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Cord Blood Transplantation from Unrelated Donors for Children with Acute Lymphoblastic Leukemia in Japan: The Impact of Methotrexate on Clinical Outcomes

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Cord blood transplantation (CBT) from an unrelated donor is recognized as one of the major treatment modalities in allogeneic stem cell transplantation (SCT) for children with hematologic malignancies. We analyzed the clinical outcomes of CBT for children with acute lymphoblastic leukemia (ALL) in Japan and identified the risk factors for the transplant outcomes. From 1997 to 2006, 332 children with ALL underwent CBT from unrelated donors, 270 of which had no prior transplant. Their disease statuses at transplant were first complete remission (CR) (n = 120), second CR (n = 71), and more advanced stages (n = 75). As preconditioning for SCT, total body irradiation (TBI) was given to 194 patients and, for the prophylaxis of graft-versus-host disease (GVHD), methotrexate (MTX) was given to 159 patients. The cumulative incidents of neutrophil and platelet recovery (>20 K) were 88.5% and 78.4%, respectively. The incidents of grade II-IV, III-IV acute GVHD (aGVHD), and chronic GVHD (cGVHD) were 45.6%, 20.4%, and 19.2%, respectively, and treatment-related mortality was 22.6%. The 5-year event-free survival (EFS) and overall survival (OS) at CR1, CR2, and advanced status were 47.4%, 45.5%, 15.0%, and 63.7%, 59.7%, and 20.7%, respectively. Multivariate analysis revealed that MTX with calcineurin inhibitor (CNI) was associated with decreased incidence of grade II-IV GVHD (CNI alone: hazard ratio [HR] = 1.74, 95% confidence interval [CI] = 1.06-2.83, $P = .027$; CNI + prednisolone (PSL), HR = 1.61, 95% CI = 1.03-2.50, $P = .036$), III-IV aGVHD (CNI alone: HR = 3.02, 95% CI = 1.55-5.91, $P = 0.001$; CNI + PSL, HR = 1.89, 95% CI = 0.93-3.83, $P = .078$), or cGVHD (CNI alone: HR = 1.78, 95% CI = 0.83-3.82, $P = .143$; CNI + PSL, HR = 2.44, 95% CI = 1.24-4.82, $P = .01$), compared with CNI alone or CNI + PSL. At an advanced stage of disease, GVHD prophylaxis with MTX + CNI is associated with improved OS compared with CNI alone (CNI alone: HR = 3.20, 95% CI = 1.43-7.15, $P = .005$; CNI + PSL, HR = 1.47, CI = 0.67-3.20, $P = .332$). Our retrospective study showed that CBT for children with ALL is feasible and GVHD prophylaxis with MTX + CNI is associated with significant favorable outcomes in prevention of aGVHD and cGVHD as well as survival advantage in advanced cases.

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KEY WORDS: Cord blood transplantation, Acute lymphoblastic leukemia, HLA, Methotrexate

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INTRODUCTION

Multiagent chemotherapy for children with acute lymphoblastic leukemia (ALL) has achieved excellent clinical outcomes in recent years [1,2]. However, those patients who relapsed during or after chemotherapy or those with very high-risk features, such as Philadelphia chromosome-positive ALL (Ph+ALL) or infant ALL with mixed lineage leukemia (MLL) gene rearrangement, are proposed as candidates for allogeneic stem cell transplantation (SCT) [3-6] at their first remission. Patients and donors are required to be compatible in terms of human leukocyte antigen (HLA) for better transplant outcome and, if they lack an HLA-identical related donor, they have options of alternative donors, such as bone marrow transplantation (BMT), peripheral blood stem cell transplantation (PBSCT), cord blood transplantation (CBT) from an unrelated donor, or transplantation from an HLA-haploidentical family donor [7-9]. Out of these four treatment modalities, CBT has advantages such as immediate availability of a CB unit for an urgent transplant, lower risks of severe acute and chronic graft-versus-host disease (aGVHD, cGVHD), and a less stringent requirement of HLA compatibility than unrelated or haploidentical BMT. In Japan, the Japan Cord Blood Bank Network (JCBBN) was established in 1999, and 11 local cord blood banks are affiliated to JCBBN where more than 7000 CBT were performed by the end of 2010. Here, we report the clinical outcomes and risk factors of children with ALL who underwent CBT in Japan.

