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A Safety and Efficacy Study of Medium-Dose Etoposide, Cyclophosphamide and Total Body Irradiation Conditioning Before Allogeneic Stem Cell Transplantation for Acute Lymphoblastic Leukemia

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Background. Prognosis for adult patients with acute lymphoblastic leukemia (ALL) has been reported to be approximately 35% to 50%, even after allogeneic stem cell transplantation (allo-SCT). We previously reported retrospective analyses of a conditioning regimen of medium-dose etoposide, cyclophosphamide (CY), and total body irradiation (TBI) before allo-SCT for ALL. To prospectively analyze the efficacy of this conditioning regimen, we conducted a trial prospectively. **Methods.** The eligibility criteria of this study were as follows: diagnosis of ALL, aged between 15 and 50 years, in complete remission, and first SCT from HLA serologically matched donor. The primary endpoint of this study was event-free survival at 1 year after SCT, and the events were defined as death and relapse. **Results.** Fifty eligible patients were treated, and the median age of the patients was 33.5 years. Nineteen patients were Philadelphia chromosome-positive, and 47 were in first complete remission at SCT. All patients achieved neutrophil engraftment. Grade 3 to 4 acute graft-versus-host disease and extensive chronic graft-versus-host disease developed in 4 patients and 18 patients, respectively. No patient died within 100 days after SCT. One-year event-free survival was 76.0%, and 1-year overall survival was 80.0%. The cumulative incidences of relapse and non-relapse mortality at 1-year after SCT were 10.0% and 14.0%, respectively. **Conclusions.** Medium-dose etoposide + CY + TBI is an effective conditioning before allo-SCT for adult patients with ALL, enabling good disease control without an increase in nonrelapse mortality. A phase 3 trial comparing this regimen with the standard CY + TBI regimen for adult patients with ALL is warranted.

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Prognosis for adult patients with acute lymphoblastic leukemia (ALL) is poor,[1-5] and 5-year survival rates were reported to be approximately 35% to 50%.[6] Allogeneic hematopoietic stem cell transplantation (allo-SCT) is therefore performed in most possible cases.[7,8] However, even in patients who received allo-SCT conditioned with a standard

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regimen of cyclophosphamide (CY) with total body irradiation (TBI) in first complete remission (CR), 5- to 6-year survival rates were shown to be approximately 35% to 40% by prospective trials using a genetic randomization method, and the main cause of death was relapse.[9-11] We previously reported a good outcome of medium-dose etoposide (ETP, VP-16) + CY + TBI, in which a total of 30 mg/kg of ETP was added to CY + TBI, as a conditioning regimen before allo-SCT for adult patients with ALL in our institution,[12,13] and we also reported a superior outcome of medium-dose ETP + CY + TBI to CY + TBI using an SCT registry database in Japan.[14,15] However, those studies were retrospective, and we therefore conducted a multicenter single-arm prospective study in Japan to confirm the safety and efficacy of this conditioning for adult patients with ALL.

PATIENTS AND METHODS

Patient Eligibilities

The eligibility criteria of this study were as follows: (1) diagnosis of ALL or acute biphenotypic leukemia, defined by criteria proposed by the European Group for the Immunological Classification of Leukemias; (2) age between 15 and 50 years; (3) in hematological CR; (4) Eastern Cooperative Oncology Group performance status of 0 to 2; (5) adequate functions of main organs, including the liver, kidneys, lungs, and heart; and (6) HLA serologically A, B, DR, 6 of 6 matched related or unrelated donor. Stem cell source was limited to bone marrow or peripheral blood. Patients who received previous autologous or allo-SCT, and those with Burkitt leukemia were ineligible for this study. Patients with active infection or psychiatric disorders were excluded. Both the protocol review committee and the institutional review board of each institution approved the study. Written informed consent was obtained from all of the patients. The study was registered to the University Hospital Medical Information Network Clinical Trials Registry (UMIN trial number, 000001672).

