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Biological significance of HLA locus matching in unrelated donor bone marrow transplantation

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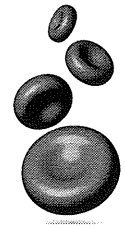
RESEARCH ARTICLE

The role of hematopoietic stem cell transplantation for relapsed and refractory Hodgkin lymphoma



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The optimal treatment strategy with the use of hematopoietic stem cell transplantation (HSCT) for relapsed and refractory Hodgkin lymphoma (HL) remains unclear. We performed a retrospective analysis using registry data from the Japanese Society for Hematopoietic Cell Transplantation. Adult patients with HL who underwent a first autologous or a first allogeneic HSCT between 2002 and 2009 were included. Patients who underwent HSCT in first complete remission (CR) were excluded. Autologous and allogeneic HSCT were performed in 298 and 122 patients, respectively. For autologous HSCT, overall survival at 3 years (3yOS) was 70%, and sex, age, disease status, and performance status (PS) at HSCT were prognostic factors. OS was favorable even in patients who underwent autologous HSCT in disease status other than CR. For allogeneic HSCT, 3yOS was 43%, and sex and PS at HSCT were prognostic factors. Disease status at HSCT, previous autologous HSCT, and conditioning intensity did not affect OS. Moreover, graft-versus-host disease did not affect progression-free survival or relapse/progression rate. A first allogeneic HSCT without a previous autologous HSCT was performed in 40 patients. 3yOS was 45%, and was significantly inferior to that in patients who underwent their first autologous HSCT. This result was retained after the correction by the different patient characteristics according to the type of HSCT. In conclusion, autologous HSCT is effective in prolonging survival in patients with relapsed and refractory HL. Allogeneic HSCT might be beneficial even to relapsed HL after autologous HSCT, although establishing the role of allogeneic HSCT remains a challenge.

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Introduction

Most patients with Hodgkin lymphoma (HL) can expect to be cured with standard chemotherapy with or without radiotherapy. However, 2–5% and 5–10% of patients have a primary refractory disease, and 10–15% and 25–30% of patients experience relapse after conventional chemotherapy in early-stage HL and advanced-stage HL, respectively [1,2]. For these patients, several studies have demonstrated that autologous hematopoietic stem cell transplantation (HSCT) can prolong survival [3–5]. In a randomized trial, Schmitz *et al.* reported longer time to treatment failure in patients with chemosensitive relapsed HL who underwent autologous HSCT, compared to those who underwent only conventional chemotherapy [5]. However, the optimal treatment strategy for chemoresistant HL has not been established, and the role of allogeneic HSCT for HL remains unclear [6]. Therefore, we performed a retrospective analysis using registry data from the Japanese Society for Hematopoietic Cell Transplantation (JSHCT) to clarify the roles of both autologous and allogeneic HSCT for relapsed and refractory HL.

Methods

Data source. Patients with HL aged more than 15 years who underwent a first autologous or a first allogeneic HSCT between 2002 and 2009 were included in this study. Clinical data for these patients were obtained from the Transplant Registry Unified Management Program (TRUMP) [7], which is the registry data of the JSHCT. We excluded patients who underwent HSCT in first complete remission (CR), since previous randomized studies have not supported the benefit of HSCT in first CR [8,9]. This study was planned by the Adult Lymphoma Working Group of JSHCT, and was approved by the data management committee of TRUMP and by the institutional review board of Nagoya University School of Medicine.

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Conflict of interest: Nothing to report

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TABLE I. Patient Characteristics

		Autologous HSCT (n = 298)	Allogeneic HSCT (n = 122)
Patient characteristics at diagnosis			
Sex	Male	200 (67%)	80 (66%)
	Female	98 (33%)	42 (34%)
Clinical stage at diagnosis	1	15 (5%)	3 (2%)
	2	115 (39%)	42 (34%)
	3	83 (28%)	29 (24%)
	4	83 (28%)	44 (36%)
B symptoms at diagnosis	–	176 (59%)	52 (43%)
	+	116 (39%)	61 (50%)
Previous autologous HSCT	–		40 (33%)
	+		82 (67%)
Patient characteristics at HSCT			
	Median follow-up days for survivors (range) [days after HSCT]	935 (14–3094)	948 (104–3214)
	Median age at HSCT (range) [year]	34 (16–75)	31 (16–68)
	Median duration between diagnosis and HSCT (range) [days]	672 (48–6313)	899 (77–5106)
Disease status at HSCT	CR	103 (35%)	24 (20%)
	PR	76 (25%)	16 (13%)
	other status	119 (40%)	82 (67%)
PS at HSCT	0	180 (60%)	51 (42%)
	1	97 (33%)	47 (39%)
	2	15 (5%)	9 (7%)
	3	2 (1%)	4 (3%)
	4	1 (1%)	3 (2%)
Stem cell source	BM	2 (1%)	56 (46%)
	PB	295 (98%)	53 (43%)
	BM+PB	1 (1%)	1 (1%)
	CB	–	11 (9%)
Donor relationship	MR		47 (39%)
	MMR		20 (16%)
	MUR		21 (17%)
	MMUR		19 (16%)
	CB		11 (9%)
Conditioning regimen including TBI	–	278 (93%)	57 (47%)
	+	8 (3%)	61 (50%)
Intensity of conditioning regimen	MAC		37 (30%)
	RIC		76 (62%)

HSCT, hematopoietic stem cell transplantation; CR, complete remission; PR, partial remission; PS, performance status; BM, bone marrow; PB, peripheral blood; CB, cord blood; MR, human leukocyte antigen (HLA)-matched related; MMR, HLA-mismatched related; MUR, HLA-matched unrelated; MMUR, HLA-mismatched unrelated; TBI, total body irradiation; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning.

Statistical considerations. Differences between groups were examined using Fisher's exact test for categorical variables. Overall survival (OS) and progression-free survival (PFS) were calculated using the Kaplan–Meier method, whereas the relapse/progression rate (RR) and non-relapse/progression mortality (NRM) were calculated using Gray's method considering each other event as a competing risk [10].

To evaluate the influence of factors for OS, proportional-hazards modeling was used for univariate and multivariate analyses. Factors with a P value of <0.10 in univariate analyses were subjected to multivariate analyses using the backward stepwise selection of covariates. Finally, P values of <0.05 were considered statistically significant. Different patients' characteristics according to the type of HSCT that patients underwent as their first HSCT were considered with Fisher's exact test in univariate analyses and a logistic regression analysis using the backward stepwise selection of covariates in multivariate analyses.

All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University) [11], which is a graphical user interface for R (The R Foundation for Statistical Computing, version 2.13.0, Vienna, Austria).

Results

Patient characteristics

Two hundred ninety-eight patients who underwent their first autologous HSCT and 122 patients who underwent their first allogeneic HSCT were included in this study. Eighty-two of the 122 patients (67%) who underwent their first allogeneic HSCT had previously received autologous HSCT, including seven patients who had a planned allogeneic HSCT following autologous HSCT. The characteristics of the patients are summarized in Table I.

Outcome of a first autologous HSCT

With a median follow-up time from HSCT of 935 days (range: 14–3094 days) for survivors, OS from HSCT in the 298 patients who underwent autologous HSCT was 85% at 1 year and 70% at 3 years (Fig. 1A). Through the univariate and multivariate analyses, female, younger age, disease status of CR, and better performance status (PS) at HSCT were significantly associated with better OS (Table II). OS was 89%, 90%, and 79% at 1 year, and 85%, 61%, and 62% at 3 years in patients who underwent autologous HSCT in CR, partial remission (PR), and the disease status other than CR/PR, respectively (Fig. 1B).

PFS, RR, and NRM at 1 year and 3 years were 68%, 25%, and 6%, and 59%, 32%, and 8%, respectively (Fig. 1C,D). Through the univariate and multivariate analyses, female, disease status of CR, and better PS at HSCT were significantly associated with better PFS (Table III).

Seven of 298 patients (2%) who underwent their first autologous HSCT developed a secondary malignancy. Two patients had a secondary solid tumor (colon cancer and brain tumor at 214 days and 1695 days from HSCT, respectively), and a patient who developed colon cancer died of it. Five patients had a secondary hematological malignancy (myelodysplastic syndrome at 78, 88, and 287 days from HSCT, and diffuse large B-cell lymphoma at 249 and 755 days from HSCT, respectively). None of them died directly of their secondary hematological malignancies.

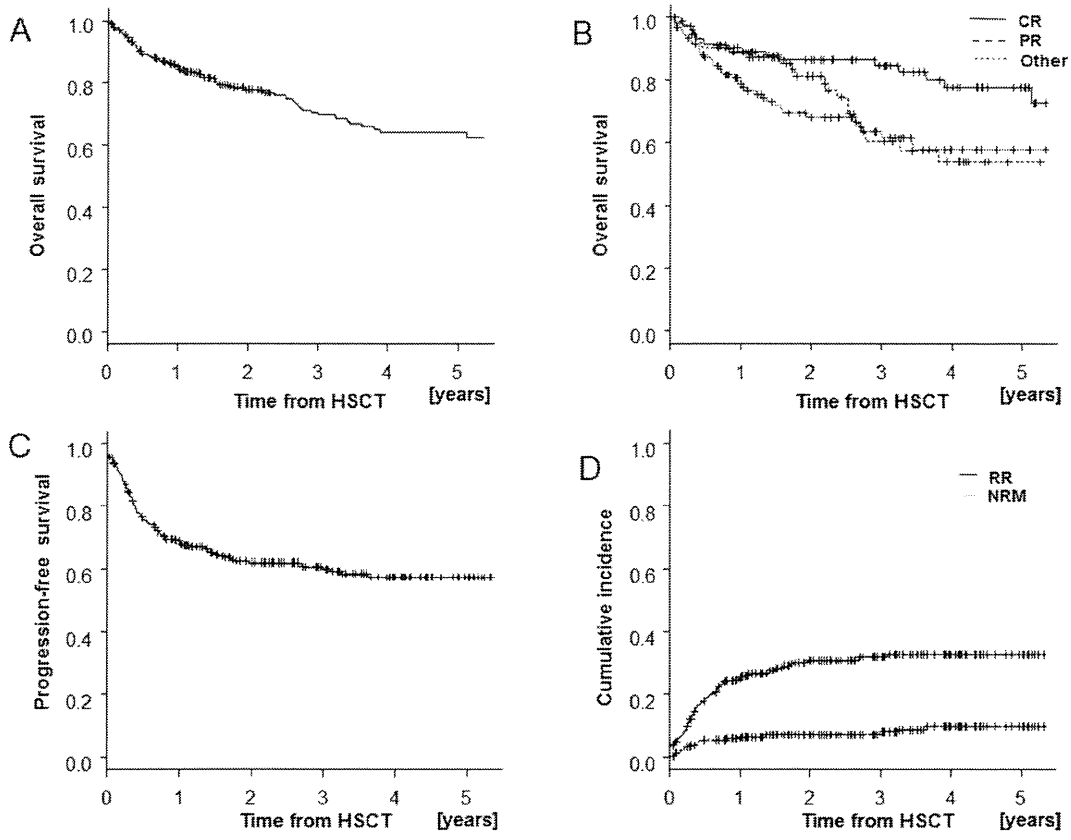

Figure 1. Overall survival from transplantation in all patients who underwent autologous HSCT (A) and in patients who underwent autologous HSCT in various disease statuses (B). Progression-free survival (C), relapse/progression rate (RR), and non-relapse/progression mortality (NRM) (D) in patients who underwent autologous HSCT.