PATIENTS AND METHODS

Patient and Donor Characteristics

From 1997 to 2006, 332 unrelated CBT were performed for children with ALL and 270 transplantations were undertaken as the first SCT in Japan. Because the overall survival (OS) of patients who underwent transplantation as the first SCT was significantly better than that of those with prior SCT (50.3% vs 12.7%, $P < .001$), we restricted this analysis to only patients with no prior SCT in order to interpret the exact risk factors of CBT. The patient and donor characteristics are shown in Table 1. Patients transplanted at first complete remission (CR1) (n = 120) include 41 infant ALL and 17 patients with Ph +ALL.

The HLA typing of cord blood units was performed in each CB bank by low-resolution molecular typing of HLA-A and B, combined with high-resolution molecular typing of DRB1. The high-resolution molecular typing for 3 loci of HLA-A, B, and DRB1 was performed in 187 patients.

In JCBBN, CB units of 0 to 2 HLA antigen mismatches with the patient were allowed for transplantation, and the minimum number of nucleated cells recommended for transplantation was 2×10^7/kg of patient body weight at cryopreservation.

Transplantation

All CB units were provided from the 11 local CB banks affiliated to JCBBN, and all transplant institutions were required to meet the minimum requirements of JCBBN in terms of experience of allogeneic SCT. The numbers of transplanted cells, preconditioning, as well as GVHD prophylaxis are shown in Table 1. Supportive care after transplantation, such as gut decontamination, empirical administration of antibiotics, prophylaxis or treatment of cytomegalovirus (CMV) infection, was performed according to each institutional protocol. Grading of GVHD was performed according to the standard criteria [10].

Definition and Statistics

The median duration of follow-up was 438 days (range: 10-3293 days). In this study, rates of neutrophil and platelet engraftment, incidents of aGVHD and cGVHD, leukemic relapse, nonrelapse mortality (NRM), and probabilities of event-free survival (EFS) and OS were analyzed. The variables evaluated included recipient age, sex, sex mismatch, disease status at transplants (CR1/CR2 vs advanced disease), ABO compatibility, HLA matching by low- and high-resolution typing, number of nucleated cells, colony-forming unit-granulocyte-macrophage (CFU-GM) and CD34-positive cells of the cord blood units at cryopreservation conditioning regimens with total body irradiation (TBI), administration of granulocyte-colony stimulating factor (G-CSF), GVHD prophylaxis (calcineurin inhibitor [CNI] alone, CNI + methotrexate [MTX] versus CNI + prednisolone [PSL]), mixed lineage leukemia (MLL) gene rearrangement, t(4;11), and transplantation year. Because the information of high-resolution DNA typing was only available for a limited number of patients, it was not included in the multivariate analysis. The day of neutrophil engraftment was defined as the first day of 3 consecutive days with absolute neutrophil count (ANC) $\geq 500/mm^3$, and that of platelet engraftment was the first day of platelet count over $20,000/mm^3$ without transfusion. The treatment-related mortality was defined as all causes of nonleukemic deaths after transplantation. The EFS was defined as patients who are alive in CR with engraftment. The probabilities of OS and EFS were calculated by the method of Kaplan and Meier. The log-rank test was used for group comparisons. Time-to-event outcomes for neutrophil and platelet engraftment, treatment-related mortality, relapse,

