Original Article

Yonsei Med J 2020 Jun;61(6):452-459
https://doi.org/10.3349/ymj.2020.61.6.452

Yonsei Medical Journal
YMJ
pISSN: 0513-5796 · eISSN: 1976-2437

Reduced-Intensity Conditioning with Busulfan and Fludarabine for Allogeneic Hematopoietic Stem Cell Transplantation in Acute Lymphoblastic Leukemia

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Purpose: Allogeneic hematopoietic stem cell transplantation (HSCT) with optimal conditioning has helped better long-term survival in acute lymphoblastic leukemia (ALL). This study investigated the efficacy and safety of reduced-intensity conditioning (RIC) with busulfan and fludarabine in adult ALL patients unfit for myeloablation.

Materials and Methods: Records of 78 patients who underwent HSCT with RIC consisting of 3.2 mg/kg/day of busulfan for 2 or 3 days and 30 mg/m^2/day of fludarabine for 5 or 6 days were analyzed.

Results: The median age at diagnosis was 49 years. Over a median follow-up of 22 months, 2-year estimates of relapse-free survival (RFS) and overall survival were 57.4% and 68.7%, respectively. Multivariate analysis showed a trend of improved RFS in patients with chronic graft-versus-host disease (GVHD) (hazard ratio, 0.53; 95% confidence interval, 0.26–1.08; p=0.080). The cumulative incidences of relapse and non-relapse mortality were 42.9% and 19.6%, respectively and one case of central nervous system relapse was noted. No hepatic veno-occlusive disease was reported. Grade II–IV acute GVHD and any grade chronic GVHD occurred in 21.1% and 41.7%, respectively.

Conclusion: RIC with busulfan and fludarabine is an effective and safe conditioning regimen for adult ALL patients unfit for myeloablation.

Key Words: Fludarabine, busulfan, lymphoblastic leukemia, stem cell transplantation, transplantation conditioning

Received: January 22, 2020 **Revised:** April 2, 2020 **Accepted:** April 22, 2020
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•The authors have no potential conflicts of interest to disclose.

INTRODUCTION

Although treatment outcomes for adolescents and young adults with acute lymphoblastic leukemia (ALL) have significantly improved with the introduction of pediatric-inspired regimens for Philadelphia-negative ALL (Ph-ALL) and tyrosine kinase inhibitors for Philadelphia-positive ALL (Ph+ALL), treatment outcomes in adult ALL remain unsatisfactory.[1] While the complete remission (CR) rate of adult ALL is over 80% with conventional regimens, nearly half of all patients with adult ALL experience disease relapse.[2] Chemotherapy for relapsed adult ALL results in a CR rate of 44%, median overall survival (OS) of 6.3 months, and 5-year OS of 7%.[3]

Allogeneic hematopoietic stem cell transplantation (HSCT) using an optimal conditioning regimen at the first CR appears to be a reasonable therapeutic approach with which to prevent relapse, exhibiting well-known graft-versus-leukemia effect, and to improve the discouraging survival outcomes of adult ALL. Pre-transplant conditioning as a part of HSCT has been used to induce sufficient immunosuppression to prevent graft rejection and to eradicate residual disease. Although no well-designed prospective studies have compared different conditioning regimens, common regimens for adult ALL include myeloablative regimens, incorporating either total body irradiation (TBI) plus chemotherapy or high dose combination chemotherapy, including cyclophosphamide, cytarabine, melphalan, and busulfan.[2] An advantage of TBI is the lessening of leukemia in the central nervous system (CNS). However, a major concern regarding conditioning with TBI is the increased severe gastrointestinal, hepatic, and pulmonary toxicities resulting in high non-relapse mortality (NRM). In addition, high dose intravenous busulfan and cyclophosphamide (BuCy), which is the most widely used non-TBI based myeloablative conditioning, has been shown to be associated with excessive NRM, which negates the anti-leukemic effect of HSCT, especially in older adults and in patients with comorbidities.[4]

The major roles of the purine analog fludarabine in allogeneic HSCT are immunosuppression and killing of tumor cells by the inhibition of lymphocyte proliferation and promotion of lymphocyte apoptosis.[4,5] In addition, fludarabine-mediated inhibition of the repair process of alkylator-induced DNA damage has been proposed.[6] These properties have increased the popularity of conditioning regimens including busulfan and fludarabine (BuFlu) for allogeneic HSCT.[7-10] However, previous studies have primarily focused on myeloablative BuFlu regimens.[7,8,11] This study evaluated the efficacy and safety of reduced-intensity conditioning (RIC) with BuFlu for frontline allogeneic HSCT in adult ALL.