TABLE II. Prognostic Factors for Overall Survival in Patients Who Underwent Their First Autologous HSCT

		Univariate analysis		Multivariate analysis	
		Relative Risk (95% C.I.)	*P* value	Relative Risk (95% C.I.)	*P* value
Sex	**Male**	1	0.074	1	0.049
	Female	0.62 (0.37–1.05)		0.58 (0.34–1.00)	
Clinical stage at diagnosis	**1,2**	1	0.031		
	3,4	1.66 (1.05–2.64)			
B symptoms at diagnosis	–	1	0.088		
	+	1.47 (0.94–2.28)			
Age at HSCT	**<40**	1	0.042	1	0.011
	≥40	1.57 (1.02–2.43)		1.72 (1.09–2.72)	
Disease status at HSCT	**CR**	1		1	0.006
	PR	2.01 (1.08–3.73)	0.014	2.28 (1.20–4.33)	
	Other status	2.27 (1.30–3.96)		2.50 (1.40–4.46)	
PS at HSCT	**0,1**	1		1	
	2–4	9.94 (5.32–18.56)	<0.001	9.89 (5.19–18.83)	<0.001

C.I., confidence interval; HSCT, hematopoietic stem cell transplantation; CR, complete remission; PR, partial remission; PS, performance status.

Outcome of a first allogeneic HSCT

With a median follow-up time from HSCT of 948 days (range: 104–3214 days) for survivors, OS from HSCT in the 122 patients who underwent their first allogeneic HSCT was 61% at 1 year and 43% at 3 years (Fig. 2A). If we consider only the 75 patients who underwent allogeneic HSCT after relapse following autologous HSCT, OS was 66% at 1 year and 42% at 3 years (Fig. 2B). Through the univariate and multivariate analyses, sex and PS at HSCT were significantly associated with better OS (Table IV). The history of previous autologous HSCT [relative risk (RR), 95% confidence interval (C.I.): 1.017 (0.62–1.66), $P = 0.95$] and conditioning intensity [RR (95% C.I.): 0.72 (0.43–1.20), $P = 0.20$] did not affect OS.

PFS, RR, and NRM at 1 year and 3 years were 45%, 27%, and 29%, and 31%, 37%, and 32%, respectively (Fig. 2C,D). Through the univariate and multivariate analyses, female and better PS at HSCT were significantly associated with better PFS (Table V). We evaluated the influence of graft-versus-host disease (GVHD). In 87 patients who were surviving without relapse/progression at least 60 days after HSCT, 62 and 46 patients experienced acute GVHD in any grade and Grade II–IV acute GVHD, respectively. In 66 patients who were surviving without relapse/progression at least 150 days after HSCT, 31 patients experienced chronic GVHD. The presence of acute GVHD in any grade, Grade II–IV acute GVHD, and chronic GVHD did not influence PFS ($P = 0.710$, $P = 0.460$, and $P = 0.834$, respectively),

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TABLE III. Prognostic Factors for Progression-Free Survival in Patients Who Underwent Their First Autologous HSCT

		Univariate analysis		Multivariate analysis	
		Relative Risk (95% C.I.)	*P* value	Relative Risk (95% C.I.)	*P* value
Sex	Male	1	0.090	1	0.023
	Female	0.68 (0.44–1.06)		0.61 (0.39–0.96)	
Clinical stage at diagnosis	1,2	1	0.071		
	3,4	1.44 (0.97–2.15)			
B symptoms at diagnosis	–	1	0.055		
	+	1.46 (0.99–2.15)			
Disease status at HSCT	CR	1	0.013	1	0.004
	PR	2.04 (1.22–3.42)		2.10 (1.24–3.57)	
	Other status	1.82 (1.14–2.91)		1.96 (1.21–3.20)	
PS at HSCT	0,1	1	<0.001	1	<0.001
	2–4	5.14 (2.79–9.47)		4.72 (2.53–8.81)	

C.I., confidence interval; HSCT, hematopoietic stem cell transplantation; CR, complete remission; PR, partial remission; PS, performance status

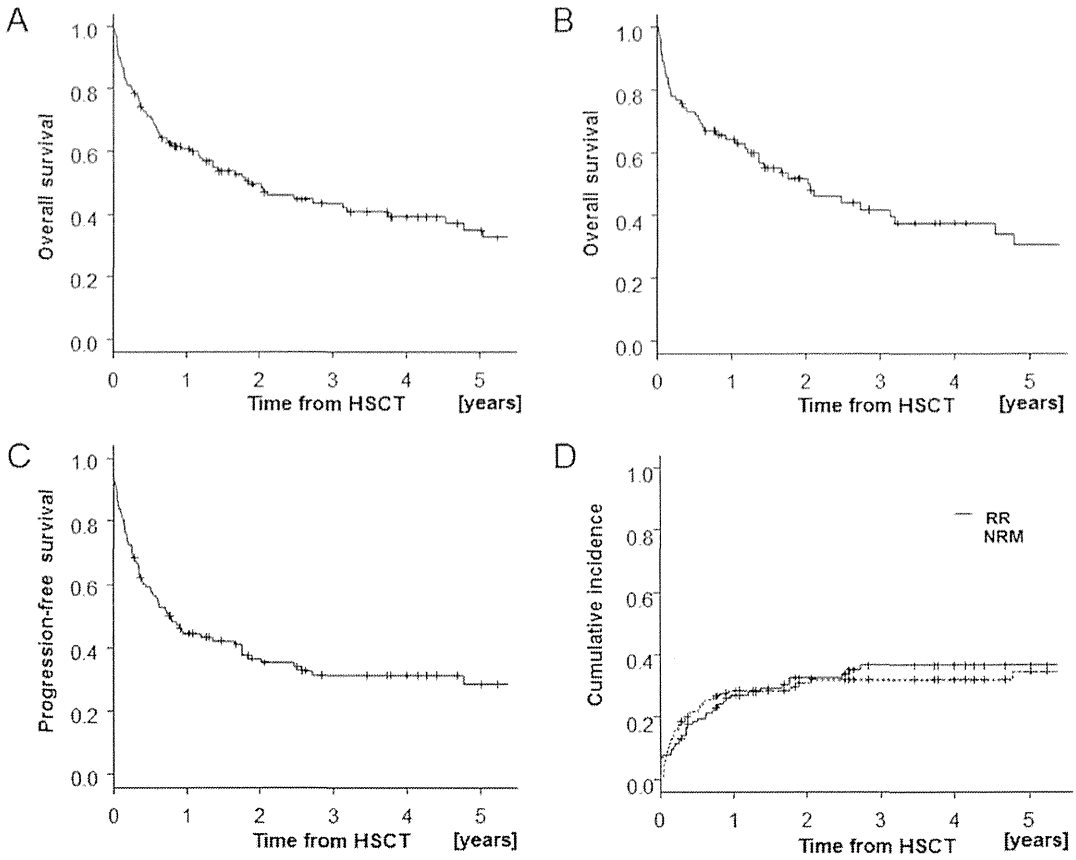

Figure 2. Overall survival from transplantation in all patients who underwent allogeneic HSCT (A) and in patients who underwent allogeneic HSCT after relapse following autologous HSCT (B). Progression-free survival (C), relapse/progression rate (RR), and non-relapse/progression mortality (NRM) (D) in patients who underwent allogeneic HSCT.

RR ($P = 0.136$, $P = 0.170$, and $P = 0.551$, respectively), and NRM ($P = 0.319$, $P = 0.068$, and $P = 0.588$, respectively).

Only one patient had a secondary malignancy (solid tumor; detailed information was not obtained).

Outcome of a first allogeneic HSCT without a previous autologous HSCT

A first allogeneic HSCT without a previous autologous HSCT was performed in 40 patients. OS from HSCT in these patients was 53% at 1 year and 45% at 3 years, and was significantly inferior to that in patients who underwent their first autologous HSCT (Fig. 3). Through the univariate (Table VI) and multivariate analyses, patients who underwent allogeneic HSCT as a first HSCT were more likely to have B symptoms at diagnosis and undergo HSCT in the worse disease status. The performance of allogeneic HSCT as a first HSCT was significantly associated with worse OS, even after the correction by the presence of B symptoms at diagnosis and disease status at HSCT.

Discussion

Many studies have reported the efficacy of autologous HSCT for relapsed and/or refractory HL. In our study, 3y OS was 70% in all patients who underwent autologous HSCT (Fig. 1A), and was

TABLE IV. Prognostic Factors for Overall Survival in Patients Who Underwent Their First Allogeneic HSCT

		Univariate analysis		Multivariate analysis	
		Relative risk (95% C.I.)	*P* value	Relative risk (95% C.I.)	*P* value
Sex	**Male**	1	0.011	1	0.018
	Female	0.49 (0.28–0.85)		0.49 (0.28–0.89)	
Disease status at HSCT	**CR**	1	0.066		
	PR	1.20 (0.46–3.11)			
	Other status	2.01 (1.05–3.86)			
PS at HSCT	**0,1**	1	<0.001	1	<0.001
	2–4	3.84 (2.12–6.96)		3.58 (1.94–6.61)	
Donor relationship	**MR**	1	<0.001		
	MMR	0.97 (0.48–1.96)			
	MUR	1.11 (0.53–2.31)			
	MMUR	3.31 (1.66–6.60)			
	CB	4.42 (2.07–9.42)			
Conditioning regimen including TBI	–	1	0.033		
	+	1.68 (1.04–2.71)			

C.I., confidence interval; HSCT, hematopoietic stem cell transplantation; CR, complete remission; PR, partial remission; PS, performance status; MR, human leukocyte antigen (HLA)-matched related; MMR, HLA-mismatched related; MUR, HLA-matched unrelated; MMUR, HLA-mismatched unrelated; CB, cord blood; TBI, total body irradiation.

