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Feasibility of Non-TBI Conditioning with Busulfan and Fludarabine for Allogeneic Stem Cell Transplantation in Lymphoid Malignancy

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Background/Aims: This retrospective study evaluated the transplantation outcomes of patients with adult lymphoid malignancies who received chemotherapy-based conditioning with busulfan and fludarabine (BuFlu) and busulfan and cyclophosphamide (BuCy2).
Methods: Thirty-eight patients (34 with acute lymphoblastic leukemia and 4 with lymphoblastic lymphoma) were included in the current study. The conditioning regimen was BuCy2 for 14 patients and BuFlu for the remaining 24 patients. Eight and 13 patients were high risk disease in the BuCy2 and BuFlu groups, respectively.
Results: The cumulative incidence of grade II-IV acute graft-versus-host disease (GVHD) was 56.5% and 55.2% and that of extensive chronic GVHD 17.0% and 55.6% (p = 0.018) for the BuFlu and BuCy2 groups, respectively. The 3-year relapse rate was 27.8% and 31.4% and 3-year overall survival 34.3% and 46.8% for the BuFlu and BuCy2 groups, respectively. Treatment-related mortality (TRM) was significantly lower in the BuFlu group (16.9%) than in the BuCy2 group (57.1%, p = 0.010). In multivariate analyses, the BuFlu regimen was identified as an independent favorable risk factor for TRM (hazard ratio [HR], 0.036; p = 0.017) and extensive chronic GVHD (HR, 0.168; p = 0.034).
Conclusions: Our BuFlu regimen would appear to be an acceptable conditioning option for lymphoid malignancies, including high-risk diseases. It was safely administered with a lower TRM rate than BuCy2 conditioning.

Keywords: Precursor cell lymphoblastic leukemia-lymphoma; Busulfan; Drug therapy; Fludarabine

INTRODUCTION

Chemotherapy-based conditioning is widely used for myeloid leukemia due to its ease of administration, reduced incidence of long-term sequelae compared to total body irradiation (TBI) conditioning, and equivalent transplantation outcomes with regard to treatment-related mortality (TRM), relapse, and leukemia-free survival (LFS) [1-5]. However, while this holds true for myeloid leukemias, there is some controversy in the case of acute lymphoblastic leukemia (ALL) due to a less potent anti-tumor effect at sanctuary sites [6,7].

Busulfan and cyclophosphamide (BuCy2) regimens have been standard treatments for achieving myeloablative conditioning since the publication of studies by the European Group for Blood and Marrow Transplantation

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(EBMT) and Center for International Blood and Marrow Transplant Research (IBMTR) that demonstrated the equivalence of BuCy2 and CyTBI regimens [1,2]. However, the major concern with BuCy2 conditioning is the high TRM caused by cyclophosphamide metabolites [8,9]. Meanwhile, fludarabine has considerable and synergistic efficacy in both immunosuppression and tumor-cell killing when administered with an alkylating agent, and is widely used as an alternative to cyclophosphamide in reduced-intensity and myeloablative conditioning [10-13].

This retrospective study evaluated the transplantation outcomes of patients with adult lymphoid malignancies who received chemotherapy-based conditioning with busulfan and fludarabine (BuFlu), adopted to avoid the sequelae of TBI conditioning and the toxicity of standard BuCy2 conditioning.

METHODS

Patients and transplantation procedures

We retrospectively reviewed the data for 38 patients with lymphoid malignancies who underwent allogeneic stem cell transplantation (SCT) at Kyungpook National University Hospital, Daegu, Korea between December 1998 and November 2009. The median follow-up after transplantation was 255 days (range, 9 to 2,804) and all of the patients received chemotherapy-based conditioning with BuCy2 or BuFlu. BuCy2 conditioning was used for patients who underwent transplantation before 2005 and BuFlu was used for those who received transplants after 2005. This study was approved by the local institutional review boards.

Until January 2008, human leukocyte antigen (HLA) typing was performed through serologic testing for class I alleles (A, B, and C) and DNA-based typing for HLA class II molecules (DRB1). However, since then, HLA-typing has been achieved through high-resolution genotyping of HLA class I and II alleles. Prophylaxis against acute graft-versus-host disease (GVHD) consisted of methotrexate (MTX) plus cyclosporine for sibling donors and tacrolimus for unrelated donors. Most patients were scheduled to receive MTX (15 mg/m^2 on day 1 and 10 mg/m^2 on days 3, 6, and 11). The last dose of MTX was omitted when mucositis higher than grade 4 or renal impairment was observed. Cyclosporine was initially administered at a dose of 5.0 mg/kg/day via continuous infusion on the day before the transplantation. The dose was reduced to 2.5 mg/kg/day via continuous infusion on day +7, and then changed to an oral dose of 3 mg/kg twice daily when tolerated. Starting on day 60, the oral cyclosporine dose was reduced by 5% per week, and then maintained at 1.5 mg/kg/day until day 270 for patients without symptomatic chronic GVHD. Tacrolimus was administered intravenously at a dose of 0.03 mg/kg/day on the day before transplantation. Through therapeutic drug monitoring, the blood level of tacrolimus was maintained within the range of 15-20 ng/mL for the first months and 10-15 ng/mL thereafter. Patients subsequently received a daily oral dose three to four times higher than the last intravenous dose. For the purpose of *in vivo* T cell depletion (TCD), anti-thymocyte globulin (ATG) was used in patients with HLA-mismatched or unrelated donors.

