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...
(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 busulfan and fludarabine (BuFlu), adopted to avoid the sequelae of TBI conditioning and the toxicity of standard BuCy2 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/m² for 6 days (total dose, 180 mg/m²/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

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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 mycophenolate 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 × 10⁹/L, while platelet engraftment was defined as the first day of at least three consecutive days on which a platelet count of ≥ 20 × 10⁹/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 × 10⁹/L for B-lineage cells and ≥ 30 × 10⁹/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 (× 10⁸/kg)                  | 7.81 (1.34-17.96) | 9.79 (0.36-18.43) | 0.757  |
| CD34+ cells (× 10⁶/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.
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,

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**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.
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).

| Variables | BuCy2 | BuFlu | p value |
|-----------|-------|-------|---------|
| No. of patients | 14 | 24 | |
| Engraftment | | | |
| ANC ≥ 0.5 × 10⁹/L, day | 14 (10-27) | 12 (9-18) | 0.073 |
| Platelet ≥ 20 × 10⁹/L, day | 13 (10-33) | 12 (7-26) | 0.125 |
| Acute GVHD, grade II-IV | 7 (50.0) | 7 (29.2) | 0.199 |
| Chronic GVHD⁴, type | | | |
| De novo | 2 (22.2) | 0 | 0.088 |
| Quiescent | 3 (33.3) | 7 (41.2) | |
| Progressive | 2 (22.2) | 1 (5.9) | |
| Chronic GVHD⁴, 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.

²26 patients (9 + 17) who survived longer than 3 months after transplantation were analyzed.

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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

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%).
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. 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 |           | p value | Multivariate |           | p value |
|----------------|------------|-----------|---------|--------------|-----------|---------|
| Sex            | 1.709      | 0.521-5.606 | 0.377   |              | 0.199    | 0.052-0.752 | 0.017 |
| Age > 35 yr    | 1.340      | 0.407-4.409 | 0.630   |              | 0.036    | 0.000-23.294 | 0.314 |
| Transplantation year, > 2006 vs. ≤ 2005 | 0.479 | 0.140-1.640 | 0.241 |             | 0.201 | 0.053-0.759 | 0.028 |
| Diagnosis, LL vs. ALL | 0.723 | 0.092-5.668 | 0.758 |             | 0.330 | 0.071-1.535 | 0.158 |
| IP type, T-cell vs. B-cell | 1.455 | 0.443-4.775 | 0.536 |         | 0.567 | 0.071-3.155 | 0.509 |
| CG risk, high  | 0.713      | 0.184-2.762 | 0.624   |              | 0.661 | 0.013-2.289 | 0.509 |
| LDH, high      | 0.742      | 0.154-3.586 | 0.711   |              | 0.040 | 0.000-76.339 | 0.403 |
| EM disease     | 1.258      | 0.272-5.829 | 0.769   |              | 0.330 | 0.071-1.535 | 0.158 |
| Status, non-CR | 1.317      | 0.349-4.972 | 0.685   |              | 0.663 | 0.084-5.197 | 0.659 |
| High risk      | 1.522      | 0.444-5.214 | 0.504   |              | 2.583 | 0.680-9.806 | 0.163 |
| RIC vs. MA     | 0.036      | 0.000-23.294 | 0.314 |             | 3.931 | 1.133-13.643 | 0.031 |
| BuCy2 vs. BuCy2 | 0.201 | 0.053-0.759 | 0.018   |              | 3.951 | 1.148-13.598 | 0.029 |
| Tacrolimus vs. CSA | 0.552 | 0.146-2.083 | 0.380 |             | 0.455 | 0.137-1.511 | 0.199 |
| Donor, unrelated vs. sibling | 0.661 | 0.193-2.259 | 0.509 |              | 3.232 | 0.944-11.068 | 0.062 |
| BM vs. PBSCs   | 0.040      | 0.000-76.339 | 0.403 |         | 1.272 | 0.387-4.178 | 0.692 |
| TCD            | 0.330      | 0.071-1.535 | 0.158   |              | 0.324 | 0.084-1.245 | 0.101 |
| HLA mismatch   | 0.663      | 0.084-5.197 | 0.659   |              | 0.719 | 0.155-3.338 | 0.673 |
| CD34 > 5 (× 10⁶/kg) | 2.583 | 0.680-9.806 | 0.163 |             | 3.931 | 1.133-13.643 | 0.031 |
| 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   |              | 0.036 | 0.000-23.294 | 0.314 |
| haGVHD         | 3.232      | 0.944-11.068 | 0.062 |             | 1.272 | 0.387-4.178 | 0.692 |
| aGVHD ≥ 2      | 0.324      | 0.084-1.245 | 0.101   |              | 0.040 | 0.000-76.339 | 0.403 |
| cGVHD          | 0.719      | 0.155-3.338 | 0.673   |              | 0.330 | 0.071-1.535 | 0.158 |