Conditioning Regimen and Graft-Versus-Host Disease Prophylaxis

All patients received medium-dose ETP+CY+TBI as a conditioning regimen.[12-14] This regimen consisted of ETP at a dose of 15 mg/kg once daily administered intravenously for 2 days (total dose, 30 mg/kg) and CY at 60 mg/kg once daily intravenously for 2 days (total dose, 120 mg/kg) followed by 12 Gy of TBI delivered in 4 or 6 fractions for 2 or 3 days. Graft-versus-host disease (GVHD) prophylaxis was provided with short-term methotrexate and cyclosporine or tacrolimus according to the physician's selection.

Definitions

High-risk ALL patients were defined as those with at least one of the following risk factors: chromosomal abnormality of Philadelphia chromosome, t (4;11) or 11q23 or t (1;19); aged 35 years or older; white blood cell count greater than 3.0×10^{10}/L at diagnosis for B-cell lineage or greater than 10.0×10^{10}/L at diagnosis for T-cell lineage, and time to CR longer than 5 weeks. This risk stratification was applied to patients with ALL in CR1 at SCT. The day of neutrophil engraftment, the day of platelet engraftment, and the day of reticulocyte engraftment were defined as the first of 3 consecutive days with absolute neutrophil count greater than 0.5×10^9/L, the first of 7 days with an untransfused platelet count greater than 50×10^9/L and the first of 3 consecutive days with reticulocyte count greater than 1%, respectively. Primary graft failure was defined as the lack of myeloid engraftment until day 28, and secondary graft failure was defined as a persistent loss of neutrophil engraftment after having achieved engraftment. Toxicity after SCT was graded according to the National Cancer Institute common toxicity criteria version 3 (NCI-CTC v3.0, Bethesda, MD). Acute and chronic GVHDs were graded using standard criteria.[16,17] Relapse was defined as a recurrence of leukemia, including hematological relapse, as defined being positive for more than 5% marrow blasts, cytogenetic relapse, as defined being positive for cytogenetic abnormalities in leukemic cells, and molecular recurrence,as determined by being positive for markers of minimal residual disease, such as BCR-ABL, by polymerase chain reaction method in 2 consecutive tests. Nonrelapse mortality was defined as death during a continuous remission throughout the duration of the study. Overall survival (OS) was calculated from the day of SCT until death or last follow-up. Event-free survival (EFS) was defined as survival without the event, and the event was relapse.

Statistical Analyses

The primary endpoint of this study was EFS at 1 year after SCT. The expected 1-year EFS was estimated to be 75%, and the threshold 1-year EFS was estimated to be 55%, on the basis of our previous observations.[13,14] With a statistical power of 80% and a 1-sided, type I error of 5%, the number of eligible patients required for this study was calculated to be 45 using a binomial method. The projected sample size was 50 patients in consideration that 10% of the patients would be deemed ineligible. Secondary endpoints were 1-year and 2-year OS, 2-year EFS, engraftment rates of neutrophils and platelets, regimen-related toxicity, incidence of infection, incidence of secondary malignancy, incidence and severity of acute and chronic GVHDs, relapse rate at 100 days and at 1 year, and nonrelapse mortality at 100 days and at 1 year. Survival rates were calculated by the Kaplan-Meier method. Probabilities of acute GVHD, relapse, and nonrelapse mortalities were estimated on the basis of cumulative incidence curves to accommodate the following competing events: death without GVHD and second transplantation for graft failure for acute GVHD, death for relapse, and relapse for non-relapse mortality. Comparisons of EFS and OS were made using the Cox regression model and log-rank test. All *P* values were 2-sided, and a *P* value of 0.05 was used as the cutoff for statistical significance. Data management was performed at the Center for Supporting Hematology-Oncology Trials, Nagoya, Japan. Statistical analyses were performed using STATA SE11 software (STATA Corporation, College Station, TX).

RESULTS

Patient and Transplant Characteristics

Between February 2009 and August 2011, 52 patients were enrolled from 19 transplant centers in Japan, but 2 patients who received SCT from HLA serologically mismatched donors were excluded from the analysis. Characteristics of the 50 eligible patients and transplants are summarized in Table 1. The median age of the patients was 33.5 years (range,

TABLE 1.