The prophylactic antibiotics consisted of ciprofloxacin (250 mg, twice daily, orally), metronidazole (500 mg, three times daily, orally), and fluconazole (100 mg, once daily, orally) from the initiation of conditioning. Acyclovir (600 mg, twice daily, orally) was started from day-1 and cotrimoxazole was started after engraftment. Ursodeoxycholic acid was used for veno-occlusive disease (VOD) prophylaxis from the initiation of conditioning.

Conditioning regimens

For the myeloablative BuCy2 regimen, busulfan (Busulfex, Orphan Medical Inc., Minnetonka, MN, USA) was infused intravenously at a dose of 3.2 mg/kg/day for 4 days (total dose 12.8 mg/kg, days -7 to -4), and cyclophosphamide was infused intravenously at a dose of 60 mg/kg/day for 2 days (total dose, 120 mg/kg; days, -3 to -2). For the myeloablative BuFlu regimen, busulfan was infused intravenously at a dose of 3.2 mg/kg/day for 4 days (total dose, 12.8 mg/kg; days, -6 to -3), and fludarabine was administered intravenously at a dose of 30 mg/m^2 for 6 days (total dose, 180 mg/m^2/day; days, -7 to -2). For the reduced-intensity BuFlu regimen, busulfan was infused intravenously at a dose of 3.2 mg/kg/day for 2 days (total dose, 6.4 mg/kg; days, -5 to -4); the fludarabine schedule was the same as for the standard BuFlu regimen.

GVHD grading and treatment

Diagnosis and grading of acute GVHD were performed according to the consensus conference guidelines for acute

GVHD [14]. The frontline treatment for acute GVHD was the prednisone (1-2 mg/kg/day). In patients with acute GVHD who did not respond to steroids and cyclosporine, cyclosporine was replaced with tacrolimus.

Chronic GVHD was diagnosed and graded based on published criteria [15]. The initial treatment for chronic GVHD was prednisone (1-2 mg/kg/day) and the reintroduction of cyclosporine or tacrolimus in the therapeutic range [16]. If further immunosuppressive agents were needed to control chronic GVHD, the salvage regimen included the use of mycofenolate mofetile (MMF), ATG, weekly MTX, or combination therapy [17,18]. MMF was added at a dose of 1.5 or 2 g/day and the steroids doses were tapered in refractory cases (by 0.2 mg/kg/wk). The dose of MMF was escalated to 2 g/day in patients with progressive GVHD.

Definitions

The day of stem cell infusion was defined as day 0. Myeloid engraftment was defined as the first day of a period of at least three consecutive days with an absolute neutrophil count (ANC) of ≥ 0.5×10^9/L, while platelet engraftment was defined as the first day of at least three consecutive days on which a platelet count of ≥ 20×10^9/L was achieved without transfusion. High-risk patients were defined as patients older than 35 years or those with a high white blood cell count at presentation (≥ 100×10^9/L for B-lineage cells and ≥ 30×10^9/L for T-lineage cells), along with all patients with Philadelphia chromosome. TRM was defined as mortality related to stem cell transplantation procedures, e.g., VOD, GVHD, and infection.

Statistical analysis

Continuous variables were compared using the two-sample *t* test, while categorical data were analyzed using the chi-square test. Overall survival (OS) was defined as the time from transplantation until death from any cause, and was analyzed using a Kaplan-Meyer test. Both groups were compared using a log-rank test or Breslow test. The cumulative incidence of chronic GVHD was calculated by Gray's method (with death or relapse without chronic GVHD considered as competing risks) using the R software package cmprsk. A time-dependent Cox regression model was used to identify clinical predictors of the development of chronic GVHD and TRM. Factors with *p* values of 0.1 or less in the univariate analysis were included in the multivariate analysis and the variables were analyzed using backward inclusion methods. For the statistical analyses, SPSS version 13 (SPSS Inc., Chicago, IL, USA) and R statistical software version 2.8.0 (R Foundation for Statistical Computing, Vienna, Austria; http://www.r-project.org) were used. A p value of < 0.05 was considered significant.

RESULTS

Patient characteristics

Thirty-four patients were diagnosed with ALL and four with lymphoblastic lymphoma. The conditioning regimen was BuCy2 for 14 patients and BuFlu for 24 patients. Six patients underwent reduced-intensity BuFlu conditioning (RIC). Median age in the BuCy2 and BuFlu groups was 31 years (range, 18 to 48) and 33 years (range, 22 to 53), respectively. There were 13 (54.2%) and 8 (57.1%) high-risk patients in the BuCy2 and BuFlu groups, respectively, including 1 (7.1%) and 7 (29.2%) Ph-chromosome-positive cases, respectively ($p = 0.859$). Initial extramedullary involvement was present in four (28.6%) and two (2.8%) patients in the BuCy2 and BuFlu groups, respectively ($p = 0.099$). The pretransplantation procedures were comparable regarding HLA-mismatched donors (7.1% vs. 16.7%, $p = 0.370$), unrelated donors (28.6% vs. 58.3%, $p = 0.076$), and peripheral blood stem cells (85.7% vs. 91.7%, $p = 0.564$). The other patient characteristics are summarized in Table 1.

