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

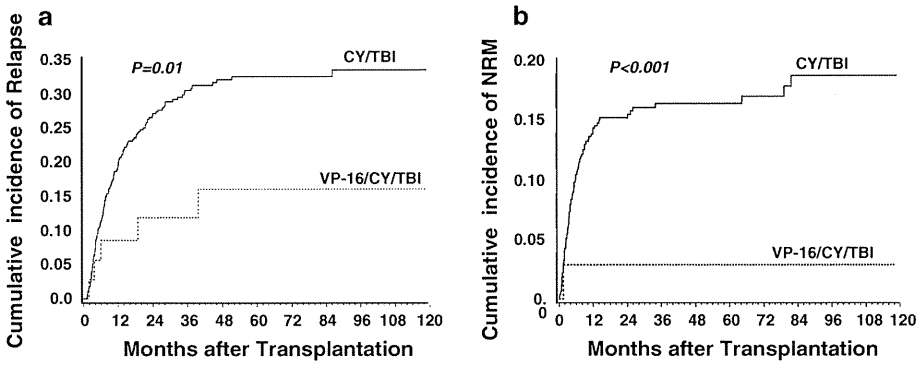

Fig. 1 Cumulative incidence analyses of relapse rate and NRM after SCT according to the conditioning regimens. Cumulative incidences of **a** relapse ($P = 0.01$) and **b** NRM ($P < 0.01$) were higher for patients who received CY/TBI than for those who received VP/CY/TBI. Relapse rate and NRM were considered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

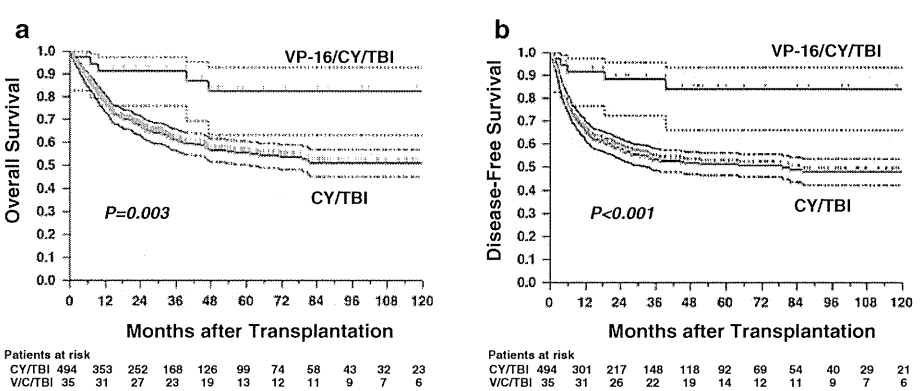

Fig. 2 Overall survival and disease-free survival after SCT according to the conditioning regimens. Probabilities of **a** OS (VP/CY/TBI vs. CY/TBI: 91.0 vs. 68.0% at 2 years, $P = 0.003$) and **b** DFS (VP/CY/TBI vs. CY/TBI: 88.1 vs. 57.9% at 2 years, $P < 0.001$) were both higher in patients who received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

Clinical Impact of Pre-transplant Pulmonary Impairment on Survival After Allogeneic Hematopoietic Stem Cell Transplantation

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Abstract We retrospectively analyzed the clinical outcomes of patients with pulmonary impairment before undergoing allogeneic hematopoietic stem cell transplantation (HSCT) for the first time. Among 297 evaluable patients who underwent their first HSCT, 23 had restrictive, obstructive or mixed ventilatory impairment (n=9, 13 and 1, respectively). Males predominated among the patients with pulmonary impairment (p=0.037) and received a reduced intensity conditioning (RIC) regimen more frequently, although the difference did not reach statistical significance (p=0.05). Among 23 patients with pulmonary impairment, 9 underwent post-transplant pulmonary function tests and obstructive ventilatory impairment progressed only in 2 patients, both of whom developed bronchiolitis obliterans. Kaplan-Meier estimates of 3-year overall (OS) among patients with and without pulmonary impairment were 57% and 47%, respectively, and those of relapse-free survival (RFS) were 70%, and 68%, respectively, with no significant differences between the groups (OS, p=0.235; RFS, p=0.287). The rates of non-relapse mortality also did not significantly differ (p=0.835). Our results suggest that allogeneic HSCT is safe for patients with pulmonary impairment. The lower frequency of fatal pulmonary complications after HSCT and the RIC regimen might contribute to favorable survival rates.

Keywords Fatal pulmonary complication · Obstructive ventilatory impairment · Restrictive ventilatory impairment · Reduced intensity conditioning regimen

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Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) can potentially cure various hematological diseases and numerous technical advances in HSCT allow HSCT to be applied even for elderly patients and/or those with mild organ dysfunction. However, vital organs must be evaluated using computed tomography (CT), echocardiography, magnetic resonance imaging and pulmonary function tests (PFT) to screen patients for HSCT eligibility because of the risk of life-threatening complications. Since pulmonary complications after HSCT represent major causes of morbidity and mortality, several reports have described post-transplant pulmonary complications such as infection, chronic GVHD of the lung, and changes in pulmonary function. In fact, several reports have described that pulmonary function parameters worsen after HSCT, although some of them are partially reversible [1–5]. Some investigations have also associated abnormal pre-transplant PFT with post-transplant pulmonary complications such as air flow obstruction [6–8]. However, the impact of pre-transplant pulmonary impairment on survival has not yet been determined. Thus, we retrospectively analyzed the clinical outcomes of patients with pulmonary impairment before HSCT.

Patients and Methods

Among the 319 patients who underwent a first allogeneic HSCT at our hospital between January 2004 and December 2009, we retrospectively reviewed the records of 297 who were evaluable. Those who underwent a second HSCT were excluded from the analysis. The median age of the patients was 42 (range: 16–67) years. All patients underwent spirometry immediately before HSCT, as well as pre-transplant evaluation by echocardiography, systemic CT and brain MRI. Pulmonary function parameters including vital capacity (VC), total lung capacity, residual volume, forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1) and peak expiratory flow were measured, but we did not routinely determine the diffusing capacity of the lung for carbon monoxide (DLCO).%VC was defined as the ratio of predicted VC and FEV1% as a ratio of FVC. Restrictive, obstructive and mixed ventilatory impairment was defined as%VC<80%, FEV1%<70% and both%VC and FEV1%<80% and <70%, respectively [1, 9].

Patients underwent preparative therapies according to the primary disease and type of transplantation. Most patients with a lymphoid malignancy were conditioned with a myeloablative regimen comprising total body irradiation (TBI, 12 Gy) plus cytarabine (8 g/m^2) and cyclophosphamide (CY, 120 mg/kg). Most patients with myeloid malignancy were conditioned with a regimen comprising busulfan (BU, 16 mg/kg orally or 12.8 mg/kg intravenously) and CY (120 mg/kg). Total lymphoid irradiation (TLI, 7 Gy) was added to the BU/CY regimen for patients undergoing human leukocyte antigen (HLA)-mismatched or unrelated transplantation. Patients with severe aplastic anemia were also conditioned using a regimen that included TLI. Most patients undergoing the reduced intensity conditioning (RIC) regimen, received fludarabine (125 mg/m^2) and melphalan (120 mg/m^2), or fludarabine (180 mg/m^2) and busulfan (6.4 mg/kg intravenously), and 1 day TBI (4Gy). Prophylaxis for acute graft versus host disease (GVHD) consisted of a short course of methotrexate and cyclosporin A or tacrolimus (FK). Patients undergoing either unrelated or HLA-mismatched transplantation also received FK.

