received CY/TBI ($P = 0.01$ for relapse, $P < 0.01$ for NRM). After a median follow-up period of 36.9 months, 5-year overall survival (OS) rates were 82.2% in the VP/CY/TBI group and 55.2% in the CY/TBI group. OS, and disease-free survival (DFS) in the VP/CY/TBI group were shown to be significantly better by multivariate analysis [hazard ratio: 0.21 (95% confidence interval: 0.06–0.49) for DFS, hazard ratio: 0.25 (95% confidence interval: 0.08–0.59) for OS]. VP/CY/TBI was associated with a lower relapse rate and no increase in NRM, resulting in better survival than that in CY/TBI for adult ALL patients.

Keywords Acute lymphoblastic leukemia · Stem cell transplantation · Conditioning regimen · Medium-dose VP/CY/TBI

1 Introduction

The prognosis of adult acute lymphoblastic leukemia (ALL) is dismal, [1–10] and allogeneic hematopoietic stem cell transplantation (alloSCT) is therefore performed in most cases. However, even in patients who received alloSCT conditioned with a standard regimen of cyclophosphamide with total body irradiation (CY/TBI), the prognosis has not been satisfactory due to a high rate of relapse. [11–16] Although VP-16 (VP) has been used as an alternative to CY or as an agent added to the standard regimen, the dose of VP was high (50–60 mg/kg or 1.5 mg/m^2) and the high rate of non-relapse mortality (NRM) was problematic [17–23]. Recently, we and others have reported excellent outcomes for adult patients with ALL who underwent alloSCT conditioned with 30–40 mg/kg VP added to CY/TBI (VP/CY/TBI). [24–26] In this paper, 30–40 mg/kg VP is called as "medium-dose VP". Although the conditioning regimen is one of the most important factors in alloSCT, there have been few studies in which conditioning regimens for ALL were compared, and there has been no study in which the outcomes of VP/CY/TBI and CY/TBI were compared. We therefore retrospectively compared the outcomes for patients who received VP/CY/TBI and patients who received CY/TBI, and we also investigated risk factors for relapse, NRM, disease-free survival (DFS) and overall survival (OS) to obtain useful information for selecting a conditioning regimen.

2 Patients and methods

2.1 Collection of data and data source

Clinical data for patients who received the VP/CY/TBI regimen were collected from six centers in Hokkaido, Japan, and data for patients who received CY/TBI were collected from the Japan Society for Hematopoietic Cell Transplantation database (Transplant Registry Unified Management Program) and the Japan Marrow Donor Program database. [27] Data for 35 patients who received VP/CY/TBI and data for 494 patients who received CY/TBI and who met all of the following criteria were analyzed: SCT performed between 1993 and 2007, first time for SCT, aged 15–59 years, diagnosed as having ALL/lymphoblastic lymphoma or acute biphenotypic leukemia, first or second CR (CR1 or CR2) at SCT, bone marrow (BM) or peripheral blood stem cells (PBSC) as stem cell source, and HLA-phenotypically 6 loci matched (A, B and DR loci) related donor (MRD) or unrelated donor (MUD). Patients who met at least one of the following criteria were excluded: secondary SCT, Burkitt leukemia/lymphoma, cord blood as stem cell source, secondary leukemia or T-cell depletion. Data on use of VP and the dose of VP were lacking in almost all of the patients in the registry data. The dose of VP was a key factor for VP/CY/TBI conditioning and we collected patients for our analysis by precise criteria including the same dose of conditioning. Therefore, we could not analyze the patients who received VP/CY/TBI conditioning from the registry data. This study was conducted with the approval of the Institutional Review Board of Hokkaido University Hospital.

2.2 Conditioning regimens and transplantation procedures

CY/TBI consisted of CY at 60 mg/kg once daily administered intravenously (i.v) for 2 days (total dose: 120 mg/kg) combined with fractionated TBI of 12 Gy (either 4 or 6 fractions). In this group, the days on which CY or TBI were administered differed depending on the center. Medium-dose VP/CY/TBI consisted of VP at a dose of 15 mg/kg once daily i.v. for 2 days (total dose: 30 mg/kg) and CY/TBI. [24, 25] VP, CY and TBI were administered on days −7 to −6, days −5 to −4 and days −3 to −1, respectively. Patients who received ATG, campath-1H or cytotoxic agents other than CY or VP in the conditioning regimen were excluded from the analysis. GVHD prophylaxis and other SCT procedures were performed according to the decision of the clinicians of each center.