Patients and transplants characteristics (n = 50)

Variables		n	%
Age	median (range)	33.5	(17-49)
	≥35	24	(48.0%)
	≥40	15	(30.0%)
Sex	Male	27	(54.0%)
	Female	23	(46.0%)
Diagnosis	B-ALL	41	(82.0%)
	T-ALL	7	(14.0%)
	Acute biphenotypic leukemia	2	(4.0%)
Cytogenetics	Normal	14	(28.0%)
	Philadelphia	19	(38.0%)
	Other abnormalities	17	(34.0%)
WBC count at diagnosis[a]	$>3\times10^{10}$/L (B-cell)	16	(39.0%)
	$>10\times10^{10}$/L (T-cell)	6	(85.7%)
Time to CR[b]	>35 days	16	(34.0%)
Disease risk[c]	Standard	7	(15.6%)
	High	38	(84.4%)
Disease status at transplantation	CR1	47	(94.0%)
	CR2	3	(6.0%)
HCT-CI score	0	35	(70.0%)
	1	8	(16.0%)
	2	3	(6.0%)
	3-4	4	(8.0%)
ECOG PS at transplantation	0	42	(84.0%)
	1-2	8	(16.0%)
Karnofsky PS at transplantation	100%	42	(84.0%)
	90%	5	(10.0%)
	70%-80%	3	(6.0%)
Donor	HLA-matched related donor	26	(52.0%)
	HLA-matched unrelated donor	24	(48.0%)
CMV serostatus	Recipient+/Donor+	25	(50.0%)
	Recipient+/Donor-	6	(12.0%)
	Recipient–/Donor+	9	(18.0%)
	Recipient–/Donor–	7	(14.0%)
	Donor: unknown	3	(6.0%)
Stem cell source	Bone marrow	40	(80.0%)
	Peripheral blood stem cell	10	(20.0%)
GVHD Prophylaxis	CSA + MTX	30	(60.0%)
	TAC + MTX	20	(40.0%)

[a] Two patients with Acute biphenotypic leukemia were excluded from this analysis.

[b] Two patients with Acute biphenotypic leukemia were excluded, and one patient with ALL cannot be assessed.

[c] Patients with ALL and CR1 at SCT (n = 45) were included this risk stratification.

HCT-CI, The Hematopoietic Cell Transplant-Co-morbidity Index; PS, performance status; CSA, cyclosporin A; MTX, methotrexete; TAC, tacrolimus.

17-49 years). Nineteen (42.0%) of the patients were Philadelphia chromosome-positive, and all of the patients with Philadelphia chromosome received tyrosine kinase inhibitors (imatinib, n = 15; dasatinib, n = 4) as a part of the pretransplant treatment. At diagnosis, 8 of the patients had extramedullary disease, including 3 patients with central nervous system (CNS) disease. Forty-seven (94.0%) of the patients were in first CR (CR1) at SCT, and 3 (6.0%) were in second CR (CR2). Twenty-six (52.0%) of the patients underwent SCT from an HLA-A–, –B–, –DR–matched related donor and 24 (48.0%) underwent SCT from an HLA-A, –B–, –DR–matched unrelated donor. Thirty-eight of the patients were assessed for HLA-Cw. HLA-Cw was matched in 34 patients, and the other 4 patients underwent SCT from an HLA-Cw 1 locus-mismatched donor. Data for HLA-DQ and HLA-DP were not available. Data for cytomegalovirus (CMV) serostatus were available for all patients. Thirty-two of the patients were seropositive for CMV, and 18 of the patients were seronegative. Data for CMV serostatus were available for 47 donors, and 34 of the donors were seropositive, and 13 patients were seronegative. Data for serostatus of Epstein-Barr virus were not available in this study. Forty (80.0%) of the patients received bone marrow, and 10 (20.0%) received peripheral blood stem cells (PBSC). The PBSC were from a related donor in all cases because donation of PBSC from unrelated donors was not permitted during this study in Japan. All patients received medium-dose ETP + CY + TBI as a conditioning regimen according to the protocol.