MATERIALS AND METHODS

Patient population

Data from 78 consecutive ALL patients aged between 19 and 65 years who underwent allogeneic HSCT using RIC with BuFlu at 13 centers between March 2010 and August 2018 were retrospectively analyzed. Patients diagnosed with acute leukemia of ambiguous lineage, primary nodal lymphoblastic involvement, and advanced pre-transplant disease status that included beyond first CR, relapsed disease, or refractory disease were excluded. The patients received induction regimens with different combinations of conventional chemotherapeutics, including cyclophosphamide, doxorubicin/daunorubicin, vincristine, corticosteroids, methotrexate, cytarabine, and L-asparaginase. In cases of Ph+ALL, imatinib was continuously added to the induction chemotherapy. This study was approved by the Institutional Review Board of Chonnam National University Hwasun Hospital (CNUHH-2017-026).

Ethical approval

All procedures performed in studies involving human participants were conducted in accordance with the ethical standards stipulated by the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Definitions

CR was defined as <5% blasts in bone marrow aspirates with full hematologic recovery in the peripheral blood (neutrophil count >1×10^3/μL and platelet count >100×10^3/μL). Disease relapse was defined as recurrence of marrow blasts >5% or the occurrence of extramedullary lesions confirmed histologically. Relapse risk was assessed based on the following clinical and genetic features: white blood cell counts >30000/μL for B cell ALL and >100000/μL for T cell ALL and high-risk cytogenetic abnormalities that included hypodiploidy (30–39 chromosomes), near tri/tetraploidy, *KMT2A* gene (11q23) rearrangement, t(4;11), t(8;14), t(1;19), or *E2A/PBX1* fusion transcript, and complex karyotype (≥5 chromosomal abnormalities). Neutrophil and platelet engraftment day was defined as the time from transplant to the first achievement of 2 consecutive days with an absolute neutrophil count >500/μL after nadir and a platelet count >20000/μL without transfusion with an increasing trend. Acute and chronic graft-versus-host disease (GVHD) was evaluated according to conventional consensus criteria.[12,13] Toxicity assessment was conducted according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0 (https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03/Archive/CTCAE_4.0_2009-05-29_QuickReference_8.5x11.pdf).

Transplantation procedures

All patients who achieved a CR by induction chemotherapy proceeded to frontline allogeneic HSCT as soon as the donor was

available. Serologic human leukocyte antigen (HLA) typing was sufficient for choosing matched sibling donors, and high resolution DNA typing was used to identify unrelated matched donors or haploidentical donors. RIC with BuFlu consisted of 3.2 mg/kg/day of intravenous busulfan for 2 or 3 days (total of 6.4–9.6 mg/kg) and 30 mg/m^2/day of fludarabine for 5 or 6 days (total of 150–180 mg/m^2). The decision of whether to apply RIC typically depended on the treating physician. The main reasons for the selection of the RIC regimen included age limit as per center policy (>40–50 years) and comorbidities at the time of the transplantation, such as prior severe infectious complications, pancreatitis, intolerance to induction chemotherapies, and unstable psychological status in younger patients. Grafts comprised peripheral blood stem cells (PBSCs) mobilized by granulocyte colony-stimulating factor (G-CSF) and umbilical cord blood. Measures for prophylactic antimicrobials, GVHD prevention, G-CSF administration after stem cell infusion, and prevention of hepatic veno-occlusive disease (VOD) were guided by the protocols of each transplantation center. Total dosages of anti-thymocyte globulin for T-cell depletion ranged from 4 to 9 mg/kg depending on the donor source.

Statistical analyses

Patients and transplantation characteristics are reported using descriptive statistics, including median, range, and proportion. Relapse free survival (RFS) was defined as the time from transplantation to relapse or death from any cause, whichever occurred first. OS was defined as the time from transplantation to death from any cause. Survival curves were plotted using the Kaplan-Meier method and compared using the Log-rank test. Competing risk analysis was used to estimate the cumulative incidences of NRM and relapse, and Gray's test was used to compare estimates. Multivariate analysis was conducted using the Cox proportional hazards model to evaluate the impact of several covariates on survival outcomes. The variables included in Cox analysis for survival outcomes were age (≥40 years), lactate dehydrogenase above the upper limit of the normal range, sex, high-risk features at presentation, time from diagnosis to HSCT exceeding 6 months, HLA disparity, busulfan dose, and the development of acute/chronic GVHD. Multivariate analysis was performed with variables displaying p values <0.1 in univariate analysis. The Fine and Gray method was used to perform proportional hazard regression analysis for competing events. Statistical significance was defined as a p value <0.05. All statistical analyses were performed using R version 3.4.4 (R Foundation for Statistical Computing, Vienna, Austria; the CRAN project, https://cran.r-project.org/) and EZR software version 1.40 (http://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmed.html).[14]