2.3 Definitions

Neutrophil engraftment and platelet engraftment were defined as the first of 3 days with absolute neutrophil count $>0.5 \times 10^9$/l and the first of 7 days with an untransfused platelet count $>50 \times 10^9$/l, respectively. Toxicity after SCT was graded by the National Cancer Institute (NCI) common toxicity criteria (NCI, Bethesda,

MD, USA). Acute GVHD (AGVHD) and chronic GVHD (CGVHD) were graded by standard criteria. [28, 29] Relapse was defined as a recurrence of underlying diseases. NRM was defined as death during a continuous remission throughout the duration of the study. OS was calculated from the day of SCT until death or last follow-up. DFS was defined as survival in a state of continuous remission.

2.4 Endpoint and statistical analysis

The primary endpoint of this study was to compare relapse, NRM and survival in adult patients with ALL who received CY/TBI and those who received VP/CY/TBI and determine prognostic factors for survival. Descriptive statistical analysis was performed to assess patient characteristics and transplantation procedure, using the Chi-square test or Fisher's exact test as appropriate for categorical variables and the 2-sided Wilcoxon rank sum test for continuous variables. The probabilities of OS and DFS were estimated using the Kaplan–Meier method. Relapse rate and NRM rates were estimated using cumulative incidence analysis and considered as competing risks, and the Pepe and Mori test was used for group comparison of cumulative incidence [30]. Data for the day of relapse were not available in 9 patients who relapsed after SCT, and all of those patients who received CY/TBI. For strict assessment of VP/CY/TBI, 1 day before the last follow-up day was used as the day of relapse of these patients in the Kaplan–Meier method and cumulative incidence analysis, and these results were checked using sensitivity analysis. The effects of various patient and disease categorical variables on survival probabilities were studied using the log-rank test. All P values were two-sided and a P value of 0.05 was used as the cutoff for statistical significance. This study was a retrospective analysis that potentially included bias, and we therefore need to adjust the difference of variables using matched-pair analysis or multiple regression analysis. We considered that the latter was statistically better for our analysis for the following reasons: Selection of matching parameters included intentional bias, and if we used "matching", accuracy of parameter estimation would be reduced due to the reduction of the number of control patients. For adjusting the difference of background, probabilities of relapse, NRM, OS and DFS were estimated using the Cox proportional-hazards regression model, with consideration of other significant clinical variables in the final multivariate models. The variables considered were conditioning regimen, year in which SCT was performed, patient's age at SCT, patient's sex, disease status at SCT, donor (MRD or MUD) and HLA-allele matching. HLA-identical siblings were included in the "HLA-allele match" group.

3 Results

3.1 Patients and transplantation characteristics

Patients and SCT characteristics are summarized in Table 1. The median age of the patients was 34 years (range 15–59 years). Cytogenetic study was performed in 475 (89.8%) of the patients, and 270 (56.8%) of the evaluable patients had chromosomal abnormalities, including poor-risk cytogenetics of Philadelphia chromosome (Ph, $n = 148$, 31.2%), MLL-related abnormalities ($n = 7$, 1.5%), t(1;19) ($n = 10$, 2.1%), −7 ($n = 5$, 1.1%) and +8 ($n = 2$, 0.4%). Data on use of tyrosine kinase inhibitors (TKI) for Ph-positive patients before SCT were lacking due to the limitation of registry data. In the 148 Ph-positive patients, 127 patients received SCT after 2001, the year in which imatinib was approved in Japan, suggesting that Ph-positive patients came to be able to receive SCT by administration of TKI. Four hundred and forty-two patients (83.6%) were in CR1 at SCT and 87 patients (16.4%) were in CR2 at SCT. In the 127 Ph-positive patients who were diagnosed after 2001, twenty-three patients received SCT in molecular remission and 34 patients were not in molecular remission, and data on molecular status were not available for 70 patients. Five of the 8 patients with Ph who received VP/CY/TBI were diagnosed after 2001. Four of those five patients were in molecular remission before SCT and the other patient was not in molecular remission. Two hundred and fifty-eight patients (48.8%) underwent SCT from an MRD and 271 patients (51.2%) underwent SCT from an MUD. Four hundred and thirty-three patients (81.9%) received BM and 95 patients (18.0%) received PBSC, and PBSC were from an MRD in all cases because donation of PBSC from unrelated donors is not permitted in Japan. Although patients who received VP/CY/TBI (VP/CY/TBI: median age of 28 years; CY/TBI: median age of 34 years, $P = 0.02$) were younger, other factors such as Ph, SCT in CR2, and donor status were not significantly different between the two groups.