TABLE V. Prognostic Factors for Progression-Free Survival in Patients Who Underwent Their First Allogeneic HSCT

		Univariate analysis		Multivariate analysis	
		Relative risk (95% C.I.)	*P* value	Relative risk (95% C.I.)	*P* value
Sex	**Male**	1	0.028	1	0.045
	Female	0.55 (0.32–0.94)		0.56 (0.32–0.99)	
Disease status at HSCT	**CR**	1	0.054		
	PR	1.83 (0.74–4.53)			
	Other status	2.29 (1.16–4.51)			
PS at HSCT	**0,1**	1		1	<0.001
	2–4	3.00 (1.64–5.49)	<0.001	2.83 (1.55–5.19)	
Donor relationship	**MR**	1	0.005		
	MMR	0.77 (0.37–1.59)			
	MUR	0.89 (0.45–1.77)			
	MMUR	2.10 (1.10–3.99)			
	CB	3.10 (1.43–6.69)			

C.I., confidence interval; HSCT, hematopoietic stem cell transplantation; CR, complete remission; PR, partial remission; PS, performance status; MR, human leukocyte antigen (HLA)-matched related; MMR, HLA-mismatched related; MUR, HLA-matched unrelated; MMUR, HLA-mismatched unrelated; CB, cord blood.

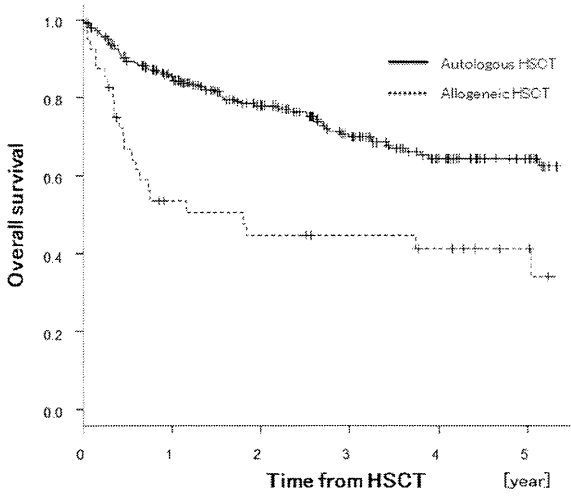

Figure 3. Among patients who had not received HSCT previously, overall survival in those who underwent allogeneic HSCT was significantly inferior to that in those who underwent autologous HSCT.

comparable to that in previous studies [4,12,13]. In addition, OS was favorable even in patients who underwent autologous HSCT in the disease status other than CR, although the disease status was associated with OS, which was similar to the results in many other studies [4,12,13]. We have to consider that clinical response was evaluated without positron-emission tomography (PET) in most of our patients. Part of patients who were evaluated as non-remission based on computed tomography (CT) might have undergone autologous HSCT in CR with PET-based assessment. The difference between OS and PFS might be attributed to the effectiveness of salvage therapy including allogeneic HSCT, which was difficult to evaluate precisely because information as to the performance of allogeneic HSCT after relapse/progression was lacking in a part of patients.

Allogeneic HSCT should be effective in prolonging OS even for relapsed HL after autologous HSCT, compared to conventional therapy [14]. In our study, 3y OS was 43% in all patients who underwent allogeneic HSCT, including those who had a previous autologous HSCT (Fig. 2A). Recently, many studies have mainly used reduced-intensity conditioning for allogeneic HSCT, and most of these patients had previously undergone autologous HSCT [15–17]. Sureda *et al.* compared the outcomes of allogeneic HSCT for patients with relapsed and refractory HL with myeloablative conditioning to those with reduced-intensity conditioning [18]. They reported that a lower NRM and a better OS were observed in patients who had been treated with reduced-intensity conditioning. In our study, the conditioning intensity did not influence OS. However, if we considered only patients who previously had undergone autologous HSCT,

TABLE VI. Characteristics of Patients Who Underwent HSCT Without Previous HSCT

		Autologous HSCT (n =298)	Allogeneic HSCT (n = 40)	P value
Patient characteristics at diagnosis				
Sex	Male	200 (57%)	24 (60%)	0.378
	Female	98 (43%)	16 (40%)	
Clinical stage at diagnosis	1,2	130 (44%)	13 (33%)	0.232
	3,4	166 (56%)	26 (65%)	
B symptoms at diagnosis	−	176 (59%)	13 (33%)	0.003
	+	116 (39%)	25 (63%)	
Patient characteristics at HSCT				
Age at HSCT	<40	170 (57%)	27 (68%)	0.235
	≥40	128 (43%)	13 (32%)	
Disease status at HSCT	CR	103 (35%)	4 (10%)	<0.001
	PR	76 (25%)	8 (20%)	
	Other status	119 (40%)	28 (70%)	
PS at HSCT	0,1	277 (93%)	29 (73%)	0.004
	2–4	18 (6%)	8 (20%)	

CR, complete remission; PR, partial remission; PS, performance status.

patients who were treated with reduced-intensity conditioning tended to have a better OS (P = 0.08). In patients who underwent allogeneic HSCT, PS at HSCT, instead of the disease status at HSCT, was significantly associated with OS in this study. The fact that the disease status was significantly associated with PS at allogeneic HSCT (P = 0.038, Fisher's exact test) might offset the influence of the disease status at HSCT on OS. Similarly, donor relationship was the significant factor for OS in a univariate analysis, but not in a multivariate analysis. Some clinical factors, other than the donor availability, might influence the donor selection, and weaken the association between donor relationship and OS. However, we could not detect such factors. In our study, sex was significantly associated with OS even through a multivariate analysis. It was demonstrated that the disadvantage of male sex, which was a known adverse prognostic factor in HL at diagnosis [19], persisted even after the performance of allogeneic HSCT.

Some studies have suggested the existence of a graft-versus-Hodgkin lymphoma effect [18,20,21]. In our study, the presence of acute or chronic GVHD did not affect PFS and RR. Acute or chronic GVHD were not associated with PFS and RR even if we analyzed only patients who underwent allogeneic HSCT in the disease status other than CR or patients who underwent allogeneic HSCT with reduced-intensity conditioning (data not shown). A graft-versus-Hodgkin lymphoma might have a very limited effect in our patients who underwent allogeneic HSCT for very advanced HL. We did not have enough patients to evaluate the role of donor lymphocyte infusion.

Recently, brentuximab vedotin has been shown to offer promising results, without severe adverse effects, even in patients with relapsed HL after autologous HSCT [22]. It has also been reported that the administration of brentuximab vedotin might be safe and effective both before and after allogeneic HSCT [23,24]. Considering that a graft-versus-Hodgkin lymphoma effect might have only a limited effect, the combination of allogeneic HSCT and brentuximab vedotin following HSCT deserve evaluation as new treatment strategy to prevent relapse after allogeneic HSCT.

The role of allogeneic HSCT as a first HSCT remains to be determined. Akpek *et al.* compared autologous HSCT and allogeneic HSCT from an HLA-matched sibling for patients with relapsed and refractory HL who had not received HSCT previously [20]. They demonstrated that there were no significant differences in OS and RR. On the other hand, OS in patients who underwent allogeneic HSCT was significantly inferior to that in patients who underwent autologous HSCT in our study. This might be attributed to a difference in the patients' background because the selection of autologous or allogeneic HSCT was at the discretion of each institution. In fact, patients who had allogeneic HSCT as a first HSCT underwent HSCT in the worse disease status, compared to those who had autologous HSCT. Moreover, chemosensitivity or number of salvage chemotherapy before HSCT might influence the difference in OS, although we could not obtain enough data as to these factors. Allogeneic HSCT without previous autologous HSCT might be a reasonable option in selected patients with chemorefractory HL, such as young patients with good PS. Prospective studies will be needed to establish the role of allogeneic HSCT in specific situations.

In conclusion, autologous HSCT is effective in prolonging survival in patients with relapsed and refractory HL. Allogeneic HSCT might be beneficial even to relapsed HL after autologous HSCT, although establishing the role of allogeneic HSCT remains a challenge.

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Comparison of Continuous and Twice-Daily Infusions of Cyclosporine A for Graft-Versus-Host-Disease Prophylaxis in Pediatric Hematopoietic Stem Cell Transplantation

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Background. Cyclosporine A (CsA) is used widely for graft-versus-host disease (GVHD) prophylaxis in hematopoietic stem cell transplantation (HSCT); however, the optimal schedule of its administration has not been established. Although comparative studies of adult patients undergoing HSCT have demonstrated enhanced efficacy and safety of twice-daily infusion (TD) compared with continuous infusion (CIF) of CsA, to our knowledge, similar studies have not yet been performed in pediatric groups. ***Procedure.*** A self-administered questionnaire was used to retrospectively compare the clinical outcome and incidence of CsA-associated adverse events of 70 pediatric acute myelogenous leukemia patients who were receiving CsA by TD (n = 36) or CIF (n = 34) as GVHD prophylaxis for their first allogeneic HSCT. ***Results.*** The cumulative incidences of grade II–IV acute GVHD and chronic GVHD, as well as the overall survival and event-free survival rates, did not differ significantly between the TD and CIF groups; however, the incidence of severe hypertension was significantly higher in the CIF group than the TD group. ***Conclusions.*** The analysis presented here indicates that TD and CIF administration of CsA have similar prophylactic effect on pediatric GVHD and suggest that TD is associated with a lower rate of toxicity than CIF in pediatric patients undergoing HSCT. Pediatr Blood Cancer 2015;62:291–298. © 2014 Wiley Periodicals, Inc.

Key words: cyclosporine; graft-versus-host disease; hematopoietic stem cell transplantation; pediatric

INTRODUCTION

The immunosuppressive drug cyclosporine A (CsA), which is usually combined with short-term treatment with methotrexate (MTX), is used widely for the prophylaxis of graft-versus-host disease (GVHD). Traditionally, CsA is typically administrated intravenously in the early period after allogeneic hematopoietic stem cell transplantation (HSCT), after which the treatment is converted to oral administration [1].

Target CsA concentrations of 250–450 ng/ml are widely accepted for continuous infusion (CIF) of CsA [2]: however, these concentrations are not sufficient to prevent GVHD in adult patients undergoing HSCT. Although CIF of CsA at higher target concentrations (450–550 ng/ml) is more effective at preventing GVHD, these concentrations are associated with adverse effects, including hypertension and acute nephrotoxicity [3,4]. The immunosuppressive effect of CsA, which occurs via calcineurin inhibition, is concentration-dependent rather than time-dependent and its greatest pharmacodynamic effect occurs within the first 2 or 3 hr after exposure [5,6]. Hence, twice-daily infusion (TD) of CsA is used during renal, liver, and heart transplantation to reduce the occurrence of graft rejections [7].

TD administration of CsA with peak concentration monitoring has also been employed as an optimized GVHD prophylaxis regimen for adult patients undergoing HSCT [8–10]. However, the dose, target blood level, and mode of intravenous infusion vary

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Conflict of interest: Nothing to declare.

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among transplant institutions, and the optimal schedule of CsA administration has not yet been established. Furthermore, the comparative studies of the efficacy of various modes of CsA treatment have not yet been performed in pediatric HSCT despite of the wide use of both TD and CID modes. Therefore, the aim of this study was to evaluate the efficacy and safety of the TD and CIF modes of CsA administration for the treatment of pediatric HSCT. For this aim, we analyzed the data of pediatric patients with acute myelogenous leukemia (AML) as a single disease entity, which is one of the most popular pediatric hematological malignancies.