Table 1. Patient and Donor Characteristics

Total Number of Patients			270
Age (year)	median (range)		5 (0-15)
Body weight (kg)	median (range)		18 (4-60)
Sex	male/female		156/114
Duration from diagnosis to transplantation (days)	median (range)		249 (94-3670)
Disease status at transplantation (patients)	CR1		120
	CR2		71
	advanced		75
	unknown		4
Cytogenetics (patients)	Philadelphia chromosome		31
	MLL gene rearrangement		73
	t(4;11)		40
Preparative regimen (patients)	TBI regimen		194
		TBI + CY + VP16	68
		TBI + CY ± others	67
		TBI + L-PAM ± others	56
		Others	3
	non-TBI regimen		76
		BU + CY ± others	55
		Others	21
G-CSF (patients)	+		249
	−		21
GVHD prophylaxis (pts)	CNI only	cyclosporine	29
		tacrolimus	12
	CNI + MTX	cyclosporine + MTX	83
		tacrolimus + MTX	66
	CNI + PSL	cyclosporine + PSL	36
		tacrolimus + PSL	11
	ATG + CNI ± MTX		7
	others		15
	none		11
Number of cells at cryopreservation, median (range)	Nucleated cell ($\times 10^7$/kg)	(n = 270)	5.00 (1.35-24.91)
	CFU-GM ($\times 10^3$/kg)	(n = 258)	34 (0.87-473.2)
	CD34 ($\times 10^5$/kg)	(n = 207)	1.49 (0.17-15.02)
Blood type of donor and recipient (pts)	match		89
	minor		77
	major		103
	unknown		1
Sex of donor and recipient (pts)	M to M		78
	F to F		64
	M to F		50
	F to M		78
HLA disparity in low resolution (patients)	No. of disparities	GVHD direction	Rejection direction
	0	54	57
	1	168	167
	2	47	45
	unknown	1	1
HLA disparity in high resolution (patients)	No. of disparities	GVHD direction	Rejection direction
	0	21	23
	1	56	58
	2	77	72
	3	28	29
	4	4	4
	5	1	1
	unknown	83	83

G-CSF indicates granulocyte-colony stimulating factor; GVHD, graft-versus-host disease; TBI, total body irradiation; CNI, calcineurin inhibitor; MTX, methotrexate; PSL, prednisolone; CY, cyclophosphamide.

and GVHD were estimated using cumulative incidence curves. The competing risk of engraftment is death before engraftment, that of GVHD is death without GVHD or relapse, and that of relapse is death without relapse. The Cox proportional-hazards regression model was used for multivariate analysis of clinical variables. P values $<.05$ were considered statistically significant. Risk factors with a P value $<.1$ in each univariate analysis were included in the multivariate analysis. STATA version 10 (Stata Corporation, College Station, TX) and NCSS 2004 (Number Cruncher Statistical Systems, Kaysville, UT) were used for the statistical analysis of data.

RESULTS

Neutrophil Engraftment

Neutrophil engraftment was obtained in 239 patients. The probability of neutrophil engraftment

was 88.5% (95% confidence interval [CI], 84.8%-92.4%) by day 90, and the median number of days to reach ANC over 500/mm^3 was 22. In univariate analysis, younger versus older than 1 year old (92.3% vs 87.6%, $P = .001$), higher versus lower than 3 $\times$ 10^7/kg of nucleated cells (89.5% vs 84.6%, $P = .003$), higher versus lower than the median number of CFU-GM (90.8% vs 86.8%, $P < .001$), higher versus lower than the median number of CD34$^+$ cells (1.5 $\times$ 10^5/kg, 89.7% vs 85.1%, $P < .001$), 0-1 versus 2 Ag HLA mismatches in either GVHD (89.2% vs 85.1%, $P = .008$) or rejection (90.2% vs 80.0%, $P = .004$) direction, allelic 0-1 versus 2 or more HLA mismatches in either GVHD (92.2% vs 87.3%, $P = .009$) or rejection (92.6% vs 86.8%, $P = .006$) direction by high-resolution typing, CR1 or CR2 versus advanced status at transplantation (89.5% vs 85.3%, $P = .022$), and presence versus absence of G-CSF (91.2% vs 57.1%, $P < .001$) were significantly associated with higher neutrophil engraftment rate. The presence or absence of MTX did not affect the neutrophil engraftment (data not shown). In multivariate analysis, favorable predictive factors of neutrophil engraftment were higher number of CD34$^+$ cells, administration of G-CSF, and HLA disparity of 0-1 antigen for rejection direction (Table 2).

Platelet Engraftment

Platelet engraftment over 20,000/mm^3 was obtained in 202 patients, and the probability of platelet engraftment by day 180 was 78.4%. In univariate analysis, younger versus older than 1 year old (82.8% vs 77.1%, $P = .004$), higher versus lower than the median number of nucleated cells (78.8% vs 76.6%, $P = .008$), higher versus lower than the median number of CFU-GM (82.8% vs 73.7%, $P = .004$), higher versus lower than the median number of CD34$^+$ cells (84.9% vs 71.8%, $P < .001$), disease status of CR1 or CR2 versus advanced (83.8% vs 63.3%, $P < .001$), and presence versus absence of G-CSF (80.1% vs 57.8%, $P = .017$) were significantly associated with higher platelet engraftment rate. Multivariate analysis revealed that a higher number of CD34$^+$ cells and CR1 or CR2 at transplantation were favorable prognostic factors for platelet engraftment (Table 2).