3.2 Transplantation outcomes

3.2.1 Engraftment

Five hundred and twenty-two patients (98.7%) achieved neutrophil engraftment and there was no difference between the groups [CY/TBI: $n = 487$ (98.6%), VP/CY/TBI: $n = 35$ (100%), $P = 0.43$, Table 2]. In both groups, median day of neutrophil engraftment was day 16 [CY/TBI: day 16 (range, days 8–49), VP/CY/TBI: day 16 (range, days 8–26), $P = 0.49$]. Platelet engraftment could be assessed in 472 patients, and 445 patients (94.1%) achieved platelet engraftment. There was no difference

Table 1 Patients and transplants characteristics

Variables	Total $n = 529$	CY/TBI $n = 494$	VP/CY/TBI $n = 35$	P
Age				
Median (range)	34 (15–59)	34 (15–59)	28 (15–58)	0.02
More than 35 years	251 (47.4%)	240 (48.6%)	11 (31.4%)	0.06
Sex				
Male	303 (57.3%)	282 (57.1%)	21 (60.0%)	0.86
Years of SCT				
Before 2001	189 (35.7%)	176 (35.6%)	13 (37.1%)	0.78
After 2002	340 (64.3%)	318 (64.4%)	22 (62.9%)	
Lineage				
B-cell	375 (70.9%)	352 (71.3%)	23 (65.7%)	0.11
T-cell	64 (12.1%)	55 (11.1%)	9 (25.7%)	
Biphenotype	16 (3.0%)	13 (2.6%)	3 (8.6%)	
Diagnosis				
ALL/LBL	495 (93.6%)	463 (93.7%)	32 (91.4%)	0.48
ABL	34 (6.4%)	31 (6.3%)	3 (8.6%)	
Ph[a]				
Yes	148 (31.2%)	140 (31.3%)	8 (29.6%)	1.00
WBC at diagnosis[b]				
High	125 (23.6%)	121 (24.5%)	4 (11.4%)	0.06
Disease status				
CR1	442 (83.6%)	414 (83.8%)	28 (80.0%)	0.64
CR2	87 (16.4%)	80 (16.2%)	7 (20.0%)	
Donor				
MRD	258 (48.8%)	242 (49.0%)	16 (45.7%)	0.92
HLA-allele matched	251 (47.4%)	235 (47.6%)	16 (45.7%)	
HLA-allele mismatched	1 (0.2%)	1 (0.2%)	0 (0.0%)	
HLA-allele unknown	6 (1.1%)	6 (1.2%)	0 (0.0%)	
MUD	271 (51.2%)	252 (51.0%)	19 (54.3%)	
HLA-allele mathched	191 (36.1%)	180 (36.4%)	11 (31.4%)	
HLA-allele mismatched	76 (14.4%)	70 (14.2%)	6 (17.1%)	
HLA-allele unknown	4 (0.8%)	2 (0.4%)	2 (5.7%)	
HLA-allele				
Match	442 (83.6%)	415 (84.0%)	27 (77.1%)	0.61
Mismatch	77 (14.6%)	71 (14.4%)	6 (17.1%)	
Unknown	10 (1.9%)	8 (1.6%)	2 (5.7%)	
Stem cell[c]				
BM from MRD	162 (30.6%)	152 (30.8%)	10 (28.6%)	0.94
PBSC from MRD	95 (18.0%)	89 (18.0%)	6 (17.1%)	
BM from MUD	271 (51.2%)	252 (51.0%)	19 (54.3%)	
GVHD prophylaxis				
CSP + MTX	341 (64.5%)	312 (63.2%)	29 (82.9%)	0.28
TK + MTX	140 (26.5%)	134 (27.1%)	6 (17.1%)	

ALL acute lymphoblastic leukemia, *ABL* acute biphenotypic leukemia, *LBL* lymphoblastic lymphoma, *Ph* Philadelphia chromosome, *WBC* white blood cell, *CR1* first complete remission, *CR2* second complete remission, *MRD* HLA-matched related donor, *MUD* HLA-matched unrelated donor, *BM* bone marrow, *PBSC* peripheral blood stem cell, *CSP* cyclosporin A, *MTX* methotrexete, *TK* tacrolimus

[a] Cytogenetic study was performed in 475 patients

[b] Definition of high WBC count; $>3.0 \times 10^{10}$/l for B lineage and $>10 \times 10^{10}$/l for T lineage

[c] One patient in the CY/TBI group received both BM and PBSC from MRD

between the two groups [CY/TBI: $n = 411$ (93.8%), VP/CY/TBI: $n = 34$ (97.1%), $P = 0.76$]. In both groups, median day of platelet engraftment was day 26 [CY/TBI: day 26 (range, days 9–235), VP/CY/TBI: day 26 (range, days 12–74), $P = 0.76$].