Continuous baseline characteristics were compared using the Mann–Whitney test and categorical characteristics were compared using the χ^2 test. Overall survival (OS) was calculated from the first transplantation to final follow-up or death. Relapse-free survival (RFS) was calculated from the first transplantation to the last follow-up or the date when underlying diseases relapsed. Non-relapse mortality

Table 1 Patients' characteristics

		Patients with normal pulmonary function n=274	Patients with pulmonary impairment n=23	Total n=297	P value
Age (y)	Median (range)	42 (16–67)	47 (18–66)	42 (16–67)	0.738
Gender	Male	154	18	172	0.037
	Female	121	5	126	
Regimen intensity	Myeloablative	243	17	260	0.05
	Reduced intensity	31	6	37	
TBI in conditioning regimen	Yes	118	17	135	0.113
	No	156	6	162	
HLA	Matched	179	19	198	0.091
	Mismatched	95	4	99	
Donor	Related	77	3	80	0.118
	Unrelated	197	20	217	
Diagnosis	Acute myeloid leukemia	117	11	128	0.329
	Myelodysplastic syndrome	37	3	40	
	Acute lymphoblastic leukemia	53	1	54	
	Chronic myeloid leukemia	23	1	24	
	Multiple myeloma	5	1	6	
	Other lymphoid malignancies[a]	19	4	23	
	Aplastic anemia and myelofibrosis	20	2	22	

[a] Other lymphoid malignancies include non-Hodgkin's lymphoma, Hodgkin's lymphoma and adult T cell leukemia/lymphoma.

HLA human leukocyte antigen; *TBI* total body irradiation

Table 2 Clinical characteristics of 23 patients with pulmonary impairment

Patient No	Age/ Gender	Primary disease	Conditioning regimen	Type of pulmonary impairment	Type of HSCT	Smoking history (number/ day *year)	Cause of pulmonary impairment	%VC before HSCT (%)	FEV1% before HSCT (%)	%VC after HSCT (%)	FEV1% after HSCT (%)	Inspection date (days after HSCT)	LONIPCs	Outcome (months)	Cause of death
1	53/M	AML	BU+Flud	M	UBMT	Yes (20*20)	Pulmonary tuberculosis	52.3	51.7				-	6†	Relapse
2	24/M	NHL	CA+CY	R	UBMT	No	Unknown	59	93.4				-	5†	Cardiomyopathy
3	54/M	AML	BU+CY	R	UBMT	Yes (30*30)	Unknown	63	79				-	6†	NA
4	32/F	HD	BU+CY	R	UBMT	No	Unknown	64.2	93				-	55	
5	21/M	AML	BU+CY	R	RPBSCT	No	Unknown	67.2	86.7	83.7	110.6	82	BO	57	
6	18/F	AML	BU+CY	R	UBMT	No	Unknown	72.6	96.3				-	9†	Relapse
7	47/F	AML	BU+CY	R	UBMT	Yes (15*26)	Unknown	75.8	88.9				-	16	
8	54/M	ATLL	L-PAM +Flud	R	UBMT	No	Metastatic calcification	76.1	71.7	79.6	88.6	145	-	8†	Relapse
9	18/M	AML	BU+CY	R	UBMT	No	Unknown	76.3	78.2				-	3†	Relapse
10	34/F	MM	CY	R	UBMT	No	Unknown	78.8	80.3	70.7	94.4	300	-	59	
11	54/M	ATLL	CY	O	UBMT	Yes	Emphysema, asthma	95	58.8				-	5†	Bacterial pneumonia
12	50/M	MDS	BU+CY	O	UBMT	NA	Emphysema, atelectasis	96.7	67.7				-	39	
13	61/M	AML	L-PAM +Flud	O	UCBSCT	Yes, (50*40)	Emphysema	99.2	67.7	92.7	72.9	64	-	13	
14	26/M	SAA	CY	O	UBMT	Yes, (10*3)	Unknown	105.2	63.8				-	71	
15	33/M	AML	IVBU+CY	O	UBMT	NA	Unknown	107.4	68.9				-	12†	Relapse
16	66/M	MDS	L-PAM +Flud	O	UCBSCT	Yes, (25*45)	Emphysema	110.3	67.7				-	4†	Engraftment failure
17	23/M	SAA	Flud+CY +ATG	O	UBMT	No	Unknown	112.6	64.1	106.2	65	172	-	20	
18	46/M	AML	CA+CY	O	RPBSCT	Yes	Emphysema	115.2	68.8				-	49†	Relapse
19	54/M	AML	BU+CY	O	UBMT	Yes, (40*30)	Emphysema	119.7	63				-	4†	Relapse
20	57/M	CML	BU+CY	O	RBMT	Yes, (30*35)	Emphysema	122.2	64.8	74.3	41.6	154	BO	67	
21	56/F	MDS	BU+CY	O	UBMT	No	Unknown	129.5	64.5	94.4	49.6	314	BO	18	
22	38/M	ALL	CA+CY	O	UBMT	Yes, (20*20)	Mycobacterium avium infection	137	69.8	129	77.2	440	OP	67	
23	62/M	AML	BU+Flud	O	UBMT	Yes, (50*45)	Emphysema	151.3	65.9	145	72.1	105	-	7†	Fungal pneumonia

M mixed; *O* obstructive; *R* restrictive. *HSCT* hematopoietic stem cell transplantation; *VC* vital capacity; *FEV* forced expiratory volume; *LONIPCs* late onset non-infectious pulmonary complications; *AML* acute myeloid leukemia; *NHL* non-Hodgkin's lymphoma; *HL* Hodgkin's lymphoma; *ATLL* adult T cell leukemia/lymphoma; *MM* multiple myeloma; *MDS* myelodysplastic syndrome; *SAA* severe aplastic anemia; *CML* chronic myelogenous leukemia; *ALL* acute lymphoblastic leukemia; *BU* busulfan; *Flud* fludarabine; *CA* cytosine arabinoside; *CY* cyclophosphamide; *L-PAM* melphalan; *ATG* anti-thymocyte globulin; *UBMT* unrelated bone marrow transplantation; *RPBSCT* related peripheral blood stem cell transplantation; *UCBSCT* unrelated cord blood stem cell transplantation; *RBMT* related bone marrow transplantation; *BO* bronchiolitis obliterans; *OP* organizing pneumonia; *NA* not available.

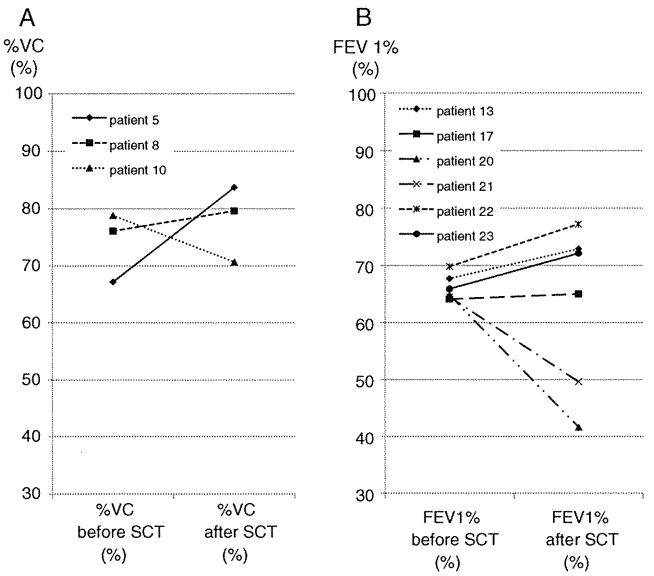


Fig. 1 Changes in pulmonary function. **a**,%VC in patients with restrictive pulmonary impairment; **b**, FEV1% in patients with obstructive pulmonary impairment

(NRM) was defined as death in the absence of relapse. Outcomes were estimated using Kaplan-Meier methods and compared using the log-rank test. All data were analyzed using PSPP II software (SPSS, Chicago, IL). All statistical tests were two-sided, and $p<0.05$ was considered statistically significant.

