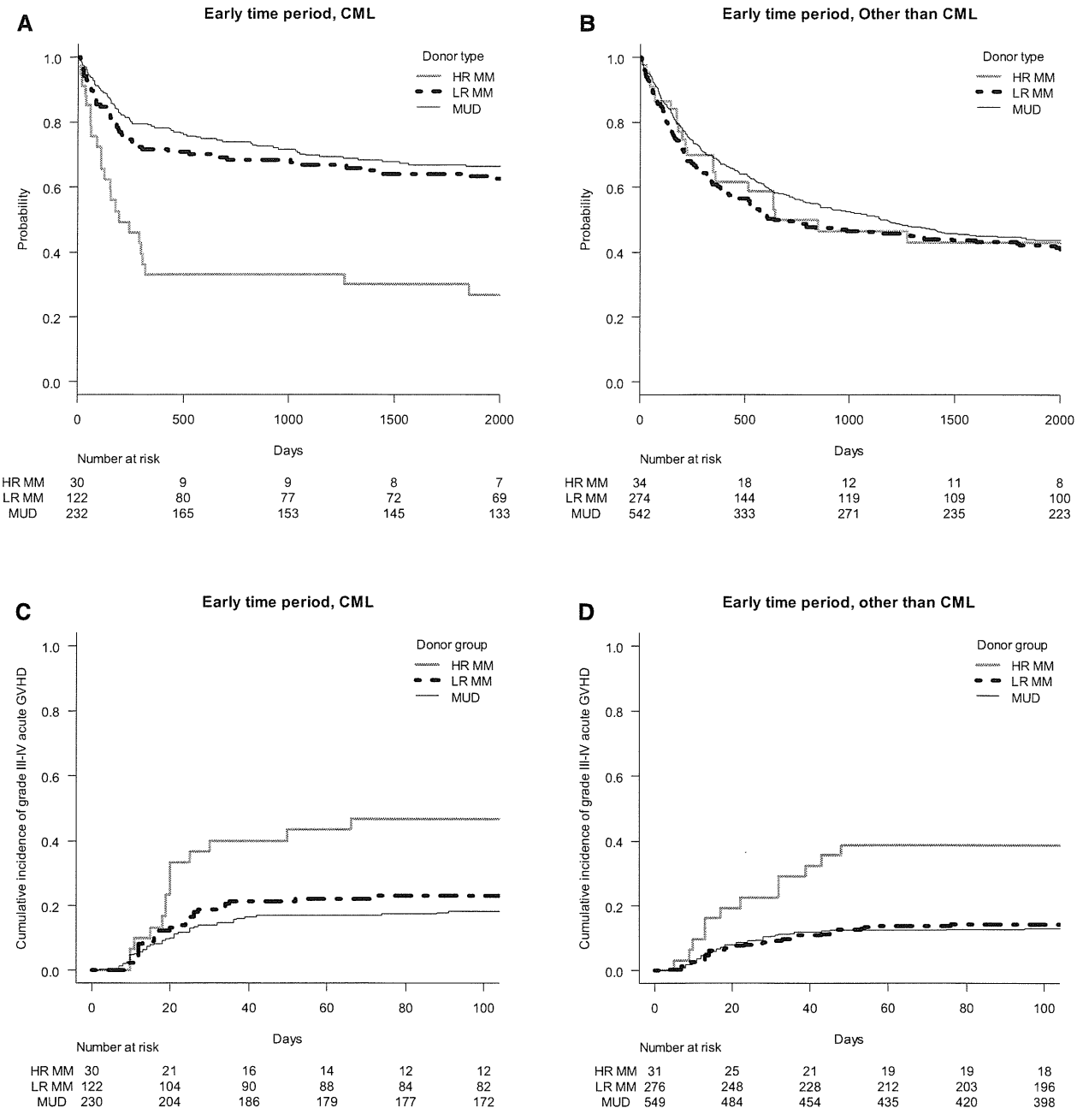

Figure 3. Adjusted overall survival (A,B) and the cumulative incidence of grade III to IV acute GVHD (C,D) grouped according to the underlying disease in the early time period. CML, chronic myelogenous leukemia; HR-MM, high-risk mismatch; LR-MM, low-risk mismatch; MUD, matched unrelated donor.

grade III to IV acute GVHD between the HR-MM and LR-MM groups was not statistically significant (HR, 1.06; 95% CI, .58 to 1.93; $P = .85$ and HR, .40; 95% CI; .10 to 1.64; $P = .21$, respectively). The presence of LR-MM significantly adversely affected the incidence of grade III to IV acute GVHD in the mid and late periods (HR, .64; 95% CI, .46 to .89; $P = .008$ and HR, .56; 95% CI, .39 to .80; $P = .0014$, respectively, for the MUD group).

Similarly, the presence of HR-MM significantly affected the incidence of grade II to IV acute GVHD compared with LR-MM only in the early time period (HR, 1.53; 95% CI, 1.05 to 2.24; $P = .028$), and not in the mid and late periods (HR, .92; 95% CI, .61 to 1.37; $P = .67$ and HR, .79; 95% CI, .40 to 1.58; $P = .51$, respectively).

Overall Survival

After adjusting for recipient age, recipient sex, presence of ABO-major mismatch, disease, disease risk, and GVHD prophylaxis, we again confirmed that survival in the HR-MM group was significantly inferior to that in the LR-MM group (HR, 1.46; 95% CI, 1.06 to 2.01; $P = .019$) in the early time period, whereas there was no significant difference between the MUD and LR-MM groups (HR, .86; 95% CI, .73 to 1.01; $P = .063$) (Table 3). On the other hand, the difference in survival between the HR-MM and LR-MM groups was not statistically significant in the mid and late time periods (HR, 1.06; 95% CI, .75 to 1.48; $P = .75$ and HR, .82; 95% CI, .42 to 1.62; $P = .58$, respectively). The difference in survival between the MUD and LR-MM groups was consistent among

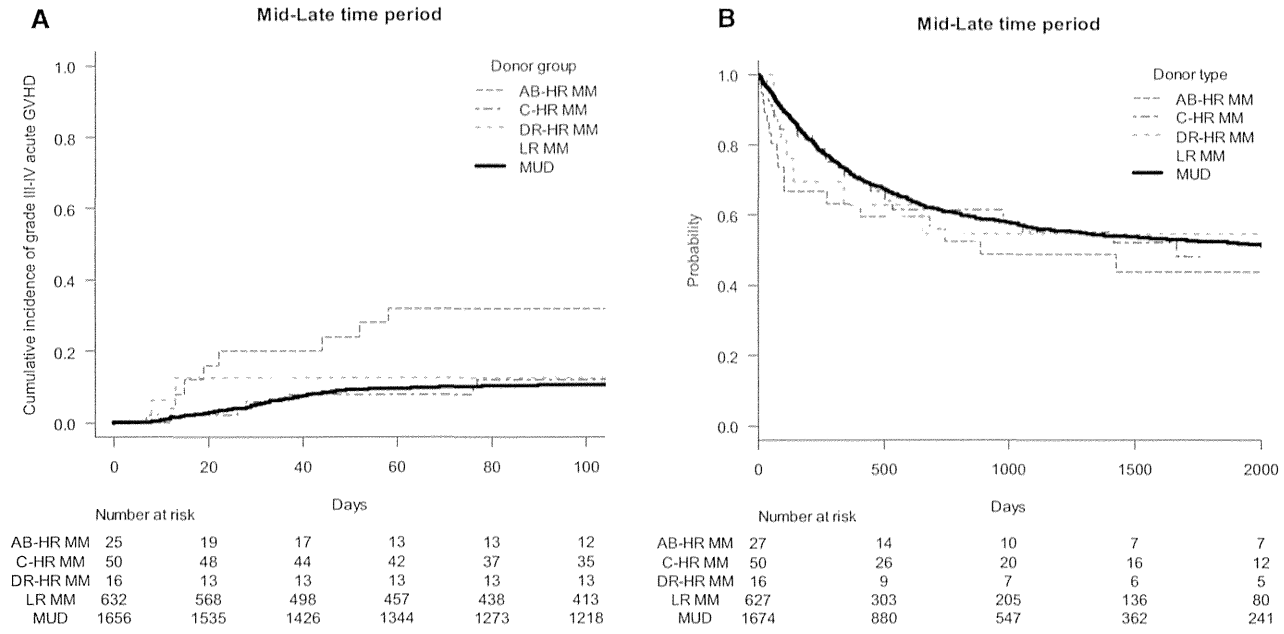

Figure 4. The cumulative incidence of grade III to IV acute GVHD (A) and adjusted overall survival (B) grouped according to the HLA mismatch loci between the donor and recipient in the mid or late time period. AB-HR MM, high-risk mismatch at the HLA-A or -B locus; C-HR MM, high-risk mismatch at the HLA-C locus; DR-HR MM, high-risk mismatch at the DRB1 locus; LR-MM, low-risk mismatch; MUD, matched unrelated donor.

the 3 time periods but statistically significant only in the mid period (HR, .83; 95% CI, .69 to .98; $P = .032$). Figure 2 shows the overall survival curves grouped according to the HLA-mismatch groups in each time period, adjusted for other significant factors by the mean of covariates method.

Disease-specific Effects of HR-MM in the Early Period

The number of patients with CML was significantly higher in the early period than in the mid and late periods. Therefore, we evaluated the disease-specific impact of HR-MM in the early period. As shown in Figures 3A and B, the presence of HR-MM had an adverse impact on overall survival only in patients with CML, although HR-MM showed a similar adverse impact on the incidence of grade III to IV acute GVHD regardless of the underlying disease (Figure 3C, D). Of the 24 CML patients who died after HSCT with HR-MM, 23 died without relapse of CML, and 10 of these patients died without grade III to IV acute GVHD.

Impact of HR-MM at Each Locus

To evaluate the impact of HR-MM at each locus in the mid and early periods, we combined the 2 periods together to

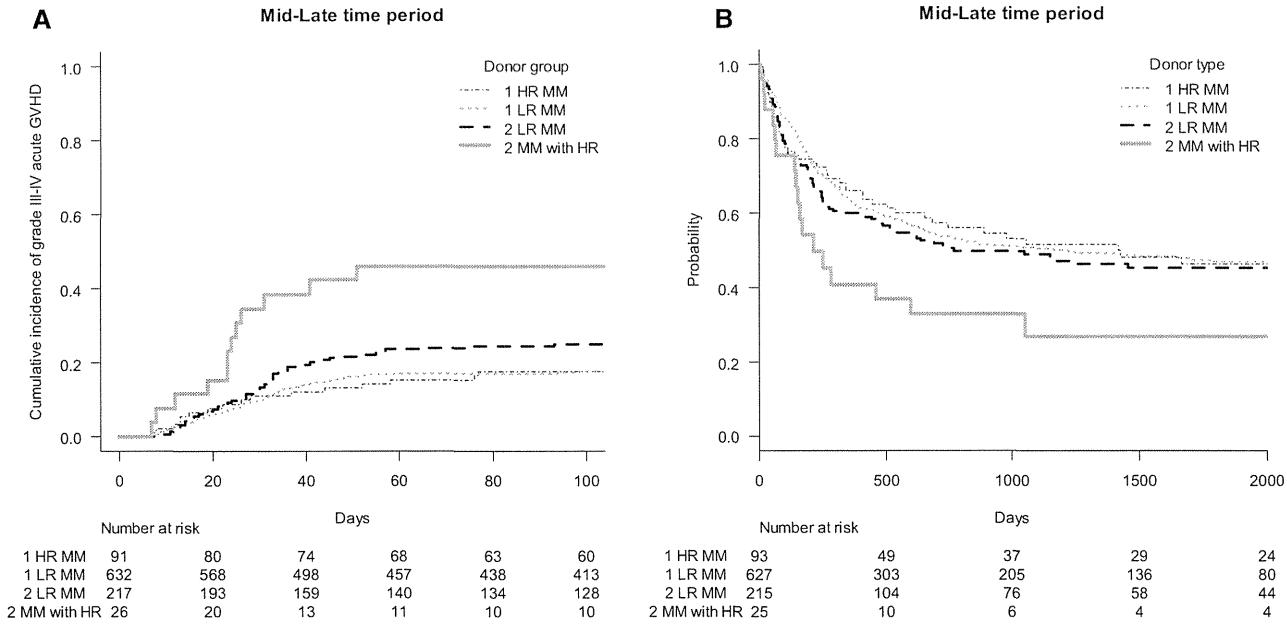

Figure 5. The cumulative incidence of grade III to IV acute GVHD (A) and adjusted overall survival (B) grouped according to the HLA mismatch between the donor and recipient in the mid or late time period. 1HR-MM, 1 high-risk mismatch; 1LR-MM, 1 low-risk mismatch; 2LR-MM, 2 low-risk mismatches; 2MM with HR, 2 allele mismatches including at least 1 HR-MM.