3.2.2 *Graft-versus-host disease*

Except for three patients who died early after engraftment and three patients whose data for AGVHD were not available, all patients who achieved engraftment were

Table 2 Engraftment and GVHD

Variables	Total	CY/TBI	VP/CY/TBI	*P*
Neutrophil engraftment				
Yes	522 (98.7%)	487 (98.6%)	35 (100.0%)	0.43
No	7 (1.3%)	7 (1.4%)	0 (0.0%)	
Day, median (range)	16 (8–49)	16 (8–49)	16 (8–26)	0.49
Platelet engraftment[a]				
Yes	445 (94.1%)	411 (93.8%)	34 (97.1%)	0.76
No	28 (5.9%)	27 (6.2%)	1 (2.9%)	
Day, median (range)	26 (9–235)	26 (9–235)	26 (12–74)	0.76
Acute GVHD[b]				
Yes	325 (63.0%)	300 (62.4%)	25 (71.4%)	0.28
No	191 (37.0%)	181 (37.6%)	10 (28.6%)	
Grade				
I	132 (25.6%)	120 (24.9%)	12 (34.3%)	0.46
II	135 (26.2%)	124 (25.8%)	11 (31.4%)	
III	44 (8.5%)	42 (8.7%)	2 (5.7%)	
IV	14 (2.7%)	14 (2.9%)	0 (0.0%)	
II–IV	193 (37.4%)	180 (37.4%)	13 (37.1%)	0.78
III–IV	58 (11.2%)	56 (11.6%)	2 (5.7%)	0.37
Onset day, median (range)	21 (1–117)	21 (1–117)	19 (7–59)	0.79
Chronic GVHD[c]				
Yes	208 (44.9%)	193 (44.9%)	15 (45.5%)	0.92
No	255 (55.1%)	237 (55.1%)	18 (54.5%)	
Grade				
Limited	79 (17.1%)	74 (17.2%)	5 (15.2%)	0.91
Extensive	126 (27.2%)	116 (27.0%)	10 (30.3%)	
Unknown	3 (0.6%)	3 (0.7%)	0 (0.0%)	
Onset day, median (range)	120 (26–797)	120 (26–797)	100 (48–201)	0.21

[a] Platelet engraftment was assessed in 473 patients (data for 56 patients were not available)

[b] Except for three patients who died early after engraftment and three patients whose data for AGVHD were not available, all patients who achieved engraftment were assessed for AGVHD ($n = 516$)

[c] Chronic GVHD was assessed in 463 patients who were alive at day 100 after SCT and whose data were available (data for 19 patients were not available)

assessed for AGVHD ($n = 516$, Table 2). AGVHD, grade II–IV AGVHD and grade III–IV AGVHD occurred in 325 (63.0%), 193 (37.4%) and 58 (11.2%) of the evaluable patients, respectively, and median onset day was 21 (range, days 1–117). No patients who received VP/CY/TBI developed grade IV AGVHD. CGVHD was assessed in 463 patients who were alive at day 100 after SCT and whose data were available (data for 19 patients not available). CGVHD occurred in 208 (44.9%) of the evaluable patients at median onset day of 120 (range, days 26–797), and extensive CGVHD occurred in 126 patients (27.2%). Incidences, grade and onset days of AGVHD and CGVHD were not different between the two regimen groups.

3.2.3 Relapse and NRM

One hundred and forty-eight patients relapsed with median day of 219 [range, days 32–1539, VP/CY/TBI: $n = 5$, median day 218 (range, days 113–1193), CY/TBI: $n = 143$, median day 218 (range, days 32–1539)]. Eighty-one patients died due to NRM with median day of 126 (range, days 2–2452). In these patients, causes of NRM were infection ($n = 19$), rejection ($n = 2$), AGVHD ($n = 8$), CGVHD ($n = 3$), bleeding ($n = 4$), hepatic veno-occlusive disease/thrombotic microangiopathy ($n = 5$), second malignancies ($n = 4$) and organ failure ($n = 37$; lung, $n = 19$; liver, $n = 6$; heart, $n = 3$; kidney, $n = 3$). Only one patient who received VP/CY/TBI died due to NRM (interstitial pneumonia of unknown cause) at day 46. Cumulative incidences of relapse and NRM were higher for patients who received CY/TBI than for those who received VP/CY/TBI with statistical significance ($P = 0.01$ for relapse and $P < 0.01$ for NRM, Fig. 1).

In multivariate analyses adjusted by other factors, there were significantly lower rates of relapse and NRM using VP/CY/TBI [hazard ratio (HR): 0.34 (95% confidence interval (CI): 0.10–0.81) for relapse, HR: 0.16 (95% CI: 0.01–0.72) for NRM (Table 3)]. T-cell lineage and disease status at SCT (CR1) were also determined to be significant factors for lower risk of relapse, and Ph negativity showed marginal significance. Disease status at SCT (CR1) and HLA-allele match were determined to be significant factors