Survival

The survival rate for all patients at 3 years was 41.8% (95% confidence interval [CI], 27.5 to 63.4) with a median follow-up duration of 982 days (95% CI, 634 to 1,329). The 3-year OS rate was 53.8% (95% CI, 33.7 to 86.0) for standard-risk patients and 30.6% (95% CI, 14.3 to 65.1) for the high-risk patients ($p = 0.213$) (Fig. 1), while the 3-year relapse rate was 14.7% for the standard-risk patients and 43.3% for the high-risk patients ($p = 0.109$). Patients with chronic GVHD had a better 3-year OS rate (64.0%) than those without chronic GVHD (24.0%, $p = 0.002$) (Fig. 1). The 3-year OS for the BuCy2 and BuFlu groups was 34.3% and 46.8%, respectively ($p = 0.279$) (Fig. 2), while the 3-year event-free survival (EFS) rate was 25.7% and 47.1%, respectively ($p = 0.215$) (Fig. 2). Relapse occurred

Table 1. Patient characteristics

Characteristics	BuCy2	BuFlu	*p* value
No. of patients	14	24	
Age, yr	31 (18-48)	33 (22-53)	0.366
Gender			
Male	9 (64.3)	14 (58.3)	0.717
Female	5 (35.7)	10 (41.7)	
Diagnosis			
ALL	12 (85.7)	22 (91.7)	0.564
LL	2 (14.3)	2 (8.3)	
Immunophenotype			
B-cell	5 (35.7)	15 (62.5)	0.111
T-cell	9 (64.3)	9 (37.5)	
Cytogenetic risk group			
Standard	10 (71.4)	12 (50.0)	0.412
High	3 (21.4)	10 (41.7)	
NE	1 (7.1)	2 (8.3)	
Ph chromosome	1 (7.1)	7 (29.2)	0.108
Use of imatinib	0	6 (25.0)	
Extramedullary			
CNS	4 (28.6)	2 (8.3)	0.099
Disease status			
CR1	6 (42.9)	18 (75.0)	0.163
CR2	3 (21.4)	2 (8.3)	
Primary refractory	2 (14.3)	3 (12.5)	
Relapsed	3 (21.4)	1 (4.2)	
Risk group			
Standard	6 (42.9)	11 (45.8)	0.859
High	8 (57.1)	13 (54.2)	
HLA			
Full match	13 (92.9)	20 (83.3)	0.370
One mismatch	1 (7.1)	1 (4.2)	
Haploidentical	0	3 (12.5)	
Donor			
Sibling	10 (71.4)	10 (41.7)	0.076
Unrelated	4 (28.6)	14 (58.3)	
Stem cell source			
PB	12 (85.7)	22 (91.7)	0.564
BM	2 (14.3)	2 (8.3)	
Conditioning			
MA	14 (100)	18 (75.0)	0.067
RIC	0	6 (25.0)	
GVHD prophylaxis			
Cyclosporine	11 (78.6)	11 (45.8)	0.049
Tacrolimus	3 (21.4)	13 (54.2)	
In vivo TCD	4 (28.6)	9 (37.5)	0.576
MNCs ($\times 10^8$/kg)	7.81 (1.34-17.96)	9.79 (0.36-18.43)	0.757
CD34+ cells ($\times 10^6$/kg)	4.95 (1.85-15.14)	5.79 (1.53-19.20)	0.518

Values are presented as mean (range) or number (%).

Bu, busulfan; Cy, cyclophosphamide; Flu, fludarabine; ALL, acute lymphoblastic leukemia; LL, lymphoblastic lymphoma; NE, not evaluable; CNS, central nervous system; CR, complete remission; HLA, human leukocyte antigen; PB, peripheral blood; BM, bone marrow; MA, myeloablative; RIC, reduced-intensity conditioning; GVHD, graft-versus-host disease; TCD, T-cell depletion; MNC, mononuclear cells.