Results

Table 1 summarizes the patients' characteristics. Of 23 patients with pulmonary impairment before HSCT, 9, 13 and 1 had restrictive, obstructive and mixed ventilatory impairment, respectively. The median ages were 47 (range: 18–66) and 42 (rage: 16–67) years for those with and without pulmonary impairment, respectively. Age, use of TBI, HLA disparity, donor type and diagnosis did not significantly differ between patients with and without pulmonary impairment. Male patients predominated in the

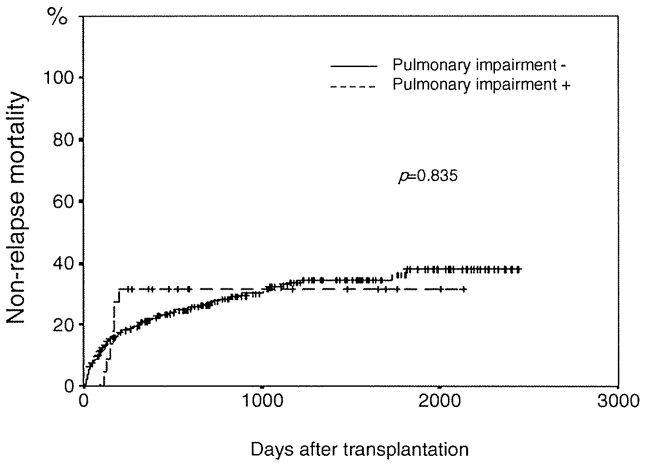
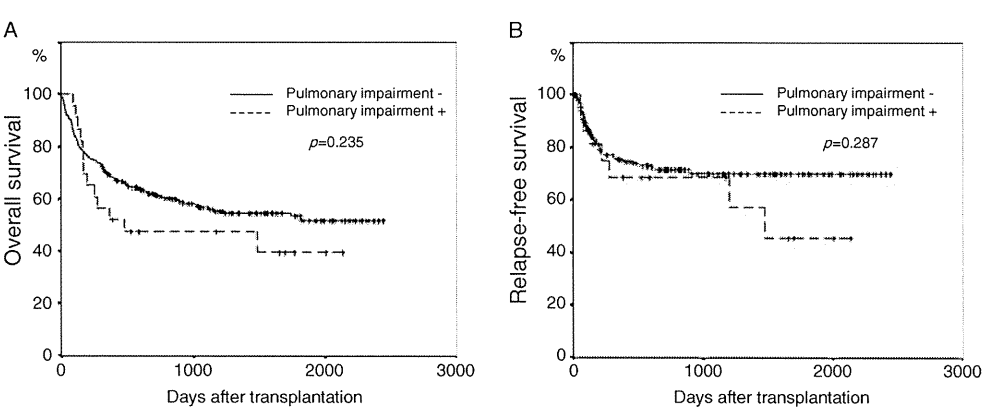


Fig. 3 Non-relapse mortality among patients with (solid line) or without (dotted line) pulmonary impairment

group with pulmonary impairment ($p=0.037$) and underwent the RIC regimen more frequently, although the value did not reach statistical significance ($p=0.05$). Nine of the 13 patients with obstructive ventilatory impairment had a smoking history. Emphysema was the main cause of the pulmonary impairment in 8 patients, of whom 7 had a smoking history, whereas the causes remained obscure in 8 patients with restrictive ventilatory impairment among whom only three had a smoking history (Table 2). Spirometry was performed after HSCT in 9 of 23 patients with pulmonary impairment (restrictive and obstructive ventilatory impairment, $n=3$ and $n=6$, respectively). Spirometric findings did not significantly change in most patients after HSCT (Fig. 1a and b). The FEV1% obviously worsened after HSCT in patient Nos. 20 (64.8% to 41.6%) and 21 (64.5% to 49.6%), both of whom developed bronchiolitis obliterans (BO), which is a sign of chronic GVHD. Twelve patients with pulmonary impairment died during the study (disease relapse, $n=7$; pulmonary complications, $n=2$; Table 2).

Kaplan-Meier estimates of 3-year OS and RFS were 57% and 70%, respectively for patients with normal pulmonary function and 47% and 68%, respectively for

Fig. 2 Overall (**a**) and relapse-free (**b**) survival in patients with (solid line) or without (dotted line) pulmonary impairment



those with pulmonary impairment (no significant differences: OS, p=0.235; RFS, p=0.287; Fig. 2a and b) and NRM also did not significantly differ between the groups (p=0.835, Fig. 3).

Discussion

We retrospectively analyzed the clinical outcomes of patients with pulmonary impairment before HSCT. Among 297 evaluable patients, 23 (7.7%) had pulmonary impairment before HSCT. Although both OS and RFS tended to be inferior to those in patients with normal pulmonary function, the differences did not reach statistical significance. Thus, our results suggest that patients with pulmonary impairment can safely undergo allogeneic HSCT. The predominance of males with pulmonary impairment seemed attributable to smoking rates, which are about 4-fold higher among men than women in Japan. Among the 13 patients with obstructive ventilatory impairment, emphysema was the most frequent cause (n=8), and most (n=7) of these had a history of smoking.

Several reports have described a negative impact of HSCT on pulmonary function, especially among patients with pre-transplant pulmonary impairment [6–8]. One study found a higher risk of pulmonary complications after HSCT among patients with pre-transplant pulmonary impairment, although the incidence of fatal pulmonary complications was not significantly increased [10]. Of the 12 patients who died during the period of the present study, 7 died of disease relapse and only two died of pulmonary complications. Patients with pulmonary impairment tended to receive the RIC regimen more frequently although the difference did not reach statistical significance (p=0.05, Table 1). The RIC regimen is less toxic and non-myeloablative regimens minimally impact post-transplant pulmonary function in patients with CML [11]. Although the periods from HSCT to post-transplant PFT were quite variable in our cohort (median, 172 days after HSCT; range, 64–440 days; Table 2), FEV1% deteriorated in only two of the nine patients who received PFT after HSCT. These two developed BO as a sign of chronic GVHD, which seemed to be a main cause of a decline in PFT. Thus, the RIC regimen and a lower frequency of fatal pulmonary complication after HSCT might contribute to favorable patient survival and post-transplant pulmonary function.

Goldberg et al. studied early non-relapse mortality after HSCT and found that FEV1, performance status, serum creatinine and serum bilirubin were independent factors associated with early toxic death [12]. Furthermore, worsening FEV1 and DLCO are key definitions of comorbidity in the hematopoietic cell transplantation specific comorbidity index (HCT-CI) that can predict non-relapse mortality and survival [13]. Although the NRM in our study also did not significantly differ (Fig. 3), this might be partly due to a difference in the method by which pulmonary function was evaluated. We evaluated obstructive impairment using FEV1%, whereas both of the above reports used %FEV as a ratio of predicted FEV. Of 13 patients with obstructive ventilatory impairment, only 4 of them had a %FEV <80% (data not shown). However, emphysema was the cause of the obstructive ventilatory impairment in 9 of 13 of our patients; thus, evaluation based on FEV1% reflected respiratory status in our cohort. The HCT-CI scores other than pulmonary function were 0 or 1 in all patients with pulmonary impairment (score 0 in 14 patients, 1 in 9 patients, data not shown). This also might contribute to better outcome.

In summary, we analyzed clinical outcomes in patients with pulmonary impairment. Our data demonstrated a favorable clinical outcome and that HSCT can be safe in appropriate conditions depending on other comorbidities and the toxicity of conditioning regimen as well. A minimal number of fatal pulmonary complications after HSCT and the RIC regimen might have contributed to the favorable outcome. However, since this retrospective study investigated a small cohort, further studies are warranted to confirm the clinical impact of pre-transplant pulmonary function on patient survival.

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Conflict of interest The authors declare no competing financial interests.

