Article

Advantages of a higher busulfan dose intensity in fludarabine-combined conditioning for patients with acute myeloid leukemia undergoing cord blood transplantation

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Abstract

Busulfan is an alkylating agent that is commonly used as conditioning in allogeneic hematopoietic stem cell transplantation for acute myeloid leukemia (AML). However, a consensus has not yet been reached regarding the optimal dose of busulfan in cord blood transplantation (CBT). Therefore, we herein conducted a large nationwide cohort study to retrospectively analyze the outcomes of CBT in patients with AML receiving busulfan at intermediate (6.4 mg/kg iv; BU2) or higher doses (12.8 mg/kg iv; BU4) within a fludarabine/intravenous busulfan regimen (FLU/BU). Among 475 patients who underwent their first CBT following FLU/BU conditioning between 2007 and 2018, 162 and 313 received BU2 and BU4, respectively. A multivariate analysis identified BU4 as a significant factor for longer disease-free survival (HR, 0.85; 95% CI, 0.75-0.97; P = 0.014) and a lower relapse rate (HR, 0.84; 95% CI, 0.72-0.98; P = 0.030). No significant differences were observed in non-relapse mortality between BU4 and BU2 (HR, 1.05; 95% CI, 0.88-1.26; P = 0.57). Subgroup analyses showed that BU4 provided significant benefits for patients transplanted in non-complete remission (CR) and those younger than 60 years. The present results suggest that higher busulfan doses are preferable in CBT, particularly for non-CR and younger patients.

Introduction

Allogeneic hematopoietic stem cell transplantation (HCT) is a potentially curative treatment for patients with acute myeloid leukemia (AML). Busulfan is an alkylating agent that is commonly used as conditioning in HCT [1]. The fludarabine/intravenous busulfan (FLU/BU) regimen is an efficacious conditioning regimen that is widely used for elderly or frail patients [2,3,4,5]. In the FLU/BU regimen, busulfan is commonly administered at intermediate (6.4 mg/kg iv; BU2) or higher doses (12.8 mg/kg iv; BU4). Retrospective studies compared BU2 with BU4 for bone marrow transplantation (BMT) and peripheral blood stem cell transplantation (PBSCT), and the findings obtained suggested that a higher busulfan dose intensity reduced relapse [6,7, 8]. However, a higher busulfan dose intensity has also been reported to increase non-relapse mortality (NRM) [6,9]. A retrospective analysis of BMT/PBSCT using Japanese registry data showed that BU4 reduced relapse, but increased NRM, which resulted in no significant differences in overall survival (OS) or disease-free survival (DFS) from BU2 [10], suggesting no apparent difference in prognosis regarding the use of BU2 or BU4 for BMT/PBSCT. On the other hand, limited information is currently available and a consensus has not yet been reached on the impact of busulfan dosages on cord blood transplantation (CBT).

In CBT, FLU/BU is also a representative conditioning regimen for AML in Japan. (Figure S1) The impact of the intensity of conditioning in CBT may differ from that in BMT/PBSCT. Lower intensity conditioning in CBT is associated with a high rate of graft failure [11], as well as the potentially higher incidence of post-HCT relapse. Therefore, we conducted a large nationwide study to retrospectively analyze the outcomes of CBT with BU2 and BU4 in AML patients using the Japanese transplant registry database.

Materials and methods

Data collection

The clinical data of patients were collected from the Transplant Registry Unified Management Program (TRUMP) of the Japanese Society for Transplantation and Cellular Therapy (JSTCT) and the Japanese Data Center for Hematopoietic Cell Transplantation [12,13]. Patients with AML were selected from the database if they had undergone their first CBT following FLU/BU conditioning (6.4 mg/kg iv; BU2 or 12.8 mg/kg iv; BU4) between 2007 and 2018 and were aged 16 years or older at the time of transplantation. CBT donors included HLA-A, -B, and -DR antigen fully matched or 1-2 antigen mismatched donors. Double-unit CBT was not included in the present study. This study was designed by the Adult Acute Myeloid Leukemia Working Group of the JSTCT and approved by the Institutional Review Board of Kyoto University and the TRUMP Data Management Committee of the JSTCT. Informed consent was obtained from each patient.