RESULTS

Patients and transplantation characteristics

A total of 78 patients underwent allogeneic HSCT using RIC

Table 1. Patient and Transplantation Characteristics

Characteristics	Total (n=78)
Age at diagnosis (yr)	
Median (range)	49 (19–64)
17–39	23 (29.5)
40–59	47 (60.2)
≥60	8 (10.3)
Male/Female	39 (50)/39 (50)
Diagnosis	
B-ALL, Ph (-)	27 (34.6)
B-ALL, Ph (+)	44 (56.4)
T-ALL	6 (7.7)
MPAL	1 (1.3)
ECOG PS 0–1/2	76 (97.4)/2 (2.6)
High risk features	
High risk cytogenetics*	17 (21.8)
Clinically high risk†	22 (28.2)
Standard risk	39 (50)
CNS involvement at presentation (n=73)	1 (1.3)
Extramedullary involvement other than CNS (n=77)	1 (1.3)
Time from diagnosis to HSCT	
Median (range), months	4.9 (2.9–14.6)
<6	58 (74.4)
≥6	20 (25.6)
Stem cell source	
Matched sibling	23 (29.5)
Unrelated	38 (48.7)
Haploidentical	15 (19.2)
Cord blood	2 (2.6)
HLA parity	
Full matched	42 (53.8)
Mismatched	35 (44.9)
Busulfan dose (mg/kg)	
6.4	51 (65.4)
9.6	27 (34.6)
ATG use	64 (82.1)
GVHD prophylaxis (n=76)	
CSA/MTX	57 (73.1)
TAC/MTX	19 (24.4)

ALL, acute lymphoblastic leukemia; MPAL, mixed phenotype acute leukemia; ECOG PS, Eastern Cooperative Oncology Group Performance Status; CNS, central nervous system; HSCT, stem cell transplantation; HLA, human leukocyte antigen; ATG, anti-thymocyte globulin; GVHD, graft-versus-host disease; CSA, cyclosporine A; MTX, methotrexate; TAC, tacrolimus.
Data are presented as number (%).
*Hypodiploidy (30–39 chromosomes), near tri/tetraploidy, *KMT2A* gene (11q23) rearrangement, t(4;11), t(8;14), t(1;19), or *E2A/PBX1* fusion transcript and complex karyotype (≥5 chromosomal abnormalities), †White blood cell count >30000/μL for B cell ALL and >100000/μL for T cell ALL.

https://doi.org/10.3349/ymj.2020.61.6.452

with BuFlu in the first remission. Baseline characteristics of the patients and transplant procedures are summarized in Table 1. The median age at diagnosis was 49 years (range, 19–64). Approximately 30% of patients were <40 years old, and only 10% were >60 years old. B lymphoblastic ALL accounted for 90% of the cases. At presentation, about half of the patients carried high risk features determined based on the aforementioned criteria. Fifty-six percent of subjects were Philadelphia-positive. There was one case each for CNS and bone involvement at diagnosis. Most patients received a transplant within 6 months after initial diagnosis. Most grafts were PBSCs, except two double cord blood grafts. Approximately half of the patients received grafts from unrelated donors, while 19.2% of the patients underwent haploidentical transplantation. Two different schedules of intravenous busulfan (2 vs. 3 days of 3.2 mg/kg/day) were used depending on the discretion of the treating physician. Cyclosporine was more frequently used than tacrolimus, combined with low dose methotrexate, for prophylaxis of GVHD.

Table 2. Transplantation-Related Adverse Events

Adverse event	n=78
Hepatic VOD	0 (0)
Cytomegalovirus (n=58)	26 (45)
Hemorrhagic cystitis* (n=76)	4 (5)
Bacteremia	5 (6)
Clostridium difficile colitis	5 (6)
Fungal pneumonia	3 (4)
Pneumocystis jiroveci pneumonia	2 (3)
Unspecified pneumonia	4 (5)
Herpes zoster	2 (3)
Hepatic candidiasis	1 (1)
Engraftment syndrome	1 (1)
Acute GVHD	24 (30)
Grade I	7 (9)
Grade II	6 (7.7)
Grade III	8 (10.3)
Grade IV	3 (4)
Chronic GVHD	32 (41)
Limited	13 (17)
Extensive	19 (24)

VOD, veno-occlusive disease; GVHD, graft-versus-host disease.
Data are presented as number (%).
*BK virus (n=3), Adenovirus (n=1).

Engraftments

All but one of the patients achieved primary neutrophil and platelet engraftments at median Day 12 (range, 6–26) and Day 13 (range, 9–57), respectively. No significant differences in engraftment across subgroups were evident according to sex, age, HLA disparity, and busulfan dose. Two patients experienced secondary engraftment failure and died of infection at Days +79 and 120.