increase statistical power because the impact of HR-MM on acute GVHD and survival tended to be similar in these 2 time periods. The presence of HR-MMs at the HLA-A/B (HLA-A or -B), HLA-C, and HLA-DRB1 loci was not associated with significantly different survival compared with the LR-MM group (HR, 1.23; 95% CI, .76 to 1.98; $P = .41$; HR, .96; 95% CI, .65 to 1.44; $P = .86$; and HR, .95; 95% CI, .45 to 2.02; $P = .89$, respectively. Figure 4A). However, the incidence of grade III to IV acute GVHD was higher in patients who had HR-MM at the HLA-A/B locus than in those with LR-MM, although this difference was not statistically significant (HR, 1.78; 95% CI, .86 to 3.66; $P = .12$; HR, .63; 95% CI, .28 to 1.41; $P = .26$; and HR, .69; 95% CI, .15 to 3.12; $P = .63$ for HLA-A/B, HLA-C, and HLA-DRB1, respectively.) (Figure 4B).

Comparison of One HR-MM and Two LR-MMs

To evaluate whether a donor with 1 HR-MM or a donor with 2 LR-MMs should be preferred, we added patients with 2 LR-MMs and those with 2 allele mismatches including at least 1 HR-MM to the dataset, and we compared the outcome of HSCT from these donors with that of HSCT from a donor with 1 LR-MM as a reference in the combined mid and late periods.

The presence of 2 LR-MMs was associated with a significantly higher incidence of grade III to IV acute GVHD (HR, 1.44; 95% CI, 1.04 to 2.00; $P = .030$), but the impact of 1 HR-MM was not statistically significant (HR, .94; 95% CI, .56 to 1.59; $P = .83$) (Figure 5A). However, the impact of 2 LR-MMs was not associated with inferior survival. The HR for survival of 1 HR-MM and 2 LR-MMs were 1.05 (95% CI, .78 to 1.42; $P = .75$) and 1.12 (95% CI, .90 to 1.39; $P = .33$), respectively (Figure 5B).

On the other hand, the presence of 2 allele mismatches including at least 1 HR-MM was associated with an extremely poor outcome; HR, 3.61 (95% CI, 1.96 to 6.66; $P < .001$) for grade III to IV acute GVHD and HR, 2.02 (95% CI, 1.25 to 3.26; $P = .0040$) for overall survival. These results suggested that the impact of HR-MM may change according to the presence or absence of an additional allele mismatch. In fact, there was a statistically significant interaction between the presence of HR-MM and the presence of an additional allele mismatch ($P = .020$). The likelihood ratio test revealed that the prognostic value of Fine and Gray's proportional hazards model for acute GVHD was significantly improved by adding the interaction term to the model ($P = .024$).

DISCUSSION

In this study, we reevaluated the clinical impact of HR-MMs in unrelated HSCT. We confirmed that the presence of HR-MMs was associated with a significantly higher incidence of grade III to IV acute GVHD and significantly inferior survival in the early transplantation time period. However, in the mid and late periods, ie, after 2002, there was no statistically significant difference in overall survival or the incidence of grade III to IV acute GVHD between patients with HR-MMs and those with LR-MMs. The methods used for the statistical analyses were somewhat different than those in a previous study, but this is not the major reason for the different results, as the significant impact of HR-MMs on survival and acute GVHD was reproduced in the early time period. Another possible explanation is a bias caused by the availability of information about HR-MMs. After the publication of a paper that reported the importance of HR-MM, physicians may have tended to intensify prophylaxis against GVHD in unrelated HSCT with HR-MMs, and, thereby, the impact of HR-MMs might have become less significant. However, this is not the case because the impact of HR-MMs was already not apparent in the mid time period, before the paper was published. We also considered that the difference in the underlying disease might have influenced the effect of HR-MMs. The proportion of patients with CML decreased from 30.7% in the early period to 10.4% and 3.6% in the mid and late periods, respectively. Therefore, we analyzed the impact of HR-MMs grouped according to the underlying disease in the early period. The effect of HR-MMs on survival was observed only in patients with CML (Figure 3A,B). However, HR-MMs had an adverse effect on the incidence of grade III to IV acute GVHD regardless of the underlying disease (Figure 3C,D). Therefore, the different effects of HR-MMs on the incidence of grade III to IV acute GVHD among the time periods could not be explained solely by the underlying diseases. We could not clarify the reason for this different effect, but the changes in the transplantation procedure, including prophylaxis against GVHD, might have reduced the clinical impact of HR-MM. In fact, the incidence of grade III to IV acute GVHD decreased from 42.6%, 16.8%, and 14.5% in the HR-MM, LR-MM, and MUD groups, respectively, in the early time period to 17.6%, 17.7%, and 10.6% in the mid or late period. Improved survival in patients who developed severe acute GVHD might also reduce the effect of HR-MMS on survival. The 1-year survival in patients who developed grade III to IV acute GVHD improved from 32.1% in the early period to 44.4% in the mid and late time periods. This change may have resulted from the progress in supportive care, including strategies against fungal or viral infections.

Another important finding is that the impact of HR-MM was significantly enhanced by the presence of an additional allele mismatch in the mid and late time periods. This fact may be explained by a hypothesis that the HR-MM biologically increases the graft-versus-host (GVH) reaction, but the recent improvement in GVHD prophylaxis has masked its effect, if HR-MM exists as a single allele mismatch, whereas the adverse impact of HR-MM is not suppressed even by recent methods of GVHD prophylaxis when an additional allele mismatch is present. Based on these findings, interaction terms should be incorporated into the statistical model when the impact of HR-MMs is analyzed in datasets that include HSCT with multiple allele mismatches.

A major limitation of this study is the small number of patients with HR-MMs, especially in the late time period. We cannot deny the possibility that an important effect of HR-MMs might be overlooked because of the poor statistical power. The lack of a significant difference in the incidence of grade III to IV acute GVHD between unrelated HSCT with HR-MMs at the HLA-A/B locus and HSCT with LR-MM should be interpreted with caution, because of the small number of patients. Furthermore, it was impossible to evaluate the effect of each mismatch combination, as the number of patients with each mismatch combination was most often fewer than 10. HR-MMs associated with at least a 20% incidence of grade III to IV acute GVHD in the mid and late periods included A*0206-A*0201 (4 of 14), A*0206-A*0207 (3 of 4), B*1501-B*1507 (1 of 1), C*0801-C*0303 (4 of 15), and C*1402-C*0304 (1 of 5), but the number of patients in each pair was too small to draw any definitive conclusions.

When we consider the impact of HR-MMs, especially at the HLA-C locus, we should also consider the effect of a killer immunoglobulin-like receptor ligand (KIR) mismatch [13,14]. Among the 50 patients with HR-MMs at the HLA-C locus in the mid and late periods, 20 had a KIR mismatch in the GVH direction, whereas 30 did not. The incidence of grade III to IV acute GVHD was 5% and 16.7%, respectively, but this

difference was not statistically significant ($P = .24$). The incidence of grade III to IV acute GVHD in the 21 patients who had LR-MMs and a KIR mismatch in the GVH direction was 15.0%. We could not conclude that a KIR mismatch had an impact in this study because of the small number of patients with a KIR mismatch in the GVH direction.

We should note that the results of the current study are applicable to patients who receive bone marrow graft after a myeloablative conditioning regimen. The impact of HR-MMs may change according to the stem cell source or the conditioning regimen. Therefore, further analyses are required to evaluate the impact of HR-MMs in peripheral blood stem cell transplantation and reduced-intensity conditioning transplantation.

In conclusion, this retrospective study revealed that the clinical impact of HR-MMs became less significant after 2002. Although HR-MMs may have a biological impact, their effect may be controlled by recent methods for GVHD prophylaxis when they exist as a single allele mismatch. It may still be prudent to avoid a donor with HR-MMs, especially at the HLA-A or -B locus, if a donor with the other mismatch combination is available. However, in the absence of MUD or an unrelated donor with a LR-MM, a donor with a single HR-MM could be a viable option for unrelated HSCT, and it is preferred over a donor with 2 LR-MMs. In addition, we should be aware that the clinical impact of risk factors may change over time periods, and therefore, we should repeatedly confirm the validity of risk factors.

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Continuing increased risk of oral/esophageal cancer after allogeneic hematopoietic stem cell transplantation in adults in association with chronic graft-versus-host disease

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Background: The number of long-term survivors after hematopoietic stem cell transplantation (HSCT) showed steady increase in the past two decades. Second malignancies after HSCT are a devastating late complication. We analyzed the incidence of, risk compared with that in the general population, and risk factors for secondary solid cancers.

Patients and methods: Patients were 17 545 adult recipients of a first allogeneic stem cell transplantation between 1990 and 2007 in Japan. Risks of developing secondary solid tumors were compared with general population by using standard incidence ratios (SIRs).

Results: Two-hundred sixty-nine secondary solid cancers were identified. The cumulative incidence was 0.7% [95% confidence interval (CI), 0.6%–0.9%] at 5 years and 1.7% (95% CI, 1.4%–1.9%) at 10 years after transplant. The risk was significantly higher than that in the general population (SIR = 1.8, 95% CI, 1.5–2.0). Risk was higher for oral cancer (SIR = 15.7, 95% CI, 12.1–20.1), esophageal cancer (SIR = 8.5, 95% CI, 6.1–11.5), colon cancer (SIR = 1.9, 95% CI, 1.2–2.7), skin cancer (SIR = 7.2, 95% CI, 3.9–12.4), and brain/nervous system cancer (SIR = 4.1, 95% CI, 1.6–8.4). The risk of developing oral, esophageal, or skin cancer was higher at all times after 1-year post-transplant. Extensive-type chronic graft-versus-host disease (GVHD) was a significant risk factor for the development of all solid tumors (RR = 1.8, $P < 0.001$), as well as for oral (RR = 2.9, $P < 0.001$) and esophageal (RR = 5.3, $P < 0.001$) cancers. Limited-type chronic GVHD was an independent risk factor for skin cancers (RR = 5.8, $P = 0.016$).

Conclusion: Recipients of allogeneic HSCT had a significantly higher ~2-fold risk of developing secondary solid cancers than the general population. Lifelong screening for high-risk organ sites, especially oral or esophageal cancers, is important for recipients with active, or a history of, chronic GVHD.

Key words: secondary solid cancers, late effect, hematopoietic stem cell transplantation

introduction

Hematopoietic stem cell transplantation (HSCT) is a curative treatment of choice for malignant and non-malignant hematological disorders [1]. The annual number of allogeneic HSCT has increased steadily over the past three decades worldwide [2–6]. Progress in transplant procedures in addition to this steady increase in the number of HSCT procedures worldwide has contributed to an increase in the number of long-term survivors.