Definitions

OS was defined as the time from CBT to death or the last date of the follow-up. DFS was defined as the time from CBT to death, relapse, or the last date of the follow-up. Relapse was defined as the loss of complete remission (CR) in patients who had achieved CR

once; patients who had never achieved CR after CBT were categorized as relapse on day 0. NRM was defined as the time to death without disease progression. Neutrophil engraftment was defined as the first date of three consecutive absolute neutrophil counts $> 0.5\times 10^9$/L. Platelet engraftment was defined as the first date of three consecutive absolute platelet counts $> 20 \times 10^9$/L without platelet transfusion. Acute and chronic graft-versus-host disease (GVHD) were graded according to standard criteria [14,15].

Statistical Analysis

Patient characteristics were compared between the two groups (BU2 or BU4) using Fisher's exact test or the chi-squared test for categorical variables, and the t-test for continuous variables. The probabilities of OS and DFS were estimated using the Kaplan-Meier method and compared among groups with the Log-rank test. The probabilities of relapse, NRM, acute/chronic GVHD, and neutrophil/platelet engraftment were estimated using the cumulative incidence curve [16], and compared using Gray's test, considering death without relapse as a competing event for relapse, relapse as a competing event for NRM, and death as a competing event for acute/chronic GVHD and neutrophil/platelet engraftment [17].

Multivariate analyses of OS and DFS were performed using the Cox proportional

hazards model, whereas multivariate analyses of relapse, NRM, and acute/chronic GVHD were conducted using the Fine and Gray regression model [18]. The following covariates were considered: patient age at transplantation, patient sex, performance status, disease status, cytogenetic risk, AML type (de novo/secondary), hematopoietic cell transplantation-comorbidity index (HCT-CI), transplant period, HLA disparity (0, 1, or 2), GVHD prophylaxis (cyclosporine A- or tacrolimus-based), the use of anti-thymocyte globulin (ATG), and the use of low-dose total body irradiation (TBI). Cytogenetic risk was classified according to the criteria described in detail in a previous study [19]. All of the above variables were included in the multivariate analysis of OS, DFS, relapse, and NRM, and their statistical interactions were tested. All of the above factors except cytogenetic risk and AML type (de novo/secondary) were introduced into the multivariate analysis of GVHD.

All P values were 2 sided, and $P < 0.05$ was considered to indicate a significant difference. All statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R (R Foundation for Statistical Computing, Vienna, Austria) [20].

Results

Patient characteristics

Patient characteristics are listed in Table 1. Among 475 patients who met the inclusion criteria, 162 were classified into the BU2 group and 313 into the BU4 group. The median follow-up times for survivors in these groups were 5.6 years (interquartile range, 2.6 to 8.7 years) and 5.0 years (interquartile range, 2.7 to 7.0 years), respectively. Median ages were 62 years (range, 20-78) and 60 years (range, 20-79) in the BU2 and BU4 groups, respectively, and were significantly different (P=0.002). There was a significantly higher proportion of non-CR patients at the time of transplantation in the BU4 group (P=0.016), and significantly higher proportion of de novo AML patients in the BU4 group (P=0.014), and no significant differences were observed in cytogenetic risk of AML.

No significant differences were observed in donor-recipient HLA disparity or cord blood total nucleated cells, and ABO blood type mismatches. Furthermore, no significant differences were noted in the use of low-dose TBI (2-4 Gy), GVHD prophylaxis (cyclosporine A- or tacrolimus-based), or ATG.

OS, DFS, relapse, and NRM

Three-year OS rates in the BU2 and BU4 groups were 33.4% (95% CI, 26.1 to 40.9%)

and 27.9% (95% CI, 22.9 to 33.2%), respectively (P = 0.53, Figure 1a). Three-year DFS rates in the BU2 and BU4 groups were 31.0% (95% CI, 23.9 to 38.4%) and 27.2% (95% CI, 22.2 to 32.4%), respectively (P = 0.92, Figure 1b). The cumulative incidence of relapse at 3 years was 40.6% in the BU2 group (95% CI, 32.9-48.2%) and 40.9% in the BU4 group (95% CI, 35.3-46.4%) (P=0.91, Figure 1c). The cumulative incidence of NRM at 3 years was 28.4% in the BU2 group (95% CI, 21.5-35.7%) and 31.9% in the BU4 group (95% CI, 26.6-37.2%) (P=0.83, Figure 1d). Although no significant differences were observed in OS, DFS, relapse, or NRM between the BU2 and BU4 groups in the univariate analysis, the multivariate analysis identified BU4 as a significant factor for longer DFS (HR, 0.85; 95% CI, 0.75-0.97; P = 0.014, Table 2) and a lower relapse rate (HR, 0.84; 95% CI, 0.72-0.98; P = 0.030, Table 2). In the multivariate analysis, no significant differences were noted in OS or NRM between the BU2 and BU4 groups (HR, 0.96; 95% CI, 0.84-1.08; P = 0.48, and HR, 1.05; 95% CI, 0.88-1.26; P = 0.57, Table 2). We calculated P for interaction of variables for busulfan dose intensity in OS. (Table S1)

To account for the effect of TBI, we present the results of the same univariate analysis in the group with low-dose TBI. (Figure S2), and performed multivariate analysis of low-dose TBI group and no TBI group. (Table S2).