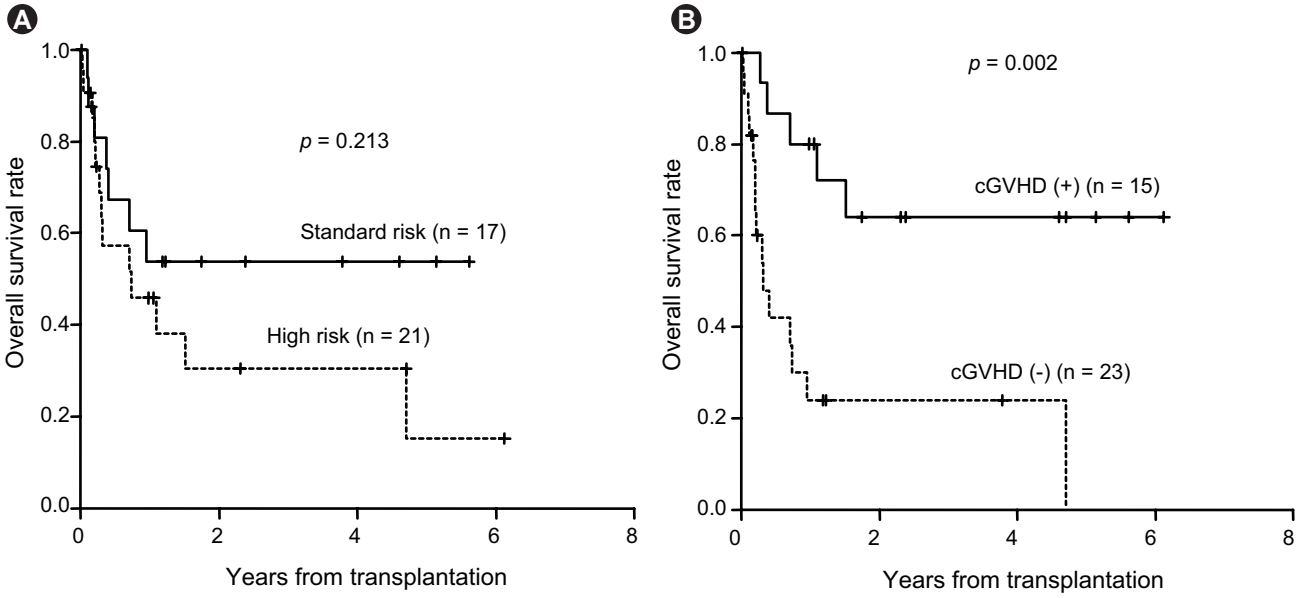

Figure 1. Survival rates according to risk group and presence of graft-versus-host disease (GVHD). (A) Overall survival according to risk group. The 3-year overall survival (OS) rate was 53.8% (95% confidence interval, 33.7 to 86.0) for standard-risk patients and 30.6% (95% confidence interval, 14.3 to 65.1) for high-risk patients. (B) Overall survival according to presence of chronic GVHD. Patients with chronic GVHD showed a better 3-year OS rate (64.0%) than those without chronic GVHD (24.0%).

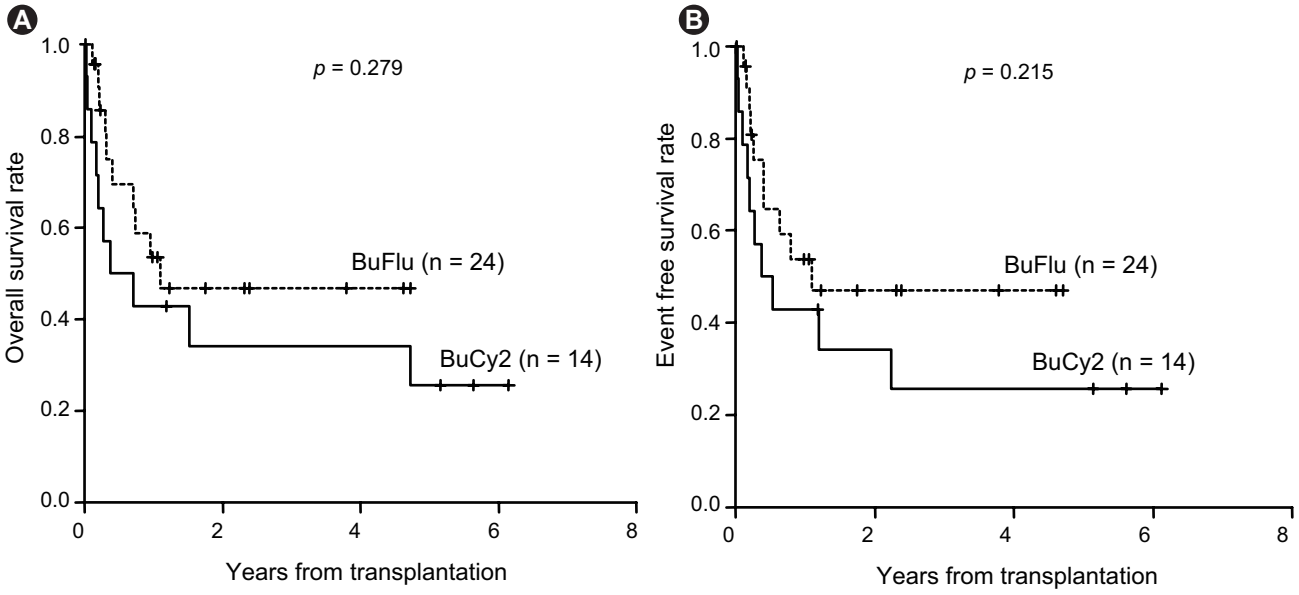

Figure 2. Survival analyses according to conditioning regimen. (A) Overall survival. The 3-year overall survival rates for the busulfan-cyclophosphamide (BuCy2) and busulfan-fludarabine (BuFlu) groups were 34.3% and 46.8%, respectively. (B) Event-free survival. The 3-year event-free survival rates for the BuCy2 and BuFlu groups were 25.7% and 47.1%, respectively.

in three patients (21.4%) in the BuCy2 group and six patients (25.0%) in the BuFlu group (p = 0.803) (Table 2). The cumulative incidence of 3-year relapse for the BuCy2 and BuFlu groups was 27.8% and 31.4%, respectively (p = 0.476) (Fig. 3).

Graft-versus-host disease

The incidence of acute GVHD did not differ between the two groups. Grade II-IV acute GVHD developed in 7 (50.0%) of the 14 patients in the BuCy2 group at a median of 15 days (range, 9 to 22) and in 7 (29.2%) of the 24 patients in the BuFlu group at a median of 24 days (range,

Table 2. Transplantation outcomes

Variables	BuCy2	BuFlu	*p* value
No. of patients	14	24	
Engraftment			
ANC ≥ 0.5 × 10^9/L, day	14 (10-27)	12 (9-18)	0.073
Platelet ≥ 20 × 10^9/L, day	13 (10-33)	12 (7-26)	0.125
Acute GVHD, grade II-IV	7 (50.0)	7 (29.2)	0.199
Chronic GVHD[a], type			
De novo	2 (22.2)	0	0.088
Quiescent	3 (33.3)	7 (41.2)	
Progressive	2 (22.2)	1 (5.9)	
Chronic GVHD[a], severity			
Limited	2 (22.2)	6 (35.3)	0.054
Extensive	5 (55.6)	2 (11.8)	
Veno-occlusive disease			
Moderate	1 (7.1)	4 (16.7)	0.058
Severe	2 (14.3)	0	
CMV reactivation	11 (78.6)	12 (50.0)	0.082
Treatment-related mortality	8 (57.1)	3 (12.5)	0.008
Relapse	3 (21.4)	6 (25.0)	0.803
Central nervous system relapse	1	1	
Death	10 (71.4)	10 (41.7)	0.076

Values are presented as mean (range) or number (%).
Bu, busulfan; Cy, cyclophosphamide; Flu, fludarabine; ANC, absolute neutrophil count; GVHD, graft-versus-host disease; CMV, cytomegalovirus.
[a]26 patients (9 + 17) who survived longer than 3 months after transplantation were analyzed.