GVHD

The cumulative incidents of grade II-IV and III-IV aGVHD were 45.6% (95% CI, 40.0%-51.9%) and 20.4% (95% CI, 16.1%-25.8%), respectively. In univariate analysis, HLA-mismatched donor versus matched donor in GVHD direction by low resolution (49.3% vs 31.5%, $P = .023$) and high resolution (51.2% vs 14.3%, $P = .003$), and presence versus absence of TBI (51.6% vs 30.3%, $P = .003$) were significantly associated with the development of grade II-IV aGVHD, and MTX + CNI showed a trend of impact on the development of grade II-IV aGVHD (40.3% in MTX + CNI, 53.7% in CNI alone, and 63.8% in CNI + PSL, $P = .096$). GVHD prophylaxis with MTX + CNI was the only significant predictive factor for decreased incidence of grade III-IV GVHD (14.1% in MTX + CNI, 27.7% in CNI + PSL and 36.7% in CNI alone, $P = .011$) (Figure 1). Multivariate analysis revealed that TBI was significantly associated with increased incidence of grade II-IV aGVHD, and GVHD prophylaxis with MTX + CNI was significantly associated with decreased incidence of grade II-IV and III-IV aGVHD (Table 2).

The cumulative incidence of the development of cGVHD was 19.2% (95% CI, 15.0%-24.6%), and the incidence of cGVHD was significantly reduced in HLA-matched donor in low resolution for rejection direction compared with that in the GVHD direction (12.2% vs 22.6%, $P = .002$), as well as GVHD prophylaxis with MTX + CNI compared with that with CNI alone or CNI + PSL (16.1% vs 22.5%, or 29.3%, respectively, $P = .03$) (Figure 1). In multivariate analysis, HLA mismatch for rejection direction in low resolution and GVHD prophylaxis with CNI + PSL were the significant risk factors for the development of cGVHD (Table 2). In our study population, only 7 patients were given anti-T cell globulin (ATG) for GVHD prophylaxis. The cumulative incidence of grade II-IV aGVHD and cGVHD in this population was 14.7%, respectively, and 5 patients died of either relapse or transplantation-related complications.

Transplant-Related Mortality (TRM)

The cumulative incidence of TRM after CBT was 22.6% (95% CI, 17.7%-27.8%). Univariate analysis showed that HLA mismatch of 2 or more loci versus 0-1 in high-resolution typing for either GVHD direction (23.2% vs 10.4%, $P = .03$) or rejection direction (23.1% vs 11.2%, $P = .03$), advanced disease status versus CR1 or CR2 (35.3% versus 17.4%, $P < .001$), and GVHD prophylaxis other than MTX + CNI (15.1% in MTX + CNI, 29.3% in CNI alone, and 31.4% in CNI + PSL, $P = .01$) were significantly associated with a higher incidence of TRM. Multivariate analysis revealed that advanced disease status at transplantation was a risk factor for TRM (Table 2).

Leukemic Relapse

Eighty-six patients relapsed between 8 and 976 days (median 182) after CBT. The cumulative incidence of leukemic relapse at 3 years was 35.2% (95% CI, 29.8%-42.1%). Advanced disease versus CR1 or CR2 (48.8% vs 30.6%, $P < .001$) and presence