MATERIALS AND METHODS

Study Design and Data Collection

Using data for patients with AML provided by the Transplant Registry Unified Management Program (TRUMP) [11], which includes data from the Japan Cord Blood Bank Network (JCBBN) and the Japan Society for Hematopoietic Cell Transplantation (JSHCT), the following criteria were used to select candidates for the self-administered questionnaire: (i) children with a diagnosis of AML who were younger than 18 years old; (ii) children in which allogeneic transplantation was performed for the first time during January 2006 and December 2009; (iii) children administered CsA for GVHD prophylaxis; and (iv) children administered CsA for more than 28 days after the first transplant. The data were extracted from the database in the Japan Society for Stem Cell Transplantation Registry and 99 cases from 58 institutions were selected as candidates. The questionnaire was distributed to gather additional information about the mode of CsA administration, the daily dose; the blood concentration of CsA; and CsA-associated adverse effects, including hypertension, renal toxicity, hyperglycemia, hyperbilirubinemia, thrombotic microangiopathy (TMA), hepatic veno-occlusive disease of liver (VOD), and encephalopathy. Of the 58 transplant institutions surveyed, 44 (75.9%) responded and data for 70 patients with AML were included in the study. This study was approved by the Data Management Committee of the Nationwide Survey of the JSHCT, and the institutional ethics committees of Kyoto University Hospital and Nagoya University Hospital.

Based on the recommendation outlined in a previous report [12], myeloablative conditioning (MAC) was classified as a regimen including at least 5 Gy of total body irradiation (TBI) as a single fraction, at least 8 Gy or TBI in fractionated doses, or oral or intravenous administration of busulfan at doses greater than 8 mg/kg. All other conditioning regimens were classified as nonmyeloablative reduced intensity conditioning (RIC). For transplantation using related bone marrow (BM) or peripheral blood (PB), or unrelated cord blood (CB), HLA matching was assessed using serological data for the HLA-A, HLA-B, and HLA-DR loci. For transplantation using unrelated BM, HLA matching was assessed using allelic data for HLA-A, HLA-B, and HLA-DRB1.

Endpoints

The primary endpoint of this study was to compare the cumulative incidences of grade II-IV and grade III-IV acute GVHD, and CsA-associated adverse events between the TD and CIF groups. Other endpoints were to compare the overall survival (OS) and event-free survival (EFS) rates, and the cumulative incidences of chronic GVHD, non-relapse mortality (NRM), and relapse between the TD and CIF groups. Acute and chronic GVHD was diagnosed and graded by the attending physicians of each hospital according to the consensus criteria [13,14]. Hypertension, renal toxicity, hyperglycemia, and hyperbilirubinemia were evaluated using the National Cancer Institute's Common Terminology Criteria for Adverse Events (version 4.0), and severe adverse events were defined as grade 2 and higher. Diagnosis of VOD, TMA, and encephalopathy were made based on characteristic clinical findings, positive laboratory data, or positive radiological findings by the attending physicians at each hospital.

Statistical Analysis

The characteristics of patients in the TD and CIF groups were compared using Fisher's exact test for categorical variables and two-sample Wilcoxon's test for continuous variables. OS and EFS rates were estimated using the Kaplan–Meier method [15], and the groups were compared using the log-rank test. The cumulative incidences of grade II–IV acute GVHD, chronic GVHD, NRM, and relapse were estimated, and the groups were compared using the log-rank test. Competing events were engraftment failure, relapse, or NRM without GVHD for acute and chronic GVHD, death without relapse for relapse, and relapse for NRM. To determine prognostic factors associated with the development of grade II–IV acute GVHD and chronic GVHD, log-rank test and a Cox regression test were used. The following variables were examined in the univariate analysis: mode of CsA administration, patient age, sex match, stage of AML, HSCT type, ABO match, conditioning regimen, and CMV serostatus. Factors with $P < 0.2$ in log-rank tests were included in the Cox regression model. To determine prognostic factors associated with the development of severe hypertension, Fisher's exact test and a logistic regression test were used. The following variables were examined in the univariate analysis: mode of CsA administration, patient age, occurrence of grade 1 hypertension before HSCT, use of melphalan (Mel), use of $\geq$8 Gy of TBI, conditioning regimen, use of prednisolone or methylprednisolone for GVHD prophylaxis and/or treatment, and HSCT type. Factors with $P < 0.2$ in Fisher's exact tests were included in the logistic regression model. All statistical analyses were performed using Stata software (version 12; StataCorp, TX). The authors had full access to the data and assume responsibility for their integrity. The P values were two-sided and $P < 0.05$ was considered significant for all analyses.

RESULTS

Characteristics of the Patients

Of 70 pediatric patients with AML, 36 (51.4%) and 34 (48.6%) received TD and CIF of CsA, respectively. The characteristics of the patients and the associated clinical data are listed in Table I. Most of the patients received MAC (58 of 70 patients; 82.9%), and most underwent short-term treatment with MTX in combination with CsA (63 of 70 patients; 90.0%). Prednisolone was administrated to only two patients (2.9%). There were no significant differences between any of the baseline characteristics of the TD and CIF groups (Table I). The median time to switch to oral administration of CsA in the TD and CIF groups were 41 days (range, 20–73 days) and 36 days (range, 21–84 days), respectively. In the TD group, CsA was administrated over two (n = 15), three (n = 19), four (n = 1), or

TABLE I. Characteristics of the 70 Patients Included in the Study

Variable	TD (n = 36)	%	CIF(n = 34)	%	*P*-value
Recipient age (years), median (range)	9 (0–17)		10 (1–17)		0.120
Patient sex					
Male	20	55.6	20	58.8	0.813
Female	16	44.4	14	41.2	
Sex match					
Match	15	41.7	13	38.2	0.323
Male to female	10	27.8	5	14.7	
Female to male	7	19.4	13	38.2	
Missing	4	11.1	3	8.8	
Diagnosis					
M0	0	0	2	5.9	0.663
M1	5	13.9	7	20.6	
M2	8	22.2	8	23.5	
M3	0	0	1	2.9	
M4	5	13.9	3	8.8	
M5a	7	19.4	2	5.9	
M5b	0	0	1	2.9	
M6	1	2.8	1	2.9	
M7	7	19.4	6	17.6	
With MD	1	2.8	2	5.9	
Others	2	5.6	1	2.9	
De novo					
De novo	33	91.7	31	91.2	1.000
Secondary	3	8.3	3	8.8	
WBC at diagnosis (/μl), median (range)	19,700 (1,300–405,900)		9,750 (610–290,000)		0.428
Stage					
1CR	21	58.3	17	50.0	0.562
2CR	3	8.3	6	17.6	
NCR	12	33.3	11	32.3	
HSCT type					
MR-BM/CB	15	41.7	13	38.2	0.641
MR-PB	3	8.3	7	20.6	
MMR-BM/PB	4	11.1	4	11.8	
MU-BM	2	5.6	2	5.9	
U-CB	12	33.3	8	23.5	
ABO match					
Matched	23	63.9	18	52.9	0.811
Minor mismatched	4	11.1	6	17.6	
Major mismatched	4	11.1	4	11.8	
Major-minor mismatched	5	13.9	6	17.6	
Conditioning regimen					
MAC	31	86.1	28	82.4	0.750
RIC	5	13.9	6	17.6	
GVHD prophylaxis					
+MTX	34	94.4	29	85.3	0.153
+PSL	0	0	1	2.9	
+MTX, PSL	1	2.8	0	0	
CsA alone	1	2.8	4	11.8	
CMV serostatus					
Negative donor to negative patient	5	13.9	4	11.8	0.924
Positive donor to negative patient	2	5.6	2	5.9	
Negative donor to positive patient	6	16.7	4	11.8	
Positive donor to positive patient	12	33.3	15	44.1	
Unknown	11	30.6	9	26.5	
Follow-up (days), median (range)	700.5 (56–1,599)		567.5 (69–1,409)		0.282

MD, myelodysplasia; WBC, white blood cell; 1CR, first complete remission; 2CR, second complete remission; NCR, no complete remission; MR-BM/CB, HLA-matched related bone marrow/cord blood; MR-PB, HLA-matched related peripheral blood stem cells; MMR-BM/PB, HLA-mismatched related bone marrow/peripheral blood stem cells; MU-BM, HLA-matched unrelated bone marrow; U-CB, unrelated cord blood; MAC, myeloablative conditioning; RIC, reduced intensity conditioning; MTX, methotrexate; PSL, prednisolone.

five hours (n = 1). None of the patients underwent *in vivo* or *ex vivo* T cell depletion.

Treatment Outcome

The median follow-up duration was 590.5 days (range, 56–1599 days). The OS (Fig. 1A) and EFS (Fig. 1B) rates did not differ significantly between the TD and CIF groups. Furthermore, there were no significant differences in the cumulative incidences of grade II–IV acute GVHD (Fig. 1C) and chronic GVHD (Fig. 1D) between the TD and CIF groups. The differences in the cumulative incidences of grade II–IV acute GVHD or chronic GVHD were also not significant when the dataset was limited to patients treated with CsA and MTX (data not shown). There were no significant differences in the cumulative incidence of grade III–IV acute GVHD between the TD and CIF groups (grade III–IV acute GVHD at day 100: TD group, 0%; CIF group, 3.0 ± 3.0%; $P = 0.303$). The cumulative incidences of relapse and NRM did not differ