Secondary malignancies, including new solid cancers, are an important cause of late mortality. Several studies have reported that survivors of HSCT have a 2–3-fold increased risk of

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developing new solid cancers compared with an age-, sex-, region-, and calendar-year-adjusted population and the risk among long-term survivors ranges from 1% to 6% at 10 years after transplantation [7–14]. Identified risk factors include exposure to radiation as a part of the conditioning regimen and chronic graft-versus-host disease (GVHD), and the latter has been shown to be strongly correlated with the development of squamous cell carcinoma [8, 10, 12, 15–17]. However, a recent long-term follow-up analysis of patients who were transplanted after myeloablative doses of busulfan and cyclophosphamide without total body irradiation (TBI) found a similar increased incidence of 0.6% at 5 years and 1.2% at 10 years after transplantation [13]. We conducted a nationwide, retrospective cohort study with a large and different cohort from those used in previous reports from North America and Europe, to determine the incidence and risks of developing secondary solid cancers.

methods

data source and collection of data

The recipient clinical data were collected by the Japan Society for Hematopoietic Cell Transplantation (JSHCT) using the Transplant Registry Unified Management Program, as described previously [18]. The JSHCT collect recipients' baseline, disease, transplant, and transplant outcome information who received HSCT in the previous year. Patient information regarding survival, disease status, and long-term complications including chronic GVHD and second malignancies are renewed annually. This study was approved by the data management committee of the JSHCT, as well as the institutional review board of Nagoya University Graduate School of Medicine.

patients

Adult patients (at least 16 years of age) who received a first HSCT between 1990 and 2007 were considered as subjects for the present study. Those who were inherently susceptible to developing cancer [Fanconi anemia ($N = 3$) and congenital immunodeficiency ($N = 12$)] were excluded. Three-hundred five recipients (1.7%) were excluded because of insufficient follow-up data. The study included 17 545 recipients; 5358 recipients of related bone marrow, 3587 recipients of related peripheral blood stem cells (including 134 bone marrow and peripheral blood stem cells combined), 6508 recipients of unrelated bone marrow, and 2092 recipients of unrelated cord blood.

statistical analysis

Standard incidence ratios (SIRs) were calculated to determine whether the number of recipients in the present cohort who developed secondary solid tumor after receiving a HSCT was different than that in the general population (supplementary method, available at *Annals of Oncology* online). Cumulative incidences of solid cancer or GVHD were estimated by taking into account the competing risk of death among patients who did not develop a second malignancy or GVHD [19]. The influence of potential risk factors was estimated by using the Cox proportional hazard model [20]. A stepwise multivariate approach was used to identify the most important predictor with respect to the development of secondary solid cancers. The variables considered were age at transplant, patient sex, donor-type (related versus unrelated), graft source, TBI as part of the conditioning regimen, reduced-intensity conditioning, grade 2–4 acute GVHD, and chronic GVHD. The model was stratified into four categories according to the primary disease; acute myeloid leukemia, acute lymphoblastic leukemia, chronic myeloid leukemia, and others. Acute and chronic GVHD were considered as time-dependent covariates. TBI and chronic GVHD were frequent risk factors and were always kept in the model. Risk factors for high-risk cancer sites with adequate numbers of events for analyses were also analyzed: oral cavity/pharynx, esophagus, colon, and skin. The models for high-risk cancer sites were stratified according to the primary disease as described, and patient age at transplantation (<19, 20–29, 30–39, 40–49, 50–59, and >60), and also adjusted by patient age as a continuous variable. All P-values were two-sided.

results

patient and transplant characteristics

Table 1 shows the patient characteristics, their disease, and transplant regimens for 17 545 recipients of a first HSCT. The cumulative incidences of grade 2–4 acute GVHD at 150 days and chronic GVHD at 2 years post-transplant were 35% [95% confidence interval (CI), 35%–36%] and 41% (95% CI, 40%–41%), respectively. The observation period reached 69 465 person-years among the subjects for analyses. Of the 17 545 recipients, 5864 had survived for 5 or more years, and 2192 recipients had survived 10 or more years at the time of analysis (Table 2).

incidence and types of secondary solid cancers

The cumulative incidence of solid cancers was 0.7% (95% CI, 0.6–0.9) at 5 years, 1.7% (95% CI, 1.4–1.9) at 10 years, and 2.9% (95% CI, 2.5–3.4) at 15 years after transplantation (Figure 1). Two-hundred sixty-nine solid cancers were identified. Multiple solid cancers were observed in 11 patients. Nineteen recipients were diagnosed within 1-year post-transplantation (Table 2).

risk compared with the general population

HSCT recipients had a 1.8-fold higher risk of invasive solid cancers compared with the general population (95% CI, 1.5–2.0). SIR was significantly higher for cancers of the oral cavity/pharynx (SIR = 15.7), esophagus (SIR = 8.5), colon (SIR = 1.9), skin (SIR = 7.2), and brain/nervous system (SIR = 4.1; Table 2). The risks of developing secondary cancers of the oral cavity/pharynx, esophagus, and skin were significantly higher than those in the general population throughout all periods after 1 year (Figure 2). The risk for developing colon cancer was elevated during the period of 1–4 years (SIR = 2.7), whereas the risks for developing cancer of the pancreas (SIR = 4.5) were elevated during the period of 5–9 years. Recipients were at higher risk of developing cancers of the rectum (SIR = 3.6) and the brain/nervous system (SIR = 19.1) after 10 years post-transplantation. The risk of developing secondary solid cancers of all types compared with the general population increased with the time since transplantation. This trend was observed for oral/pharynx and esophageal cancer (Table 2; Figure 2).

recipients' age at transplantation and risks for developing secondary solid cancers

SIRs were also analyzed according to the recipient's age at transplantation (Table 3). Compared with the general population in Japan, the SIRs were significantly increased for all solid cancers, oral/pharynx, esophagus, liver, bronchus/lung, and brain/nervous system for recipients who were 16–19 years of age at transplant, all solid cancers, oral/pharynx, and esophagus for recipients who

Table 1. Patient, disease, and transplant characteristics

Characteristics	Number	Percent
Total number	17 545	
Year of transplant		
1990–1994	1630	9
1995–1999	3750	21
2000–2004	7078	40
2005–2007	5087	29
Patient sex		
Male	10 386	59
Female	7149	41
Missing	10	<1
Patient age		
Median (range)	40 (16–85)	
16–19	1399	8
20–29	3506	20
30–39	3787	22
40–49	4167	24
50–59	3549	20
≥60	1137	6
Diagnosis		
Acute myeloid leukemia	6096	35
Acute lymphoblastic leukemia	3334	19
Chronic myeloid leukemia	2514	14
Myelodysplastic syndromes	1716	10
Adult T-cell leukemia	591	3
Other leukemia	130	1
Myeloproliferative disorders	224	1
Non-Hodikin's lymphoma	1652	9
Hodikin's lymphoma	46	<1
Other lymphoma/type missing	54	<1
Multiple myeloma	210	1
Aplastic anemia	745	4
Pure red cell aplasia	4	<1
Paroxysmal nocturnal hemoglobinuria	20	<1
Solid tumor	109	1
Others	86	<1
Data missing	14	<1
Donor		
Related, siblings	7825	45
Related, other relatives	941	5
Related, data missing	179	1
Unrelated	8600	49
Stem cell source		
Bone marrow	11 866	68
Peripheral blood	3453	20
Bone marrow and peripheral blood	134	1
Cord blood	2092	12
Conditioning regimen		
Myeloablative		
Cyclophosphamide + TBI ± other	8298	47
Other TBI regimen	1321	8
Busulfan + cyclophosphamide ± other	2798	16
Other non-TBI regimen	778	4
Reduced intensity		
Fludarabine + busulfan ± other	1527	9
Fludarabine + cyclophosphamide ± other	503	3
Fludarabine + melphalan ± other	1480	8
Other RIST	631	4
Data missing	209	1
GVHD prophylaxis		
No	85	<1
Cyclosporine A + sMTX	10 091	58
Cyclosporine A ± other	1175	7
Tacrolimus + sMTX	4682	27
Tacrolimus ± other	876	5
Other	323	2
Data missing	312	2

TBI, total body irradiation; sMTX, short-term methotrexate.

were 20–29 years of age at transplant, all solid cancers, oral/pharynx, esophagus, and gallbladder for recipients who were 30–39 years of age at transplant, all solid cancers, oral/pharynx, esophagus, and skin for recipients who were 40–49 years of age at transplant, all solid cancers, oral/pharynx, esophagus, colon, and skin for recipients who were 50–59 years of age at transplant (Table 3).

risk factors for the development of secondary solid cancers

Extensive-type chronic GVHD and age at transplantation were important risk factors for the development of secondary solid cancers (Table 4). The risk was not increased in recipients who received TBI for conditioning. The results were similar when subjects were limited to those who received myeloablative conditioning (RR = 1.5, $P = 0.069$ for limited-type chronic GVHD, RR = 1.9, $P < 0.001$ for extensive-type chronic GVHD, and RR = 0.9, $P = 0.751$ for TBI). Risk factor analyses for high-risk organs with more than 10 cancer cases revealed that extensive-type chronic GVHD was an independent risk factor for cancers in the oral cavity/pharynx and esophagus. Limited-type chronic GVHD was a risk factor for cancers of skin (Table 4). For secondary cancers which developed within 1-year post-transplant, the only risk factor identified was older age at transplant (age 60 years or older; supplementary Table, available at *Annals of Oncology* online).

discussion

Our main objective was to determine the incidence of, the risk compared with the general population, and risk factors for secondary solid tumors after allogeneic stem cell transplantation in a large cohort of adult recipients. Allogeneic HCT recipients were at higher risk of developing cancers of the oral cavity, esophagus, colon, and skin. The incidence and SIR of developing all solid cancers continued to increase with follow-up, which suggested a continuous increase as follow-up progressed. Our data are important since we included a large number of subjects and person-years of follow-up, in a transplant cohort that is different from those in previously reported large studies.

Table 2. Standard incidence ratio, ratio of observed versus expected number of secondary solid cancers according to duration post-transplant

	Time since transplantation (years)								Total		
	<1		1–4		5–9		10 or longer				
Number of recipients	17 545		10 210		5864		2192		17 545		
Person-years at risk	12 803		30 599		18 845		7218		69 465		
Secondary cancer sites	O	SIR	O	SIR	O	SIR	O	SIR	O/E	SIR	95% CI
All solid cancers	19	0.7	97	1.5*	90	2.0*	63	3.1*	269/153.6	1.8*	1.5–2.0
Oral/pharynx	0	0.0	16	9.5*	27	23.4*	21	38.5*	64/4.1	15.7*	12.1–20.1
Esophagus	0	0.0	13	6.5*	17	12.6*	11	16.8*	41/4.8	8.5*	6.1–11.5
Stomach	2	0.4	7	0.6	6	0.8	1	0.3	16/26.0	0.6	0.4–1.0
Colon	2	0.8	16	2.7*	5	1.2	4	2.2	27/14.3	1.9*	1.2–2.7
Rectum	0	0.0	1	0.2	0	0.0	5	3.6*	6/10.7	0.6	0.2–1.2
Liver	1	0.6	5	1.4	0	0.0	2	1.8	8/8.6	0.9	0.4–1.8
Gallbladder	2	5.1	2	2.1	2	3.0	0	0.0	6/2.3	2.6	1.0–5.7
Pancreas	0	0.0	2	1.0	6	4.5*	1	1.6	9/4.7	1.9	0.9–3.7
Broncus/lung	3	1.2	4	0.6	9	2.1	3	1.5	19/15.1	1.3	0.8–2.0
Skin	2	7.0	6	8.1*	3	5.7*	2	8.4*	13/1.8	7.2*	3.9–12.4
Female breast	0	0.0	3	0.3	1	0.1	3	0.9	7/24.5	0.3	0.1–0.6
Cervix uteri	1	1.3	4	2.0	1	0.7	1	1.6	7/4.8	1.5	0.6–3.0
Corpus uteri	2	3.7	1	0.7	2	1.8	0	0.0	5/3.6	1.4	0.4–3.2
Ovary	0	0.0	1	0.7	1	1.0	1	2.2	3/3.6	0.8	0.2–2.4
Prostate	1	1.2	0	0.0	1	0.6	1	1.4	3/5.4	0.6	0.1–1.6
Bladder	1	1.9	3	2.4	0	0.0	0	0.0	4/2.9	1.4	0.4–3.5
Kidney	0	0.0	1	0.6	1	0.9	0	0.0	2/4.1	0.5	0.1–1.8
Brain/nervous system	1	3.4	1	1.4	1	2.1	4	19.1*	7/1.7	4.1*	1.6–8.5
Thyroid	0	0.0	2	1.1	2	1.5	0	0.0	4/4.5	0.9	0.2–2.3
Other[a]	1		9		4		3		17		

[a]Other sites included two testicular cancers, four connective tissue cancers, four bone cancers, one larynx cancer, one malignant salivary gland tumor, one duodenum papilla cancer, one germ cell tumor, one carcinomatous pleurisy of origin unknown, and two squamous cell carcinomas of unknown origin.
*$P < 0.05$.

