japan Society for Hematopoietic Cell Transplantation (JSHCT) [13], a total 51 cases were analyzed (Tables I and II). Patients were selected according to the following criteria: (1) diagnosed with MDS or MPN (the disease type was based on the FAB classification); (2) salvage allogeneic HSCT was performed for graft failure or relapse after the first allogeneic HSCT; (3) aged 15 years or younger at the time of salvage HSCT; and (4) both the first and salvage HSCTs were performed between 1982 and 2011.

Myeloablative conditioning was defined as total body irradiation (TBI) of 8 Gy or higher or the administration of busulfan (BU) at a

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Conflict of interest: Nothing to declare.

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TABLE I. Data of Patients With Second HSCT for Relapse

UPN	Age[a]	Disease type	Donor and HLA disparity[b]		Conditioning		Time to relapse (days)	Interval to second HSCT (days)	Outcome after second HSCT		
			First HSCT	Second HSCT	First HSCT	Second HSCT			Relapse	Alive/dead	Follow-up (days)
4	7	RAEBt	RD(match)/BM	RD(match)/BM	BU	TBI	266	371	Yes	Dead	1,089
5	3	JMML	UD(1MM)/BM	CB(1MM)	BU	TBI	183	238	No	Dead	89
9	12	RAEB	RD(match)/BM	UD(match)/BM	BU	RIC	726	910	No	Alive	383+
11	0	JMML	UD(match)/BM	CB(1MM)	RIC	RIC	0	117	Yes	Dead	437
12	9	RAEBt	RD(match)/BM	UD(1MM)/BM	TBI	RIC	76	398	No	Dead	20
19	4	RAEBt	RD(match)/BM	RD(match)/BM	TBI	BU	323	392	Yes	Dead	712
21	1	JMML	RD(match)/BM	RD(match)/BM	BU	TBI	148	380	No	Alive	1,359+
24	5	JMML	RD(match)/BM	CB(1MM)	BU	TBI	774	975	No	Dead	6
32	8	RAEBt	RD(match)/BM	RD(match)/PB	BU	RIC	872	1,286	Yes	Alive	4,810+
34	6	JMML	RD(match)/BM	CB(2MM)	BU	TBI	95	212	Yes	Dead	1,226
37	2	NA	RD(match)/BM	CB(1MM)	BU	TBI	240	611	No	Dead	40
38	8	RAEBt	RD(match)/BM	RD(match)/BM	BU	BU	85	121	No	Alive	3,366+
39	2	CMMoL	RD(match)/BM	RD(match)/BM	TBI	BU	188	448	Yes	Dead	92
41	0	JMML	RD(match)/BM	RD(1MM)/BM	BU	BU	90	238	No	Dead	52
43	2	JMML	RD(match)/BM	CB(1MM)	BU	TBI	125	466	No	Alive	3,339+
44	1	JMML	RD(match)/BM	RD(1MM)/BM	BU	TBI	308	378	No	Alive	4,581+
46	10	RAEBt	UD(match)/BM	UD(match)/BM	BU	TBI	75	466	No	Dead	19
49	7	NA	UD(match)/BM	UD(match)/BM	BU	TBI	1,656	1,835	Yes	Dead	2,113
51	2	JMML	RD(1MM)/BM	RD(match)/PB	TBI	BU	163	260	No	Alive	2,062+
52	0	JMML	RD(match)/BM	RD(match)/PB	BU	TBI	92	250	No	Alive	5,386+
58	2	JMML	CB(1MM)	CB(1MM)	TBI	BU	190	330	No	Dead	24
59	3	CMMoL	CB(match)	RD(match)/BM	BU	TBI	14	108	No	Alive	3,858+

[a]Age at diagnosis (years). [b]MM indicates the number of serological mismatch. RAEB, refractory anemia with excess blasts; RAEBt, RAEB in transformation; JMML, juvenile myelomonocytic leukemia; NA, data not available; RD, related donor; UD, unrelated donor; CB, cord blood; BM, bone marrow; PB, peripheral blood stem cell; BU, myeloablative with busulfan; TBI, myeloablative with total body irradiation.

dose higher than 8 mg/kg. All other regimens were considered as reduced intensity (non-myeloablative) conditioning [14]. Engraftment was defined as the first day of 3 consecutive days with an absolute neutrophil count of 500/μL or greater.

Statistical Analysis

The overall survival (OS) probability was calculated using Kaplan–Meier estimates. The incidence of engraftment and non-relapse mortality (NRM) were expressed as cumulative incidence curves and were used to adjust for death before engraftment and relapse for competing risks, respectively. Univariate analyses of OS were performed using the log-rank test. All statistical analyses were performed with R software 2.13.0.

RESULTS

Patients

The characteristics of the 51 patients and HSCT analyzed in the present study are shown in Tables I and II. Indications for salvage HSCT were relapse for 22 patients (Table I) and graft failure for 29 patients (Table II). The median age at diagnosis was 3 years (range: 0–12) for the relapsed patients and 1 year (0–15) for the patients with graft failure. The median follow-up period in surviving patients was 5.0 years (range: 1.0–14.8) after salvage HSCT.

The estimated OS probability and standard error 3 years after salvage HSCT was 53.2 ± 7.2% for all 51 patients. Of 22 HSCT for relapse, nine were performed in 2000 or before, and 22 were performed after 2000. The OS was not statistically different between each era, 44.4 ± 16.6% and 52.7 ± 14.1%, respectively ($P = 0.65$). Seven HSCT for graft failure were performed in 2000 or before, and 22 were performed after 2000. The OS was also not different statistically, 42.9 ± 18.7% and 62.0 ± 10.8%, respectively ($P = 0.33$).