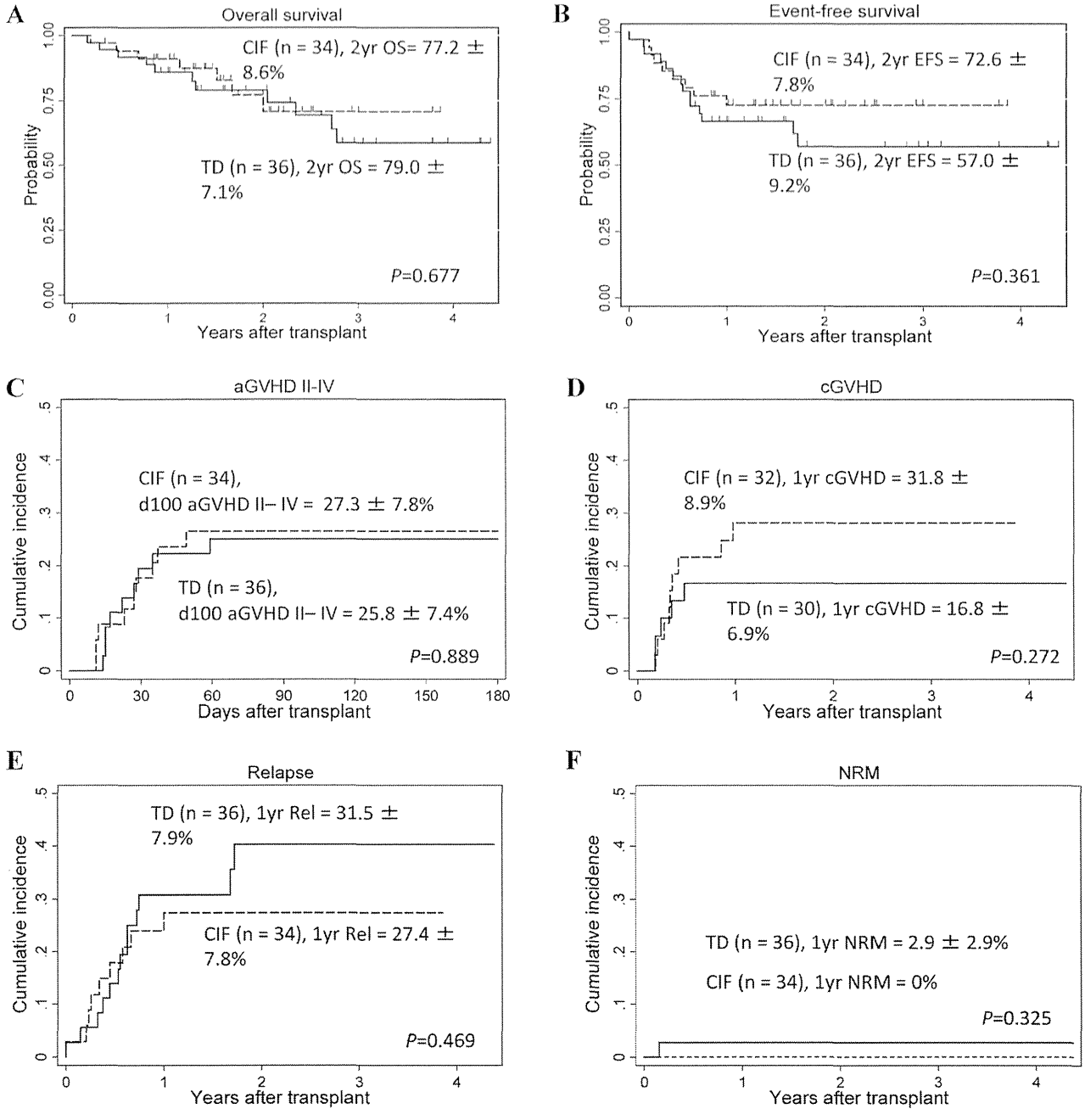

Fig. 1. The overall survival (**A**) and event-free survival (**B**) rates, and the cumulative incidences of grade II–IV acute GVHD (**C**) and chronic GVHD (**D**) among patients grouped by the mode of CsA administration. The cumulative incidences of relapse (**E**) and non-relapse mortality (**F**) among patients grouped by the mode of CsA administration. The solid and dashed lines indicate the TD and CIF groups, respectively.

significantly between the TD and CIF groups (Fig. 1E and F). Next, a univariate analysis was performed to evaluate the impact of potential confounding factors on the development of grade II–IV acute GVHD. Stage of AML, HSCT type, and CMV serostatus were identified as risk factors for grade II–IV acute GVHD; however, a multivariate analysis using a Cox regression test demonstrated that no independent risk factors were identified (Table II). For chronic GVHD, stage of AML, HSCT type, and conditioning regimen were identified as risk factors; however, a multivariate analysis demonstrated that no independent risk factors were identified.

Incidence of CsA-associated Adverse Events

The incidences of CsA-associated adverse events in the TD and CIF groups during the first 28 days after transplantation were compared. For each adverse event, patients who had grade 2 or higher toxicity before transplantation were excluded from the analysis. The incidence of severe hypertension was significantly higher in the CIF group than the TD group; however, the incidences of severe renal toxicity, hyperglycemia, and hyperbilirubinemia, TMA, VOD, and encephalopathy did not differ significantly between the two groups (Table III).

Univariate and Multivariate Analyses of Factors Related to the Development of Severe Hypertension

Univariate analysis was performed to evaluate the impact of potential confounding factors on the development of severe hypertension. As shown in Table IV, CIF administration of CsA, grade 1 hypertension before HSCT, the use of melphalan, and conditioning regimen were identified as risk factors for severe hypertension. A multivariate analysis using a logistic regression test was then performed to identify independent risk factors for the development of severe hypertension. CIF administration of CsA was identified as the sole independent significant risk factor.

Daily Doses and Trough Blood Concentration of CsA

In a previous study of adult patients receiving CsA, the incidence of grade II–IV acute GVHD was significantly higher and renal toxicity was significantly less frequent in the CIF group than the TD group [8]. In the adult study, patients in the TD group received a higher dose of CsA than those in the CIF group and the trough blood concentrations in these two groups were maintained at 150–300 ng/ml and 250–400 ng/ml, respectively [8]. To enable a direct comparison of the results, the daily doses and trough blood

TABLE II. Univariate and Multivariate Analyses of the Effects of Pre-transplantation Factors on the Incidence of Grade II-IV Acute GVHD in the 70 Patients Included in the Study

				Multivariate analysis	
Characteristics	Factors (n)	Grade II-IV acute GVHD	Univariate analysis *P*-value	Odds ratio (95% CI)	*P*-value
CsA mode	TD (36)	27.3 ± 7.8	0.889	1.02 (0.40–2.58)	0.973
	CIF (34)	25.8 ± 7.4			
Age group	0–9 (34)	21.2 ± 7.1	0.284	N.E.	N.E.
	10–17 (36)	31.4 ± 7.9			
Sex match	Match (28)	25.0 ± 8.2	0.294	N.E.	N.E.
	Male to female (15)	40.7 ± 12.9			
	Female to male (20)	27.8 ± 10.6			
	Missing (7)	0			
Stage	1CR (38)	15.8 ± 5.9	0.015	1.58 (0.95–2.63)	0.075
	2CR (9)	55.6 ± 16.6			
	NCR (23)	33.3 ± 10.3			
HSCT type	MR-BM/CB (28)	25.0 ± 8.2	0.011	1.00 (0.75–1.34)	0.987
	MR-PB (10)	0			
	MMR-BM/PB (8)	62.5 ± 17.1			
	MU-BM (4)	66.7 ± 27.2			
	U-CB (20)	21.0 ± 9.4			
ABO match	Matched (41)	20.6 ± 6.5	0.455	N.E.	N.E.
	Minor mismatched (10)	40.0 ± 15.5 %			
	Major mismatched (8)	37.5 ± 17.1			
	Major-minor mismatched (11)	27.3 ± 13.4			
Conditioning regimen	MAC (59)	28.1 ± 6.0	0.536	N.E.	N.E.
	RIC (11)	18.2 ± 11.6			
CMV serostatus	Negative donor to negative patient (9)	11.1 ± 10.5	0.159	1.19 (0.80–1.70)	0.390
	Positive donor to negative patient (4)	0			
	Negative donor to positive patient (10)	30.0 ± 14.5			
	Positive donor to positive patient (27)	42.3 ± 9.7			
	Unknown (20)	15.8 ± 8.4			

N.E., not evaluated; 1CR, first complete remission; 2CR, second complete remission; NCR, no complete remission; MR-BM/CB, HLA-matched related bone marrow/cord blood; MR-PB, HLA-matched related peripheral blood stem cells; MMR-BM/PB, HLA-mismatched related bone marrow/peripheral blood stem cells; MU-BM, HLA-matched unrelated bone marrow; U-CB, unrelated cord blood; MAC, myeloablative conditioning; RIC, reduced intensity conditioning.

TABLE III. The Incidences of Complications (≥grade 2 and ≥grade 3) in Patients Grouped by the Mode of CsA Administration

Complication	CsA mode	Cases	≥grade2	*P*-value	≥grade3	*P*-value
Hypertension	TD	36	2 (5.5%)	0.021	0 (0%)	0.010
	CIF	34	9 (26.5%)		6 (17.6%)	
Hyperglycemia	TD	36	3 (8.3%)	0.466	0 (0%)	0.225
	CIF	33	5 (15.2%)		2 (6.1%)	
Renal toxicity	TD	36	6 (16.7%)	0.261	1 (2.8%)	1
	CIF	34	2 (5.9%)		1 (2.9%)	
Hyperbilirubinemia	TD	36	1 (2.8%)	0.608	0 (0%)	0.478
	CIF	33	2 (6.1%)		1 (3.0%)	

concentrations of CsA were evaluated in the 70 patients included in this study during the first 28 days after transplantation.

No significant differences in the daily doses of CsA were observed between the TD and CIF groups at days 7, 14, 21, and 28 (Fig. 2A). The trough blood concentrations of CsA in the TD group at days 7, 14, 21, and 28 were 122.9 ± 68.1 ng/ml, 158.7 ± 71.5 ng/ml, 187.2 ± 102.5 ng/ml, and 190.6 ± 93.0 ng/ml, respectively. The corresponding concentrations in the CIF group were 294.8 ± 83.3 ng/ml, 350.9 ± 138.3 ng/ml, 335.8 ± 132.3 ng/ml, and 310.5 ± 119.0 ng/ml, respectively. At days 7, 14, 21, and 28, trough concentrations of CsA below 150 ng/ml occurred in 58.3%, 52.9%, 38.2% and 31.0% of patients in the TD group, respectively. Trough concentrations below 250 ng/ml occurred in 17.6%, 14.7%, 23.5% and 30.0% of patients in the CIF group at days 7, 14, 21, and 28, respectively. These data indicate that, compared with the CIF group, a significantly higher percentage of patients in the TD group were treated with a lower dose of CsA during the first two weeks after transplantation than that reported in a previous study of CsA administration to adults undergoing HSCT[8] ($P = 0.009$ and $P = 0.002$ at days 7 and 14, respectively) (Fig. 2B).