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...
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 at presentation. Recent studies have indicated that the graft-versus-leukemia (GVL) effect may play a significant role in preventing CNS relapse in patients with lymphoid

### Table 5. Patients with extramedullary disease

| Patients | Initial EMD | Cytogenetics | IP | Status | Treatment | GVHD | Relapse | Current status |
|----------|-------------|--------------|----|--------|-----------|------|---------|---------------|
| M/18     | CNS         | Normal       | T  | Ref2   | IT        | Extensive | BM     | Dead (D + 551) |
| F/28     | CNS, retinopathy | Normal | T  | Ref3   | IT        | NE     | NE     | Dead (D + 9)   |
| F/28     | CNS mass    | Ph+ Pre-B, T | Ref | RT + IT | Limited BM, pelvic mass | 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 | ITb   | No        | CNS mass | Dead (D + 1719) |
| M/46     | -           | -11q:2q-6q- Pre-B | Ref | ITb   | 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.

*Pelvic mass disappeared after radiotherapy and dasatinib treatment.*

*Prophylactic intrathecal treatment.*

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http://www.kjim.or.kr
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.

REFERENCES

1. Ringden O, Labopin M, Tura S, et al. A comparison of busulfan versus total body irradiation combined with cyclophosphamide as conditioning for autograft or allograft bone marrow transplantation in patients with acute leukemia: Acute Leukaemia Working Party of the European Group for Blood and Marrow Transplantation (EBMT). Br J Haematol 1996;93:637-645.

2. Litzow MR, Perez WS, Klein JP, et al. Comparison of outcome following allogeneic bone marrow transplantation with cyclophosphamide-total body irradiation versus busulphan-cyclophosphamide conditioning regimens for acute myelogenous leukemia in first remission. Br J Haematol 2002;119:1115-1124.

3. Blaise D, Maraninchi D, Archimbaud E, et al. Allogeneic bone marrow transplantation for acute myeloid leukemia in first remission: a randomized trial of a busulfan-Cytocan versus Cytosan-total body irradiation as preparative regimen: a report from the Group d’Etudes de la Greffe de Moelle Osseeuse. Blood 1992;79:2578-2582.

4. Devergie A, Blaise D, Attal M, et al. Allogeneic bone marrow transplantation for chronic myeloid leukemia in first chronic phase: a randomized trial of busulphan-cytosan versus cytosan-total body irradiation as preparative regimen: a report from the French Society of Bone Marrow Graft (SFGM). Blood 1995;85:2263-2268.

5. Socie G, Clift RA, Blaise D, et al. Busulfan plus cyclophosphamide compared with total-body irradiation plus cyclophosphamide before marrow transplantation for myeloid leukemia: long-term follow-up of 4 randomized studies. Blood 2001;98:3569-3574.

6. Bunin N, Aplenc R, Kamani N, Shaw K, Cnaan A, Simms S. Randomized trial of busulfan vs total body irradiation containing conditioning regimens for children with acute lymphoblastic leukemia: a Pediatric Blood and Marrow Transplant Consortium study. Bone Marrow Transplant 2003;32:543-548.

7. Davies SM, Ramsay NK, Klein JP, et al. Comparison of prepa- rative regimens in transplants for children with acute lymphoblastic leukemia. J Clin Oncol 2000;18:340-347.

8. DeLeve LD. Cellular target of cyclophosphamide toxicity in the murine liver: role of glutathione and site of metabolic activation. Hepatology 1996;24:830-837.

9. Shulman HM, Luk K, Deeg HJ, Shuman WB, Storb R. Induction of hepatic veno-occlusive disease in dogs. Am J Pathol 1987;126:114-125.

10. Terenzi A, Aristei C, Aversa F, et al. Efficacy of fludarabine as an immunosuppressor for bone marrow transplantation conditioning: preliminary results. Transplant Proc 1996;28:3101.