Transplantation-related adverse events

Among 67 patients with medical records of oral mucositis, 92% experienced oral mucositis, of which 45%, 42%, and 13% had grade I, II, and III oral mucositis, respectively. None of the patients experienced grade IV oral mucositis, and all cases were completely reversible with supportive care. There was no case of hepatic VOD. Reactivation of cytomegalovirus developed in 26 patients at a median of 29 days post-transplantation (range, 14–100 days). Until Day +100, 24 events of acute GVHD occurred. The cumulative incidences of all grade and II–IV acute GVHDs were 30.1% [95% confidence interval (CI), 20.2–40.7%] and 21.1% (95% CI, 12.5–31.3%), respectively; and there was no mortality from acute GVHD. The cumulative incidence of chronic GVHD was 41.7% (95% CI, 28.1–52.8%): of these patients, approximately 60% were in the extensive stage. Four patients died from the progression of chronic GVHD at Days +233, 494, 558, and 800. Major transplantation-related adverse events are summarized in Table 2.

Relapse, NRM, and survivals

Twenty-seven relapses and 11 NRMs occurred in the follow-up period. Three deaths resulted from engraftment failure, four from infectious complications, and four from chronic GVHD. The cumulative incidences of 2-year relapse and NRM were

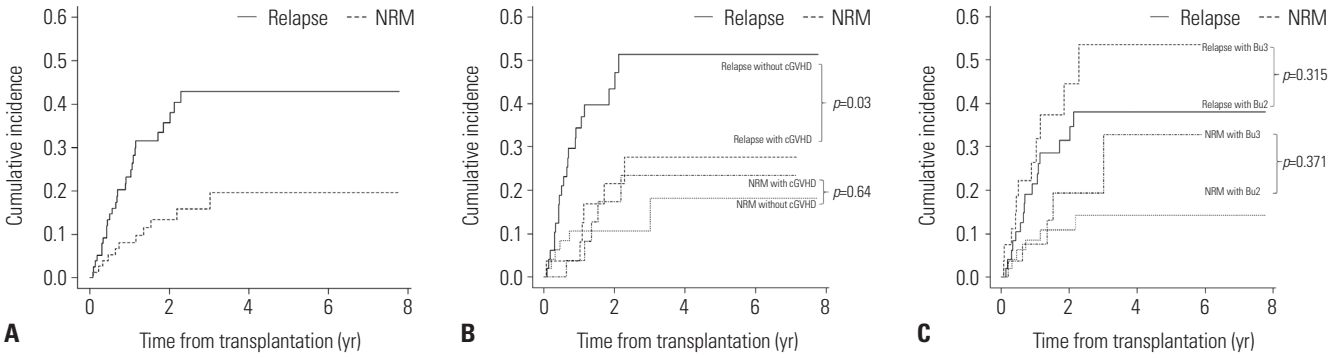

Fig. 1. Cumulative incidences of relapse and non-relapse mortality (NRM) (A) in all populations and their subgroups, (B) according to development of chronic graft-versus-host disease (cGVHD), and (C) according to busulfan dose. Bu2, 2 days of intravenous busulfan; Bu3, 3 days of intravenous busulfan.

35.7% (95% CI, 24.0–47.5%) and 13.4% (95% CI, 6.4–22.8%), respectively (Fig. 1A). Among patients with bone marrow relapse, one patient had a simultaneous leptomeningeal relapse that was confirmed by magnetic resonance imaging and cerebrospinal fluid cytology. Early mortality within 6 months post-transplantation in four patients resulted from engraftment failure and infectious complications. The impacts of the development of chronic GVHD and the busulfan dose on relapse

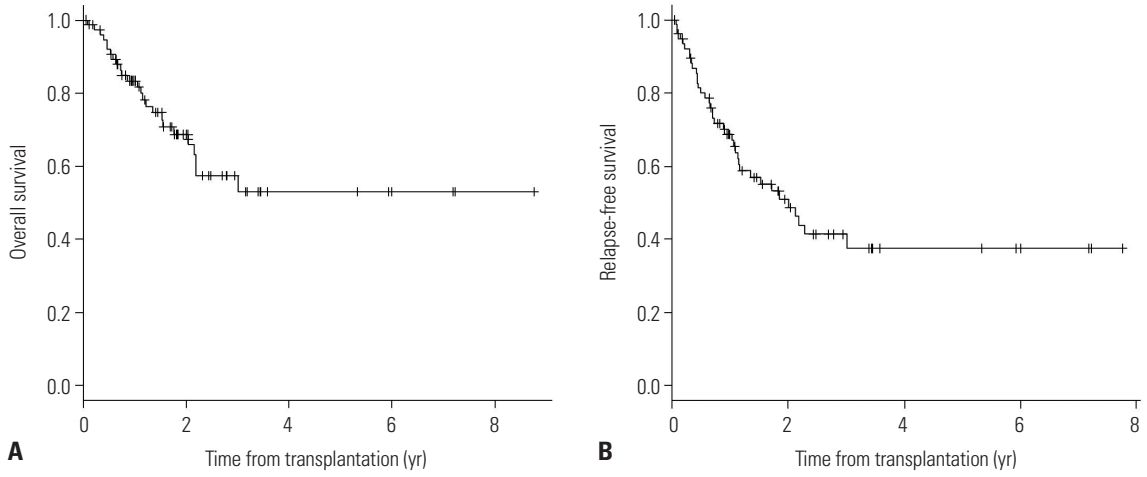

Fig. 2. Kaplan-Meier plots for (A) overall survival and (B) relapse-free survival.