DISCUSSION

Because uncontrolled variables, such as patient age and underlying disease, may influence the incidence or severity of acute GVHD, it is necessary to evaluate the efficacy and safety of different types of GVHD prophylaxis within homogenous groups of patients. To achieve this aim, a nationwide survey was performed to select pediatric AML cases who had recently received their first allogeneic transplantation and had been treated with CsA for GVHD prophylaxis. Historically, CsA was administered to most pediatric patients via CIF; however, the mode of CsA administration in Japan has gradually shifted to TD over the last few years. Consequently, the 70 patients selected for inclusion in this study were divided approximately equally between the TD and CIF groups, which enabled a reliable comparison of the effect of CIF and TD

TABLE IV. Univariate and Multivariate Analyses of the Effects of Pre-transplantation Factors on the Incidence of Severe Hypertension (HT) in the 70 Patients Included in the Study

				Multivariate analysis	
Characteristics	Factors (n)	≥grade2	Univariate analysis *P*-value	Odds ratio (95% CI)	*P*-value
CsA mode	TD (36)	2 (5.6%)	0.022	7.99 (1.37–46.4)	0.021
	CIF (34)	9 (26.5%)			
Age group	0–9 (34)	6 (17.6%)	0.750	N.E.	N.E.
	10–17 (36)	5 (13.9%)			
Grade 1 hypertension before HSCT	Yes (65)	9 (13.8%)	0.173	6.26 (0.69–57.2)	0.173
	No (5)	2 (40.0%)			
Mel	Yes (31)	7 (22.6%)	0.196	0.35 (0.06–1.86)	0.217
	No (39)	4 (10.3%)			
TBI ≥8 Gy	Yes (43)	6 (14.0%)	0.739	N.E.	N.E.
	No (27)	5 (18.5%)			
PSL/mPSL for GVHD prophylaxis and/or treatment	Yes (14)	4 (28.6%)	0.212	N.E.	N.E.
	No (56)	7 (12.5%)			
Conditioning regimen	MAC (59)	7 (11.9%)	0.063	3.45 (0.56–21.4)	0.182
	RIC (11)	4 (36.4%)			
SCT type	MR-BM/CB (28)	2 (7.1%)	0.325	N.E.	N.E.
	MR-PB (10)	2 (20.0%)			
	MMR-BM/PB (8)	2 (25.0%)			
	MU-BM (4)	0 (0%)			
	U-CB (20)	5 (25.0%)			

N.E., not evaluated; Mel, melphalan; TBI, total body irradiation; PSL, prednisolone; mPSL, methylprednislone; MAC, myeloablative conditioning; RIC, reduced intensity conditioning; MR-BM/CB, HLA-matched related bone marrow/cord blood; MR-PB, HLA-matched related peripheral blood stem cells; MMR-BM/PB, HLA-mismatched related bone marrow/peripheral blood stem cells; MU-BM, HLA-matched unrelated bone marrow; U-CB, unrelated cord blood.

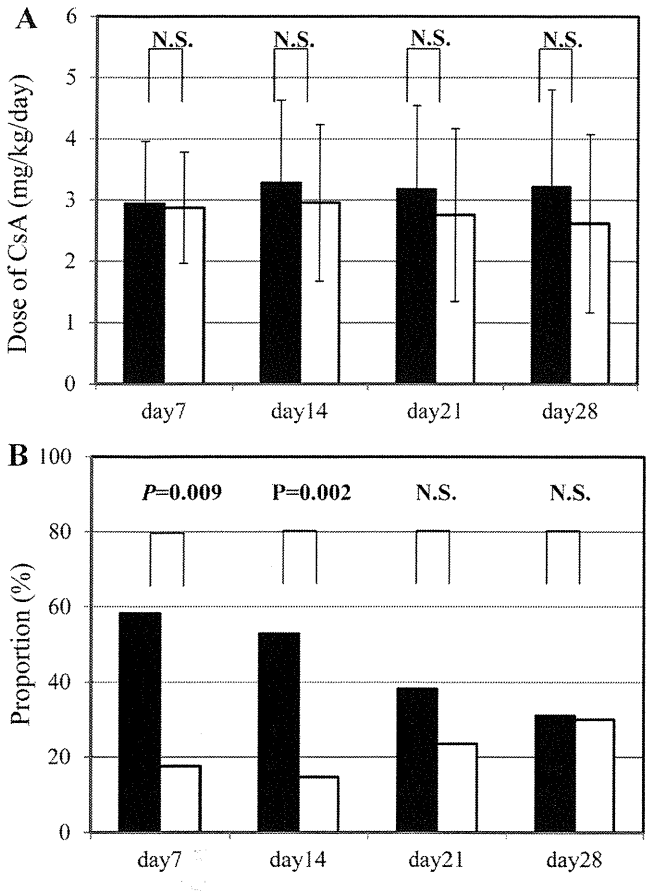

Fig. 2. (**A**) The daily doses of CsA administrated to patients in the TD (solid bars) and CIF (open bars) during the first 28 days after transplantation. (**B**) The percentages of patients in the TD (solid bars) and CIF (open bars) groups with trough concentration of CsA below 150 ng/ml (TD group) or 250 ng/ml (CIF group) during the first 28 days after transplantation. N.S., not significant. The data are presented as the mean ± SD.

administration of CsA among relatively homogenous pediatric populations.

In a previous study of adults undergoing HSCT, renal dysfunction was significantly less frequent in the CsA CIF group than the TD groups [8]. By contrast, in the current study, the incidences of CsA-associated adverse events, including renal dysfunction, were comparable in the TD and CIF groups. A possible explanation for the lack of increased renal dysfunction in the TD group observed here is that a large proportion of the pediatric TD patients (>50% in the first 14 days after transplantation) had trough concentration of CsA less than those reported in the adult study. By contrast, a significantly smaller proportion of pediatric patients in the CIF group had trough concentrations lower than those reported in the adult study. Alternatively, the pharmacokinetics and adverse effects of CsA may differ between pediatric and adult patients. Notably, CIF of CsA was identified as the sole independent risk factor for the development of severe hypertension, although TMA and encephalopathy, both of which are closely related to CsA-associated hypertension, occurred rarely in both the TD and CIF groups. Clinicopathological findings, as well as animal model studies, have indicated that CsA-induced acute reversible nephrotoxicity, caused by vasoconstriction of the afferent arterioles, might trigger the development of chronic irreversible damage to renal vessels, interstitial tubules, and glomeruli [16]. Furthermore, hypertension can persist long-term in some HSCT survivors [17], and the presence of multiple cardiovascular risk factors, including hypertension, is associated with an increased risk of late cerebrovascular disease and coronary artery disease after HSCT [18]. TD administration of CsA to pediatric patients undergoing HSCT may reduce the risk of late-occurring sequelae in long-term survivors.

Unlike a comparative previous study in adults [8], the analysis presented here fails to demonstrate the superiority of TD over CIF of CsA for the prevention of acute GVHD in pediatric patients undergoing HSCT. The lower incidence of acute GVHD in pediatric patients undergoing HSCT than adult patients undergoing HSCT, reported previously [19], may be related to similar efficiencies of different types of GVHD prophylaxis in children. Alternatively, it is possible that the peak concentrations of CsA did not reach levels sufficient to induce beneficial effects in a considerable proportion of the pediatric patients in the TD group. The limitations of this study include a retrospective analysis of small numbers of patients within the groups. Therefore, prospective randomized controlled studies are required to evaluate the efficiency and safety of TD administration alongside measurements of the peak concentration of CsA in pediatric patients undergoing HSCT.

In summary, this study demonstrates that TD is a potentially promising mode of CsA administration to pediatric HSCT patients, since the incidence of severe hypertension was lower in the TD group than the CIF group. Additional prospective studies of larger pediatric populations, including long-term follow-ups, are required to validate the efficacy and safety of TD administration of CsA.

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Granulocyte colony-stimulating factor combined regimen in cord blood transplantation for acute myeloid leukemia: a nationwide retrospective analysis in Japan

Cord blood transplantation (CBT) from an unrelated donor has been increasingly used as an alternative transplant method for adult patients without human leukocyte antigen (HLA)-compatible related or unrelated donors.[1-4] However, the main disadvantage of CBT is still the limited cell dose, especially in adults, and this might contribute to a higher incidence of graft failure and delayed hematopoietic recovery, leading to higher transplant-related mortality (TRM) or overall mortality after CBT.

The purpose of a conditioning regimen prior to allogeneic hematopoietic stem cell transplantation (allo-HSCT) for hematologic malignancies is disease eradication and immunosuppression to overcome graft rejection. Although the standard myeloablative conditioning regimen prior to allo-HSCT has been total body irradiation (TBI) or busulfan combined with cyclophosphamide (CY) for patients with adult acute myeloid leukemia (AML), the role of an intensified conditioning regimen has been analyzed extensively in order to reduce the rate of post-transplant relapse and improve survival.[5-7] However, the majority of these studies analyzed patients receiving allo-HSCT using bone marrow (BM) or mobilized peripheral blood (PB) as a stem cell source. Therefore, an optimal myeloablative conditioning regimen prior to CBT for adult AML still has to be determined.

Granulocyte colony-stimulating factor (G-CSF) stimulates proliferation, differentiation, and functional activation of neutrophils. In clinical use, G-CSF is most commonly used for reducing the duration of neutropenia after chemotherapy and HSCT, and for the mobilization of hematopoietic stem/progenitor cells from the BM into PB

Table 1. Characteristics of patients, cord blood units, and transplantation.

	Total	TBI≥10Gy+Ara-C +CY	TBI≥10Gy+Ara-C /G-CSF+CY	TBI≥10Gy+other	TBI<10Gy+other or non-TBI	P
Number of patients	438	163	80	156	39	
Age						0.61
16-39 years	226(52 %)	74(45 %)	40(50 %)	81(52 %)	17(44 %)	
40-55 years	212(48 %)	89(54 %)	40(50 %)	75(48 %)	22(56 %)	
Sex						0.82
Male	217(50 %)	83(50 %)	42(53 %)	73(47 %)	19(49 %)	
Female	221(50 %)	80(49 %)	38(48 %)	83(56 %)	20(51 %)	
Disease status at CBT *						0.32
Standard risk	214 (49 %)	74(45 %)	45(56 %)	79(51 %)	16(41 %)	
High risk	221(50 %)	87(53 %)	35(44 %)	76(49 %)	23(59 %)	
Unknown	3(<1 %)	2(1 %)	0	1(<1 %)	0	
GVHD prophylaxis						<0.001
Cyclosporine A+methotrexate	304(69 %)	107(66 %)	74(93 %)	100(64 %)	23(59 %)	
Tacrolimus+methotrexate	134(31 %)	56(34 %)	6(8 %)	56(39 %)	16(41 %)	
Number of nucleated cells						0.71
$<2.5\times10^7$/kg	204(47 %)	70(43 %)	40(50 %)	75(48 %)	19(49 %)	
$\geq2.5\times10^7$/kg	200(46 %)	79(48 %)	33(41 %)	70(45 %)	18(37 %)	
Unknown	34(8 %)	14(9 %)	7(9 %)	11(7 %)	2(5 %)	
Number of $CD34^+$cells						0.23
$<1\times10^5$/kg	279(64 %)	110(67 %)	43(54 %)	101(64 %)	25(64 %)	
$\geq1\times10^5$/kg	144(33 %)	52(32 %)	34(43 %)	46(29 %)	12(31 %)	
Unknown	15(3 %)	1(<1 %)	3(4 %)	9(6 %)	2(5 %)	
HLA disparities†						0.24
0	40(9 %)	9(6 %)	7(9 %)	19(1 %)	5(1 %)	
1	148(34 %)	64(39 %)	22(28 %)	50(1 %)	12(1 %)	
≥2	250(57 %)	90(55 %)	51(64 %)	87(1 %)	22(1 %)	
ABO incompatibility						0.11
Match	152(35 %)	67(41 %)	29(36 %)	42(27 %)	14(39 %)	
Major/bidirectional mismatch	175(25 %)	30(18 %)	22(28 %)	47(30 %)	11(28 %)	
Minor mismatch	110(40 %)	66(40 %)	29(36 %)	66(42 %)	14(39 %)	
Unknown	1(<1 %)	0	0	1(<1 %)	0	
Year of CBT						<0.001
1998-2002	56(13 %)	12(7 %)	16(20 %)	24(15 %)	4(10 %)	
2003-2005	158(36 %)	40(25 %)	32(40 %)	64(41 %)	22(56 %)	
2006-2008	224(51 %)	111(68 %)	32(40 %)	68(44 %)	13(33 %)	