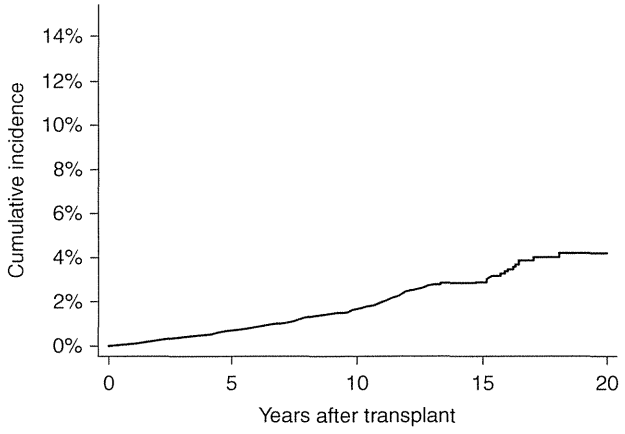

Figure 1. Cumulative incidence of developing a secondary solid cancer. The cumulative incidence of solid cancers was 0.7% [95% confidence interval (CI), 0.6–0.9] at 5 years, 1.7% (95% CI, 1.4–1.9) at 10 years, and 2.9% (95% CI, 2.5–3.4) at 15 years after transplantation.

Extensive-type chronic GVHD has repeatedly been shown to be a significant risk factor for the development of secondary solid tumor and is highly correlated with squamous cell carcinoma [8, 9, 12, 15, 16]. Extensive-type chronic GVHD was also shown to be a significant risk factor for oral cancer in our study. Extensive-type chronic GVHD was shown to be a significant risk factor for esophageal cancer, which was found to be increased in recipients compared with the general population in our study as well as in two other smaller Japanese cohorts in previous studies [11, 14]. Subjects were shown to be at a higher risk for the development of cancers of the oral cavity or esophagus at all time periods after 1 year. Data were not obtained for affected organ sites of chronic GVHD in JSHCT data collection prior to transplants in 2006. Therefore, we could not investigate whether oral or esophageal cancers were related to the chronic GVHD of the same organ. However, results of risk factor analyses for cancer sites of oral, esophagus, colon, and skin which showed high associations of extensive-type chronic GVHD and oral or esophagus cancer, limited-type chronic GVHD, and skin cancer showed that development of secondary solid tumors were likely to be influenced by GVHD-affected sites. Lifelong screening for oral, pharynx, or esophageal cancers for recipients with active or resolved chronic GVHD is important after 1-year post-transplant. The prognosis of solid cancers is highly influenced by the stage of the cancers when they are first detected. Our findings support recently published recommended screening guidelines [21, 22].

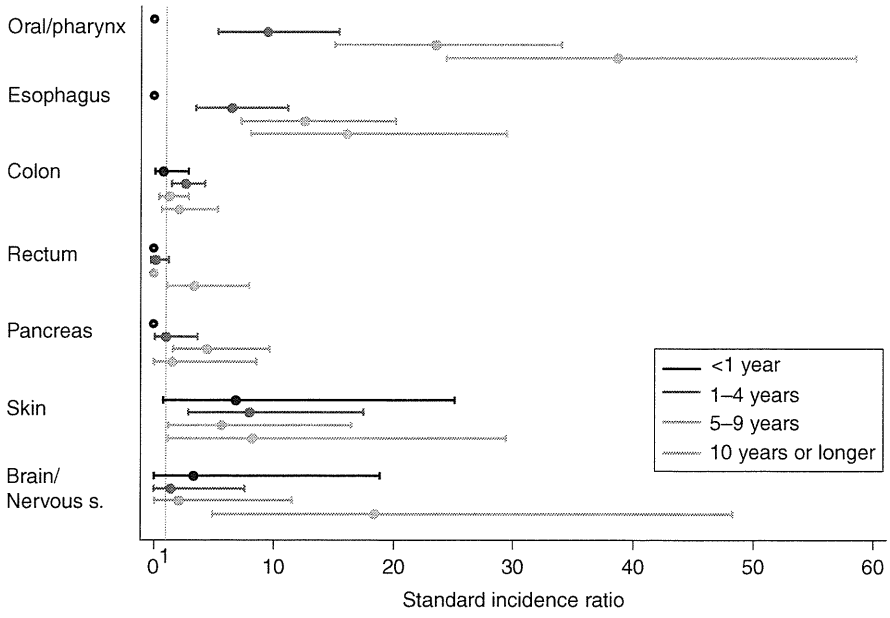

Figure 2. Trends of standard incidence ratios (SIRs) and its 95% confidence intervals (CIs) of high-risk secondary solid cancer sites according to time since transplant. The SIR and 95% CIs for <1, 1–4, 5–9, and 10 years or longer post-transplant were 0.0, 9.5 (5.4–15.4), 23.4 (15.4–34.0), and 38.5 (23.8–58.9) for oral/pharynx cancer, 0.0, 6.5 (3.5–11.2), 12.6 (7.3–20.2), and 16.8 (8.4–30.1) for esophageal cancer, 0.8 (0.1–2.9), 2.7 (1.5–4.3), 1.2 (0.4–2.9), and 2.2 (0.6–5.7) for colon cancer, 0.0, 0.2 (0.0–1.3), 0.0, and 3.6 (1.2–8.4) for rectum cancer, 0.0, 1.0 (0.1–3.7), 4.5 (1.6–9.7), and 1.6 (0.0–8.9) for pancreatic cancer, 7.0 (0.8–25.1), 8.1 (3.0–17.5), 5.7 (1.2–16.7), and 8.4 (1.0–30.3) for skin cancer, and 3.4 (0.1–19.0), 1.4 (0.0–7.7), 2.1 (0.1–11.6), and 19.1 (5.2–49.0) for cancers of brain/nervous system, respectively.

Table 3. Standard incidence ratio according to recipient's age at transplant

	Recipient's age at transplantation											
	16–19		20–29		30–39		40–49		50–59		60 or older	
Number-of-recipients	1399		3506		3787		4167		3549		1137	
Person-years at risk	7083		17 912		17 303		16 198		9126		1843	
Secondary cancer sites	O	SIR	O	SIR	O	SIR	O	SIR	O	SIR	O	SIR
All solid cancers	18	17.0*	28	4.1*	51	2.4*	71	1.4*	79	1.5*	22	1.0
Oral/pharynx	7	140.0*	11	50.7*	19	36.5*	13	10.1*	12	8.1*	2	3.9
Esophagus	1	350.0*	3	131.0*	13	48.5*	10	7.0*	13	5.9*	1	1.1
Stomach	1	13.3	0	0.0	1	0.3	7	0.8	5	0.5	2	0.5
Colon	0	0.0	0	0.0	3	2.0	6	1.3	12	2.1*	6	2.6
Rectum	1	33.1	0	0.0	0	0.0	1	0.3	4	0.9	0	0.0
Liver	1	66.4*	1	8.1	0	0.0	2	0.8	3	0.8	1	0.6
Gallbladder	0	0.0	0	0.0	2	12.0*	1	1.5	2	2.1	1	2.0
Pancreas	0	0.0	0	0.0	2	5.5	1	0.7	4	2.0	2	2.3
Broncus/lung	1	44.3*	0	0.0	2	1.6	7	1.6	7	1.1	2	0.7
Skin	1	28.6	1	6.3	0	0.0	6	11.6*	4	7.4*	1	4.0
Female breast	0	0.0	1	0.7	1	0.2	1	0.1	3	0.5	1	0.9
Cervix uteri	0	0.0	1	1.2	3	1.9	2	1.4	1	1.4	0	0.0
Corpus uteri	0	0.0	1	5.2	0	0.0	2	1.4	2	1.6	0	0.0
Ovary	0	0.0	1	3.2	0	0.0	1	0.7	0	0.0	1	6.4
Prostate	0	0.0	0	0.0	0	0.0	2	2.4	0	0.0	1	0.5
Bladder	0	0.0	0	0.0	0	0.0	2	2.3	2	1.7	0	0.0
Kidney	0	0.0	0	0.0	0	0.0	2	1.4	0	0.0	0	0.0
Brain/nervous system	2	23.9*	1	3.8	1	2.7	1	2.0	1	2.6	1	9.1
Thyroid	0	0.0	2	3.9	0	0.0	1	0.7	1	0.9	0	0.0

*$P < 0.05$.

Table 4. Risk factors for second solid cancers among >1 year survivors after hematopoietic stem cell transplantation

Solid cancer	Risk factor	Number of patients with second cancer	RR	95% CI	*P*-value
All second solid cancers[a]		249			
	Total body irradiation	151	0.9	0.7–1.1	0.294
	Chronic GVHD				
	Limited type	45	1.4	1.0–1.9	0.087
	Extensive type	93	1.8	1.4–2.4	<0.001
	Age at transplant (years)				
	16–29	45	1.0		
	30–39	46	1.6	1.0–2.4	0.042
	40–49	68	2.5	1.7–3.7	<0.001
	50–59	71	5.5	3.7–8.2	<0.001
	60 or older	19	7.9	4.4–14.1	<0.001
Oral cancer[b]		64			
	Total body irradiation	38	1.0	0.8–1.3	0.957
	Chronic GVHD				
	Limited type	10	1.4	0.6–2.9	0.440
	Extensive type	29	2.9	1.6–5.1	<0.001
Esophageal cancer[b]		41			
	Total body irradiation	22	0.6	0.3–1.1	0.108
	Chronic GVHD				
	Limited type	7	2.1	0.8–5.9	0.151
	Extensive type	25	5.3	2.4–11.8	<0.001
Colon cancer[b]		26			
	Total body irradiation	12	0.5	0.2–1.2	0.144
	Chronic GVHD				
	Limited type	6	1.7	0.6–4.9	0.353
	Extensive type	10	1.6	0.6–4.2	0.329
	Grade 2–4 acute GVHD	12	2.0	0.9–4.4	0.101
Skin cancer[b]		13			
	Total body irradiation	12	1.2	0.8–1.6	0.377
	Chronic GVHD				
	Limited type	6	5.8	1.4–23.9	0.016
	Extensive type	2	1.8	0.3–8.9	0.500

RR, relative risk; CI, confidence interval; TBI, total body irradiation; GVHD, graft-versus-host disease.
[a]Stratified for primary disease (acute myeloid leukemia, acute lymphoblastic leukemia, chronic myeloid leukemia, and other).
[b]Stratified for primary disease (acute myeloid leukemia, acute lymphoblastic leukemia, chronic myeloid leukemia, and other) and patient age groups (<19, 20–29, 30–39, 40–49, 50–59, and >60). Adjusted for patient age as a continuous variable.

The incidence of secondary solid tumors in our study was similar to those in previously reported large studies [8, 9, 12, 13]. Rizzo et al. [12] reported that the incidence of secondary solid cancers among 28 874 transplant recipients and 85 583 person-years at risk was 1% at 10 years and 2.2% at 15 years, which were very similar to our results using the same statistical method for cumulative incidence, while treating death before secondary solid tumor as a competing risk. Majhail et al. [13] reported that the incidence of secondary solid cancers after HSCT using non-TBI, busulfan-cyclophosphamide conditioning was also ~1.2% at 10 years. The oral cavity was the most prominent high-risk cancer site compared with the general population, as in previous reports [8, 9, 12, 13]. Despite regional and racial differences in cancer incidence and cancer sites in the general population, the impact of HSCT on secondary cancer was similar.