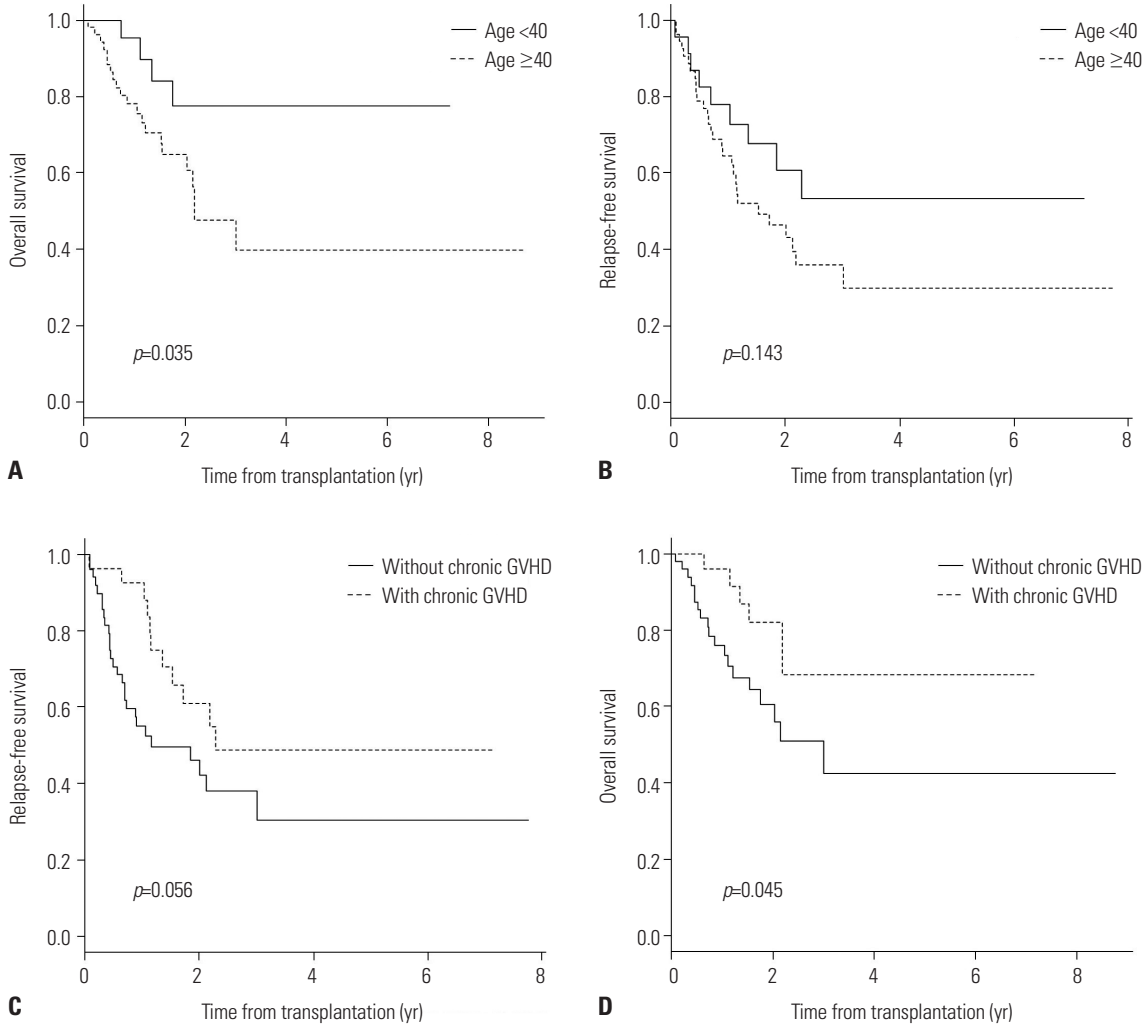

Fig. 3. Survival outcomes according to age ≥40 years (A and B) and development of chronic graft-versus-host disease (GVHD) (C and D).

https://doi.org/10.3349/ymj.2020.61.6.452

and NRM are presented in Fig. 1B and C. Only the development of chronic GVHD was associated with a decreased cumulative incidence of relapse [hazard ratio (HR), 0.35; 95% CI, 0.14–0.84; p=0.019]. There were no significant differences in age (43.7±13.6 vs. 46.6±13.1, p=0.355) and the proportion of cytogenetically high-risk patients (25.9% vs. 19.6%, p=0.723) between the high and low busulfan dose groups, and a higher dose of busulfan did not lower the relapse rate.