Disease status at CBT was classified as standard risk or high risk; complete remission without poor prognostic karyotype according to the MRC10 criteria was classified as standard risk, whereas patients in all other situations were classified as high risk. †The number of HLA disparities was defined as low resolution for HLA-A,-B, and –DR in graft-versus-host direction. Ara-C: cytosine arabinoside; CBT: cord blood transplantation; CY: cyclophosphamide; G-CSF: granulocyte colony-stimulating factor; GvHD: graft-versus-host disease; HLA: human leukocyte antigen; TBI: total body irradiation.

for HSCT. Furthermore, since administration of G-CSF increases the susceptibility to cytarabine arabinoside (Ara-C) through induction of cell cycle entry of dormant leukemia cells,[8,9] the efficacy of concomitant use of G-CSF and chemotherapy has been analyzed.[10,11] Several studies, as well as our own single institute studies, have demonstrated that G-CSF combined with myeloablative conditioning prior to allo-HSCT could be safely and effectively used for patients with myeloid malignancies in a single arm trial.[9,12,13] However, there has been no comparative study of transplant outcomes for AML after allo-HSCT following a conditioning regimen with or without G-CSF. This retrospective study is the first to assess the effect of a G-CSF combination in a myeloablative conditioning regimen for CBT on the transplant outcome in adult AML patients in Japan. Patients and study methods are described in the *Online Supplementary Appendix*.

Characteristics of patients and cord blood units are shown in Table 1. There was a significant difference in cumulative incidence of neutrophil recovery among the four groups in univariate analysis (P<0.001) (Figure 1A). In the multivariate analysis, the hazard risk of neutrophil engraftment was significantly higher in the TBI≥10Gy+Ara-C/G-CSF+CY group (P<0.001) and lower in the TBI≥10Gy+other group (P=0.03) and TBI<10Gy+other or non-TBI group (P<0.001) compared with the TBI≥10Gy+Ara-C+CY group (Table 2). Among patients achieving neutrophil engraftment, neutrophil recovery times were significantly shorter in the TBI≥10Gy+Ara-C/G-CSF+CY group compared with the TBI≥10Gy+Ara-C+CY group (P<0.001). There was a significant difference in cumulative incidence of platelet recovery among the four groups in univariate analysis (P<0.001) (Figure 1B). Multivariate analysis showed no significant difference between the TBI≥10Gy+Ara-C+CY group and TBI≥10Gy+Ara-C/G-CSF+CY group (P=0.14). However, the hazard risk of platelet engraftment was significantly lower in the TBI≥10Gy+other group (P<0.001) and TBI<10Gy+other or non-TBI group (P<0.001) compared with the TBI≥10Gy+Ara-C+CY group (Table 2). Among patients achieving platelet engraftment, there was no significant difference in platelet recovery times among the four groups (P=0.32).

Among patients in the entire cohort, the cumulative incidence of TRM at 100 days and at one year was 17% (95%CI: 13%-20%) and 22% (95%CI: 18%-26%), respectively. There was no significant difference in cumulative incidence of TRM at one year among the four groups in univariate analysis (P=0.19) (Figure 1C). Multivariate analysis of TRM, adjusting for other variables, showed no significant difference between the TBI≥10Gy+Ara-C+CY group and the TBI≥10Gy+Ara-C/G-CSF+CY group (P=0.67), TBI≥10Gy+other group (P=0.25), or TBI<10Gy+other or non-TBI group (P=0.95) (Table 2). The cumulative incidence of relapse at three years was 30% (95%CI: 25%-35%) in the entire cohort. There was no significant difference in cumulative incidence of relapse at three years among the four groups (P=0.05) (Figure 1D). In multivariate analysis, the hazard risk of relapse was lower in the TBI≥10Gy+Ara-C/G-CSF+CY group (P=0.03), but not in the TBI≥10Gy+other group (P=0.94) and TBI<10Gy+other or non-TBI group (P=0.73) compared with the TBI≥10Gy+Ara-C+CY group (Table 2).

Among the entire cohort, the probability of disease-free survival (DFS) and overall survival (OS) at three years was 44% (95%CI: 39%-49%) and 52% (95%CI: 46%-57%), respectively. There was a significant difference in the probability of DFS at three years among the four groups in univariate analysis (P=0.001) (Figure 1E). The probability of

Table 2. Multivariate analysis of transplant outcomes.

Outcomes	N. of patients	HR (95 % CI)	P
Neutrophil engraftment			
Conditioning regimen			
TBI≥10Gy+Ara-C+CY	163	1	Reference
TBI≥10Gy+Ara-C/G-CSF+CY	80	1.57(1.17-2.11)	0.002
TBI≥10Gy+other	156	0.76(0.58-0.98)	0.03
TBI<10Gy+other or non-TBI	39	0.46(0.27-0.78)	0.004
Number of CD34$^+$cells			
<1×10^5/kg	279	1	Reference
≥1×10^5/kg	144	1.56(1.23-1.98)	<0.001
Platelet engraftment			
Conditioning regimen			
TBI≥10Gy+Ara-C+CY	163	1	Reference
TBI≥10Gy+Ara-C/G-CSF+CY	80	1.25(0.92-1.71)	0.14
TBI≥10Gy+other	156	0.54(0.39-0.73)	<0.001
TBI<10Gy+other or non-TBI	39	0.40(0.23-0.67)	<0.001
Number of CD34$^+$cells			
<1×10^5/kg	279	1	Reference
≥1×10^5/kg	144	1.58(1.22-2.06)	<0.001
Transplant-related mortality			
Conditioning regimen			
TBI≥10Gy+Ara-C+CY	163	1	Reference
TBI≥10Gy+Ara-C/G-CSF+CY	80	0.86(0.44-1.68)	0.67
TBI≥10Gy+other	156	1.31(0.82-2.10)	0.25
TBI<10Gy+other or non-TBI	39	1.02(0.46-2.25)	0.95
Age			
<40 years	226	1	Reference
≥40 years	212	1.64(1.08-2.49)	0.01
Disease status at CBT			
Standard risk	214	1	Reference
High risk	221	1.81(1.20-2.72)	0.004
Relapse			
Conditioning regimen			
TBI≥10Gy+Ara-C+CY	163	1	Reference
TBI≥10Gy+Ara-C/G-CSF+CY	80	0.45(0.21-0.95)	0.03
TBI≥10Gy+other	156	0.98(0.61-1.57)	0.94
TBI<10Gy+other or non-TBI	39	1.14(0.53-2.44)	0.73
Disease status at CBT			
Standard risk	214	1	Reference
High risk	221	3.28(2.16-4.98)	<0.001
Treatment failure			
Conditioning regimen			
TBI≥10Gy+Ara-C+CY	163	1	Reference
TBI≥10Gy+Ara-C/G-CSF+CY	80	0.57(0.36-0.91)	0.01
TBI≥10Gy+other	156	1.24(0.90-1.70)	0.17
TBI<10Gy+other or non-TBI	39	1.24(0.75-2.02)	0.39
Disease status at CBT			
Standard risk	214	1	Reference
High risk	221	3.10(2.29-4.19)	<0.001
Overall mortality			
Conditioning regimen			
TBI≥10Gy+Ara-C+CY	163	1	Reference
TBI≥10Gy+Ara-C/G-CSF+CY	80	0.52(0.31-0.87)	0.01
TBI≥10Gy+other	156	1.19(0.84-1.69)	0.31
TBI<10Gy+other or non-TBI	39	1.25(0.74-2.12)	0.39
Disease status at CBT			
Standard risk	214	1	Reference
High risk	221	2.68(1.93-3.71)	<0.001

The only significant variables other than conditioning regimen were described in each end point. Variables considered in multivariate analysis were conditioning regimen (TBI≥10Gy+Ara-C+CY vs. TBI≥10Gy+Ara-C/G-CSF+CY vs. TBI≥10Gy+other vs. TBI<10Gy+other or non-TBI), age (<40 vs. ≥40 years), patients' gender (male vs. female), disease status at CBT (standard risk vs. high risk), GVHD prophylaxis (cyclosporine A with methotrexate vs. tacrolimus with methotrexate), cord blood nucleated cell count (<2.5×10^7/kg vs. ≥2.5×10^7/kg), cord blood CD34$^+$ cell count (<1×10^5/kg vs. ≥1×10^5/kg), HLA disparities (0 vs. 1 vs. ≥2), donor–recipient ABO compatibility (match vs. major/bidirectional mismatch vs. minor mismatch), and year of CBT (1998-2002 vs. 2003-2005 vs. 2006-2008). Ara-C: cytosine arabinoside; CBT: cord blood transplantation; CI: confidence interval; CY: cyclophosphamide; G-CSF: granulocyte colony-stimulating factor; HR: hazard ratio; TBI: total body irradiation.