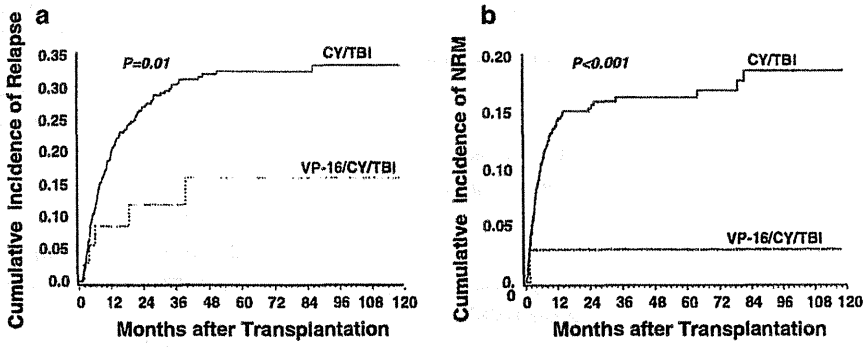

Fig. 1 Cumulative incidence analyses of relapse rate and NRM after SCT according to the conditioning regimens. Cumulative incidences of **a** relapse ($P = 0.01$) and **b** NRM ($P < 0.01$) were higher for patients who received CY/TBI than for those who received VP/CY/TBI. Relapse rate and NRM were considered as competing risks

Table 3 Multivariate analysis for prognostic factors for relapse, NRM, DFS and OS

Variables	Relapse			NRM			DFS			OS		
	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
Conditioning regimen												
CY/TBI	1.00			1.00			1.00			1.00		
VP/CY/TBI	0.34	(0.10–0.81)	0.01	0.16	(0.01–0.72)	0.01	0.21	(0.06–0.49)	<0.01	0.25	(0.08–0.59)	<0.01
Year of SCT												
Before 2001	1.00			1.00			1.00			1.00		
After 2002	0.89	(0.61–1.31)	0.55	0.60	(0.36–1.00)	0.05	0.80	(0.58–1.10)	0.16	0.76	(0.54–1.06)	0.11
Age												
More than 35 years	1.00			1.00			1.00			1.00		
Less than 34 years	1.19	(0.82–1.72)	0.35	0.73	(0.44–1.20)	0.21	1.02	(0.75–1.39)	0.88	0.94	(0.68–1.30)	0.70
Sex												
Male	1.00			1.00			1.00			1.00		
Female	0.94	(0.65–1.33)	0.72	0.80	(0.49–1.28)	0.35	0.88	(0.65–1.18)	0.38	0.95	(0.69–1.31)	0.76
Lineage												
B	1.00			1.00			1.00			1.00		
T	0.77	(0.58–0.98)	0.04	0.79	(0.55–1.09)	0.15	0.93	(0.68–1.22)	0.59	0.83	(0.58–1.14)	0.26
Ph												
Positive	1.00			1.00			1.00			0.40		
Negative	0.70	(0.47–1.05)	0.08	0.94	(0.55–1.67)	0.83	0.70	(0.51–0.99)	0.04	0.75	(0.52–1.07)	0.11
Disease status at SCT												
CR2	1.00			1.00			1.00			0.75		
CR1	0.44	(0.29–0.70)	<0.01	0.44	(0.26–0.78)	0.01	0.43	(0.30–0.63)	<0.01	0.41	(0.28–0.61)	<0.01
Donor												
Unrelated	1.00			1.00			1.00			0.56		
Related	0.79	(0.54–1.14)	0.21	1.01	(0.59–1.73)	0.98	0.84	(0.61–1.16)	0.30	0.77	(0.54–1.09)	0.14
HLA-allele disparity												
Mismatch	1.00			1.00			1.00			1.00		
Match	0.96	(0.56–1.72)	0.89	0.38	(0.21–0.68)	<0.01	0.62	(0.42–0.95)	0.03	0.56	(0.36–0.88)	0.01

Abbreviations are same as Tables 1 and 2

NRM non-relapse mortality, *DFS* disease-free survival, *OS* overall survival, *HR* hazard ratio, *CI* confidence interval

for lower risk of NRM, and years of SCT performed after 2002 showed marginal significance.

We could not compare the incidences of second malignancies in the two regimen groups due to insufficiency of data for secondary malignancies in the CY/TBI group. However, no patients in the VP/CY/TBI group had developed second malignancies after a median follow-up period of 48.4 months.