With a median follow-up of 22 months among survivors (range, 3–105 months), OS and RFS were 53% (95% CI, 36.6–66.9) and 37.6% (95% CI, 24–51.1), respectively (Fig. 2). The 2-year and 3-year OS estimates were 68.7% (95% CI, 55.4–78.8%) and 57.4% (95% CI, 42.1–70.0%), respectively. The 2-year and 3-year RFS estimates were 57.4% (95% CI, 42.1–70.0%) and 41.3% (95% CI, 28.2–54.0%), respectively. Survival outcomes did not differ between Ph+ALL and Ph-ALL patients. The median RFS of Ph+ B-ALL patients was higher than that of Ph- B-ALL patients, although the difference was not significant (25.5 months vs. 13.6 months, p=0.733). OS was shorter in patients ≥40 years of age, compared with that in younger patients (median OS, 26.2 months vs. not reached, p=0.035) (Fig. 3A and B). Survival was greater for patients with chronic GVHD than for patients without chronic GVHD (median OS, not reached vs. 36.1 months, p=0.045; median RFS, 27.4 months vs. 13.9 months, p=0.056) (Fig. 3C and D). Time to transplantation, sex, and high-risk features did not result in significant differences in survival outcome. In multivariate analysis for OS and RFS, a trend of improved RFS was noted in patients who developed chronic GVHD (HR, 0.53; 95% CI, 0.26–1.08; p=0.08) (Table 3).

DISCUSSION

Allogeneic HSCT is now considered the standard approach for preventing relapse at the first or second remission in the treatment of adult ALL. Over the past two decades, efforts to improve transplantation outcomes in adults with ALL have focused on refinement of the conditioning regimen to reduce transplantation-related mortality and relapse incidence. The choice of an optimal conditioning regimen for each patient is still challenging because clinical trials to date have been quite heterogeneous in terms of conditioning and enrolled patients. Before the recognition of the graft-versus-leukemia effect, myeloablative doses of TBI or busulfan combined with other chemotherapeutic agents, mainly cyclophosphamide (Cy/TBI and BuCy), were widely used to eradicate remnant tumor cells.[4] Several studies comparing myeloablative Cy/TBI with BuCy suggested similar survival outcomes; however, there were in-

Table 3. Univariate and Multivariate Cox Regression Analyses for Overall Survival and Relapse-Free Survival

Variables	Univariate		Multivariate	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value
Overall survival				
Age ≥40 years (yes vs. no)	2.99 (1.02–8.75)	0.045	2.05 (0.62–6.68)	0.233
LDH above ULN (yes or no)	1.35 (0.40–4.52)	0.625	-	-
Sex (male vs. female)	0.79 (0.36–1.74)	0.561	-	-
Clinically high-risk (yes vs. no)	0.87 (0.36–2.08)	0.746	-	-
Cytogenetically high-risk (yes vs. no)	2.17 (0.90–5.24)	0.085	1.76 (0.71–4.32)	0.216
Time to HSCT (≥6 months vs. <6 months)	1.88 (0.80–4.41)	0.144	-	-
HLA mismatch (yes or no)	0.59 (0.26–1.32)	0.198	-	-
Busulfan dose (9.6 mg/kg vs. 6.4 mg/kg)	1.02 (0.44–2.39)	0.957	-	-
Acute GVHD (yes or no)	0.42 (0.16–1.13)	0.085	0.70 (0.23–2.09)	0.525
Chronic GVHD (yes or no)	0.40 (0.16–1.01)	0.053	0.56 (0.21–1.48)	0.245
Relapse-free survival				
Age ≥40 years (yes vs. no)	1.74 (0.82–3.69)	0.148	-	-
LDH above ULN (yes or no)	1.32 (0.51–3.39)	0.563	-	-
Sex (male vs. female)	0.69 (0.36–1.31)	0.256	-	-
Clinically high-risk (yes vs. no)	0.67 (0.32–1.43)	0.304	-	-
Cytogenetically high-risk (yes vs. no)	1.90 (0.92–3.94)	0.082	1.78 (0.86–3.70)	0.118
Time to HSCT (≥6 months vs. <6 months)	1.66 (0.81–3.38)	0.164	-	-
HLA mismatch (yes or no)	1.12 (0.59–2.13)	0.726	-	-
Busulfan dose (9.6 mg/kg vs. 6.4 mg/kg)	1.65 (0.86–3.15)	0.131	-	-
Acute GVHD (yes or no)	0.70 (0.34–1.41)	0.318	-	-
Chronic GVHD (yes or no)	0.51 (0.25–1.03)	0.061	0.53 (0.26–1.08)	0.080