12 to 64) (p = 0.199) (Table 2). The cumulative incidence of grade II-IV acute GVHD 100 days after transplantation was 56.5% in the BuCy2 group and 55.2% in the BuFlu group (p = 0.130) (Fig. 4). Among patients who survived longer than 3 months after transplantation, limited and extensive chronic GVHD developed in two (22.2%) and five (55.6%) patients, respectively, in the BuCy2 group, and in six (35.3%) and two (11.8%) patients, respectively, in the BuFlu group (p = 0.054) (Table 2). The cumulative incidence of extensive chronic GVHD after transplantation in the BuFlu group (17.0%) was lower than that in the BuCy2 group (55.6%; p = 0.018) (Fig. 4).

In univariate analyses, complete remission (CR) (hazard ratio [HR], 3.999; p = 0.077), the BuFlu regimen (HR, 0.168; p = 0.034), and hyperacute GVHD (HR, 5.847; p = 0.054) were identified as factors affecting TRM. Meanwhile, multivariate analysis identified the BuFlu regimen (HR, 0.144; 95% CI, 0.026 to 0.803; p = 0.027) as an independent favorable risk factor for the development of extensive chronic GVHD (Table 3).

Treatment-related mortality

TRM affected eight patients (57.1%) in the BuCy2 group and three patients (12.5%) in the BuFlu group (p = 0.008) (Table 2). The cumulative incidence of TRM was significantly lower in the BuFlu group (16.9%) than in the BuCy2 group (57.1%, p = 0.010) (Fig. 3). Causes of TRM in the BuCy2 group included infection (two patients, 14.3%), chronic GVHD (two, 14.3%), VOD (two, 14.3%), and brain hemorrhage (two, 14.3%); in the BuFlu group, they included infection (two patients, 8.3%) and VOD (one, 4.2%).

In univariate analyses, the BuFlu regimen (HR, 0.201; p = 0.018), VOD (HR, 3.931; p = 0.031), and hyperacute GVHD (HR, 3.232; p = 0.062) were identified as significant factors affecting TRM. Multivariate analysis identified the BuFlu regimen (HR, 0.199; 95% CI, 0.052 to 0.752; p = 0.017) as an independent favorable risk factor

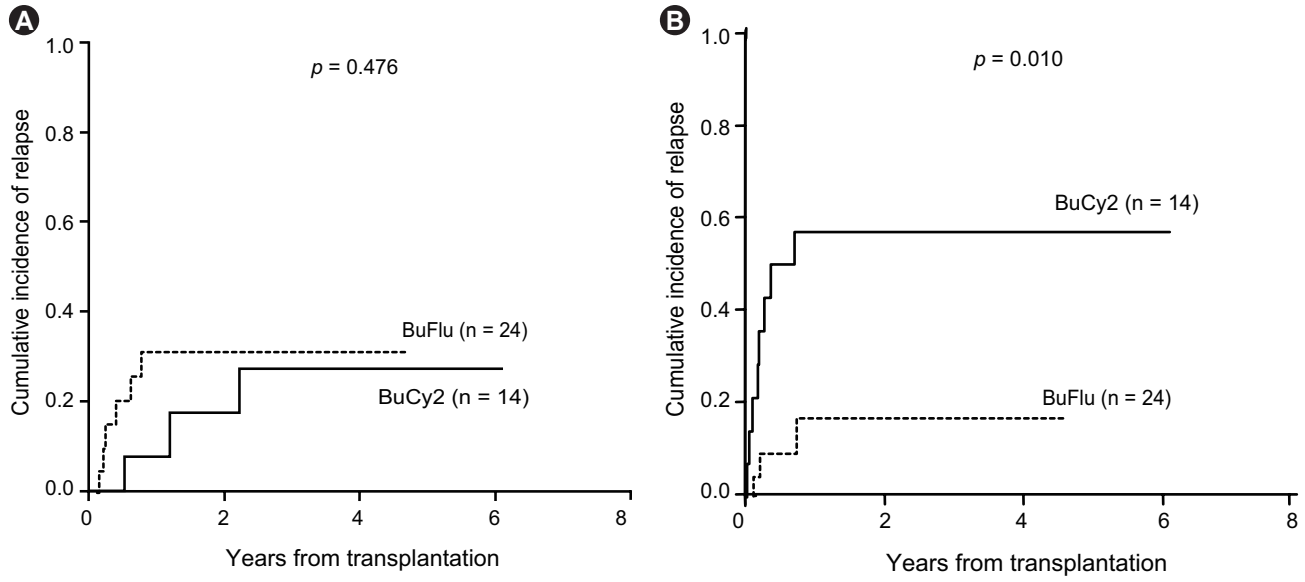

Figure 3. Relapse and treatment-related mortality (TRM) according to conditioning regimen. (A) Cumulative incidence of relapse. The cumulative incidence of 3-year relapse for the busulfan-cyclophosphamide (BuCy2) and busulfan-fludarabine (BuFlu) groups was 27.8% and 31.4%, respectively. (B) Treatment-related mortality. The cumulative incidence of TRM was significantly lower for the BuFlu group (16.9%) than for the BuCy2 group.