3.2.4 *Survival*

The median follow-up period for survivors was 36.9 months (range 1.2–181.0 months; CY/TBI: 34.9 months vs. VP/CY/TBI: 51.6 months, $P = 0.02$). Two-year OS and 5-year OS were 91.0 and 82.2%, respectively, in patients who received VP/CY/TBI, and they were 68.0 and 55.2%, respectively, in patients who received CY/TBI. Mortality rate within 100 days after SCT, which mainly indicated early death due to regimen-related toxicity, was not increased in patients who received VP/CY/TBI (100-day mortality rate: 6.5% in the CY/TBI group vs. 2.9% in the VP/CY/TBI group). The survival curve reached a plateau at 47 months after SCT in the VP/CY/TBI group and at 82 months in the CY/TBI group. OS and DFS were significantly better in patients who received the VP/CY/TBI regimen [OS: log-rank $P = 0.003$, DFS: log-rank $P < 0.001$ (Fig. 2)]. Among patients in CR1, OS and DFS were significantly better in patients who received the VP/CY/TBI regimen [OS: log-rank $P = 0.02$, DFS: log-rank $P = 0.006$]. Although the number of patients was small, significance of better survival was shown in patients in CR2 [OS: log-rank $P = 0.04$, DFS: log-rank $P = 0.03$]. Better OS and DFS using VP/CY/TBI were verified by multivariate analysis using a Cox regression model [HR: 0.21 (95% CI: 0.06–0.49) for DFS, HR: 0.25 (95% CI: 0.08–0.59) for OS (Table 3)]. CR2 at SCT and HLA-allele mismatch donor were also determined to be risk factors for DFS and OS. Ph positivity was determined to be a risk factor for DFS but not for OS, and year in which SCT was performed, sex, advanced age, lineage and unrelated donor were not risk factors for DFS and OS. Our analysis included patients older than 50–55 years of age, who usually have no indication for myeloablative SCT, and we therefore also performed multivariate analysis for OS in the limited patients under 50 years of age ($n = 471$). This analysis also showed that VP/CY/TBI was better than CY/TBI in this age group ($P < 0.001$, HR: 0.20, 95% CI: 0.05–0.53). We used age as a variable in multivariate analysis and the cut-off was 35 years, which has frequently been reported as a prognostic factor for ALL. Other cut-off of age in multivariate analysis also showed that VP/CY/TBI resulted in better survival than did CY/TBI and age was not determined to be a significant prognostic factor (data not shown). Although disease status at SCT could be assessed in only 75 patients by the PCR method, patients in PCR-negative CR at SCT ($n = 37$) showed better OS and DFS than did those in PCR-positive CR at SCT ($n = 38$) by univariate analysis (log-rank $P < 0.01$ for OS and DFS).

4 Discussion

VP has been shown to have anti-leukemia activity and has been used in conditioning regimens for ALL. [17–26, 31] Although it has been reported that VP-containing regimens showed superior disease control, VP-containing regimens also showed increased risk of NRM (24–47%), especially in patients of advanced age, [17] and pulmonary toxicity and liver toxicity were the main causes of death. [17–23] We previously reported the safety and efficacy of medium-dose VP/CY/TBI as a conditioning regimen for alloSCT in adult patients with ALL, [24, 25] in which the dose of VP (30 mg/kg) was smaller than that in other studies including VP in the conditioning regimens (60 mg/kg or 1.5–1.8 g/m^2).

In the current study, we focused on comparison of the standard regimen of CY/TBI and VP/CY/TBI for adult patients with ALL with the aim of obtaining useful information for selecting a conditioning regimen before SCT using a large number of homogenous patients selected by precise criteria. This study showed that VP/CY/TBI enabled very good disease control without increase in NRM, resulting in better survival than that with CY/TBI. Although the number of patients who received VP/CY/TBI was limited and patients who received VP/CY/TBI were younger than those who received CY/TBI, the number of control patients who received CY/TBI was sufficient to compare the outcomes of the regimens. Also, age was not determined to be a risk factor for survival and these results were verified by multivariate analysis.

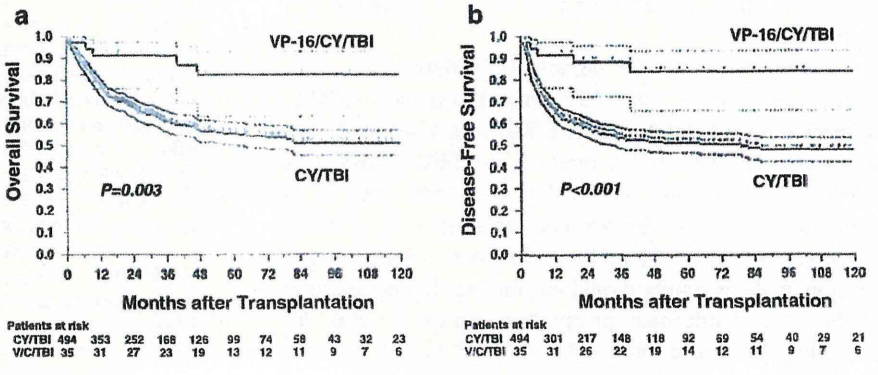

Fig. 2 Overall survival and disease-free survival after SCT according to the conditioning regimens. Probabilities of **a** OS (VP/CY/TBI vs. CY/TBI: 91.0 vs. 68.0% at 2 years, $P = 0.003$) and **b** DFS (VP/CY/TBI vs. CY/TBI: 88.1 vs. 57.9% at 2 years, $P < 0.001$) were both higher in patients who received VP/CY/TBI. *Blocked lines* show survival curves and *dotted lines* show 95% confidence intervals