Survival

After 1-year follow-up of the final enrollment and at a median follow-up of 719 days after SCT (range, 379-1218 days), 34 patients were alive. No patient died within 100 days after SCT. The 1-year EFS and 2-year EFS rates were 76.0% (95% confidence interval [95% CI], 61.6%-85.6%) and 64.9% (95% CI, 49.6%-76.6%), respectively (Figure 1A). The 1-year OS and 2-year OS rates were 80.0% (95% CI, 66.0-88.7%) and 67.0% (95% CI = 51.8%-78.4%), respectively (Figure 1B). In the 19 patients with Philadelphia chromosome, 12 patients were alive, 3 patients relapsed, and 4 patients died without relapse. Subgroup analyses were performed for EFS using the Cox regression model, and the results are summarized in Table 2. Development of CMV antigenemia and development of grade 3 to 4 acute GVHD were determined to be risk factors for EFS. The CMV serostatus of recipients and that of donors were not determined to be a risk factor for CMV antigenemia or EFS.

Engraftment

All patients achieved neutrophil engraftment at a median day of 16 (range, 11-24 days) after SCT. Granulocyte colony-stimulating factor was administered for 48 patients, and median duration of granulocyte colony-stimulating factor administration was 17.5 days (range, 10-27 days) after SCT. Data for chimerism analyses were available for 42 patients, and all patients achieved complete donor chimerism. Forty-four (88.0%) of the patients achieved platelet engraftment at a median day of 30.5 (range, 13-279 days) after SCT. Data for engraftment of reticulocytes were available for 41 patients, and all patients achieved reticulocyte engraftment at a median day of 24 (range, 13-52 days). Secondary graft failure occurred in 3 patients.

Regimen-Related Toxicities

Table 3 shows a summary of regimen-related toxicities of NCI-CTC grade 3 to 4 toxicities before engraftment, other regimen-related toxicities and GVHD. The NCI-CTC grade 3 to 4 stomatitis, diarrhea, and nausea/vomiting were common, occurring in 30 patients (60.0%), 13 patients (26.0%), and 19 patients (38.0%), respectively. Other grade 3 toxicities developed in only 7 episodes. Febrile neutropenia were also common, occurring in 36 patients (72.0%), and pathogens of the fever could be detected in 14 patients.

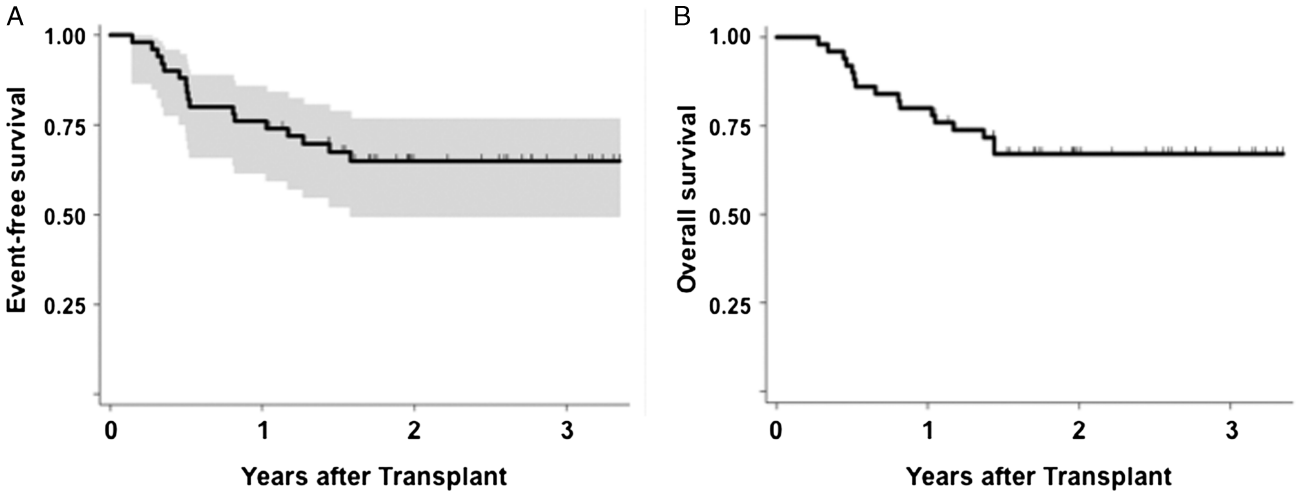

FIGURE 1. Kaplan-Meier curves of estimated event-free survival (A) and overall survival (B). The gray area in Figure 2A shows 95% confidence intervals.