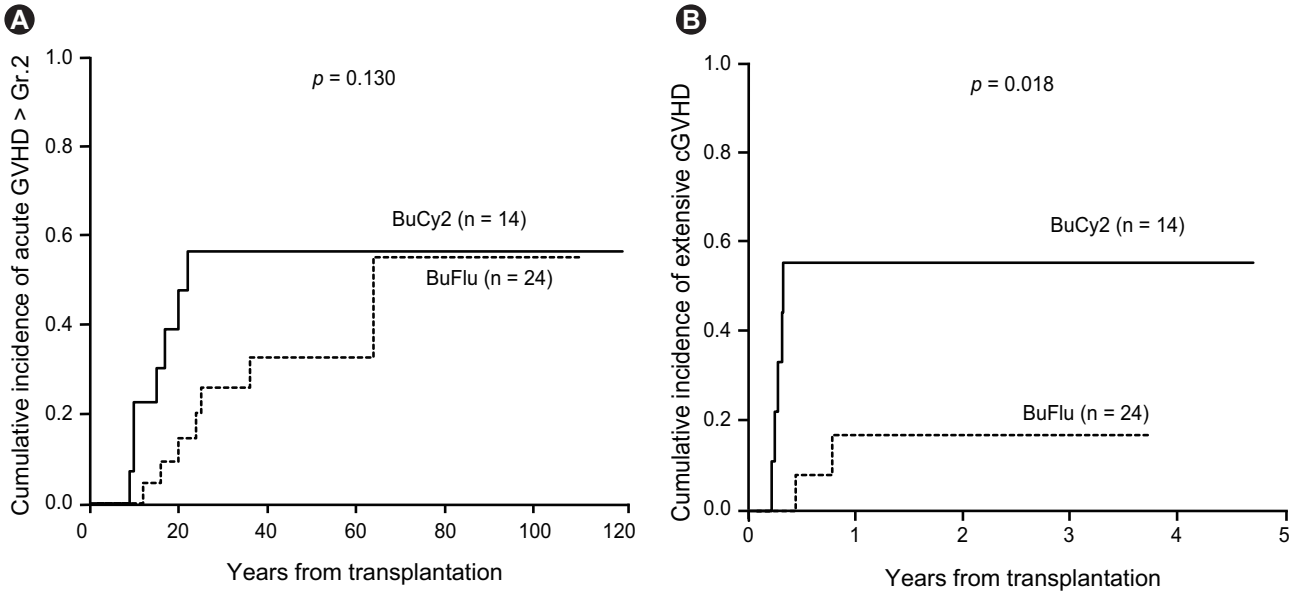

Figure 4. Graft-versus-host disease (GVHD) according to conditioning regimen. (A) Cumulative incidence of grade II-IV acute GVHD. The cumulative incidence of grade II-IV acute GVHD at 100 days after transplantation was 56.5% for the busulfan-cyclophosphamide (BuCy2) group and 55.2% for the busulfan-fludarabine (BuFlu) group. (B) Cumulative incidence of extensive chronic GVHD. Among patients who survived longer than 3 months after transplantation, the cumulative incidence of extensive chronic GVHD after transplantation was lower in the BuFlu group (17.0%) than in the BuCy2 group (55.6%).

for TRM, and VOD (HR, 3.951; 95% CI, 1.148 to 13.598; p = 0.029) as an unfavorable risk factor for TRM (Table 4).

Extramedullary disease

Among the six patients who presented with extramedullary involvement at the time of diagnosis, the five

Table 3. Factors affecting the development of extensive chronic GVHD

	Univariate analysis			Multivariate analysis		
Variables	HR	95% CI	*p* value	HR	95% CI	*p* value
Sex	0.469	0.089-2.457	0.370			
Age > 35 yr	1.960	0.437-8.791	0.380			
Transplantation year, > 2006 vs. ≤ 2005	0.269	0.051-1.420	0.122			
Diagnosis, LL vs. ALL	1.082	0.130-9.008	0.942			
IP type, T-cell vs. B-cell	0.849	0.189-3.817	0.831			
CG risk, high	0.829	0.149-4.595	0.830			
LDH, high	1.407	0.168-11.761	0.752			
EM disease	2.870	0.540-15.264	0.216			
Status, non-CR	3.999	0.861-18.560	0.077	5.119	0.928-28.230	0.061
High risk	1.598	0.355-7.201	0.542			
RIC vs. MA	0.036	0.000-159.988	0.439			
BuFlu vs. BuCy2	0.168	0.032-0.874	0.034	0.144	0.026-0.803	0.027
Tacrolimus vs. CSA	0.256	0.031-2.134	0.208			
Donor, unrelated vs. sibling	0.922	0.206-4.135	0.915			
BM vs. PBSCs	1.007	0.118-8.626	0.995			
TCD	0.208	0.025-1.733	0.146			
HLA mismatch	0.043	0.000-8464	0.613			
CD34 > 5 ($\times 10^6$/kg)	1.270	0.282-5.720	0.756			
VOD	0.040	0.000-1513	0.550			
CMV	2.629	0.315-21.913	0.372			
haGVHD	5.847	0.973-35.127	0.054	4.122	0.502-33.812	0.187
aGVHD ≥ 2	1.064	0.237-4.774	0.936			