Salvage HSCT for Relapse

The OS rate after salvage HSCT for patients with relapsed MDS (n = 22) was 49.0 ± 10.8% at 3 years (Fig. 1A). Seven patients relapsed following salvage HSCT after a median time of 1.3 years, which resulted in a cumulative incidence of relapse of 29.8 ± 10.7% and NRM incidence of 28.6 ± 10.2% at 3 years. Our cohort included 11 cases of JMML and 7 cases of advanced MDS (RAEB or RAEBt), and the survival curves of these two disease types were superimposed onto each other (Fig. 1B).

Figure 1C shows relationship between the two HSCT conditioning regimens and outcomes. Sixteen of 22 patients relapsed following BU-based myeloablative HSCT, and TBI-based myeloablative salvage HSCT was performed on 12 of these patients, three of whom relapsed and four died without evidence of relapse. Salvage HSCT with non-myeloablative conditioning was performed on four patients, two of whom relapsed after salvage HSCT and one died before relapse.

Eight patients received salvage HSCT from the same donor as the first HSCT, four of whom are still alive without disease.

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TABLE II. Data of Patients With Second HSCT for Graft Failure

			Donor and HLA disparity[b]		Conditioning				Outcome after second HSCT		
UPN	Age[a]	Disease type	First HSCT	Second HSCT	First HSCT	Second HSCT	Interval to second HSCT (days)	Engraftment	Relapse	Alive/dead	Follow-up (days)
1	0	JMML	CB(1MM)	UD(1MM)/BM	BU	Unknown	204	Yes	No	Dead	715
3	2	JMML	UD(1MM)/BM	CB(1MM)	BU	RIC	35	Yes	Yes	Dead	246
7	5	JMML	CB(2MM)	UD(match)/BM	BU	TBI	213	Yes	Yes	Dead	240
8	2	JMML	RD(1MM)/BM	RD(1MM)/BM	TBI	BU	168	No	No	Dead	275
10	1	JMML	CB(1MM)	CB(1MM)	BU	RIC	38	Yes	No	Alive	2,137+
13	1	RA	RD(match)/BM	RD(match)/PB	RIC	RIC	522	Yes	No	Alive	403+
15	5	JMML	CB(1MM)	CB(1MM)	BU	RIC	25	No	Yes	Alive	1,194+
18	5	RA	RD(match)/BM	RD(match)/BM	RIC	BU	111	No	Yes	Dead	1,462
20	1	JMML	UD(match)/BM	CB(1MM)	BU	RIC	65	Yes	No	Alive	1,405+
25	7	RA	CB(1MM)	RD(2MM)/PB	RIC	RIC	34	Yes	No	Dead	31
26	5	RA	RD(3MM)/BM	RD(3MM)/PB	TBI	RIC	42	Yes	No	Dead	99
27	1	JMML	RD(1MM)/PB	RD(1MM)/PB	BU	RIC	28	Yes	Yes	Alive	3,898+
28	0	JMML	CB(1MM)	RD(1MM)/BM	BU	RIC	33	Yes	No	Alive	434+
29	0	CMMoL	RD(match)/BM	RD(match)/BM	TBI	RIC	1,076	Yes	Yes	Dead	569
30	9	RA	RD(match)/BM	RD(match)/BM	BU	TBI	200	Yes	No	Alive	2,135+
31	7	JMML	RD(3MM)/PB	RD(3MM)/PB	TBI	BU	70	Yes	Yes	Dead	232
33	0	JMML	UD(match)/BM	UD(match)/BM	BU	TBI	238	Yes	No	Alive	1,167+
40	2	JMML	CB(match)	RD(2MM)/BM	TBI	RIC	54	No	No	Dead	21
42	0	JMML	UD(match)/BM	RD(2MM)/PB	TBI	BU	46	Yes	No	Dead	1,459
45	15	RAEBt	UD(match)/BM	CB(1MM)	RIC	RIC	62	Yes	Yes	Alive	425+
47	3	JMML	CB(match)	RD(1MM)/BM	BU	RIC	75	Yes	No	Alive	1,708+
48	0	JMML	UD(match)/BM	CB(match)	BU	RIC	49	Yes	No	Alive	1,961+
50	1	JMML	UD(match)/BM	UD(match)/BM	TBI	BU	140	No	No	Dead	42
53	0	JMML	UD(1MM)/BM	CB(1MM)	BU	RIC	48	Yes	No	Alive	447+
54	2	JMML	CB(match)	UD(match)/BM	BU	BU	281	Yes	Yes	Dead	149
55	NA	JMML	UD(1MM)/BM	CB(1MM)	BU	TBI	49	Yes	No	Alive	1,197+
56	1	JMML	RD(match)/BM	RD(match)/PB	BU	TBI	144	Yes	No	Alive	3,944+
57	0	NA	CB(1MM)	CB(2MM)	BU	TBI	456	Yes	Yes	Dead	78
62	0	JMML	UD(match)/BM	CB(1MM)	BU	RIC	30	Yes	No	Alive	1,648+

[a]Age at diagnosis (years). [b]MM indicates the number of serological mismatch. RA, refractory anemia; RAEBt, refractory anemia with excess blasts in transformation; JMML, juvenile myelomonocytic leukemia; NA, data not available; RD, related donor; UD, unrelated donor; CB, cord blood; BM, bone marrow; PB, peripheral blood stem cell; BU, myeloablative with busulfan; TBI, myeloablative with total body irradiation.