Hepatic veno-occulusive disease and transplant-related thrombotic microangiopathy occurred in 1 patient (2.0%) and 3 patients (6.0%), respectively. Another patient was treated as having transplantation-related thrombotic microangiopathy clinically, although she did not meet the criteria of transplantation-related thrombotic microangiopathy.

Infection and Secondary Malignancy

Cytomegalovirus antigenemia developed in 25 (51.0%) of the 49 evaluated patients. The CMV colitis developed in 2 patients, and no patient developed other CMV diseases. The incidence of CMV antigenemia and that of CMV disease were compared according to CMV serostatus of the patients, CMV serostatus of the donors and relationships of CMV serostatus between the patient and donor, and there were no differences between any of the groups. Only age of the patient (more than 40 years of age) was determined to be a risk factor for CMV antigenemia (hazard ratio, 3.93; 95%CI, 1.04-14.89; P = 0.044). Other viral infections including herpes simplex virus, varicella zoster virus, and human herpes virus-6 developed in 2 patients, in 5 patients and in 2 patients, respectively. Only 4 patients developed fungal infections, including aspergillus infections in 3 patients and candida infection in 1 patient. Two patients developed Epstein-Barr virus–associated posttransplant lymphoproliferative disorders. No other secondary malignancies were observed during the follow-up period.

TABLE 2.

Subgroup analyses on event-free survival

Variables		HR	(95% CI)	P
Sex	Male vs female	1.70	(0.63-4.61)	0.294
Age	≥40 vs 40<	1.22	(0.45-3.30)	0.697
Ph chromosome	Positive vs negative	1.00	(0.32-3.16)	0.998
Disease risk	High vs standard	0.66	(0.19-2.34)	0.522
Donor	Rel-PBSCT vs Rel-BMT	0.77	(0.14-4.22)	0.766
	UR-BMT vs Rel-BMT	2.04	(0.65-6.40)	0.223
GVHD prophylaxis	TAC+MTX vs CSA+MTX	1.46	(0.56-3.80)	0.434
CMV antigenemia	Yes vs no	3.70	(1.20-11.36)	0.023
Grade 3–4 AGVHD	Yes vs no	4.83	(1.56-14.88)	0.006
Extensive CGVHD	Yes vs no	1.53	(0.54-4.35)	0.427

HR, indicates hazard ratio; Ph, Philadelphia; R-PBSCT, related-peripheral blood stem cell transplantation; UR-BMT, unrelated bone marrow transplantation; AGVHD, acute GVHD; CGVHD, chronic GVHD.

TABLE 3.

Organ toxicities and graft-versus-host disease

		n	(%)
Toxicities before engraftment (grade 3–4)[a]			
Gastrointestinal	Stomatitis	30	(60.0%)
	Diarrhea	13	(26.0%)
	Nausea/vomiting	19	(38.0%)
Hepatic	Bilirubin	3	(6.0%)
	AST	1	(2.0%)
	ALT	0	(0.0%)
Cardiac		1	(2.0%)
Arrhythmia		0	(0.0%)
Pulmonary		2	(4.0%)
Renal/urinary		0	(0.0%)
Skin		0	(0.0%)
Neurological		0	(0.0%)
Bleeding		1	(2.0%)
Febrile neutropenia before engraftment		36	(72.0%)
Infection before engraftment		14	(28.0%)
Engraftment syndrome		3	(6.0%)
Veno-occlusive disease		1	(2.0%)
Thrombotic microangiopathy		3	(6.0%)
Hemorrhagic cystitis		5	(10.0%)
CMV antigenemia[b]		27	(55.1%)
CMV disease	overall	2	(4.0%)
	colitis	2	(4.0%)
GVHD[c]			
Acute GVHD, overall		33	(66.0%)
Acute GVHD grade 2-4		18	(36.0%)
Acute GVHD grade 3-4		4	(8.0%)
Chronic GVHD, overall		27	(56.3%)
Chronic GVHD, extensive		18	(37.5%)

[a] Toxicity is graded by NCI common toxicity criteria version 3.0.

[b] CMV antigenemia was evaluated in 49 patients.

[c] Acute GVHD and chronic GVHD were evaluated in 50 and 48 patients, respectively.

AST, aspartate aminotransferase; ALT, alanine aminotransferase.