GVHD, graft-versus-host disease; HR, hazard ratio; CI, confidence interval; LL, lymphoblastic lymphoma; ALL, acute lymphoblastic lymphoma; IP type, immunophenotype; CG, cytogenetic; LDH, lactate dehydrogenase; EM, extramedullary; CR, complete remission; RIC, reduced-intensity conditioning; MA, myeloablative; BuFlu, busulfan-fludarabine; BuCy2, busulfan-cyclophosphamide; CSA, cyclosporine; BM, bone marrow; PBSC, peripheral blood stem cells; TCD, T-cell depletion; HLA, human leukocyte antigen; VOD, veno-occlusive disease; CMV, cytomegalovirus; haGVHD, hyperacute GVHD; aGVHD, acute GVHD.

with positive cerebrospinal fluid cytology were treated with intrathecal chemotherapy, while the remaining patient, who had a central nervous system (CNS) mass, was treated with brain radiotherapy and intrathecal chemotherapy (Table 5). All six patients were treated successfully with no CNS system relapse, although four of them experienced bone marrow relapse and the patient with the CNS mass developed a pelvic mass, which was successfully treated with radiotherapy and dasatinib treatment. Extramedullary relapse was observed in two of the nine patients who relapsed. Two of the thirty-two patients without extramedullary involvement at presentation experienced recurrence in the CNS as well as the BM, even though they had received prophylactic CNS treatment. However, both of these patients presented with complex cytogenetic abnormalities at the time of diagnosis and did not show any evidence of chronic GVHD after transplantation.

DISCUSSION

Many studies have already investigated substituting busulfan for TBI to reduce the long-term side effects of TBI, and randomized studies comparing TBI-based and non-TBI-based regimens have shown comparable results for myeloid leukemias. However, experience with chemotherapy-based regimens is limited, especially

Table 4. Factors affecting the development of TRM

	Univariate			Multivariate		
	HR	95% CI	*p* value	HR	95% CI	*p* value
Sex	1.709	0.521-5.606	0.377			
Age > 35 yr	1.340	0.407-4.409	0.630			
Transplantation year, > 2006 vs. ≤ 2005	0.479	0.140-1.640	0.241			
Diagnosis, LL vs. ALL	0.723	0.092-5.668	0.758			
IP type, T-cell vs. B-cell	1.455	0.443-4.775	0.536			
CG risk, high	0.713	0.184-2.762	0.624			
LDH, high	0.742	0.154-3.586	0.711			
EM disease	1.258	0.272-5.829	0.769			
Status, non-CR	1.317	0.349-4.972	0.685			
High risk	1.522	0.444-5.214	0.504			
RIC vs. MA	0.036	0.000-23.294	0.314			
BuFlu vs. BuCy2	0.201	0.053-0.759	0.018	0.199	0.052-0.752	0.017
Tacrolimus vs. CSA	0.552	0.146-2.083	0.380			
Donor, unrelated vs. sibling	0.661	0.193-2.259	0.509			
BM vs. PBSCs	0.040	0.000-76.339	0.403			
TCD	0.330	0.071-1.535	0.158			
HLA mismatch	0.663	0.084-5.197	0.659			
CD34 > 5 (× 10^6/kg)	2.583	0.680-9.806	0.163			
VOD	3.931	1.133-13.643	0.031	3.951	1.148-13.598	0.029
CMV	0.455	0.137-1.511	0.199			
haGVHD	3.232	0.944-11.068	0.062	2.764	0.657-11.633	0.166
aGVHD ≥ 2	1.272	0.387-4.178	0.692			
cGVHD	0.324	0.084-1.245	0.101			
ecGVHD	0.719	0.155-3.338	0.673			

TRM, treatment-related mortality; HR, hazard ratio; CI, confidence interval; LL, lymphoblastic lymphoma; ALL, acute lymphoblastic lymphoma; IP type, immunophenotype; CG, cytogenetic; LDH, lactate dehydrogenase; EM, extramedullary; CR, complete remission; RIC, reduced-intensity conditioning; MA, myeloablative; BuFlu, busulfan-fludarabine; BuCy2, busulfan-cyclophosphamide; CSA, cyclosporine; BM, bone marrow; PBSC, peripheral blood stem cells; TCD, T-cell depletion; HLA, human leukocyte antigen; VOD, veno-occlusive disease; CMV, cytomegalovirus; haGVHD, hyperacute graft-versus-host disease; aGVHD, acute GVHD; cGVHD, chronic GVHD; ecGVHD; extensive chronic GVHD.

for adult patients with ALL. Moreover, there have been conflicting outcomes [1-5].

In the current study, when using non-TBI-based conditioning, the survival rate was comparable to that reported in previous studies, with an overall 3-year OS rate of 41.8% (53.8% for the standard-risk patients and 30.6% for the high-risk patients) (Fig. 1). Meanwhile, patients with chronic GVHD showed a better survival rate than those without chronic GVHD, demonstrating the strong anti-leukemia effect of GVHD. These results are supported by the results of the subgroup analysis in the EBMT trial, which found no significant differences between patients treated with BuCy2 and those treated with CyTBI in terms of TRM, the incidence of relapse, or LFS [1]. LFS among allograft recipients with intermediate-risk ALL was 43 ± 6% in the BuCy2 group and 33 ± 6% in the CyTBI group, a difference that was not statistically significant. However, there remains some controversy over the role of non-TBI conditioning in ALL patients.