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Mycophenolate and Tacrolimus for Graft-Versus-Host Disease Prophylaxis for Elderly After Cord Blood Transplantation: A Matched Pair Comparison With Tacrolimus Alone

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Background. The optimal graft-versus-host disease (GVHD) prophylaxis after umbilical cord blood transplantation has not been established. Our previous observation using single calcineurin inhibitors revealed a high incidence and severity of early immune-mediated complications, especially for older patients or those with poor performance status.
Methods. We conducted a single institute pilot study assessing the safety and effectiveness of mycophenolate mofetil (MMF) and tacrolimus (FK) combination as a GVHD prophylaxis for 29 patients (FK+MMF), and the results were compared with matched-pairs extracted from our historical database who received FK alone as GVHD prophylaxis (control).
Results. FK+MMF group showed superior engraftment rate compared with control group (cumulative incidence until day 60 posttransplant; 90%±0% vs. 69%±1%, $P=0.02$). A cumulative incidence of severe type preengraftment immune reactions was significantly decreased in FK+MMF group (16%±1%) compared with that of control group (52%±2%, $P=0.03$), and, remarkably, there was no nonrelapse mortality (NRM) observed up to day 30 posttransplant in FK+MMF group, whereas 21%±1% of NRM was observed in the control group. However, the incidences of acute and chronic GVHD, estimated overall and progression-free survivals were comparable between two groups.
Conclusions. MMF and FK in combination was well tolerated and decreased early NRM possibly by better control of preengraftment immune reactions. Subsequent NRM or disease progression needs to be overcome to further improve survival.

Keywords: Cord blood transplantation, GVHD prophylaxis, Mycophenolate mofetil, Tacrolimus, Elderly patients.

(*Transplantation* 2011;92: 366–371)

Although umbilical cord blood transplantation (UCBT) has been increasingly used as a curative treatment of hematological diseases, accompanying toxicity, especially early period posttransplant, has been a major problem (*1, 2*). Our previous observation indicated that elderly patients were more vulnerable to early toxicity posttransplant, with nonrelapse mortality (NRM) being a major cause of treatment failure (*3*). Early immune-mediated complications, termed preengraftment immune reactions (PIR), were significant factors that negatively affected overall survival (OS) (*3–5*).

Various immunosuppressive drugs have been used for graft-versus-host disease (GVHD) prophylaxis in UCBT, including mycophenolate mofetil (MMF), (*6–8*) methotrexate (MTX), (*9–11*) corticosteroids, (*11*) anti-thymocyte globulin, (*12, 13*), and sirolimus (*14*); mostly in combination with calcineurin inhibitors. So far, no available data indicate that one drug or combination is better than the other.

MMF is an inosine monophosphate dehydrogenase inhibitor that exerts its immunosuppressive effect by blocking the production of guanosine nucleotide synthesis through the

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N.U. performed research design, data analysis, and writing the manuscript; A.W., N.N., K.I., S.T., M.T., H.Y., D.K., N.M., K.M., H.A., Y.A.M., K.I., S.M., and A.Y. participated in the performance of the research; A.W. and S.T. contributed with data analysis and writing of the manuscript; and all authors reviewed the manuscript.

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de novo pathway (*15*). It has been extensively used in solid organ transplantations (*16*) and more recently, in hematopoietic cell transplantation (HCT) (*7, 17–19*). In HCT, less mucosal damage compared with MTX has been observed, (*19–21*) with a comparable incidence of GVHD, suggesting a potential advantage of MMF over MTX. It therefore seemed rational to incorporate MMF in reduced-intensity (RI) UCBT for patients at high risk for NRM. Since December 2005, MMF+tacrolimus (FK) combination was started to be used as GVHD prophylaxis in RI-UCBT as a pilot study for those who agreed to participate. The results were compared with that of those who performed RI-UCBT using FK alone extracted as matched pairs from our historical database.

RESULTS

Patients/Matched Controls

Table 1 shows the demography of the patient characteristics of two groups. A total of 89% of the control patients who had GVHD prophylaxis of FK alone were transplanted from 2004 to 2005, whereas 93% of the patients with FK+MMF were from 2006 to 2007 ($P<.0001$). The differences between the groups did not reach statistical significance in Eastern Cooperative Oncology Group (ECOG) performance status (PS), HCT-specific comorbidity index (HCT-CI) score, history of previous HCT, human leukocyte antigen (HLA) disparity to UCB, and conditioning regimen. The median FK concentrations (11.9±0.33 ng/mL in FK+MMF group vs. 12.6±0.47 ng/mL in control group, $P=0.46$) and the proportions of FK concentration more than or equal to 10 ng/mL during day 0 to the date of engraftment (72.4%±3.1% in FK+MMF group vs. 75.0%±4.0% in control group, $P=0.43$) were comparable in each group.

Engraftment

Twenty-seven patients in FK+MMF group achieved neutrophil engraftment, and all except 1 showed complete donor chimerism. The cumulative incidence of primary engraftment until day 60 posttransplant was 90%±0%, whereas that of control group was 69%±1% ($P=0.02$). Median time to engraftment was 19 days after transplantation both in FK+MMF group (range, 13–32 days) and control group (range, 12–33 days). Among the two patients in FK+MMF group who failed to engraft, one experienced disease recurrence before day 28, and the other experienced rejection of donor cells and was later found to have anti-HLA antibodies against one of the antigens expressed on donor cells. One patient in FK+MMF group who showed mixed chimerism on neutrophil engraftment, when 87.2% of total bone marrow (BM) cells were of donor origin, experienced early BM relapse of leukemia on day 30 posttransplant. There were three patients in control group who experienced hemophagocytic syndrome (HPS) early after transplant and resulted in early death before engraftment, whereas there was no such cases observed in FK+MMF group. Platelet recovery more than 20×10^9/L was observed in 17 patients, with a cumulative incidence of 59%±1% at day 100 posttransplant (median, 40 days; range, 25–70 days), whereas in control group, the cumulative incidence was 52%±1% (median, 40 days; range, 26–62 days, $P=0.69$).

TABLE 1. Patient, treatment, and donor umbilical cord blood characteristics

Characteristic	N (%) of patients FK+MMF	Control	*P*
Sex			0.38
Male	21 (72)	23 (79)	
Female	8 (28)	6 (21)	
Age (yr)			0.67
Median (range)	62 (52–70)	63 (56–69)	
Age distribution (yr)			
51–55	5 (17)	0	
56–60	4 (14)	9 (31)	
61–65	12 (41)	13 (45)	
66–70	8 (28)	7 (24)	
Diagnosis			0.11
AML/MDS	19 (66)	16 (55)	
ALL	2 (7)	5 (17)	
ML	5 (17)	5 (17)	
CML	0	3 (10)	
AA	3 (10)	0	
ECOG performance status			0.37
0	0	0	
1	22 (76)	17 (59)	
2	5 (17)	9 (31)	
3	2 (7)	3 (10)	
HCT-CI			0.25
0	9 (31)	18 (62)	
1	12 (41)	7 (24)	
2	1 (3)	1 (3)	
≥3	7 (24)	3 (10)	
Disease status			0.78
Standard risk	10 (34)	9 (31)	
High risk	19 (66)	20 (69)	
History of prior HCT			0.16
None	22 (76)	26 (90)	
Autologous	4 (14)	3 (10)	
Allogeneic	3 (10)	0	
Year of transplant			<0.0001
2004	0	11 (38)	
2005	2 (7)	12 (41)	
2006	7 (24)	6 (21)	
2007	20 (69)	4 (14)	
Conditioning regimen[a]			
Flu/Mel 140	8 (28)	1 (3)	
Flu/Mel 80-140/TBI 2-8	13 (45)	25 (86)	
Flu/Mel 80/Tespa 10	0	1 (3)	
Flu/Mel 80-140/Bu 8-16	4 (14)	0	
Flu/Bu 16	0	1 (3)	
Flu/Bu 8-16/TBI 2-4	3 (10)	1 (3)	
Flu/Bu 8/VP-16 450	1 (3)	0	
HLA disparity to UCB			0.22
0 antigen mismatch	1 (3)	1 (3)	
1 antigen mismatch	5 (17)	1 (3)	
2 antigen mismatch	23 (79)	27 (93)	
Total nucleated cell number			0.66
Median ($\times10^7$/kg)	2.4	2.31	
Range ($\times10^7$/kg)	2.0–4.5	1.91–4.76	
$CD34^+$ cell number			0.15
Median ($\times10^5$/kg)	0.9	0.81	
Range ($\times10^5$/kg)	0.11–2.32	0.11–1.9	

[a] Units for each number are as follows: Mel (mg/m^2), TBI (Gy), Tespa (mg/kg), Bu doses: oral (1 dose=1 mg/kg) or iv (1 dose=0.8 mg/kg), and VP-16 (mg/m^2).

AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukemia; ML, malignant lymphoma; CML, chronic myeloid leukemia; AA, aplastic anemia; HCT-CI, hematopoietic cell transplantation-specific comorbidity index; Flu, fludarabine; Mel, melphalan; TBI, total body irradiation; Bu, busulfan; VP-16, etoposide; and UCB, umbilical cord blood; FK, tacrolimus; MMF, mycophenolate mofetil; ECOG, Eastern Cooperative Oncology Group; HLA, human leukocyte antigen.

TABLE 2. Incidence of PIR and GVHD

	FK+MMF (N)	Control (N)
PIR (n=29)		
No. of evaluable[a]	29	28
Yes	22	23
Severe type	4	10
Acute GVHD		
No. of evaluable[b]	27	20
Grade I	4	4
Grade II	7	2
Grade III	7	5
Grade IV	4	3
Chronic GVHD		
No. of evaluable[c]	13	11
Limited	1	2
Extensive	1	2

[a] Those who showed clinical symptoms characteristic to PIR, and those who survived longer than 27 d posttransplant without PIR.
[b] Those who engrafted without disease progression.
[c] Those who survived beyond day 100 posttransplant without disease progression.
PIR, preengraftment immune reactions; GVHD, graft-versus-host disease; FK, tacrolimus; MMF, mycophenolate mofetil.

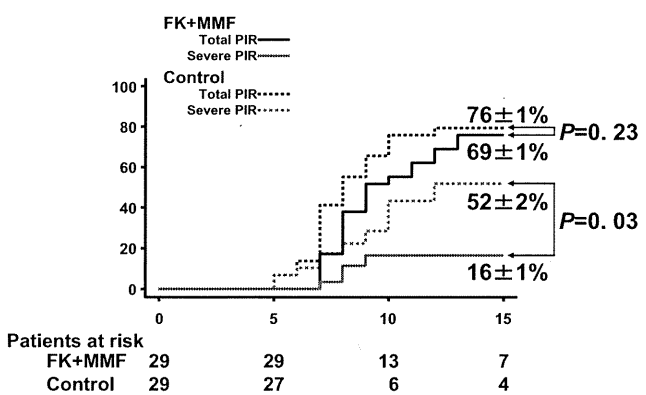

FIGURE 1. Cumulative incidences of preengraftment immune reactions (PIR) after RI-UCBT according to tacrolimus (FK)+mycophenolate mofetil (MMF) or FK alone graft-versus-host disease (GVHD) prophylaxis. The overall incidences of PIR in FK+MMF group (*black solid line*), in control group (*black dotted line*), and the incidences of severe type of PIR in FK+MMF group (*gray solid line*), and in control group (*gray dotted line*) were plotted. There was significant reduction of severe type of PIR in FK+MMF group compared with that in control group (P=0.03).

PIR and GVHD

In FK+MMF group, 22 of 29 patients experienced clinical symptoms defined as PIR, whereas in control group, 23 of 28 evaluable patients did (Table 2). Cumulative incidences of PIR in both groups were comparable each other (76%±1% in control group and 69%±1% in FK+MMF group, P=0.23, Fig. 1) and were similar to that reported in our previous publication (*3*). However, the cumulative incidence of severe type of PIR, defined by the criteria described in materials and methods section, in the FK+MMF group was lower (16%±1%) than that of control group (52%±2%) with statistical significance (P=0.03, Fig. 1).

In FK+MMF group, 22 of 27 evaluable patients developed acute GVHD, and 18 of them were grade II and higher. In control group, 14 of 20 evaluable patients had acute GVHD, and 10 of them were grade II and higher (Table 2). Cumulative incidences of grade II and higher acute GVHD at day 100 posttransplant were 63%±1% in FK+MMF and 35%±1% in control group (P=0.09). Chronic GVHD was observed in two of 13 FK+MMF group and four of 11 control group patients who survived longer than 100 days posttransplant without disease progression (Table 2). Cumulative incidences of chronic GVHD at 2 years posttransplant were 7%±0% in FK+MMF and 16%±1% in control group (P=0.35).

TABLE 3. Causes of death

	FK+MMF, N (%)	Control, N (%)
NRM	9 (45)	11 (65)
GVHD	5 (25)	3 (18)
IPS	4 (20)	1 (6)
Infection	0	5 (29)
CNS complication	0	2 (12)
Relapse/disease progression	11 (55)	6 (35)
Total	20	17

FK, tacrolimus; MMF, mycophenolate mofetil; NRM, nonrelapse mortality; GVHD, graft-versus-host disease; IPS, idiopathic pneumonia syndrome; CNS, central nervous system.

Survival, Disease Progression, and NRM

At the time of analysis, 9 FK+MMF group patients survived for a median of 980 days (range, 145–1430 days) after transplantation, whereas 12 control group patients were alive for a median of 1073 days (range, 49–2071 days). The Kaplan-Meier estimates of OS and progression-free survival (PFS) at 2 year posttransplant in FK+MMF group were 33%±9% and 21%±8%, whereas those in control group were 45%±10% and 34%±9%, respectively. The differences were not statistically significant (P=0.83 for OS, and P=0.75 for PFS).

Thirteen patients in FK+MMF group showed progression of the underlying disease at a median of 84 days (range, 19–344 days) after transplantation, and 11 of these patients died of the disease (Table 3). In control group, 9 patients did so at a median of 126 days (range, 12–1084 days) and 6 died of the disease. The cumulative incidences of disease progression at 2 years were 46%±1% in FK+MMF group and 29%±1% in control group, respectively (P=0.29).

Nine in FK+MMF group died of nonrelapse causes, whereas in control group patients, 11 NRM were observed (Table 3). GVHD and noninfectious pulmonary complications were observed in both groups as cause of death. None of the FK+MMF group died from infections as a sole reason of death, whereas five of the control group did. There was no death before day 30 posttransplant in FK+MMF group, whereas six in control group did. The cumulative incidences of NRM at day 30, 100, 365 were 0%±0%, 21%±1%, 28%±1% in FK+MMF group, and 21%±1%, 35%±1%,

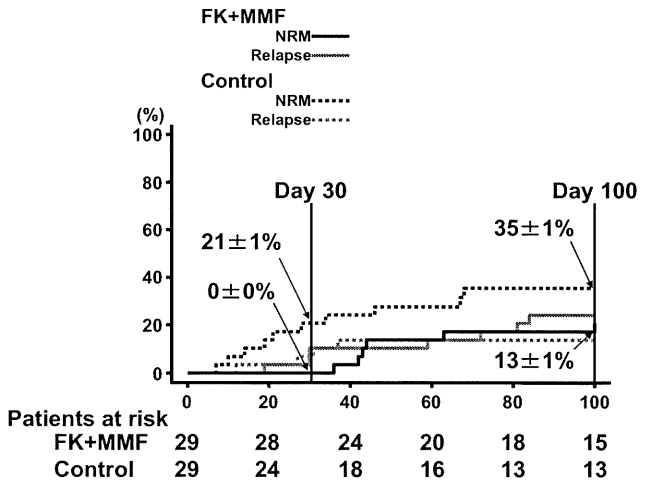

FIGURE 2. Day 100 nonrelapse mortality (NRM) and disease progression. Cumulative incidence estimates of NRM (*black line*) and disease progression (*gray line*) up to day 100 posttransplant for tacrolimus (FK)+mycophenolate mofetil (MMF) group (*solid line*) and control group (*dotted line*) were plotted. There were no NRM within 30 days posttransplant in FK+MMF group, whereas 21%±1% NRM were estimated in control group (P=0.01).