11. Chun HG, Leyland-Jones B, Cheson BD. Fludarabine phosphate: a synthetic purine antimetabolite with significant activity against lymphoid malignancies. J Clin Oncol 1991;9:175-188.

12. Chae YS, Sohn SK, Kim JG, et al. New myeloablative conditioning regimen with fludarabine and busulfan for allogeneic stem cell transplantation: comparison with BuCy2. Bone Marrow Transplant 2007;40:541-547.

13. Iravani M, Evazi MR, Mousavi SA, et al. Fludarabine and busulfan as a myeloablative conditioning regimen for alloge- neic stem cell transplantation in high- and standard-risk leukemic patients. Bone Marrow Transplant 2007;40:105-110.

14. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. Bone Marrow Transplant 1995;15:825-828.

15. Shulman HM, Sullivan KM, Weiden PL, et al. Chronic graft-versus-host syndrome in man: a long-term clinicopathologic study of 20 Seattle patients. Am J Med 1980;69:204-217.

16. Kim HJ, Chung JJ, Lee JI, et al. A case of chronic graft-versus-host-disease following allogeneic peripheral blood stem cell res- cue for poor graft function after bone marrow transplantation. Korean J Intern Med 1998;13:60-63.

17. Racigalupo A, Lamparelli T, Bruzzi P, et al. Antithymocyte glob- ulin for graft-versus-host disease prophylaxis in transplants
from unrelated donors: 2 randomized studies from Gruppo Italiano Trapianti Midollo Osseo (GITMO). Blood 2001;98:2942-2947.

18. Giaccone L, Martin P, Carpenter P, et al. Safety and potential efficacy of low-dose methotrexate for treatment of chronic graft-versus-host disease. Bone Marrow Transplant 2005;36:337-341.

19. Copelan EA, Biggs JC, Avalos BR, et al. Radiation-free preparation for allogeneic bone marrow transplantation in adults with acute lymphoblastic leukemia. J Clin Oncol 1992;10:237-242.

20. Granados E, de La Camara R, Madero L, et al. Hematopoietic cell transplantation in acute lymphoblastic leukemia: better long term event-free survival with conditioning regimens containing total body irradiation. Haematologica 2000;85:1060-1067.

21. Slattery JT, Clift RA, Buckner CD, et al. Marrow transplantation for chronic myeloid leukemia: the influence of plasma busulfan levels on the outcome of transplantation. Blood 1997;89:3055-3060.

22. Ljungman P, Hassan M, Bekassy AN, Ringden O, Oberg G. High busulfan concentrations are associated with increased transplant-related mortality in allogeneic bone marrow transplant patients. Bone Marrow Transplant 1997;20:909-913.

23. Grochow LB, Krivit W, Whiteley CB, Blazar B. Busulfan disposition in children. Blood 1990;75:1723-1727.

24. Schuler U, Schroer S, Kuhnle A, et al. Busulfan pharmacokinetics in bone marrow transplant patients: is drug monitoring warranted? Bone Marrow Transplant 1994;14:759-765.

25. Vassal G, Gouyette A, Hartmann O, Pico JL, Lemerle J. Pharmacokinetics of high-dose busulfan in children. Cancer Chemother Pharmacol 1989;24:386-390.

26. Kashyap A, Wingard J, Cagnoni F, et al. Intravenous versus oral busulfan as part of a busulfan/cyclophosphamide preparative regimen for allogeneic hematopoietic stem cell transplantation: decreased incidence of hepatic venoocclusive disease (HVOD), HVOD-related mortality, and overall 100-day mortality. Biol Blood Marrow Transplant 2002;8:493-500.

27. Imamura M, Tanaka J. Graft-versus-leukemia effect of non-myeloablative stem cell transplantation. Korean J Intern Med 2009;24:287-298.

28. Shah AJ, Lenarsky C, Kapoor N, et al. Busulfan and cyclophosphamide as a conditioning regimen for pediatric acute lymphoblastic leukemia patients undergoing bone marrow transplantation. J Pediatr Hematol Oncol 2004;26:91-97.

29. Goldstone AH, Richards SM, Lazarus HM, et al. In adults with standard-risk acute lymphoblastic leukemia, the greatest benefit is achieved from a matched sibling allogeneic transplantation in first complete remission, and an autologous transplantation is less effective than conventional consolidation/maintenance chemotherapy in all patients: final results of the International ALL Trial (MRC UKALL XII/ECOG E2993). Blood 2008;111:1827-1833.