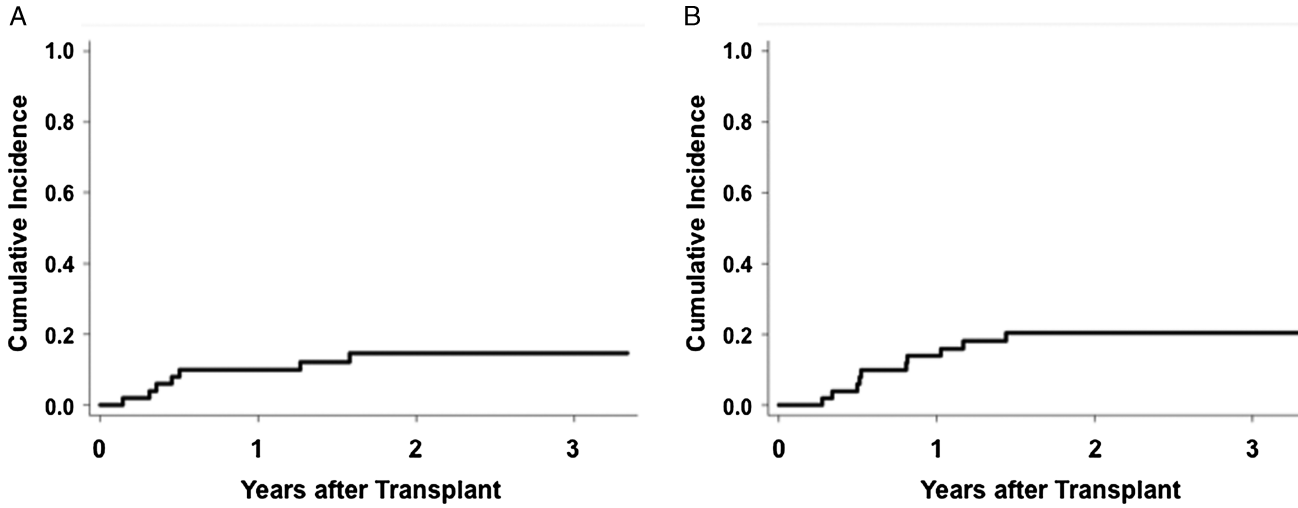

FIGURE 2. Cumulative incidences of relapse (A) and nonrelapse mortality (B).

Acute and Chronic GVHD

Acute GVHD was evaluable in all patients and developed in 33 patients (66.0%, grade I in 14 patients, grade 2 in 15, grade 3 in 3, and grade 4 in 1), and median onset day was day 18 (Table 3). Target organs of acute GVHD were skin in 32 patients (stage 1 in 6 patients, stage 2 in 12, stage 3 in 14, and no stage 4), gut in 7 patients (stage 1 in 4 patients, stage 2 in 0, stage 3 in 2, and stage 4 in 1), and liver in one patient (stage 4). No variable was determined to be a risk factor for grade 3 to 4 acute GVHD using the Cox regression model. However, SCT from unrelated donor was determined to be a risk factor for grade 2 to 4 acute GVHD by the log-rank test (related donor, 0%; unrelated donor, 16.7%; $P = 0.03$).

Among the 48 evaluable patients, 27 patients (56.3%) developed chronic GVHD (18 with extensive type and 9 with limited type) at a median day of 134, and the subtypes of onset were progressive type in 3, quiescent in 17, and de novo in 7 patients, respectively. The target organs of chronic GVHD were as follows: skin (n = 15), liver (n = 12), oral cavity (n = 11), ocular (n = 9), lung (n = 6), joint (n = 3), and other organs (n = 3).