39%±1% in control group, respectively (P=0.01, P=0.17, P=0.29, Fig. 2).

DISCUSSION

The most remarkable observation in this study was that higher rate of neutrophil recovery and no early deaths before day 30 posttransplant were observed in FK+MMF group despite the patients' poor conditions before transplant, that is, all were older than 50 years and 69% of them had some comorbidities. Although the incidence of PIR in FK+MMF group was comparable with control group, the severity of PIR was less and thus did not result in severe organ damage early after transplant. There was no death directly caused by infections in FK+MMF group. We have reported higher incidence of HPS after RI-UCBT, which has been reasoned to be the delayed engraftment or graft failure (*22*). Interestingly, majority of the suffered BM cells were donor cell dominant, indicating HPS was mediated by donor-derived immune cells. Moreover, we have reported HLA mismatch in GVH direction, not in host-versus-graft direction, affected negatively to successful engraftment (*23*). All these facts fit well to the idea that hyperimmune reactions caused by donor cord blood (CB) cells may play crucial role in high rate of early NRM. Because there were no case of HPS in FK+MMF group, MMF may have promoted engraftment by sufficiently suppressing immune reactions of CB cells and preventing development of hemophagocytosis, which may also have reduced the incidence of severe infections. The presence of this type of hyperimmune reactions after UCBT has recently been recognized by others (*24*). The differences in incidence of PIR may have been affected by agents included in pretransplant conditioning, such as antithymocyte globulin, or by GVHD prophylaxis including corticosteroids or intravenous MMF.

Despite the present observation that the combination of MMF and FK succeeded in reducing early NRM, the incidence and severity of GVHD was not altered. Because most of the patients in the present study had advanced disease status, MMF was discontinued or started to be tapered on the day of neutrophil engraftment, which may have been responsible for this results. Much longer administration of MMF has been used in the setting of matched unrelated BM/peripheral blood (PB) transplantation (*7*). In addition, MMF was administered at 15 mg/kg twice daily in this study, which is the common dosing schedule in the settings of solid organ transplant (*16*). Several recent reports from Minnesota and Seattle considered 15 mg/kg three times daily as more appropriate based on pharmacokinetic data obtained from HCT recipients (*7, 17, 25*). A serum concentration measurement of mycophenolic acid, which was not assessed in this study, is needed to determine the optimal dosing of MMF.

Although NRM early after UCBT was significantly reduced in FK+MMF group, OS and PFS at 2 year posttransplant were still comparable with those of control group. Fifty-five percent of the deaths were from disease relapse or progression. Although MMF may have a beneficial effect on early survival after transplant by reducing severe immune reactions, it may increase the risk of disease progression for those who have active disease with a high risk of disease recurrence. According to previous publications, relapse rate is comparable in CB and unrelated BM/PB recipients despite lower incidences of chronic GVHD in CB recipients (*26, 27*), early immune reactions may have impact on reducing disease relapse. Because this is a relatively small sized, retrospective study, the presence of uncontrolled bias cannot be excluded. Prospectively conducted larger studies are warranted to further confirm the results.

In conclusion, MMF, used in combination with FK as GHVD prophylaxis in elderly patients with advanced hematologic diseases with or without comorbidities, may reduce early mortality posttransplant by regulating severe PIR and thus protecting patients from severe organ damage or HPS. An optimal dosing schedule of MMF needs to be determined prospectively using more homogenous populations.

MATERIALS AND METHODS

Patients

The initial pilot study included patients aged 51 years and older who underwent RI-UCBT using MMF+FK combination as GVHD prophylaxis at our institute from December 2005 through December 2007. Patients were eligible for this study if they had any hematologic malignancies at high risk for relapse or severe aplastic anemia refractory to standard immunosuppressive therapy and were unable to find suitable related or unrelated BM/PB donors within reasonable periods relative to their disease conditions. Patients with acute leukemia could be at first remission but at high risk for relapse due to adverse cytogenetic abnormalities, have a previous hematologic disorder, or be at any status beyond first remission. Patients with myelodysplastic syndrome (MDS) had to be refractory anemia (RA) with excess of blasts or chronic myelomonocytic leukemia, or have RA with transfusion dependency or severe neutropenia. Malignant lymphoma (ML) patients had to be beyond first remission. Patients who had end-stage cardiac dysfunction (left ventricular ejection fraction <35%), pulmonary dysfunction (SpO_2 <90% in room air), or active serious infection at the time of transplantation were not eligible. All patients gave written informed consent. Twenty-nine patients were enrolled and subjected to the matched pair analysis as below.

Selection of Matched Controls and Matching Variables

A matched-pair control group (GVHD prophylaxis with FK alone) for 29 patients who used MMF+FK combination was obtained by selecting one of the most recently transplanted control patients from our historical RICBT database from 2004 to 2007 after excluding those who met exclusion criteria of the pilot study described earlier. Controls were individually matched to cases on a 1:1 ratio. Matching was attempted for the following criteria applied in the order listed: age at transplantation (51–60, 61–70 years), disease risk (standard risk vs. high risk, acute leukemia, chronic myeloid leukemia, or ML in complete remission, MDS RA, aplastic anemia patients were categorized as standard risk, and all the others were as high risk), ECOG PS (PS 0–1, 2–3), pretransplant conditioning (busulfan containing vs. others), number of serological HLA mismatch (0–1, 2), HCT-CI (0–1, ≥2), total nucleated cell dose infused (≤2.3, >2.3×10^7/kg), and $CD34^+$ cell dose infused (≤0.8, >0.8×10^5/kg). To avoid any potential selection bias, matching was blinded, and only the patient's initials and pretreatment variables were known. This retrospective analysis was approved by the institutional review board.

One hundred percent matching was achieved for age group; 97% for disease risk (high risk, 66% of FK+MMF patients vs. 69% of control patients; P=0.78); 83% for ECOG PS (≥2 score, 32% of FK+MMF patients vs. 41% of FK alone patients; P=0.16); 72% for HCT-CI (≥2 score, 28% of FK+MMF patients vs. 14% of control patients; P=0.19); and number of serological HLA mismatch (2 antigens, 79% of FK+MMF patients vs. 93% of control patients; P=0.13); 86% for pretransplant conditioning (inclusion of busulfan, 24% of FK+MMF patients vs. 7% of control patients; P=0.07); 69% for total nucleated cell dose (≤2.3×10^7/kg, 41% of FK+MMF patients vs. 45% of control patients; P=0.79); and 62% for $CD34^+$ cell dose (≤0.8×10^5/kg, 45% of FK+MMF patients vs. 48% of control patients; P=0.79). Characteristics of the studied patients in both groups were shown in Table 1. Patients' comorbidity was assessed by a previously reported scoring system (*28*).

Donor Selection

UCB units were obtained from the Japanese Cord Blood Bank Network. HLA-A, -B, and -DR antigens were identified by serologic typing. UCB grafts had at least four of six HLA-A, -B, and -DR antigens that were matched to the recipient and had a cryopreserved cell dose of at least 1.9×10^7 nucleated cells per kg of recipient body weight.