LDH, lactate dehydrogenase; ULN, upper limit of normal range; HSCT, hematopoietic stem cell transplantation; HLA, human leukocyte antigen; GVHD, graft-versus-host diseases; HR, hazard ratio; CI, confidence interval.

consistent results regarding relapse incidence and NRM.[11,15,16] BuFlu was later introduced to further reduce the toxicity of BuCy. A retrospective study comparing myeloablative Cy/TBI with BuFluTBI reported no significant differences in OS, RFS, and NRM. Estimates of 2-year OS were 69.6% and 67.9% for Cy/TBI and BuFluTBI, respectively.[7] Another retrospective study comparing myeloablative BuFlu with Cy/TBI showed that BuFlu resulted in better OS driven by improved NRM, with a similar relapse rate.[8]

Although strong evidence regarding optimal conditioning intensity in the treatment of adult ALL is lacking, RIC might be a feasible approach with which to reduce NRM and to permit older and unfit patients to receive HSCT.[17] Generally, transplant-related mortality increases with age. In one study, 5-year TRM in adults >35 years of age who underwent myeloablative HSCT ranged between 33% and 58%.[18] Presently, we report the efficacy and safety of RIC with BuFlu. Estimated 2- and 3-year OS rates were 68.7% and 57.4%, respectively, which were comparable to the values reported in the aforementioned retrospective study. However, these results should be interpreted with caution because there can be differences in survival rates over time, which was not statistically tested. In addition, heterogeneity was evident in the study population and conditioning regimen. Nonetheless, considering the relatively high proportion (50%) of patients with high-risk features in this study, the observed survival outcomes may be acceptable. Interestingly, 3 days of busulfan use did not improve clinical outcomes, although this needs to be confirmed in a larger cohort. The graft-versus-leukemia effect was reconfirmed by the observation that patients with chronic GVHD had a lower relapse incidence. Of note, relapse at the CNS was observed in only one patient, suggesting that conditioning without TBI might be feasible.

In terms of transplantation-related toxicity, RIC with BuFlu showed good safety profiles. There was no incidence of hepatic VOD. Tsirigotis, et al.[19] reported on the incidence and risk factors for hepatic VOD after administration of the RIC regimen. In their study, patients uniformly received RIC containing fludarabine combined with either oral or intravenous busulfan for 2 days. The incidence of hepatic VOD in the intravenous busulfan group was 5.3%. The reason for the absence of cases of hepatic VOD in our study is unclear. Moreover, the patients suffered less from oral mucositis. A mild form of oral mucositis (grade I or II) occurred in the majority of the patients in this study. Considering the retrospective nature of this study, and the significant variance in the criteria for grading oral mucositis, the precise incidence of mucositis needs to be confirmed in prospective trials.

There are limitations to our study, including recall bias, due to its retrospective nature. The size of the cohort was small and the duration of follow-up was relatively short. Because there were no definitive criteria for the selection of a RIC regimen across the transplantation centers, our study was prone to selection bias. Pre-transplant determination of minimal residual disease could not be conducted in our study. Survival analysis according to the minimal residual disease may provide valuable information about the role of HSCT with RIC in the treatment of adult ALL. Lastly, we did not analyze data regarding pre-transplant induction chemotherapy, which may vary between centers and affect transplantation outcomes. Despite these limitations, our results may act as a reference for BuFlu RIC.

In conclusion, our study demonstrated that the clinical outcomes and safety of RIC with BuFlu, which was effective for frontline allogeneic HSCT in adult ALL. There was a low incidence of treatment-related toxicities, including relatively low NRM. As BuFlu conditioning is increasingly used, prospective trials comparing BuFlu of different intensities or combinations are needed to determine the optimal conditioning for adult ALL.

AUTHOR CONTRIBUTIONS

Conceptualization: Seok Lee and Deok-Hwan Yang. Data curation: all authors. Formal analysis: Seung-Shin Lee, Sung-Hoon Jung , and Deok-Hwan Yang. Investigation: Deok-Hwan Yang. Methodology: Deok-Hwan Yang. Project administration: all authors. Resources: all authors. Software: Seung-Shin Lee and Deok-Hwan Yang. Supervision: Deok-Hwan Yang. Validation: Seung-Shin Lee and Deok-Hwan Yang. Visualization: Seung-Shin Lee and Deok-Hwan Yang. Writing—original draft: Seung-Shin Lee and Sung-Hoon Jung. Writing—review & editing: Seung-Shin Lee, Sung-Hoon Jung , and Deok-Hwan Yang. Approval of final manuscript: all authors.