In previous studies, TBI was reported to be a significant risk factor for the development of secondary cancer, but significant differences were not found in our study [7, 8, 10, 12, 23]. The subjects in this study were adult recipients, which may explain the different findings. Conditioning with radiation was reported to be associated with the development of secondary solid cancer in recipients at a younger age at transplant [12]. Moreover, a recent long-term follow-up analysis of patients who were transplanted after myeloablative doses of busulfan and cyclophosphamide without TBI found a similar increased incidence of secondary solid cancers as previous reports [13].

An older recipient age at transplant was a significant risk factor for the development of secondary solid tumor, as in previous studies [9, 13]. This result was not surprising since it is also the case in the general population. However, it is important to note that older patients are at higher risk of developing

secondary cancer and to promote patient education and preventive practices, since there has been a dramatic increase in the number of transplant recipients who are more than 50 years of age at transplant over the past decade. In comparison with the general population, younger patients were at a higher risk of developing a solid tumor. Several high-risk cancer sites (esophagus, liver, and bronchus/lung) in younger group did have only one observed cases, therefore, these results should not be emphasized and need to be confirmed in other studies. These sites were found to be significant because the expected numbers in general population for these sites were extremely small.

Although this study included a large number of recipients and a large number of person-years of follow-up, there are limitations. The follow-up years for older recipients were still limited, and therefore we may find a higher incidence of and risk of secondary solid cancers among recipients who are 50 years of age or older at transplant in the future. Second limitation involves possible under-reporting by recipients to transplant centers or by transplant centers to the registry. Until recently, transplant recipients have received only limited information regarding screening or the prevention of secondary solid cancers. Another limitation of this analysis was lack of central pathology review for secondary solid tumors. JSHCT data collection does not include the submission of specimen or pathology report. Since this study included transplants from 1990, central pathology review was difficult to perform at the time of analyses. In addition, limiting secondary tumors to centrally diagnosed tumors would decrease the number of identified secondary tumors; therefore, secondary solid tumors were identified as reported from transplant centers.

In conclusion, recipients of allogeneic hematopoietic stem cell transplant had a significantly higher risk of developing secondary solid cancers than the general population. Older recipients are at higher risk of developing secondary solid tumors, as in the general population. Lifelong screening is important for high-risk organ sites, especially for oral, pharynx, and esophageal cancers in recipients with active, or a history of, chronic GVHD.

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disclosure

The authors have declared no conflicts of interest.

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BRIEF REPORT
Reduced Intensity Conditioning in Allogeneic Stem Cell Transplantation for AML With Down Syndrome

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Allogeneic hematopoietic stem cell transplantation (HSCT) has not been widely used in patients with acute myeloid leukemia (AML) and Down syndrome (DS) due to fear of transplantation-related toxicity. A retrospective analysis of the outcome of allogeneic HSCT was conducted in 15 patients with AML and DS. The five patients transplanted with the reduced intensity conditioning (4 in complete remission (CR) and 1 in non-CR) had a significantly better survival rate than 10 patients transplanted with a conventional conditioning (4 in CR and 6 in non-CR) (3-year EFS (95% confidence interval): 80.0% (20.4–96.9%) vs. 10.0% (0.6%–35.8%), $P = 0.039$). Pediatr Blood Cancer 2014;61:925–927.

Key words: acute myeloid leukemia; allogeneic stem cell transplantation; Down syndrome; reduced intensity conditioning

INTRODUCTION

Patients with Down syndrome (DS) have a 10- to 20-fold increased risk of developing acute myeloid leukemia (AML), especially acute megakaryoblastic leukemia (AMKL) [1,2]. The introduction of reduced-dose chemotherapy regimens specifically designed for AML in patients with DS has improved the survival outcome [3–7]. However, approximately 15% of patients still experience induction failures or relapses of leukemia [7]. In the general population, allogeneic hematopoietic stem cell transplantation (HSCT) has been adopted as a promising treatment option for relapsed or high-risk leukemia. Nevertheless, HSCT has not been widely used in patients with DS because of the comorbidities associated with the condition and increased chemotherapy-related toxicity. Recently, reduced intensity conditioning (RIC) regimens have been extensively introduced for patients with comorbidities, such as elderly patients. Patients with DS may also be good candidates for HSCT with RIC regimens. Reports on transplant outcomes in patients with DS are very limited, with the majority of patients being transplanted with conventional conditioning regimens [8,9]. In the present study, the outcome of allogeneic HSCT was retrospectively analyzed in 15 AML patients with DS, including five patients transplanted with a RIC regimen.

PATIENTS AND METHODS

Patients

Using the Japan Society for Hematopoietic Cell Transplantation registry, 15 patients (10 males and 5 females) with DS who suffered from AML and had undergone allogeneic SCT between 1993 and 2008 were identified. The patients' characteristics, including sex, age, diagnosis, and disease status at SCT, are summarized in Table I. The patients' median age was 3 years (range, 0–14 years), and French-American-British (FAB) classifications were: M2 (n = 1), M4 (n = 1), and M7 (n = 13). Eight patients underwent transplantation in complete remission (CR) [first CR (n = 4), second CR (n = 3), and third CR (n = 1)], while seven patients underwent

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Conflict of interest: Nothing to declare.

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TABLE I. Patient Characteristics

Patient no.	Age at SCT (years)	Diagnosis	Disease status at SCT	Days from diagnosis to SCT	Donor	Serological HLA mismatched loci	Graft	ATG	RIC/MA	Conditioning regimen	GvHD prophylaxis
1	1	M7	NCR	307	Unrelated	HLA-B, DR	CB	−	MA	Bu + TEPA	MTX
2	4	M7	NCR	188	Related	HLA-B	BM	−	MA	Bu + MEL	TAC + MTX
3	3	M7	3CR	872	Related	HLA-B	BM	−	RIC	FLU + MEL + TBI 2 Gy	TAC + MTX
4	1	M7	2CR	333	Related	HLA-A, B	BM	−	MA	Bu + CA + CY	TAC + MTX
5	1	M7	1CR	117	Related	Matched	BM	−	MA	Bu + MEL	CyA + MTX
6	0	M7	NCR	237	Related	HLA-B, DR	BM	−	MA	CA + CY + TBI 8 Gy	TAC + MTX
7	4	M7	2CR	543	Related	Matched	PBSC	−	MA	Bu + CA + CY	CyA + Steroid
8	1	M7	1CR	171	Unrelated	HLA-DR	CB	−	RIC	FLU + MEL + ETP	CyA + MTX
9	2	M7	2CR	368	Unrelated	Matched	CB	−	RIC	FLU + MEL + TBI 2 Gy	TAC + MTX
10	6	M2	NCR	378	Unrelated	Matched	CB	−	MA	CA + CY + FLU + ETP + TBI 6 Gy	TAC + MTX
11	3	M7	1CR	795	Related	HLA-B	BM	−	MA	CY + TBI 10 Gy	CyA + MTX
12	3	M7	NCR	503	Related	Matched	BM	−	MA	BU + CY	TAC
13	1	M7	1CR	263	Unrelated	Matched	CB	+	RIC	FLU + MEL	TAC + MTX
14	3	M7	NCR	236	Related	Matched	PBSC	−	MA	BU + CY + MEL + TEPA	CyA + MTX
15	14	M4	NCR	724	Unrelated	Matched	BM	+	RIC	FLU + MEL + TBI 3 Gy	TAC + MTX

SCT, stem cell transplantation; HLA, human leukocyte antigen; ATG, anti-thymocyte globulin; RIC, reduced intensity conditioning; MA, myeloablative conditioning; GvHD, graft versus host disease; AML, acute myeloid leukemia; NCR, no complete remission; 1CR, first complete remission; 2CR, second complete remission; 3CR, third complete remission; CB, cord blood stem cell; BM, bone marrow; PBSC, peripheral blood stem cell; Bu, busulfan; TEPA, tespamin; MEL, melphalan; TBI, total body irradiation; CA, cytosine arabinoside; CY, cyclophosphamide; ETP, etoposide; FLU, fludarabine; MTX, methotrexate; TAC, tacrolimus; CyA, cyclosporine A.

transplantation in non-CR (NCR). The median interval from diagnosis to transplantation was 333 days (range, 117–872 days). The ethics committee of Nagoya University Graduate School of Medicine approved this study.

Transplant Procedures

Features of SCT, including donor, graft, preparative regimen, and graft versus host disease (GvHD) prophylaxis, are shown in Table I. Three children received a graft from an HLA-matched sibling [bone marrow (BM) (n = 2) and peripheral blood stem cells (PBSC) (n = 2)], four received a graft from an HLA-matched unrelated donor [BM (n = 1) and cord blood stem cells (CB) (n = 3)], two received a graft from an HLA-mismatched unrelated donor [CB (n = 2)], and five received a graft from an HLA-mismatched family member [BM (n = 5)]. Anti-thymocyte globulin (ATG) was given to two patients. Five patients received a RIC regimen (fludarabine (FLU) + melphalan (MEL)-based regimen; patients 3, 8, 9, 13, and 15), seven received a busulfan (Bu)-based regimen (patients 1, 2, 4, 5, 7, 12, and 14), and three patients received a total body irradiation (TBI)-based regimen (6–10 Gy) (patients 6, 10, and 11). A GvHD prophylaxis regimen with methotrexate (MTX) alone was used in one patient, tacrolimus (TAC) ± MTX was used in nine patients, and cyclosporine A (CyA) ± MTX ± steroid was used in five patients.

RESULTS

Engraftment and GvHD

All 15 patients achieved neutrophil engraftment between days +10 and +34. One patient (patient 9) experienced secondary graft failure on day +33. The data for acute GvHD (aGvHD) were not available for patient 11. Grade II–IV aGvHD was observed in seven patients, with two (patients 2 and 6) classified as grade III–IV aGvHD. Chronic GvHD (cGvHD) was observed in seven patients (47%), with one being an extensive-type (patient 4).

Relapse, Transplant-Related Mortality, and Survival Outcome

Six patients relapsed, and all but one died (patients 1, 6, 7, 12, and 14). Four patients died of transplant-related complications [aGvHD (patient 2), cGvHD (patient 4), secondary graft failure (patient 9), and idiopathic pneumonia (patient 11)]. At the time of this report, six patients were still alive (patients 3, 5, 8, 10, 13, and 15). The 3-year event-free survival (EFS) and overall survival (OS) were 32.0% (95% CI, 10.9–55.7%) and 38.9% (95% CI, 15.3–62.2%), respectively. Eight patients transplanted in CR showed trend of better survival rates compared to seven patients transplanted in NCR [3-year EFS, 95% CI: 46.9% (12.0–76.3%) vs. 14.3% (0.7–46.5%), $P = 0.102$]. Although 4 of 5 patients transplanted in CR, patients transplanted with the RIC regimen (FLU + MEL-based regimen) had a significantly better survival rate than patients transplanted with the other conventional conditioning regimens [3-year EFS, 95% CI: 80.0% (20.4–96.9%) vs. 10.0% (0.6–35.8%), $P = 0.039$] (Table II).