Conditioning Regimens and Postgrafting Immunosuppression

Pretransplant conditionings were primarily RI regimens including 125 to 180 mg/m^2 of fludarabine (25 mg/m^2 for 5 days or 30 mg/m^2 for 6 days). Antithymocyte globulin was not incorporated. Granulocyte colony-stimulating factor (G-CSF) was started on day 1 posttransplant. Detailed information is shown in Table 1. Immunosuppressive therapy with FK (0.03 mg/kg continuous infusion, aiming for 12 to 17 ng/mL by at least three times a week measurement) with or without MMF (15 mg/kg twice daily) were started on day −1. MMF was discontinued or started to taper down on the day of neutrophil engraftment in the absence of active GVHD.

Definition of Engraftment, Preengraftment Immune Reactions, and Endpoints

Engraftment was defined as absolute neutrophil count more than 0.5×10^9/L for 3 consecutive days. Chimerism was assessed using fluorescent in situ hybridization in sex-mismatched donor-recipient pairs, or polymerase chain reaction for a variable number of tandem repeats with donor cells detected at a sensitivity of 10% in sex-matched pairs. Whole blood or BM cells were assessed at the time of granulocyte engraftment. Complete donor-type chimerism was defined when donor cells consisted of more than 90% of analyzed cells. PIR was characterized by the presence of at least three of the following symptoms with no direct consequences of infection or adverse effects of medication six or more days before engraftment, as described previously (*4, 5*): a high fever (>38.5°C), skin eruptions, body weight gain greater than 5% of baseline, or peripheral edema. Those who had all four symptoms and at least two of the following criteria indicating severe organ damage were classified as severe type; (1) SpO_2 less than 92% or pleural/pericardial effusions present; (2) serum creatinine level more than or equal to 3 times of baseline; (3) total bilirubin level more than 3 mg/dL or asparatate aminotransferase/alanine aminotransferase levels more than three times of upper limit of normal; and (4) development of hemophagocytosis in BM.

The main parameters analyzed between groups were as follows: (1) cumulative incidences of neutrophil or platelet engraftment; (2) cumulative incidences of NRM and relapse; (3) incidences of PIR, acute and chronic GVHD; and (4) overall and progression-free survival (OS and PFS). The analysis was performed as of April, 2010. OS was calculated from the day of transplantation until death from any cause or last follow-up. PFS was calculated from the day of transplantation until relapse, second transplantation due to engraftment failure, or death from any cause or last follow-up. NRM was defined as death in the absence of disease progression. Deaths occurring after disease progression were categorized as relapse regardless of the cause of death. Infection was considered the cause of death when bacterial, viral, or fungal infection was determined to be the proximate cause of death in patients who had not relapsed. Patients underwent BM aspiration at the time of engraftment or if clinically indicated. Relapse for acute myeloid leukemia, acute lymphoblastic leukemia, MDS, or chronic myeloid leukemia was determined by flow cytometric, morphologic, or cytogenetic evidence of malignant or dysplastic cells with clonal markers similar to those observed before transplantation. Relapse for ML was defined as progressive adenopathy or BM involvement. Acute and chronic GVHD were defined and graded by standard criteria (*29*). Relapse and NRM rates were estimated using cumulative incidence analysis and were considered competing risks (*30*). Similarly, in the analysis of PIR rates, death due to other causes or relapse leading to early withdrawal of immune suppression were considered competing risks.

Statistical Methods

Chi-square test was used to compare patient characteristics between two groups in matched-pair analysis. For continuous variables, Mann-Whitney nonparametric test was used. The probabilities of OS and PFS were estimated and plotted using the Kaplan-Meier method (*31*). Cumulative incidence curves were drawn using Gray's method (*32*). The level of significance in all cases was set at P less than 0.05. The effect of various categoric variables on survival probabilities was studied with the log-rank test. A Cox proportional hazard model with limited variables because of small sample was used to determine the significance of multiple variables in determining these outcomes. All analyses were carried out using StatView statistical software for Kaplan-Meier curve, and S-PLUS software (Mathsoft, Seattle, WA) for cumulative incidence curve.

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Brief report

Successful sustained engraftment after reduced-intensity umbilical cord blood transplantation for adult patients with severe aplastic anemia

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We retrospectively analyzed 12 consecutive adult severe aplastic anemia patients who received unrelated umbilical cord blood transplantation after a reduced-intensity conditioning regimen (RI-UCBT). The conditioning regimen consisted of 125 mg/m^2 fludarabine, 80 mg/m^2 melphalan, and 4 Gy of total body irradiation. The median infused total nucleated cell number and CD34$^+$ cell number were 2.50 × 10^7/kg and 0.76 × 10^5/kg, respectively. Eleven of the 12 patients achieved primary neutrophil and platelet engraftment. All patients who achieved engraftment had complete hematologic recovery with complete donor chimerism, except for one patient who developed late graft failure 3 years after RI-UCBT. Two of the 12 patients died of idiopathic pneumonia syndrome, and the remaining 10 patients are alive, having survived for a median of 36 months. Our encouraging results indicate that RI-UCBT may become a viable therapeutic option for adult severe aplastic anemia patients who lack suitable human leukocyte antigen-matched donors and fail immunosuppressive therapy. (*Blood*. 2011;117(11):3240-3242)

Introduction

Bone marrow transplantation from a human leukocyte antigen (HLA)-matched sibling is recommended as first-line therapy for younger patients with severe aplastic anemia (SAA).[1,2] However, many patients lack HLA-matched sibling donors. Bone marrow transplantation from an HLA-matched unrelated donor has been an alternative therapeutic option for patients who fail one or more courses of immunosuppressive therapy, but high rates of graft failure (GF), graft-versus-host disease (GVHD), and infection still remain to be solved.[3] The number of unrelated umbilical cord blood transplantations (UCBTs) has been increasing.[4] However, little information has been available on whether UCBT is feasible for SAA patients. We reported successful urgent UCBT using reduced-intensity (RI) conditioning for a 70-year-old SAA patient in 2003.[5] Here we present successful sustained engraftment of 11 consecutive patients with SAA who received RI-UCBT with the same RI conditioning regimen after the first report.

Methods

This study included 12 consecutive adult patients with acquired SAA who underwent RI-UCBT at our institute from September 2002 through January 2009. The patients' characteristics and umbilical cord blood (UCB) units are summarized in Table 1. Their median age was 49 years (range, 20-70 years). Four cases of severe, 6 of very severe, and 2 of fulminant type were included according to criteria as previously reported.[2,6] Fulminant type was defined as no neutrophils in the peripheral blood at diagnosis despite administration of granulocyte-colony stimulating factor. Ten patients, except for the 2 patients with fulminant type, had failed at least one course of immunosuppressive therapy. All patients gave their written informed consent in accordance with the Declaration of Helsinki, and the study was approved by the Toramon Hospital Institutional Review Board. UCB units were obtained from the Japanese Cord Blood Bank Network, and single UCB unit was infused in all the studied patients. All UCB units were serologically typed for HLA-A, -B, and -DR antigen before selection and were tested by high-resolution DNA typing before transplantation. The degree of mismatch is expressed using antigen level at HLA-A and -B, and allele level at DRB1. ABO incompatibility was not incorporated as one of the factors used in CB unit selection. The median total nucleated cell number and CD34$^+$ cell number at cryopreservation were 2.50 × 10^7/kg (range, 1.83-4.39 × 10^7/kg) and 0.76 × 10^5/kg (range, 0.27-1.52 × 10^5/kg), respectively. Anti-HLA antibodies were screened before transplantation in 6 patients using a FlowPRA method (One Lambda), and LAB Screen PRA or Single Antigen (One Lambda) was used to identify HLA antibody specificities.[7,8] All patients were conditioned with 25 mg/m^2 fludarabine daily for 5 days, 40 mg/m^2 melphalan daily for 2 days, and 4 Gy of total body irradiation in 2 fractions in 1 day. GVHD prophylaxis consisted of cyclosporine in 2, tacrolimus in 2, and tacrolimus plus mycophenolate mofetil in 8. Assessment of engraftment, GF, chimerism, GVHD, and supportive care during transplantation were performed as previously reported.[9,10] Karnofsky performance status score was assessed as surrogate for quality of life of the survivors. Overall survival was estimated using the Kaplan-Meier method.