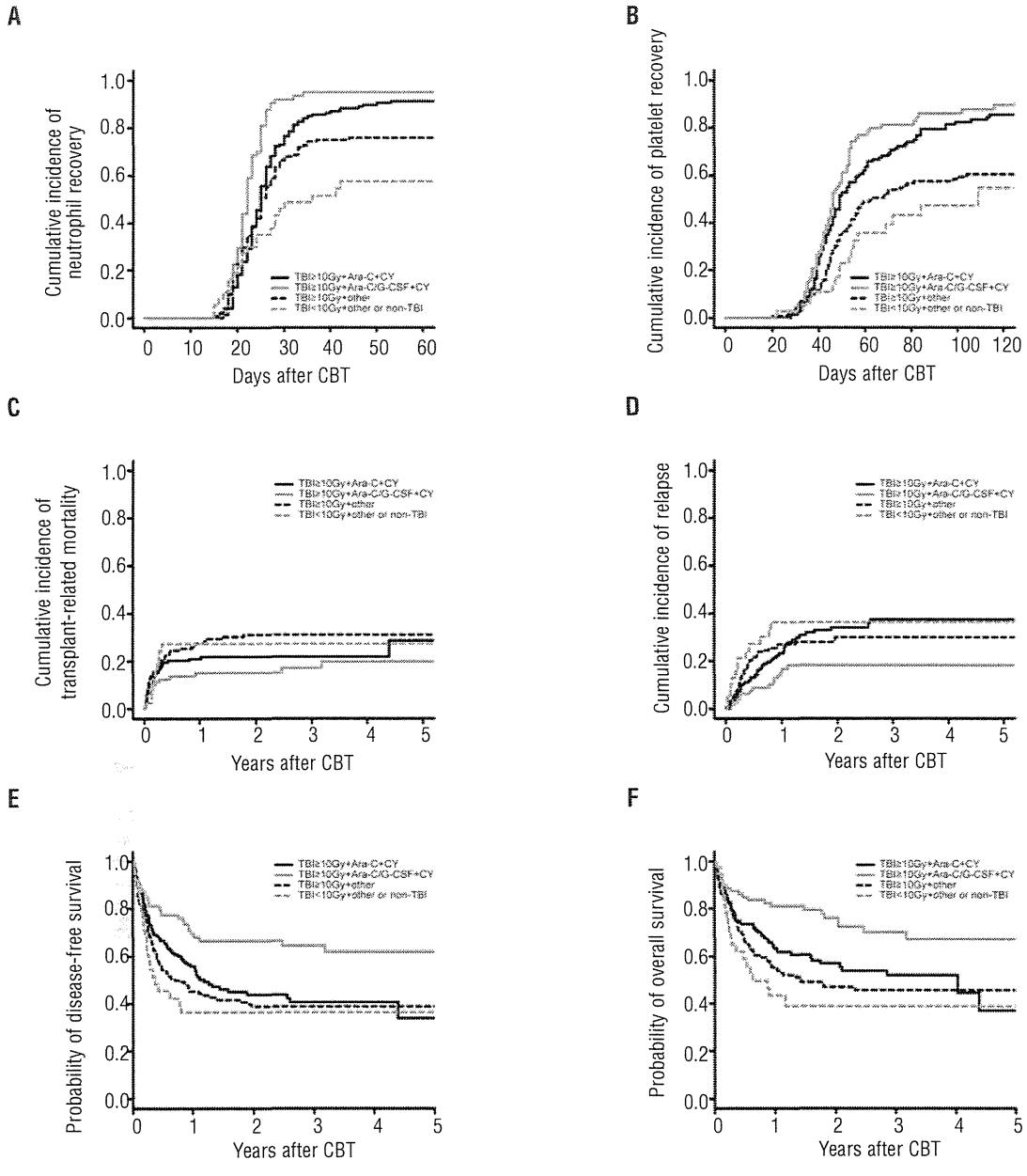

Figure 1. Cumulative incidences of neutrophil (A) and platelet (B) recovery, transplant-related mortality (TRM) (C) and relapse (D), probabilities of disease-free survival (E) and overall survival (F) after cord blood transplantation according to conditioning regimen. (A) Cumulative incidence of neutrophil recovery 42 days after CBT was 88% [95% confidence interval (CI): 81%-92%] in the TBI≥10Gy+Ara-C+CY group, 95% (95%CI: 85%-98%) in the TBI≥10Gy+Ara-C/G-CSF+CY group, 74% (95%CI: 66%-81%) in the TBI≥10Gy+other group, and 57% (95%CI: 37%-70%) in the TBI<10Gy+other or non-TBI group. Median times to neutrophil recovery were 24 days (range 17-53 days) in the TBI≥10Gy+Ara-C+CY group, 22 days (range 16-34 days) in the TBI≥10Gy+Ara-C/G-CSF+CY group, 23 days (range 15-65 days) in the TBI≥10Gy+other group, and 22 days (range 15-42 days) in the TBI<10Gy+other or non-TBI group. (B) Cumulative incidence of platelet recovery 100 days after CBT was 82% (95%CI: 74%-87%) in the TBI≥10Gy+Ara-C+CY group, 85% (95%CI: 74%-92%) in the TBI≥10Gy+Ara-C/G-CSF+CY group, 58% (95%CI: 48%-66%) in the TBI≥10Gy+other group, and 47% (95%CI: 25%-62%) in the TBI<10Gy+other or non-TBI group. Median times to platelet recovery were 46 days (range 28-168 days) in the TBI≥10Gy+Ara-C+CY group, 45.5 days (range 27-263 days) in the TBI≥10Gy+Ara-C/G-CSF+CY group, 48 days (range 20-249 days) in the TBI≥10Gy+other group, and 51 days (range 22-109 days) in the TBI<10Gy+other or non-TBI group. (C) Cumulative incidence of TRM at one year was 21% (95%CI: 15%-27%) in the TBI≥10Gy+Ara-C+CY group, 15% (95%CI: 8%-23%) in the TBI≥10Gy+Ara-C/G-CSF+CY group, 27% (95%CI: 20%-35%) in the TBI≥10Gy+other group, and 27% (95%CI: 14%-42%) in the TBI<10Gy+other or non-TBI group. (D) Cumulative incidence of relapse at three years was 37% (95%CI: 28%-46%) in the TBI≥10Gy+Ara-C+CY group, 18% (95%CI: 10%-27%) in the TBI≥10Gy+Ara-C/G-CSF+CY group, 30% (95%CI: 22%-37%) in the TBI≥10Gy+other group, and 36% (95%CI: 20%-52%) in the TBI<10Gy+other or non-TBI group. (E) Probability of disease-free survival at three years was 40% (95%CI: 31%-49%) for the TBI≥10Gy+Ara-C+CY group, 64% (95%CI: 52-74%) for the TBI≥10Gy+Ara-C/G-CSF+CY group, 38% (95%CI: 30%-47%) for the TBI≥10Gy+other group, and 36% (95%CI: 20%-51%) for the TBI<10Gy+other or non-TBI group. (F) Probability of overall survival was 52% (95%CI: 42%-60%) for the TBI≥10Gy+Ara-C+CY group, 70% (95%CI: 57%-79%) for the TBI≥10Gy+Ara-C/G-CSF+CY group, 45% (95%CI: 36%-54%) for the TBI≥10Gy+other group, and 39% (95%CI: 22%-55%) for the TBI<10Gy+other or non-TBI group. Median period of follow up for survivors (n=261) in the entire cohort was 24 months (range 1-122 months) after CBT.

DFS at three years was significantly better in the TBI≥10Gy+Ara-C/G-CSF+CY group compared with the TBI≥10Gy+Ara-C+CY group (P=0.02), the TBI≥10Gy+other group (P=0.002) and TBI<10Gy+other or non-TBI group (P=0.006). Multivariate analysis showed significantly decreased rates of treatment failure in the TBI≥10Gy+Ara-C/G-CSF+CY group compared with the TBI≥10Gy+Ara-C+CY group (P=0.01) (Table 2). In univariate analysis, there was a significant difference in the probability of OS at three years among the four groups (P=0.001) (Figure 1F). Multivariate analysis showed significantly decreased overall mortality in the TBI≥10Gy+Ara-C/G-CSF+CY group compared with the TBI≥10Gy+Ara-C+CY group (P=0.01) (Table 2). We also analyzed a subgroup of patients with standard risk (n=214) or high risk (n=221) at CBT. In standard-risk patients, the hazard risk of overall mortality (P=0.04), treatment failure (P=0.01) and relapse (P=0.002) was significantly lower in the TBI≥10Gy+Ara-C/G-CSF+CY group compared with the TBI≥10Gy+Ara-C+CY group, while that of high-risk patients was not (*Online Supplementary Table S1 and Figures S1 and S2*).

Anti-leukemia effects of allo-HSCT consist of leukemia eradication by both a conditioning regimen of chemotherapy with or without radiation and the graft-versus-leukemia (GvL) effect. Since relapse is the most common cause of death after allo-HSCT, an intensified conditioning regimen or enhancement of GvL effects is needed to reduce the incidence of relapse. Because of the difficulty in controlling the degree of GvL effects, an intensified conditioning regimen has been extensively analyzed. The several improvements to a typical conditioning regimen have included the addition of other agents to a standard myeloablative regimen, a dose escalation of drugs or TBI, or administration of drugs other than CY. Among these, the addition of other agents to a standard myeloablative regimen has been the most commonly used.[5,6] In fact, several studies have reported a decrease in the incidence of relapse following intensified conditioning, but with a higher TRM, and no improvement in survival was achieved.[5,7] Furthermore, the effect of adding high-dose Ara-C to a TBI/CY myeloablative conditioning regimen is controversial.[7] However, all of these studies analyzed patients receiving BM or mobilized PB stem cell transplantation from related or unrelated donors. This finding was not confirmed in CBT. In our study, neutrophil and platelet engraftment was significantly higher in the TBI≥10Gy+Ara-C+CY group compared with the TBI≥10Gy+other group, suggesting that the addition of Ara-C to TBI/CY was beneficial in terms of stable engraftment, but not for survival in CBT for AML.

Granulocyte colony-stimulating factor was originally identified as an agent for stimulation of neutrophil production. Although G-CSF is most commonly used to reduce the duration of neutropenia after chemotherapy, it is also commonly used for hematopoietic stem cell (HSC) mobilization for HSCT. Although the mechanism of HSC mobilization is not clearly understood, G-CSF could disrupt the contact between HSC in a BM niche, leading to HSC migration. In a mouse bone marrow transplantation (BMT) model, G-CSF prior to low-dose irradiation enhanced donor HSC engraftment.[14] This effect might be mainly due to the migration of recipient HSC from a BM niche by G-CSF treatment before transplantation. In fact, our data showed that neutrophil engraftment was significantly higher in the TBI≥10Gy+Ara-C/G-CSF+CY group compared with the TBI≥10Gy+Ara-C+CY group. These data suggest that the effect of the addition of G-CSF to a conditioning regimen could enhance neutrophil engraftment after CBT.

It has been reported that the administration of G-CSF increased the susceptibility of the cell-cycle-specific agent Ara-C in leukemia cells *in vitro* and in a xenograft model.[8,9,15] In clinical studies, several regimens have attempted to demonstrate the efficacy of concomitant use of G-CSF with chemotherapy for newly diagnosed AML.[10,11] We hypothesized that the addition of G-CSF to a conditioning regimen might improve outcome in an allo-HSCT setting. In our study, relapse was significantly lower in the TBI≥10Gy+Ara-C/G-CSF+CY group compared with the TBI≥10Gy+Ara-C+CY group. In a subgroup analysis, the effect of a G-CSF combination regimen for reduced relapse was significant in standard-risk but not high-risk patients. This is similar to a previous prospective randomized study of concomitant use of G-CSF with chemotherapy by Löwenberg *et al.*[11] Further studies are required to confirm which subgroup of patients with AML could benefit from a G-CSF combination regimen in CBT to reduce the incidence of relapse.

In conclusion, our data show that the addition of G-CSF-combined Ara-C to a TBI+CY conditioning regimen resulted in a significantly higher incidence of neutrophil engraftment and significantly better DFS and OS, and a reduced relapse rate in CBT for AML. Although these findings should be confirmed in prospective studies, a G-CSF-combined myeloablative conditioning regimen promotes better engraftment and survival results in CBT for AML.

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