Table 2. Multivariate Analysis of Risk Factors for Transplantation Outcomes

	Variable		Hazard Ratio	*P* Value	95% CI
Neutrophil engraftment	CD34 ($\times 10^5$/kg)	<1.5	1		
		≥1.5	1.7	.001	1.26-2.28
	G-CSF	no	1		
		yes	3.06	.001	1.60-5.83
	HLA disparity in low resolution (rejection direction)	0-1	1		
		2	0.62	.024	0.41-0.94
Platelet engraftment (≥20,000/mm^3)	CD34 ($\times 10^5$/kg)	<1.5	1		
		≥1.5	1.9	.001	1.35-2.66
	Disease status	CR1, CR2	1		
		advanced	0.58	.008	0.39-0.87
Acute GVHD (≥II)	TBI	no	1		
		Yes	1.859	.015	1.13-3.06
	GVHD prophyalxis	CNI + MTX	1		
		CNI only	1.74	.027	1.06-2.83
		CNI + PSL	1.61	.036	1.03-2.50
Acute GVHD (≥III)	GVHD prophylaxis	CNI + MTX	1		
		CNI only	3.02	.001	1.55-5.91
		CNI + PSL	1.89	.078	0.93-3.83
Chronic GVHD	HLA disparity in low resolution (GVHD direction)	0	1		
		1,2	2.73	.055	0.98-7.61
	GVHD prophylaxis	CNI + MTX	1	.029	
		CNI only	1.777	.143	0.83-3.82
		CNI + PSL	2.44	.01	1.24-4.82
Treatment-related mortality	Disease status	CR1, CR2	1		
		advanced	2.56	.005	1.33-4.92
Relapse	Disease status	CR1, CR2	1		
		advanced	3.16	<.001	2.04-4.89
	t(4;11)	no	1		
		yes	1.93	.014	1.14-3.26
Overall survival	Disease status	CR1, CR2	1		
		advanced	3.62	<.001	2.44-5.8
Event-free survival	Disease status	CR1, CR2	1		
		advanced	2.54	<.001	1.83-3.51

CI indicates confidence interval; G-CSF indicates granulocyte-colony stimulating factor; GVHD, graft-versus-host disease; TBI, total body irradiation; CNI, calcineurin inhibitor; MTX, methotrexate PSL, prednisolone.

versus absence of t(4;11) chromosomal abnormality (48.3% vs 33.1%, $P = .044$) were significantly associated with leukemic relapse in univariate analysis. Both of these factors were also significant in multivariate analyses (Table 2).

OS

One hundred fifty-two patients were alive after CBT, and their median number of days of survival was 961 (91-3293). The cause of death in 118 patients included relapse or progressive disease (n = 50), TRM (n = 66), and unknown reason (n = 2). The probability of projected 5-year OS for all patients was 50.3% (95% CI, 43.4%-56.8%), and it was 63.7% in CR1, 59.7% in CR2, and 20.7% at more advanced disease status (Figure 2). Univariate analysis revealed that HLA mismatch of ≥2 versus 0 or 1 for rejection direction (50.9% vs 66.0%, $P = .017$) in high-resolution typing, advanced disease versus CR1 or CR2 (20.7% vs 62.1%, $P < .001$), and GVHD prophylaxis of other than MTX + CNI (56.8% in MTX + CNI, 43.3% in CNI alone, and 40.6% in CNI + PSL, $P = .049$) were significantly associated with OS (Figure 3). In multivariate analysis, advanced disease status at transplantation was the only risk factor for OS. When multivariate analysis was restricted to the patients with advanced diseases, OS was significantly superior for patients with GVHD prophylaxis of MTX + CNI than CNI alone (CNI alone: HR = 3.20, 95% CI = 1.43-7.15, $P = .005$; CNI + PSL, HR = 1.47, CI = 0.67-3.20, $P = .332$).

EFS

The probability of projected 5-year EFS for all patients was 38.1% (95% CI, 31.8%-44.4%), and it was 47.4% in CR1, 45.5% in CR2, and 15.0% at more advanced disease status. Univariate analysis revealed that HLA mismatch of 1 or more versus 0 with high-resolution typing in either GVHD direction (33.9% vs 51.3%, $P = .047$) or rejection direction (31.5% vs 52.9%, $P = .010$), advanced disease status versus CR1 or CR2 (15.0% vs 46.6%, $P < .001$), and absence versus presence of G-CSF (22.0% vs 39.3%, $P = .028$) were significantly associated with EFS. The EFS rates of patients according to the HLA disparity in high-resolution typing in GVHD direction were 61.9% in 0 of 6 (n = 21), 47.0% in 1 of 6 (n = 56), 36.4% in 2 of 6 (n = 77), and 28.4% in 3 of 6 (n = 28) ($P = .127$). Although this was not significant, the more the HLA disparity increased, the lower the EFS became. In multivariate analysis, advanced disease