DISCUSSION

This retrospective survey identified 15 DS children with AML who had undergone transplantation in Japan between 1993 and 2008. Six of these children survived. Previous reports of

TABLE II. Clinical Outcome of Stem Cell Transplantation in 15 Children With Acute Myeloid Leukemia and Down Syndrome

Patient no.	aGvHD	cGvHD	Relapse, graft failure	Cause of death	Outcome	Observation period, months
1	II	—	Relapse, day +35	Relapse	Dead	3
2	IV	—	—	aGvHD	Dead	4
3	I	Limited	—	—	Alive	+51
4	II	Extensive	—	cGvHD	Dead	26
5	—	Limited	—	—	Alive	+211
6	III	—	Relapse, day +55	Relapse	Dead	6
7	II	—	Relapse, day +64	Relapse	Dead	5
8	—	Limited	—	—	Alive	+22
9	—	—	Secondary graft failure, day +33	Graft failure	Dead	10
10	II	—	Relapse, day +233	—	Alive	+36
11	ND	Limited	—	IP	Dead	7
12	I	Limited	Relapse, day +56	Relapse	Dead	5
13	I	—	—	—	Alive	+28
14	II	Limited	Relapse, day +123	Relapse	Dead	7
15	—	—	—	—	Alive	+47

aGVHD, acute graft versus host disease; cGVHD, chronic graft versus host disease; ND, not determined; IP, idiopathic pneumonia.

transplantation in patients with DS have mainly involved patients with ALL [8,9]. Very recently, the retrospective study of 28 transplantations for DS-AML was reported from the Center for International Blood and Marrow Transplant Research (CIBMTR) [10]. Hence, the current study represents one of the largest DS cohorts with AML who received HSCT.

German and Austrian groups reported 11 transplanted children with DS (8 ALL, 3 AML) and showed that the main cause of death was relapsed leukemia (5/11) rather than transplant-related mortality (TRM) (2/11) [9]. In the present cohort, both relapse (6/15) and TRM (4/15) had an impact on survival. However, when five patients transplanted from HLA-mismatched family donors (patients 2, 3, 4, 6, and 11) were excluded, the majority of the remaining patients died of relapse (5/10) rather than TRM (1/10).

The present analysis showed that the RIC regimen (FLU + MEL-based) had a positive effect on survival in patients with AML and DS. Previous reports have focused mainly on full conditioning regimens including recent report from CIBMTR [8–10]. Thus, this is one of the first reports of transplantation with RIC in patients with DS. Of the five patients who received the RIC regimen, only one died of graft failure (patient 9). This patient, with graft rejection, received a relatively low dose of melphalan (40 mg/m^2) compared to the other four patients (120–180 mg/m^2). To ensure engraftment, we assume that the melphalan dose should not be reduced in the conditioning regimen for HSCT in patients with DS. Considering that even patients with advanced risk (patients 3 and 15) maintained EFS for a long time, the FLU + MEL-based regimen appears to have sufficient anti-leukemia activity for patients with AML and DS.

In conclusion, this retrospective analysis of 15 patients with AML and DS who underwent transplants in Japan demonstrated that a RIC regimen was well tolerated in patients with DS. A prospective clinical trial is required to further evaluate the present findings.

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ORIGINAL ARTICLE

Risk factors and organ involvement of chronic GVHD in Japan

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Few studies have evaluated the risk factors for chronic GVHD and organ involvement associated with different graft types, including unrelated cord blood (U-CB). We retrospectively studied 4818 adult patients who received their first allogeneic transplantation and survived for at least 100 days. The incidence of chronic GVHD at 2 years was 37%. The following factors were associated with the development of chronic GVHD: female donor/male recipient, CMV-Ab seropositivity, matched related peripheral blood grafts vs matched related BM grafts, no *in vivo* T-cell depletion and the occurrence of grade II–IV acute GVHD. Among these factors, the association with acute GVHD occurrence was consistently significant across donor subtypes. The use of U-CB was not associated with chronic GVHD, but was associated with a low incidence of extensive chronic GVHD. Chronic GVHD patients who had received U-CB transplants showed less frequent involvement of the oral cavity (28% vs 55%), eye (12% vs 26%), liver (20% vs 44%), lung (11% vs 25%) and joint (0% vs 6%) than those with matched related BM grafts. In conclusion, we found that U-CB transplants were associated with a low incidence of extensive chronic GVHD and less frequent involvement of the oral cavity, eye, liver, lung and joints.

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Keywords: chronic GVHD; unrelated cord blood; acute GVHD; risk factors

INTRODUCTION

Chronic GVHD is a serious complication that affects the survival and quality of life of long-term survivors after allogeneic hematopoietic SCT.[1–3] Various pre- and post-transplant risk factors associated with chronic GVHD have been identified, mostly in transplantations using BM and PBSC grafts from related or unrelated donors.[2,3] Several studies have reported a history of acute GVHD to be a strong risk factor that is consistently associated with chronic GVHD development.[4–8] Other identified risk factors include the following: female donor and male recipient,[4,6] use of PBSC grafts,[6,9–13] older patient,[4,6–8] older donor,[6,7] transplantation from a mismatched or unrelated donor,[5,6,14] diagnosis of CML[4,7,8] and absence of anti-thymocyte globulin (ATG) use.[15]

The number of unrelated cord blood (U-CB) transplantations performed has rapidly increased during the past decade. However, few studies have compared the incidences and risk factors of chronic GVHD and its organ-specific symptoms in adult patients receiving U-CB and other available grafts, including related or unrelated BM/PBSC grafts.[16,17] Therefore, we conducted a retrospective study using national registry data involving 4818 patients who underwent allogeneic transplantation. This study aimed to evaluate the incidence and risk factors of chronic GVHD, and the prevalence of chronic GVHD organ involvement in patients who received transplantation using various types of graft, including U-CB.

MATERIALS AND METHODS

Data collection

Data for 54 072 patients who had received auto-SCT or allo-SCT by December 31, 2009 were provided by the Transplant Registry Unified Management Program (TRUMP).[18] We included 4993 adult patients who had: (1) received allogeneic transplantation for hematologic malignancies; (2) received their first SCT; (3) used the same questionnaire form involving chronic GVHD organ involvement (skin, oral cavity, eye, liver, lung, joint, intestine/genitals and other manifestations; 2006–2009 for transplantations using BM or PBSC grafts and 2007–2009 for transplantations using U-CB units); (4) achieved neutrophil engraftment; (5) survived for at least 100 days; and (6) received the following: (a) a related BM or PBSC graft (R-BM/PB), (b) an unrelated BM (U-BM) or (c) a single U-CB unit. Donation of peripheral blood by unrelated volunteers was permitted for the first time in Japan in 2011. The following patients were excluded: (1) patients who received *ex vivo* T-cell-depleted grafts ($n = 26$) and (2) patients who lacked data on acute or chronic GVHD ($n = 149$). Thus, 4818 patients were included in this study, which was approved by the TRUMP Data Management Committees and by the institutional review board of the Nagoya University Graduate School of Medicine, where this study was performed.

Histocompatibility

Histocompatibility data for the HLA-A, HLA-B and HLA-DR loci were obtained through reports acquired from the institution where the transplantation was performed or from the cord blood bank. HLA

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matching was assessed using serological data for the HLA-A, HLA-B and HLA-DR loci in R-BM/PB or U-CB transplantations, and using allelic data for the HLA-A, HLA-B and HLA-DRB1 loci in U-BM transplantations.

Statistical analysis

The physicians who performed the transplantations at each center diagnosed and classified acute and chronic GVHD according to traditional criteria.[1,19] The reported type of chronic GVHD was reclassified according to the information on its organ involvement. 'Progressive onset' of chronic GVHD was defined as chronic GVHD transitioned from active acute GVHD, 'quiescent onset' as chronic GVHD after remission of acute GVHD and '*de novo* onset' as chronic GVHD without history or acute GVHD. The intensity of conditioning regimen was classified as myeloablative or reduced intensity on the basis of the Center for International Blood and Marrow Transplant Research report and the information from the questionnaire, as previously described.[20–23] We defined the following as standard-risk diseases: AML and ALL in first or second remission; CML in the first or

Table 1. Patient characteristics

Variable	*R-BM/PB*		*U-BM*		*U-CB*		P-*value*
	n = *1859*	%	n = *2215*	%	n = *744*	%	
Recipient age, years, median (range)	46 (16–74)		47 (16–73)		51 (16–82)		<0.001
Donor age, years, median (range)	43 (10–79)		35 (20–55)[a]		—	—	—
Recipient sex							
Female	789	42	916	41	334	45	0.238
Male	1070	58	1299	59	410	55	
Sex match between recipient and donor							
Match	965	52	1251	56	227	31	<0.001
Male to female	398	21	573	26	109	15	
Female to male	496	27	389	18	131	18	
Missing	0	0	2	0	277	37	
Disease							
AML	799	43	986	45	395	53	0.004
MDS	210	11	276	12	76	10	
CML	60	3	73	3	25	3	
ALL	385	21	439	20	123	17	
ATL	110	6	131	6	29	4	
NHL	206	11	214	10	70	9	
Other diseases	89	5	96	4	26	3	
Disease risk							
Standard	1058	57	1351	61	331	44	<0.001
High	724	39	780	35	390	52	
Missing	77	4	84	4	23	3	
Source of stem cells							
BM	842	45	2215	100	—	—	—
Peripheral blood	1017	55	—	—	—	—	
Cord blood	—	—	—	—	744	100	
HLA compatibility[b]							
Matched	1486	80	1507	68	53	7	<0.001
Mismatched	373	20	708	32	691	93	
Conditioning regimen							
Myeloablative	1202	65	1505	68	436	59	<0.001
Reduced intensity	649	35	696	31	308	41	
Missing	8	1	14	1	0	0	
GVHD prophylaxis							
CsA based	1367	74	469	21	311	42	<0.001
Tac based	449	24	1737	78	425	57	
Others/missing	43	2	9	1	8	1	
Use of in vivo *T-cell depletion*							
No	1741	94	2143	97	730	98	<0.001
Yes	118	6	72	3	14	2	
CMV Ab (recipient and donor)							
Both negative	127	7	150	7	151	20	<0.001
Either positive	1561	84	2003	90	535	72	
Unknown	171	9	62	3	58	8	
Acute GVHD							
Grade II–IV	665	36	897	41	338	45	<0.001
Grade III–IV	217	12	236	11	81	11	0.578
Follow-up of survivors (years), median (range)	2.0 (0.3–4.7)		1.9 (0.3–4.8)		1.7 (0.3–3.9)		<0.001

Abbreviations: ATL = adult T-cell leukemia; MDS = myelodysplastic syndrome; NHL = non-Hodgkin's lymphoma; R-BM/PB = related BM or PBSC; Tac = tacrolimus; U-BM = unrelated BM; U-CB = unrelated cord blood. [a]Data are missing in 20 patients [b]HLA matching was assessed by serological data for HLA-A, HLA-B and HLA-DR loci in transplantation using R-BM/PB or U-CB grafts, whereas it was assessed by allelic data for HLA-A, HLA-B and HLA-DRB1 loci in transplantation using U-BM grafts.

second chronic phase or in the accelerated phase; myelodysplastic syndrome (MDS) with refractory anemia or refractory anemia with ringed sideroblasts; adult T-cell leukemia (ATL) in CR; and Hodgkin's or non-Hodgkin's lymphoma (NHL) in CR or PR. Others were defined as high-risk diseases.

The probability of developing chronic GVHD was estimated on the basis of cumulative incidence curves.[24] Competing events for chronic GVHD were death or relapse without GVHD. Groups were compared using Gray's test.[25] The Cox proportional hazards model was used to evaluate the effect of confounding variables on chronic GVHD. The following possible confounding variables were considered: recipient age; recipient sex; sex mismatch between recipient and donor (match, male (donor)/female (recipient), or female (donor)/male (recipient)); disease (CML or others); disease risk before transplantation (standard or high risk); donor type (HLA-matched related BM (MR-BM), HLA-matched related PBSCs (MR-PB), HLA-mismatched related BM (MMR-BM), HLA-mismatched related PBSCs (MMR-PB), HLA-matched unrelated BM (MU-BM), HLA-mismatched unrelated BM (MMU-BM) and U-CB); type of conditioning regimen (myeloablative or reduced intensity); type of GVHD prophylaxis (CsA based or tacrolimus based); use of *in vivo* T-cell depletion (yes or no); anti-CMV Ab detection (negative for both recipient and donor, or positive for either recipient or donor), and presence of grade II–IV acute GVHD. Confounding factors were selected in a stepwise manner from the model with a variable retention criterion of $P<0.05$. Reported factors associated with chronic GVHD (recipient age, sex mismatch, donor type, use of *in vivo* T-cell depletion and the presence of grade II–IV acute GVHD) was additionally selected as confounding factors in the analysis of chronic GVHD risk. In the subset analysis, the same variables used in the analysis for the entire cohort were added to the final model. Furthermore, the following variables were also added for the specific group: donor age, presence of an HLA mismatch and the use of PBSCs for the R-BM/PB group; donor age and presence of an HLA mismatch for the U-BM group; and presence of an HLA mismatch for the U-CB group.