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REFERENCES

1. Terwilliger T, Abdul-Hay M. Acute lymphoblastic leukemia: a comprehensive review and 2017 update. Blood Cancer J 2017;7:e577.
2. Marks DI, Alonso L, Radia R. Allogeneic hematopoietic cell transplantation in adult patients with acute lymphoblastic leukemia. Hematol Oncol Clin North Am 2014;28:995-1009.
3. Tavernier E, Boiron JM, Huguet F, Bradstock K, Vey N, Kovacsovics T, et al. Outcome of treatment after first relapse in adults with acute

https://doi.org/10.3349/ymj.2020.61.6.452

lymphoblastic leukemia initially treated by the LALA-94 trial. Leukemia 2007;21:1907-14.
4. Gyurkocza B, Sandmaier BM. Conditioning regimens for hematopoietic cell transplantation: one size does not fit all. Blood 2014;124:344-53.
5. Iravani M, Evazi MR, Mousavi SA, Shamshiri AR, Tavakoli M, Ashouri A, et al. Fludarabine and busulfan as a myeloablative conditioning regimen for allogeneic stem cell transplantation in high- and standard-risk leukemic patients. Bone Marrow Transplant 2007;40:105-10.
6. Gandhi V, Plunkett W. Cellular and clinical pharmacology of fludarabine. Clin Pharmacokinet 2002;41:93-103.
7. Speziali C, Daly A, Abuhaleeqa M, Nitta J, Abou Mourad Y, Seftel MD, et al. Fludarabine, busulfan, and low-dose TBI conditioning versus cyclophosphamide and TBI in allogeneic hematopoietic cell transplantation for adult acute lymphoblastic leukemia. Leuk Lymphoma 2019;60:639-48.
8. Gooptu M, Kim HT, Ho VT, Alyea EP, Koreth J, Armand P, et al. A comparison of the myeloablative conditioning regimen fludarabine/busulfan with cyclophosphamide/total body irradiation, for allogeneic stem cell transplantation in the modern era: a cohort analysis. Biol Blood Marrow Transplant 2018;24:1733-40.
9. Kawamura K, Kako S, Mizuta S, Ishiyama K, Aoki J, Yano S, et al. Comparison of conditioning with fludarabine/busulfan and fludarabine/melphalan in allogeneic transplantation recipients 50 years or older. Biol Blood Marrow Transplant 2017;23:2079-87.
10. Santarone S, Pidala J, Di Nicola M, Field T, Alsina M, Ayala E, et al. Fludarabine and pharmacokinetic-targeted busulfan before allografting for adults with acute lymphoid leukemia. Biol Blood Marrow Transplant 2011;17:1505-11.
11. Kebriaei P, Anasetti C, Zhang MJ, Wang HL, Aldoss I, de Lima M, et al. Intravenous busulfan compared with total body irradiation pretransplant conditioning for adults with acute lymphoblastic leukemia. Biol Blood Marrow Transplant 2018;24:726-33.
12. Schoemans HM, Lee SJ, Ferrara JL, Wolff D, Levine JE, Schultz KR, et al. EBMT-NIH-CIBMTR Task Force position statement on standardized terminology & guidance for graft-versus-host disease assessment. Bone Marrow Transplant 2018;53:1401-15.
13. Harris AC, Young R, Devine S, Hogan WJ, Ayuk F, Bunworasate U, et al. International, multicenter standardization of acute graft-versus-host disease clinical data collection: a report from the mount sinai acute gvhd international consortium. Biol Blood Marrow Transplant 2016;22:4-10.
14. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant 2013;48:452-8.
15. Sakellari I, Gavriilaki E, Chatziioannou K, Papathanasiou M, Mallouri D, Batsis I, et al. Long-term outcomes of total body irradiation plus cyclophosphamide versus busulfan plus cyclophosphamide as conditioning regimen for acute lymphoblastic leukemia: a comparative study. Ann Hematol 2018;97:1987-94.
16. Mitsuhashi K, Kako S, Shigematsu A, Atsuta Y, Doki N, Fukuda T, et al. Comparison of cyclophosphamide combined with total body irradiation, oral busulfan, or intravenous busulfan for allogeneic hematopoietic cell transplantation in adults with acute lymphoblastic leukemia. Biol Blood Marrow Transplant 2016;22:2194-200.
17. Leonard JT, Hayes-Lattin B. Reduced intensity conditioning allogeneic hematopoietic stem cell transplantation for acute lymphoblastic leukemia; current evidence, and improving outcomes going forward. Curr Hematol Malig Rep 2018;13:329-40.
18. Scott BL, Pasquini MC, Logan BR, Wu J, Devine SM, Porter DL, et al. Myeloablative versus reduced-intensity hematopoietic cell transplantation for acute myeloid leukemia and myelodysplastic syndromes. J Clin Oncol 2017;35:1154-61.
19. Tsirigotis PD, Resnick IB, Avni B, Grisariu S, Stepensky P, Or R, et al. Incidence and risk factors for moderate-to-severe veno-occlusive disease of the liver after allogeneic stem cell transplantation using a reduced intensity conditioning regimen. Bone Marrow Transplant 2014;49:1389-92.