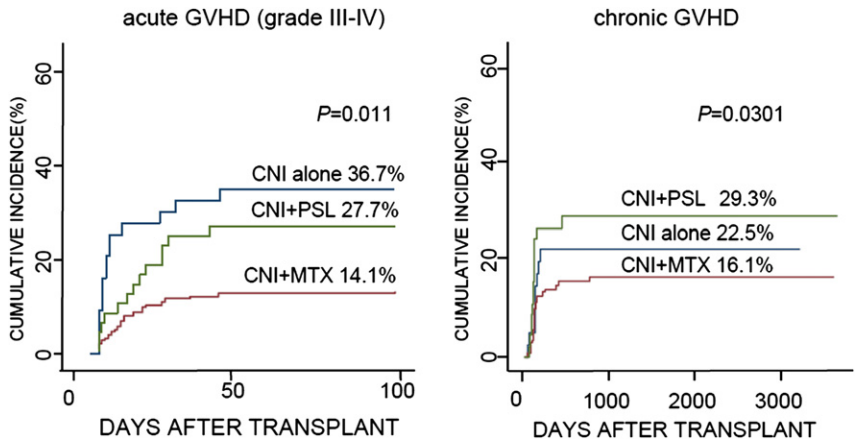

Figure 1. Cumulative incidence of aGVHD (grade III-IV) (left) and cGVHD (right). GVHD prophylaxis with CNI + MTX is associated with significantly lower incidence of aGVHD and cGVHD.

status at transplantation was significantly associated with lower EFS (Table 2).

DISCUSSION

The outcomes of CBT according to the disease status at transplantation in children with ALL were reported from a multicenter study of Eurocord. The disease-free survival (DFS) rates of those patients transplanted at complete remission and at more advanced stages were 36%-49% and 10%-18%, respectively [11-13]. In contrast to these multicenter studies, single or small numbers of institutions report better results. A study from Minnesota University reported that the EFS of children with ALL in standard and high-risk patients are 55% and 32%, respectively [14]. In the Cord Blood Transplantation (COBLT) study, the OS of children with ALL was around 60% in first and second remission [15], and a study in Denver [16] reported DFS of 62% including standard and high-risk patients. Our study is a retrospectively reviewed multicenter study with a large number of children with ALL, and the EFS or OS is comparable to that of these single-center studies.

The relevance of HLA disparity to clinical outcome in unrelated CBT has been reported by several investigators. In an International Bone Marrow Transplant Registry (IBMTR) study, the OS of serologically 6/6-matched CBT was significantly better than that of mismatched CBT, irrespective of the cell dose of the CB unit [17]. In Eurocord, the serologic disparity of HLA was reported to be important for engraftment and relapse but not for GVHD or survival, namely, serologic HLA mismatch reduced the relapse rate after transplantation. In those studies, HLA disparity in high resolution did not affect any clinical outcomes [18]. In our study, HLA disparity in low resolution affected the neutrophil engraftment and GVHD or cGVHD but not for relapse and survival. The OS according to the HLA disparity in high resolution gradually declined as the HLA disparity increased, even though this was not statistically significant in univariate analysis.

The different results regarding risk factors for relapse between our data and Eurocord may be explained by the difference of the patient population. Our study

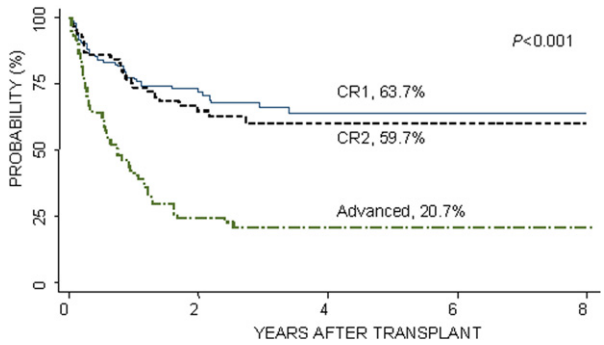

Figure 2. Probability of overall survival of patients according to disease status at transplantation. Patients with CR1 and CR2 are associated with significantly better overall survival compared with patients with advanced stage.