Hunault et al. [26] reported results of the GOELAL-02 trial in which the conditioning regimen was similar to ours (VP at 20 mg/kg for 2 days + CY/TBI), and 6-year OS in the patients who received alloSCT in CR1 from an MRD was 75%; therefore, the dose of VP in conditioning regimens seems to be very important for lowering relapse rate without increasing NRM. In the present study, the doses of CY and TBI in the VP/CY/TBI regimen were the same as those in the CY/TBI regimen, and it is therefore difficult to understand how the addition of VP to CY/TBI could "lessen" the risk of NRM even if with adjustment by multivariate analysis. This might be due to biases of the patients including age and variables that could not be included in this study such as comorbidity, molecular status of the disease at SCT and center effect. These factors were difficult to analyze in this study due to the limitation of retrospective database-based analysis. However, we do not think that additional VP increased the risk of NRM including second malignancies.

Factors other than the conditioning regimen, including disease status at SCT and HLA-allele disparity, were also determined to be prognostic factors for OS. HLA-allele mismatch was related to the occurrence of grade II–IV AGVHD, resulting in lower OS. In fact, development of grade II–IV AGVHD was determined to be a prognostic factor for worse OS, whereas development of CGVHD was determined to be a prognostic factor for better survival, when these factors were included in multivariate analysis as time-dependent variables [grade II–IV AGVHD: HR 1.62 (95% CI: 1.19–2.22), CGVHD: HR 0.52 (95% CI: 0.36–0.74)]. Although better survival due to CGVHD indicated a graft-versus-leukemia (GVL) effect for ALL and CGVHD seems to be very important for disease control, we are not able to separate the GVL effect from GVHD, and we therefore consider choice of conditioning regimen for a patient to be the key for disease control in a clinical setting [32–34]. There was no difference in HLA-allele disparity, incidence of AGVHD and incidence of CGVHD between the VP/CY/TBI and CY/TBI groups, and we therefore thought that better outcomes in the VP/CY/TBI group were achieved not by increasing the GVL effect but by the direct anti-leukemia effect of the conditioning regimen.

In conclusion, a large number of patients who were selected by precise eligibility criteria provided reliable information showing that VP/CY/TBI was associated with lower relapse rate and no increase in NRM resulting in superior survival rate and higher cure rate than those achieved by the CY/TBI regimen for adult ALL patients. However, our analysis had the limitation of a retrospective fashion, and our results should be confirmed in prospective studies. A multicenter prospective phase 2 trial for assessing the efficacy and safety of VP/CY/TBI for adult patients with ALL is now ongoing in Japan (UMIN trial number 000001672).

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Conflict of interest The authors have declared no conflict of interest.

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bjh research paper

Outcome of unrelated umbilical cord blood transplantation in 88 patients with primary immunodeficiency in Japan

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Summary

We report the results of umbilical cord blood transplantation (UCBT) performed in 88 patients with primary immunodeficiency (PID) between 1998 and 2008 in Japan; severe combined immunodeficiency (SCID, $n = 40$), Wiskott–Aldrich syndrome (WAS, $n = 23$), chronic granulomatous disease ($n = 7$), severe congenital neutropaenia (SCN, $n = 5$) and other immunodeficiencies ($n = 13$). Five-year overall survival (5-year OS) for all patients was 69% [95% confidence interval (CI), 57–78%], and was 71% and 82% for SCID and WAS, respectively. The main cause of death before day 100 was infection (17/19), while that after day 100 was graft-versus-host disease (GVHD) (5/7). Using multivariate analyses, pre-transplant infection, no conditioning, ≥2 human leucocyte antigen (HLA) mismatches or diagnosis other than SCID, SCN or WAS were all associated with poor prognosis. Reduced-intensity conditioning was associated with decreased overall mortality compared with myeloablative therapy. The cumulative incidence of grade 2–4 acute GVHD at day 100 was 28% (95% CI, 19–38%), and that of chronic GVHD at day 180 was 13% (95% CI, 7–23%). We conclude that UCBT should be considered for PID patients without an HLA-matched sibling. The control of pre-transplant infection and selection of HLA-matched donors will lead to a better outcome.

Keywords: primary immunodeficiency, severe combined immunodeficiency, Wiskott-Aldrich syndrome, cord blood transplantation, reduced-intensity conditioning.

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Allogeneic haematopoietic stem cell transplantation (HSCT) has been successfully used as a curative therapy for most severe forms of primary immunodeficiency (PID) (Zeidler *et al*, 2000; Antoine *et al*, 2003; Sakata *et al*, 2004; Rao *et al*, 2005; Kobayashi *et al*, 2006; Mazzolari *et al*, 2007; Dvorak & Cowan, 2008; Griffith *et al*, 2008; Cuvelier *et al*, 2009). Stem cell transplantation from a human leucocyte antigen (HLA)-identical family donor provides better prognosis than bone marrow transplantation from an unrelated donor (Antoine *et al*, 2003). Survival with this type of transplantation from a matched unrelated donor has improved significantly over the years in patients with severe combined immunodeficiency (SCID), whereas no improvement in survival has been observed with this transplantation in non-SCID patients (Antoine *et al*, 2003). The optimal stem cell source for PID patients with no HLA-identical sibling remains to be determined (Dvorak & Cowan, 2008; Griffith *et al*, 2008; Cuvelier *et al*, 2009).