Results and discussion

Patients' outcomes are summarized in Table 2. Eleven of the 12 patients achieved primary neutrophil and platelet engraftment. The median times to achieve neutrophil engraftment and platelet count more than 20 × 10^9/L were 18 days (range, 12-28 days) and

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Table 1. Characteristics of patient, grafts, and GVHD prophylaxis

Case no.	Age, y	Previous treatment	Interval from diagnosis to UCBT, mo	Previous transfusion times (RBCs/platelet)	Disease status at UCBT	HLA match	HLA Ab (reactive to CB)	ABO group (R/D)	TNC $\times 10^7$/kg	CD34$^+$, $\times 10^5$/kg	GVHD prophylaxis
1	70	CSA	3	11/14	SAA	4/6	NT	A/A	4.00	1.23	CSA
2	20	ATG + CSA	78	> 20/> 20	VSAA	4/6	NT	B/O	2.65	1.07	CSA
3	22	ATG + CSA, PSL	157	> 20/> 20	SAA	4/6	NT	A/O	2.26	0.27	Tac
4	26	ATG + CSA	3	> 20/> 20	VSAA	5/6	NT	A/A	2.65	0.70	Tac
5	59	ATG + CSA	8	> 20/> 20	SAA	5/6	Positive (no)	O/O	2.15	1.52	Tac + MMF
6	49	ATG + CSA, PSL	12	> 20/> 20	VSAA	3/6	NT	A/A	2.04	0.62	Tac + MMF
7	70	None	1	5/8	Fulminant	4/6	Positive (yes)	A/O	4.39	1.29	Tac + MMF
8	52	None	1	4/6	Fulminant	4/6	NT	AB/A	3.20	0.49	Tac + MMF
9	46	ATG + CSA	45	> 20/> 20	VSAA	4/6	Positive (no)	AB/O	1.83	0.42	Tac + MMF
10	49	ATG + CSA, PSL	327	> 20/> 20	VSAA	6/6	Positive (no)	B/O	2.34	0.82	Tac + MMF
11	65	CSA	6	16/> 20	VSAA	6/6	Positive (no)	A/A	3.31	0.56	Tac + MMF
12	31	ATG + CSA, PSL	215	> 20/> 20	SAA	4/6	Positive (no)	B/O	2.09	1.26	Tac + MMF

RBC indicates red blood cell; CB, cord blood; R, recipient; D, donor; TNC, total nucleated cells; CSA, cyclosporine-A; ATG, antithymocyte globulin; PSL, prednisone; VSAA, very severe aplastic anemia; NT, not tested; Tac, tacrolimus; and MMF, mycophenolate mofetil.

42 days (range, 26-64 days), respectively. All patients who achieved engraftment had complete hematologic recovery and were free from transfusion, and they showed complete donor chimerism at the time of the first chimerism analysis (median, 14 days; range, 11-73 days). One patient developed primary GF and was later found to have antibody against mismatched HLA on donor cells. Another patient developed secondary GF 3 years after UCBT. Both patients underwent a second RI-UCBT and obtained rapid donor engraftment. The negative impact of multiple transfusions before transplantation was not detected (Tables 1-2). Among 11 evaluable patients, 2 developed grade I and 5 developed grade II acute GVHD. Of the 9 patients who survived longer than 100 days after transplantation, 3 developed limited type of chronic GVHD. No patients developed grade III-IV acute GVHD and extensive type of chronic GVHD. Two of the 12 patients died of idiopathic pneumonia syndrome, and the remaining 10 patients are alive, having survived for a median of 36 months (range, 14-91 months). The probability of overall survival at 3 years was 83.3% (Figure 1). The surviving patients had high Karnofsky performance status score with a median of 90% (range, 60%-100%).

The present study demonstrated that our RI conditioning regimen allows a sufficient sustained engraftment of UCB in adult SAA patients. The RI conditioning regimen was originally developed in our institute for UCBT for various hematologic malignancies.[9] Eleven of the 12 patients achieved primary engraftment, which compares favorably with previously reported engraftment rates of UCBT for SAA.[11-16] Our RI conditioning regimen would be more potent than the others to overcome immunologic barriers for engraftment. Cell dose has been known to significantly influence the rate of engraftment after UCBT.[14] In the present study, although the cell dose was not very large, sufficient engraftment was seen. Any significant relationship between cell dose (total nucleated cell, ≥ 2.5 vs $< 2.5 \times 10^7$/kg; CD34$^+$, ≥ 0.8 vs $< 0.8 \times 10^5$/kg) and engraftment kinetics were observed (data not shown). Thus, not just cell dose but other factors, such as the intensity of the conditioning regimen and posttransplantation immunosuppression, may be important to achieve better engraftment after UCBT for SAA patients. Interestingly, all 6 patients who were screened for HLA antibodies before transplantation had HLA antibodies, and the one case who had positive HLA antibodies against an HLA on a transplanted UCB unit was the only one who failed primary engraftment. Recently, Takanashi et al reported that, in large number of UCBT for various hematologic malignancies, the

Table 2. Outcomes of 12 patients after reduced-intensity unrelated cord blood transplantation

Case no.	Days to ANC > 0.5 × 10^9/L	Days to PC > 20 × 10^9/L	% Donor chimerism (days tested, methods)	aGVHD	cGVHD	Discontinuation of IS (mo)	Complications	Survival (mo)
1	12	52	100 (14, FISH)	Grade II (skin)	No	Yes (3)	Possible IPA	Alive (91)
2	20	64	> 90 (49, PCR-STR)	Grade II (skin)	Limited	Yes (2)	No	Alive (90)
3	26	42	100 (26, FISH)	No	No	Yes (26)	Yes	Alive (69)
4	18	53	100 (18, FISH)	No	No	Yes (5)	*Pneumocystis jirovecii*, late GF, rescued by second RI-UCBT	Alive (69)
5	16	26	96.6 (14, FISH)	Grade I (skin)	Limited	Yes (14)	Norwalk virus colitis, EBV-PTLD	Alive (39)
6	28	64	99.6 (11, FISH)	No	NE	No	IPS	Dead; IPS (3)
7	No	No	48.8 (10, FISH), 4.3 (15, FISH)	NE	NE	NE	Primary GF, rescued by second RI-UCBT	Alive (32)
8	18	28	99.2 (13, FISH)	Grade II (skin, gut)	No	Yes (7)	CMV colitis, EBV-PTLD	Alive (28)
9	28	43	> 90 (14, PCR-STR)	Grade I (skin)	NE	No	HSV pneumonia, IPS	Dead; IPS (3)
10	15	27	99 (73, FISH)	No	Limited	No	No	Alive (22)
11	15	27	100 (20, FISH)	Grade II (skin, gut)	No	No	No	Alive (22)
12	13	28	100 (14, FISH)	Grade II (gut)	No	No	No	Alive (14)

ANC indicates absolute neutrophil count; PC, platelet count; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; IS, immunosuppressant; FISH, fluorescence in situ hybridization; PCR-STR, PCR of short tandem repeat; NE, not evaluable; IPA, invasive pulmonary aspergillosis; EBV-PTLD, Epstein-Barr virus–associated posttransplantation lymphoproliferative disorder; and IPS, idiopathic pneumonia syndrome.