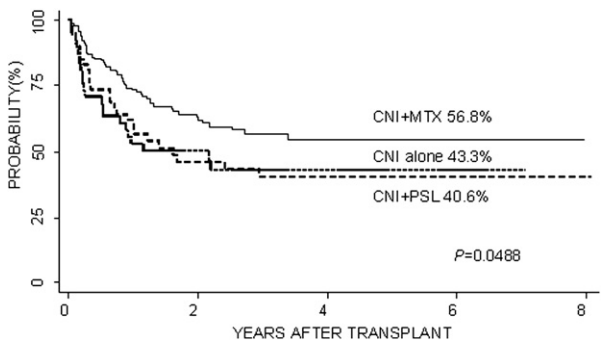

Figure 3. Probability of overall survival of patients according to GVHD prophylaxis. Patients with GVHD prophylaxis of CNI + MTX is associated with significantly better overall survival compared with patients with CNI alone or CNI + PSL.

was restricted to childhood ALL, whereas Eurocord included acute myelogenous leukemia (AML) patients [12,18], for whom a graft-versus-leukemia (GVL) effect could be more efficient than ALL patients after allogeneic SCT. Although the implications of the HLA disparity in high resolution for clinical outcome are still controversial, future study with a large number of patients could clarify the relevance of HLA disparity in high resolution on clinical outcomes.

GVHD prophylaxis after CBT is still controversial, and various methods of prophylaxis are applied in each institution or study group. In the early era of unrelated CBT, cyclosporine (CsA) and steroids with or without MTX were given as GVHD prophylaxis [19]. Subsequently, MTX was abandoned, and immunosuppression with CsA and steroids became popular in the United States and European countries. In their reports, the incidence of GVHD after CBT is 35% to 44% for grade II-IV aGVHD, 11% to 27% for grade III-IV aGVHD, and 9% to 33% for cGVHD [14,20-22], mostly by prophylaxis with CsA and steroids. GVHD prophylaxis with CNI alone after CBT was reportedly complicated with preengraftment immune reaction [23], but a Japanese retrospective study showed the superiority of GVHD prophylaxis with 2 agents compared with that of single agent in terms of DFS for patients with acute leukemia [24]. In this study, we found that the use of MTX showed favorable effects of significantly lower incidents of aGVHD and cGVHD, and in advanced cases, better OS was observed without affecting the engraftment or relapse. In Eurocord, an unfavorable effect of delayed myeloid engraftment by MTX was reported only in related CBT but not in unrelated CBT [25,26]. Another disadvantage of MTX reported by Eurocord was a higher relapse rate in unrelated CBT for children with ALL [12]. This unfavorable effect was not observed in our study, and this discrepancy could be explained by the different proportion of patients who were given ATG before SCT. In one Eurocord study for children with ALL, 88% of patients were given ATG [13], but only 7 of 270 patients (2.6%) were given ATG in our study. Because ATG reduces the incidence of aGVHD and cGVHD by purging T cells in vivo [27], GVHD prophylaxis including MTX with or without ATG needs to be analyzed in terms of transplantation outcomes including the GVL effect.

In Japan, Narimatsu [28] and Terakura [29] reported that MTX after CBT reduced the complications such as preengraftment immune reaction, engraftment syndrome, and aGVHD, as well as the incidence of treatment-related mortality and improved survival in adults. Takahashi also reported superior DFS after CBT with GVHD prophylaxis of MTX and CsA [30]. Neither of these studies found any unfavorable effects caused by MTX in unrelated CBT. In a Japanese pediatric study of CBT for AML, MTX contributed to lower TRM [31]. The critical role of MTX in unrelated CBT should be emphasized as a key drug in terms of prophylaxis for GVHD, although transplantation outcomes according to the dose and frequency of MTX administration was unable to be analyzed in this study. In our study, nobody was given mycophenolate mofetil (MMF), and the combination of CNI + MMF needs to be compared with CNI + MTX in the pediatric population.

In conclusion, CBT from an unrelated donor is feasible and effective as a treatment modality for children with ALL, and GVHD prophylaxis, which includes MTX, is critical to reduce the incidence of aGVHD and cGVHD without affecting engraftment, as well as to achieve better OS in advanced cases.

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