Relapse and Nonrelapse Mortality

Relapses occurred in 7 patients at a median day of 166 (range, 53-577 days) after SCT, and 2 patients relapsed after 1 year. The cumulative incidences of relapses were 2.0% (95% CI, 0.2%-9.2%) at 100 days after SCT, 10.0% (95% CI, 3.7%-20.1%) at 1 year, and 14.7% (95% CI, 6.4%-26.2%) at 2 years (Figure 2A). Two patients had extramedullary relapse. Among the 3 patients who had CNS leukemia at diagnosis, 2 patients relapsed, and one of them had CNS relapse. Fine and Gray's model was used to identify risk factors for relapse, and the following variables were analyzed: patients' sex, age (≥40 or <40), diagnosis (ALL or acute biphenotypic leukemia), Philadelphia chromosome chromosome, disease risk, donor (related-BMT, related-PBSCT or unrelated-BMT) and GVHD prophylaxis (cyclosporine + methotrexete or taclorimus + methotrexete). No variable was determined to be a risk factor for relapse. The cumulative incidences of nonrelapse mortality were 0.0% at 100 days after SCT, 14.0% (95% CI = 6.2%-25.0%) at 1 year and 20.4% (95% CI, 10.5%-32.6%) at 2 years after SCT (Figure 2B). Seven patients, including 6 patients with bacterial infections and 1 patient with viral infection, died due to infections.

DISCUSSION

In this study, the primary endpoint of 1-year EFS was 76.0%, and the lower limit of the CI for 1-year EFS exceeded 55% of the threshold value. Therefore, the efficacy of this regimen was confirmed by a prospective study.

In previous studies on allo-SCT using standard conditioning of CY+TBI, prognosis was not satisfactory due to a high rate of relapse in adult ALL patients.[8-11] In those studies, a genetic randomization method was mainly used to compare the survival of patients who received allo-SCT and that of patients who received chemotherapy. The efficacy of different conditioning regimens was not directly assessed in those studies. The patients included in those studies received allo-SCT from a related donor when they were in CR1, and patients who received allo-SCT when they were beyond CR2 or from unrelated donors were not included. In the LALA-94 trial, adult ALL patients in CR1 who had a related donor were allocated to allo-SCT using CY + TBI. In the patients treated with allo-SCT, 3-year nonrelapse mortality rate, 3-year relapse rate, and 3-year disease-free survival rate were 18%, 34%, and 47%, respectively,[11] indicating that relapse was the main cause of death even after allo-SCT. Ribera et al and Labar et al also reported the outcomes after allogeneic SCT using a genetic randomization method and using a conditioning regimen of CY+TBI for patients in CR1. The 5-year OS rate was reported to be 35% by Ribera et al, and 6-year OS was reported to be 41.2% by Labar et al.[9,10]

Etoposide has been shown to have antileukemic activity and has been used as an alternative to CY or as an agent added to the standard conditioning regimen.[18-24] Although it has been reported that ETP-containing regimens showed superior disease control, relatively high doses of ETP in

previous studies (60 mg/kg or 1.5-1.8 g/m^2) led to high nonrelapse mortality rates (28%-47%), mainly caused by pulmonary toxicity (pulmonary hemorrhage and interstitial pneumonitis) and liver toxicity including veno-occulusive disease. Advanced age was considered to be a risk factor for nonrelapse mortality in these patients.[24]

We considered the dose of ETP to be important for reduction of relapse without increasing nonrelapse mortality. Spitzer et al and Petersen et al reported that a lower dose of ETP led to reduction in nonrelapse mortality.[20,25] Hunault et al[26] reported excellent results of an ETP-containing regimen in the GOELAL-02 trial, in which the conditioning regimen was similar to ours (ETP 40 mg/kg + CY+TBI), and 6-year OS rate in patients who received allo-SCT in CR1 from a related donor was 75%. However, genetic randomization was also used in that study, and the study did not focus on efficacy of the conditioning regimen; hence, only patients in CR1 with a related donor were received allo-SCT in the trial.

We previously reported the outcome of patients with ALL who received CY/TBI as a conditioning regimen using an SCT database in Japan.[14] In that study, we compared the outcome of medium-dose ETP + CY + TBI with that of CY + TBI retrospectively. Eligibility criteria in that study were the same as those in the current study except for the years of SCT performed, and the characteristics of patients and transplants were comparable between that study and the current study. The 2-year EFS rate for patients who received CY + TBI in that study was 57%, and that in the current study was 65%. The medium-dose ETP + CY + TBI conditioning regimen might be superior to the CY + TBI regimen. However, this study was a single-arm prospective study, and we did not compare this conditioning regimen with CY + TBI. It was a great limitation of this study, and a phase 3 trial comparing this regimen with the standard CY+TBI is needed.