We also compared the prevalence of chronic GVHD presentation or organ involvement between MR-BM and other graft types using the χ^2 test. We further evaluated chronic GVHD-specific survival, which is defined as the time from the day of chronic GVHD diagnosis to the day of death in the absence of relapse, among patients who developed chronic GVHD. We also evaluated OS among those who developed chronic GVHD. The probability of developing chronic GVHD-specific survival or OS from the onset of chronic GVHD was estimated using the Kaplan–Meier method, and univariate comparison between groups was performed using the log-rank test. In the analysis of chronic GVHD-specific survival, patients who were alive without disease recurrence were censored at the time of their last follow-up visit and those who experienced disease recurrence were censored at the time of diagnosis of recurrence. The Cox proportional hazards model was used to evaluate the effect of presentation or of each organ's manifestation of chronic GVHD on chronic GVHD-specific survival, after adjusting for donor type and other confounding factors that were selected from the model in a stepwise manner using a variable retention criterion of $P<0.05$. We also evaluated the effect of chronic GVHD on relapse, where the occurrence of chronic GVHD was treated as a time-varying covariate.

All tests were two-sided, and *P*-values <0.05 were considered statistically significant, except for the comparison of prevalence of chronic GVHD organ involvement between MR-BM and other graft types, where *P*-values <0.008 was significant in consideration of multiple comparison. All statistical analyses were performed using Stata version 12 (Stata Corp., College Station, TX, USA) and EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan),[26,27] which is a graphical user interface for R (R Foundation for Statistical Computing, version 2.13.0, Vienna, Austria).

RESULTS

Patient characteristics

Table 1 shows patient characteristics according to the stem cell source. The median age of recipients at the time of the transplant was 47 years (range, 16–82 years) for the entire cohort, and it was significantly higher for patients in the U-CB group. High-risk diseases were more prevalent in the U-CB group. The grafts used were MR-BM ($n=687$), MR-PB ($n=799$), MMR-BM ($n=155$), MMR-PB ($n=218$), MU-BM ($n=1507$), MMU-BM ($n=708$) and U-CB ($n=744$). CsA-based GVHD prophylaxis was received by 74% of the patients in the R-BM/PB group and by only 21% of the U-BM recipients. *In vivo* T-cell depletion was used for only 4% of the entire cohort (ATG, $n=197$; alemtuzumab, $n=7$). Grade II–IV and III–IV acute GVHD occurred in 39% and 11% of the cohort, respectively.

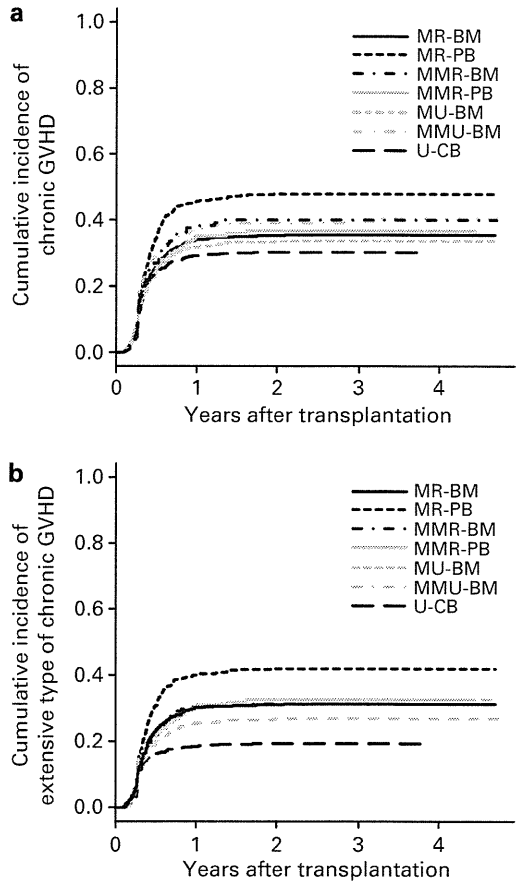

Figure 1. Cumulative incidence of chronic GVHD (**a**) and extensive type of chronic GVHD (**b**).

Chronic GVHD

The incidence of chronic GVHD at 2 years was 37% (95% confidence interval (CI), 35–38%) for the entire cohort, with a median onset of 120 days (range, 30–1203 days), 36% (32–39%) for the MR-BM group, 48% (44–51%) for the MR-PB group, 40% (32–48%) for the MMR-BM group, 37% (30–44%) for the MMR-PB group, 34% (31–36%) for the MU-BM group, 40% (36–44%) for the MMU-BM group and 30% (27–34%) for the U-CB group (Gray's test for the whole group, $P<0.001$; Figure 1a). Female/male mismatch between recipient and donor (hazard ratio (HR), 1.29; $P<0.001$), CMV Ab detection (HR, 1.26; $P=0.015$), the use of MR-PB vs MR-BM graft (HR, 1.49; $P<0.001$), the use of *in vivo* T-cell depletion (HR, 0.48; $P<0.001$) and the occurrence of grade II–IV acute GVHD (HR, 1.62; $P<0.001$) were significantly associated with chronic GVHD development (Table 2). The use of PBSC grafts was significantly associated with chronic GVHD development in the R-BM/PB group (HR, 1.42; $P<0.001$). The impact of CMV Ab positivity on chronic GVHD development was significant only for the U-CB group, but HR was consistently high across donor subtypes. The effect of sex mismatch was significant for the R-BM/PB group, but was not significant for the U-CB group. The effect of grade II–IV acute GVHD occurrence on chronic GVHD development was consistently significant across donor subtypes.

Table 2. Risk factors for chronic GVHD

Variable	*Chronic GVHD (Total)*			*Chronic GVHD (R-BM/PB)*			*Chronic GVHD (U-BM)*			*Chronic GVHD (U-CB)*		
	HR	*95% CI*	P-*value*	*HR*	*95% CI*	P-*value*	*HR*	*95% CI*	P-*value*	*HR*	*95% CI*	P-*value*
Recipient age, per 10 years	1.03	(0.99–1.06)	0.136	1.09	(1.01–1.17)	0.021	1.01	(0.96–1.07)	0.741	0.91	(0.83–1.00)	0.056
Donor age, per 10 years				1.01	(0.94–1.09)	0.730	1.04	(0.95–1.14)	0.429			
Sex match between recipient and donor												
Match	1.00			1.00						1.00		
Male to female	0.97	(0.86–1.10)	0.619	1.01	(0.83–1.23)	0.905	1.00	(0.84–1.19)	0.992	0.78	(0.51–1.19)	0.253
Female to male	1.29	(1.14–1.44)	<0.001	1.45	(1.23–1.71)	<0.001	1.16	(0.96–1.41)	0.127	1.12	(0.78–1.62)	0.535
CMV Ab (donor and recipient)												
Both negative	1.00			1.00			1.00			1.00		
Either positive	1.26	(1.05–1.52)	0.015	1.12	(0.82–1.54)	0.469	1.22	(0.90–1.66)	0.196	1.53	(1.07–2.21)	0.021
Type of donor and stem cell source												
MR-BM	1.00											
MR-PB	1.49	(1.26–1.75)	<0.001									
MMR-BM	1.21	(0.91–1.60)	0.187									
MMR-PB	1.31	(1.00–1.72)	0.054									
MU-BM	0.91	(0.78–1.07)	0.247									
MMU-BM	1.10	(0.92–1.31)	0.306									
U-CB	1.00	(0.81–1.23)	0.991									
Type of stem cell source												
BM				1.00								
PB				1.42	(1.23–1.65)	<0.001						
HLA disparity												
Match				1.00			1.00			1.00		
Mismatch				1.12	(0.92–1.36)	0.274	1.17	(1.00–1.36)	0.043	0.96	(0.55–1.69)	0.887
Use of in vivo *T-cell depletion*												
No		1.00		1.00			1.00			1.00		
Yes	0.48	(0.34–0.66)	<0.001	0.29	(0.18–0.45)	<0.001	0.85	(0.55–1.34)	0.490	0.35	(0.05–2.50)	0.293
Acute GVHD												
Grade 0–I		1.00		1.00			1.00			1.00		
Grade II–IV	1.62	(1.47–1.78)	<0.001	1.44	(1.24–1.66)	<0.001	1.73	(1.50–2.00)	<0.001	1.76	(1.34–2.31)	<0.001

Abbreviations: CI = confidence interval; HR = hazard ratio; MMR-BM = HLA-mismatched related BM; MMR-PB = HLA-mismatched related PBSCs; MMU-BM = HLA-mismatched unrelated BM; MR-BM = HLA-matched related BM; MR-PB = HLA-matched related PBSCs; MU-BM = HLA-matched unrelated BM; R-BM/PB = related BM or PBSC; U-BM; unrelated BM; U-CB = unrelated cord blood.

Extensive chronic GVHD

The incidence of extensive chronic GVHD at 2 years was 30% (29–31%) for the entire cohort, 32% (28–35%) for the MR-BM group, 42% (39–46%) for the MR-PB group, 31% (24–39%) for the MMR-BM group, 33% (26–39%) for the MMR-PB group, 27% (25–29%) for the MU-BM group, 32% (28–36%) for the MMU-BM group and 19% (17–22%) for the U-CB group (Gray's test for the whole group, $P<0.001$; Figure 1b). In addition to being a significant variable in the analysis of chronic GVHD, the use of reduced-intensity conditioning (vs myeloablative conditioning) was inversely associated with the development of extensive chronic GVHD (HR, 0.86; $P=0.019$; Table 3). Compared with MR-BM, MR-PB and MMR-PB were associated with the development of extensive chronic GVHD, whereas MU-BM and U-CB grafts were inversely associated with its development. Grade II–IV acute GVHD occurrence was the only significant variable consistently observed across all donor types.

Organ-specific chronic GVHD

Figure 2 shows the type of presentation and organ involvement associated with chronic GVHD. Among the 1716 patients who developed chronic GVHD, *de novo*, progressive and quiescent chronic GVHD presentations were observed in 467 (27%), 348 (20%) and 901 (53%) patients, respectively. Compared with the MR-BM group, progressive chronic GVHD was more frequently observed in the MMU-BM group (33% vs 15%), and quiescent chronic GVHD was more frequently observed in the U-CB group (62% vs 53%).

Limited type of skin involvement was more frequently observed in the U-CB group than in the MR-BM group (53% vs 29%). We examined the types of chronic GVHD (limited vs extensive) in patients with limited type of skin GVHD to evaluate the effect of limited type of skin GVHD on chronic GVHD type in the U-CB group. Accordingly, extensive chronic GVHD was observed in 73% of patients with limited type of skin GVHD in the MR-BM group, compared with 49% of patients in the U-CB group. Oral cavity (28% vs 55%), eye (12% vs 26%), liver (20% vs 44%), lung (11% vs 25%) and joint (0% vs 6%) involvement was less prevalent in the U-CB group than in the MR-BM group. There was no organ that was more frequently involved in the U-CB group than in the MR-BM group.