BuCy2 has been used in adult patients with ALL and has an LFS rate that is comparable to those of radiation-containing regimens [19]. In contrast, in a study conducted by Granados et al. [20], TBI was associated with a lower relapse rate and better EFS than a busulfan-based

Table 5. Patients with extramedullary disease

Patients	Initial EMD	Cytogenetics	IP	Status	Treatment	GVHD	Relapse	Current status
M/18	CNS	Normal	T	Rel2	IT	Extensive	BM	Dead (D + 551)
F/28	CNS, retinopathy	Normal	T	Rel3	IT	NE	NE	Dead (D + 9)
F/28	CNS mass	Ph+	Pre-B, T	Ref	RT + IT	Limited	BM, pelvic mass[a]	Alive (D + 358)
M/30	CNS	Normal	T	Ref	IT	Extensive	No	Alive (D + 2236)
F/38	CNS	t(7p;22q)	B	Ref	IT	No	BM	Dead (D + 113)
M/39	CNS	t(7q;12q)	Pre-B	CR4	IT	No	BM	Dead (D + 62)
M/25	-	-7,-19,+5	Pre-B, T	CR2	IT[b]	No	CNS mass	Dead (D + 1719)
M/46	-	t(1;19),2q-,6q-	Pre-B	Ref	IT[b]	No	BM , CNS	Dead (D + 112)

EMD, extramedullary disease; IP, immunophenotype; GVHD, graft-versus-host disease; CNS, central nervous system; Rel, relapse; IT, intrathecal chemotherapy; BM, bone marrow; NE, not evaluable; Ref, refractory; RT, radiotherapy; CR, complete remission.
[a]Pelvic mass disappeared after radiotherapy and dasatinib treatment.
[b]Prophylactic intrathecal treatment.

conditioning regimen. However, it is difficult to compare the results directly because Granados et al. [20] included some childhood ALL cases, which might have affected the outcomes, and used oral busulfan instead of IV busulfan. The inferior survival rate for BuCy2 patients, especially children, was likely due to the oral busulfan used in these trials. The intestinal absorption of oral busulfan is unpredictable, causing inter-patient variability in the plasma concentration. Moreover, the total plasma clearance rate is two to four times higher in children than in adults [21-25]. In contrast, intravenous preparations of busulfan produce a more predictable steady-state concentration, resulting in a lower incidence of hepatic VOD and better 100-day survival [26].

Experience with fludarabine-containing conditioning for adult ALL patients is very limited. In a study by Iravani et al. [13] using a myeloablative BuFlu conditioning regimen, the 1-year OS for ALL patients was 55.6%, with a 33.3% relapse rate at 1 year. In the current study, the outcomes for BuFlu conditioning were comparable to those for BuCy2 in terms of OS, EFS, and incidence of relapse (Figs. 2 and 3). The cumulative incidence of extensive chronic GVHD was significantly lower in the BuFlu group than in the BuCy2 group. Indeed, the BuFlu regimen was an independent favorable risk factor for the development of extensive chronic GVHD. In addition, while the higher relative incidence of TRM remains a major concern for patients receiving BuCy2 conditioning [12,13], this was not a significant issue for patients who received BuFlu conditioning (Table 4). However, interpretation of the current results requires caution because the disease status before transplantation was different in the two groups and, as BuFlu conditioning was performed more recently, improvements in supportive care may have contributed to the favorable outcome regarding TRM in the BuFlu group. CR1 was achieved in more patients in the BuFlu group (75.0%) than in the BuCy2 group (42.9%). Moreover, although only a small number of patients with the Philadelphia chromosome were included in the current study, tyrosine kinase inhibitors were used in more patients in the BuFlu group. In terms of conditioning intensity, RIC in the BuFlu group also confounded the difference in incidence of GVHD between the groups [27]. In addition, to achieve more meaningful results requires analysis of the data after distinguishing between sibling and unrelated donors and a larger sample size.

The major concern with chemotherapy-based conditioning in ALL is overcoming the blood-brain barrier. Bunin et al. [6] reported 5 cases of extramedullary relapse (23.8%) with BuCy2 and 2 cases of testicular relapse (9.1%) with TBI, but only 1 case of CNS relapse out of 52 patients who followed a BuCy2 regimen, this patient having previously received cranial irradiation to treat multiply relapsed CNS leukemia [6]. Meanwhile, in the present study, extramedullary relapse in the CNS was only observed in 2 of the 32 patients without extramedullary involvement at presentation, and both of these patients had complex cytogenetic abnormalities at presentation and exhibited no evidence of chronic GVHD after transplantation. Recent studies have indicated that the graft-versus-leukemia (GVL) effect may play a significant role in preventing CNS relapse in patients with lymphoid

malignancies who only receive chemotherapy-based conditioning for allogeneic transplantation [28]. In the international collaborative trial conducted by Goldstone et al. [29] (MRC UKALL XII/ECOG E2993), the 5-year OS rate for adult ALL patients was 53% for standard-risk patients and 41% for high-risk patients, while the 5-year relapse rate was 24% for standard-risk patients and 37% for high-risk patients, leading to speculation that allogeneic transplantation has the most potent anti-leukemic effect in adult ALL, as demonstrated by the significantly reduced relapse rate (37% with a donor vs. 63% without a donor, $p < 0.001$).

In conclusion, our BuFlu regimen would appear to be an acceptable conditioning option for lymphoid malignancies, including high-risk cases, in terms of OS and EFS. It was safely administered with a lower TRM rate compared to BuCy2 conditioning. When considering the graft-versus-leukemia effect, sanctuary site relapse after transplantation would not seem to be a significant issue in patients with lymphoid malignancies.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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