All patients achieved neutrophil engraftment in this study, and we consider that medium-dose ETP + CY + TBI conditioning is myeloablative and sufficiently immunosuppressive for engraftment of donor hematopoietic cells, as we reported previously.[14,15] Before engraftment, grade 3 gastrointestinal symptoms and febrile neutropenia were common; however, other organ toxicities, including hepatic, cardiac, and pulmonary toxicities were not common, and no patient died of organ failure or infection because of regimen-related toxicities. Although there have been reports that acute GVHD is related in part to cytokine release from damaged gastrointestinal tissues, and diarrhea due to regimen-related toxicities was common in this study, the incidence and severity of acute GVHD in this study were not increased. Incidences of other important complications after allo-SCT, including chronic GVHD, viral infections, veno-occulusive disease, and transplantation-related thrombotic microangiopathy, were not increased compared with those in previous studies.[8,11,26] Surprisingly, no patient died within 100 days after SCT, possibly due to good disease control and low incidence of severe acute GVHD and confirming that intensification of conditioning using medium-dose ETP does not increase the risk of early nonrelapse mortality due to regimen-related toxicities.

Only seven patients in this study relapsed, and the relapse rate was quite acceptable. However, two of the three patients with CNS disease relapsed and one of them relapsed in the CNS. This might be due to the low permeability of ETP into the CNS.

A unique aspect of this study was the inclusion of patients in CR2 and SCT from an unrelated donor. Some studies have shown that the outcome for ALL patients in CR2 at SCT was worse than that for patients in CR1,[27-29] and OS rates for patients in CR2 who received allo-SCT from a related donor were less than 30% in the United States[30] and approximately 40% in Japan.[31] Although only 3 patients in CR2 were included in the current study, no patient died during the follow-up period. We have reported that there was no significant difference between the outcomes of patients in CR1 and CR2 who received this conditioning,[12,13] and this conditioning resulted in better survival than CY + TBI in CR2 patients.[14] Marks et al reported that an ETP+TBI regimen showed better disease control than standard-dose CY + TBI for patients with ALL in CR2, indicating that ETP has better anti-ALL activity than that of CY.[32] Some studies have shown that ETP stimulates immune rejection against tumor cells via antigenic modification of tumor cells and cytotoxic T-cell stimulation in vivo,[33,34] and this effect was not clear when a high dose of ETP was used.[35] The medium-dose ETP + CY + TBI regimen was approximately half the dose of ETP frequently used in conditioning regimens, and this dosing might explain the lower incidence of nonrelapse mortality due to severe GVHD or regimen-related toxicities and low incidence of relapse via increasing the GVL effect.

This study included SCT from an unrelated donor. Although EFS and OS were not significantly different between SCT from an unrelated donor and SCT from a related donor, SCT from an unrelated donor had a tendency to lower EFS compared to SCT from a related donor (1-year EFS: 85% for related donor and 67% for unrelated donor, log rank $P = 0.10$). Grade 3 to 4 acute GVHD was increased in patients who received SCT from an unrelated donor according to the log rank test, and grade 3 to 4 acute GVHD had an adverse impact on EFS. Therefore, modification of GVHD prophylaxis might improve survival of patients who received SCT from an unrelated donor.

In the present study, focusing on this conditioning regimen and including patients in CR2 and SCT from an unrelated donor, the dose of ETP (30 mg/kg) was lower than those in other studies in which ETP was used. The favorable outcome with 1-year OS rate of 80.0% and 2-year OS rate of 67.0% confirms the feasibility and efficacy of medium-dose ETP + CY + TBI by a prospective multicenter trial. Although our analysis has limitations because of the small sample size and the fact that this study was not planned to compare with standard conditioning of CY + TBI, we think that this conditioning could be a useful conditioning for adult patients with ALL, especially patients who have a high risk of relapse, such as patients in CR2.

In conclusion, this study was a multicenter single arm prospective trial to confirm the safety and efficacy of the conditioning regimen of medium-dose ETP + CY + TBI for allo-SCT in adult patients with ALL. The results demonstrate that the conditioning regimen enables good disease control without an increase in nonrelapse mortality, even for patients of relatively advanced age and patients with high-risk disease. A phase 3 trial comparing this regimen with the standard CY + TBI regimen for adult patients with ALL is warranted.

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