Umbilical cord blood grafts from unrelated donors have been successfully used, primarily in children and subsequently in adults (Kurtzberg *et al*, 1996; Wagner *et al*, 1996; Gluckman *et al*, 1997; Rubinstein *et al*, 1998; Rocha *et al*, 2000, 2004; Laughlin *et al*, 2004). Theoretically, unrelated cord blood transplantation (UCBT) has the following distinct advantages in PID patients: (i) the cord blood product is rapidly accessible in most cases; (ii) the incidence and severity of graft-versus-host disease (GVHD) is not excessive, even in mismatched transplantation and (iii) the risk of latent viral transmission is low. The disadvantages of UCBT include slower haematopoietic/immunological reconstitution and graft failure, which have been observed with UCBT for malignant disorders, and naivety of lymphocytes to pathogens (Brown *et al*, 2008; Griffith *et al*, 2008; Szabolcs *et al*, 2008). Rapid immune reconstitution is particularly important in PID patients with ongoing infection who undergo UCBT.

The limited data available show that UCBT can be a curative measure in patients with SCID, Wiskott–Aldrich syndrome (WAS), chronic granulomatous disease (CGD) and severe congenital neutropaenia (SCN) (Knutsen & Wall, 2000; Bhattacharya *et al*, 2003, 2005; Fagioli *et al*, 2003; Knutsen *et al*, 2003; Kobayashi *et al*, 2006). Most of the available data have come from a single centre, and thus, detailed information on the outcome and problems associated with UCBT in PID patients is still lacking. In this study, we report the results of UCBT performed in 88 PID patients between 1998 and 2008 in Japan.

Methods

Collection of data

All UCBTs carried out for PIDs through the Japan Cord Blood Bank Network (JCBBN) between August 1998 and January 2008 was enrolled in this study. Eighty-eight patients with PID underwent UCBT during this period. All data were provided by JCBBN, which collects recipients' clinical information at day 100 after transplantation. Recipients' data on survival, disease status and long-term complications are renewed annually by administering follow-up questionnaires. Latest data acquisition was performed in November 2009. The present study was approved by the institutional ethical and data management committees of JCBBN.

Patients

A summary of patients enrolled in this study is shown in Table I. Forty patients had SCID (45%) and 48 (55%) had non-SCID. Patients with familial haemophagocytic syndrome were not included in this study. The median age at the time of transplantation was 10 months (range, 0–248 months).

Procedures

Cryopreserved, unrelated cord blood cells were used as the source of haematopoietic stem cells. The type of conditioning used and median cell dose infused are shown in Table I.

In most cases, HLA matching was performed by both serological and DNA typing for HLA-A, HLA-B and HLA-DRB1. In this study, HLA mismatch was defined according to serological or low-resolution molecular typing for HLA-A and HLA-B and high-resolution molecular typing for HLA-DRB1. Of the UCB donors, 29 (33%) were HLA fully compatible. Of the mismatched donors, 40 (45%) were 1-antigen mismatched, 15 (17%) were 2-antigen mismatched and four (5%) were 3-antigen mismatched (Table I). In 48 patients in whom high-resolution genotypical typing was performed for HLA-A, HLA-B and HLA-DRB1, 11 were fully matched, 13 were 1-antigen mismatched, 16 were 2-antigen mismatched, five were 3-antigen mismatched and three were 4-antigen mismatched.

Immunosuppressive prophylaxis against GVHD after UCBT consisted of ciclosporin A (CyA)- and tacrolimus-based regimens in 48 and 35 patients, respectively. Five patients were not administered any immunosuppressive drug after UCBT.

Various techniques including karyotyping, HLA typing and fluorescence *in situ* hybridization for the XY chromosome and variable number of tandem repeats were used to confirm the engraftment of donor cells.

Definitions

Neutrophil recovery was defined by an absolute neutrophil count of at least $0{\cdot}5 \times 10^9$/l for three consecutive days. Platelet recovery was defined by a count of at least 20×10^9/l, unsupported by transfusion for 7 d. Reticulocyte recovery was defined by a count of at least 20‰.

Patients without conditioning or with only anti-thymocyte globulin (ATG) were categorized as receiving no conditioning. Patients administered busulfan (BU)/cyclophosphamide (CY) ± total body irradiation (TBI) or total lymphoid irradiation