Progressive onset of chronic GVHD, extensive skin GVHD, intestinal or genital involvement and extensive type of chronic GVHD were significantly associated with lower chronic GVHD-specific survival rates in multivariate analysis, after adjusting for other confounders (Table 4). Lung involvement in GVHD was marginally significant. On the other hand, limited type of skin GVHD was associated with higher chronic GVHD-specific survival rates. Chronic GVHD-specific survival and OS curves showing a significant difference between the groups are shown in Figure 3 and Supplementary Figure 1. The impact of chronic GVHD on relapse is also an important issue. The occurrence of chronic GVHD

Table 3. Risk factors for extensive type of chronic GVHD

Variable	*Extensive chronic GVHD (Total)*			*Extensive chronic GVHD (R-BM/PB)*			*Extensive chronic GVHD (U-BM)*			*Extensive chronic GVHD (U-CB)*		
	HR	*95% CI*	P-*value*	*HR*	*95% CI*	P-*value*	*HR*	*95% CI*	P-*value*	*HR*	*95% CI*	P-*value*
Recipient age, per 10 years	1.10	(1.05–1.15)	<0.001	1.12	(1.03–1.21)	0.010	1.07	(1.00–1.15)	0.049	1.10	(0.96–1.26)	0.180
Donor age, per 10 years				1.02	(0.94–1.10)	0.662	1.08	(0.98–1.20)	0.136			
Sex match between recipient and donor												
Match	1.00			1.00			1.00			1.00		
Male to female	1.02	(0.89–1.16)	0.822	1.00	(0.81–1.24)	0.977	1.08	(0.90–1.31)	0.409	0.82	(0.49–1.37)	0.442
Female to male	1.32	(1.16–1.50)	<0.001	1.49	(1.25–1.77)	<0.001	1.25	(1.01–1.55)	0.042	0.88	(0.55–1.41)	0.608
CMV Ab (donor and recipient)												
Both negative	1.00			1.00			1.00			1.00		
Either positive	1.32	(1.06–1.64)	0.014	1.17	(0.83–1.64)	0.383	1.37	(0.95–1.97)	0.089	1.54	(0.97–2.44)	0.068
Type of donor and stem cell source												
MR-BM	1.00											
MR-PB	1.41	(1.19–1.58)	<0.001									
MMR-BM	1.08	(0.79–1.49)	0.614									
MMR-PB	1.35	(1.01–1.81)	0.042									
MU-BM	0.78	(0.66–0.93)	0.005									
MMU-BM	0.93	(0.77–1.13)	0.452									
U-CB	0.65	(0.51–0.83)	0.001									
Type of stem cell source												
BM				1.00								
PB				1.42	(1.21–1.66)	<0.001						
HLA disparity												
Match				1.00			1.00			1.00		
Mismatch				1.10	(0.88–1.36)	0.397	1.14	(0.96–1.35)	0.142	0.89	(0.45–1.76)	0.743
Conditioning												
Myeloablative	1.00			1.00			1.00			1.00		
Reduced intensity	0.86	(0.75–0.97)	0.019	0.90	(0.74–1.08)	0.255	0.88	(0.72–1.07)	0.206	0.64	(0.42–0.96)	0.031
Use of in vivo *T-cell depletion*												
No	1.00			1.00			1.00					
Yes	0.39	(0.26–0.58)	<0.001	0.23	(0.13–0.41)	<0.001	0.80	(0.46–1.37)	0.407			
Acute GVHD												
Grade 0–I	1.00			1.00			1.00			1.00		
Grade II–IV	1.74	(1.56–1.93)	<0.001	1.52	(1.30–1.78)	<0.001	1.91	(1.62–2.26)	<0.001	2.02	(1.43–2.86)	<0.001

Abbreviations: CI = confidence interval; HR = hazard ratio; MMR-BM = HLA-mismatched related BM; MMR-PB = HLA-mismatched related PBSCs; MMU-BM = HLA-mismatched unrelated BM; MR-BM = HLA-matched related BM; MR-PB = HLA-matched related PBSCs; MU-BM = HLA-matched unrelated BM; R-BM/PB = related BM or PBSC; U-BM; unrelated BM; U-CB = unrelated cord blood.

was significantly associated with lower incidence of relapse than the absence of chronic GVHD for the total cohort (HR 0.88, $P = 0.018$). However, we did not find any significant different impact of type, onset and organ involvement of chronic GVHD on relapse among those with chronic GVHD.

DISCUSSION

In the present study, we extensively analyzed the risk factors for chronic GVHD, particularly focusing on donor graft sources and organ involvement, using recently obtained national registry data that included a large number of U-CB transplantations. In addition to confirming previously reported chronic GVHD risk factors, we observed a lower incidence of extensive chronic GVHD in recipients of U-CB than in recipients of MR-BM. Moreover, in patients with chronic GVHD, oral cavity, eye, liver, lung and joint involvement was substantially lower in the U-CB group than in the MR-BM group.

Grade II–IV acute GVHD occurrence was a strong risk factor for chronic and extensive chronic GVHD, regardless of the donor type, which is consistent with previous findings.[4–7] The mechanism through which chronic GVHD develops is considered to be different from that of acute GVHD,[28] and the underlying mechanism by which acute GVHD strongly influences chronic GVHD development remains unknown. Acute GVHD causes thymic epithelial damage and functional deterioration, leading to a decrease in thymic output, represented by low T-cell receptor excision circle levels.[29] The association between low T-cell receptor excision circle levels and occurrence of chronic GVHD was reported in HLA-identical sibling transplantation,[30] which may partly explain the association between the history of acute GVHD and the development of chronic GVHD. The combination of female donor/male recipient was significantly associated with the development of chronic GVHD, which is also consistent with previous studies.[4,6] In the subset analysis, the combination of female donor/male recipient was significant for the R-BM/PB group, but not significant for the U-CB group. T cells transplanted from adult female donors can be activated by exposure to Y-chromosome-associated proteins and may cause chronic GVHD, but those from female U-CB units may be less activated against them.[31] Studies on the effect of the CMV Ab on chronic GVHD development have previously yielded controversial results.[2,32] In this study, we observed a significant impact of CMV seropositivity on the incidences of chronic GVHD and extensive chronic GVHD. However, the presence of antigenemia itself was not a significant factor in univariate analysis (data not shown); therefore, the mechanism through which CMV Ab affects chronic GVHD development remains unknown. We also confirmed that the use of a PBSC graft vs a BM graft constituted a strong risk factor for chronic and extensive chronic GVHD development in the R-BM/PB group. On the other

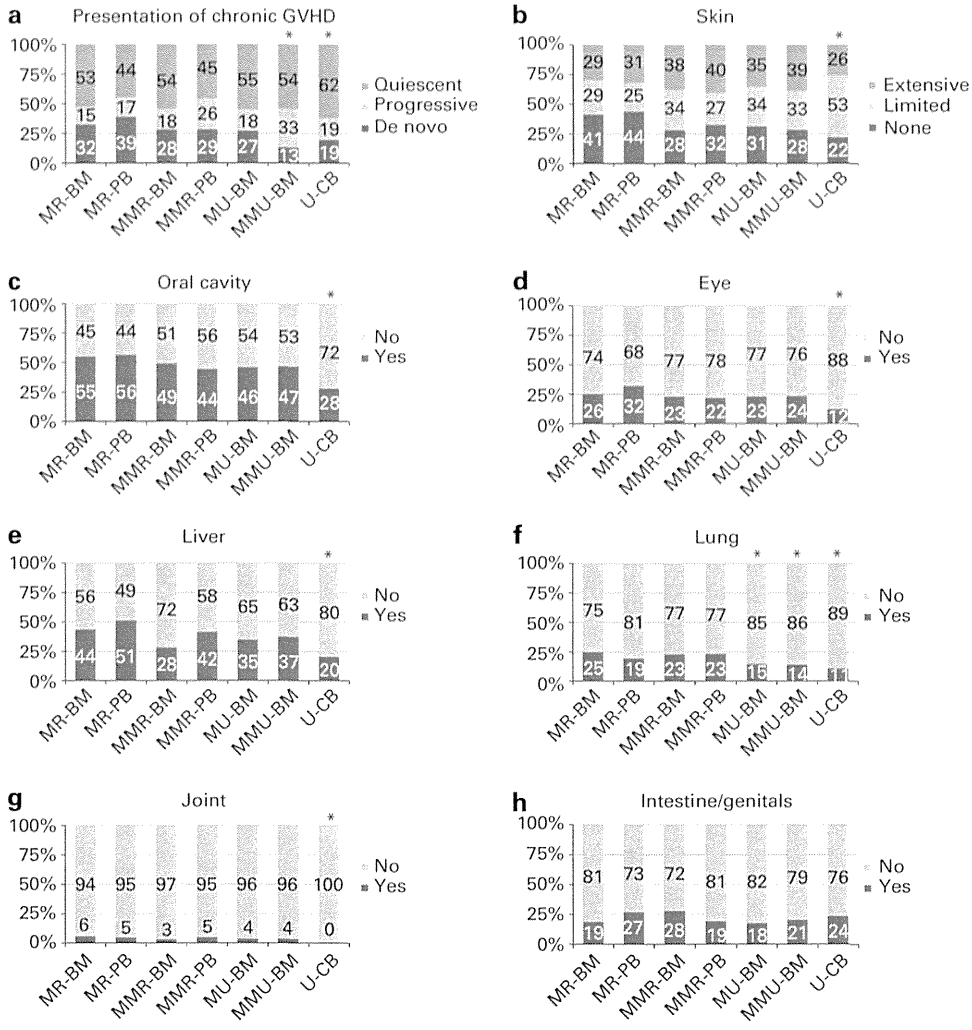

Figure 2. Presentation (**a**) and organ involvement (**b**–**h**) of chronic GVHD according to type of donor and stem cell source. Prevalence was compared between MR-BM and MR-PB, MMR-BM, MMR-PB, MU-BM, MMU-BM or U-CB. $^{*}P < 0.008$.

hand, the use of ATG was associated with a lower incidence of chronic GVHD, particularly in the R-BM/PB group. Contrary to previous reports, HLA disparity did not have a strong effect on chronic GVHD development in the R-BM/PB group. In addition, the use of MU-BM grafts was significantly associated with a lower incidence of extensive chronic GVHD. These findings may indicate that GVHD prophylaxis was intensified according to the acknowledged risk of GVHD. Therefore, we performed the same analysis after excluding the use of ATG or in the subgroup of patients who used tacrolimus or CsA as GVHD prophylaxis. However, we obtained the same result, which suggests that some other factor, such as the timing of immunosuppressive agent tapering, may be affecting the results.

In the analysis of chronic GVHD-specific survival, extensive type (vs limited type), progressive onset (vs *de novo* onset), extensive skin involvement (vs none), no skin involvement (vs limited involvement), and intestinal or genital involvement were associated with lower chronic GVHD-specific survival rate. The impact of quiescent onset chronic GVHD has been controversial,[2,33] but chronic GVHD-specific survival in the patients showing quiescent onset chronic GVHD was almost comparable to those showing *de novo* onset in line with several recent reports.[5,34] Although oral involvement was not associated with lower chronic GVHD-specific survival, which is compatible with a previous report,[35] intestinal or genital involvement was associated with lower survival rate. The use of U-CB was not associated with chronic GVHD-specific survival, even when only patients with extensive chronic GVHD were considered (data not shown). This finding suggests that chronic GVHD, if it occurs, does not behave differently regardless of the stem cell source. On the other hand, oral cavity, eye, liver, lung and joint involvement were substantially lower in the U-CB group, which contributed to the significantly lower incidence of extensive GVHD in the U-CB than in the MR-BM group. The high incidence of early TRM, such as that involving graft failure and infection, is considered a disadvantage of U-CB transplantations. However, if a patient survives the first few months following U-CB transplantation without treatment-related complications, the risk of extensive GVHD and GVHD-associated treatment-related complications would then be lower than in other transplantations. The low incidence of chronic GVHD would also contribute to the early discontinuation of immunosuppressive agents, which would allow or even promote immune reconstitution in long-term survivors of U-CB transplantation. Therefore, the choice of using U-CB as an alternative graft source might be prioritized if early treatment-related complications can be avoided through new approaches to ensure engraftment and enhance early immune reconstitution.