Acute and chronic GVHD

The cumulative incidence of acute GVHD (grades II to IV) at 150 days was 36.2% in the BU2 group (95% CI 28.3-44.2%) and 44.5% in the BU4 group (95% CI 38.5-50.3%) (*P*=0.14, Figure 2a). The cumulative incidence of acute GVHD (grades III to IV) at 150 days was 15.6% in the BU2 group (95% CI 10.4-22.6%) and 14.7% in the BU4 group (95% CI 10.8-19.2%) (*P*=0.73, Figure 2b). In the multivariate analysis, BU4 was identified as a significant factor for a higher incidence of acute GVHD (grades II to IV) (HR, 1.22; 95% CI, 1.03-1.45; *P* = 0.020, Table 3).

The cumulative incidence of chronic GVHD (extensive) at 3 years was 9.1% in the BU2 group (95% CI 4.6-15.4%) and 19.3% in the BU4 group (95% CI 14.2-25.0%) (*P*=0.022, Figure 2c). In the multivariate analysis, BU4 was identified as a significant factor for a higher incidence of chronic GVHD (HR, 1.70; 95% CI, 1.16-2.49; *P* = 0.0064, Table 3). The cumulative incidence of chronic GVHD was significantly higher in the BU4 group in the univariate and multivariate analyses.

Subgroup analyses

Since the impact of the busulfan dose intensity may differ with the disease status and age of patients, we performed a multivariate analysis of the following subgroups of patients:

CR, non-CR, younger than 60 years, and older than 60 years. Characteristics of each group of patients are listed in Table S3-6. Multivariate analysis was performed in the subgroup analysis to correct for the effects of confounding factors.

In the CR group, no significant differences were observed in OS, DFS, relapse, or NRM between the BU2 and BU4 groups (Table 4). In the non-CR group, BU4 correlated with longer DFS and a lower relapse rate (HR, 0.80; 95% CI, 0.68 -0.95; P = 0.010, and HR, 0.83; 95% CI, 0.71-0.98; P = 0.032, Table 4). No significant differences were noted in OS or NRM between the BU2 and BU4 groups (HR, 0.95; 95% CI, 0.80 - 1.13; P = 0.54, and HR, 1.10; 95% CI, 0.81-1.50; P = 0.55, Table 4).

In the younger than 60 years group, BU4 correlated with longer DFS and a lower relapse rate (HR, 0.77; 95% CI, 0.61 -0.98; P = 0.036, and HR, 0.71; 95% CI, 0.51-0.99; P = 0.046, Table 4). No significant differences were observed in OS or NRM between the BU2 and BU4 groups (HR, 0.80; 95% CI, 0.64 -1.01; P = 0.065, and HR, 0.93; 95% CI, 0.69-1.26; P = 0.64, Table 4). In the older than 60 years group, no significant differences were noted in OS, DFS, relapse, or NRM between the BU2 and BU4 groups (Table 4).

Engraftment rate

The cumulative incidence of neutrophil engraftment at 50 days was 73.9% in the BU2

group (95% CI: 66.4–80.0%) and 77.3% in the BU4 group (95% CI: 72.3-81.6%) (*P*=0.67, Figure 3a). No significant differences were noted in the cumulative incidence of neutrophil engraftment between the BU2 and BU4 groups.

The cumulative incidence of platelet engraftment at 6 months was 57.8% in the BU2 group (95% CI: 49.7–65.0%) and 60.6% in the BU4 group (95% CI: 54.9-65.8%) (*P*=0.94, Figure 3b). No significant difference was observed in the cumulative incidence of platelet engraftment between the BU2 and BU4 groups.

We then compared the impact of busulfan dose intensities on the engraftment rate in patients treated with and without TBI. In the TBI group, the cumulative incidence of neutrophil engraftment at 50 days was 75.0% in the BU2 group (95% CI: 66.7%–81.5%) and 80.7% in the BU4 group (95% CI: 75.3%-85.0%) (*P*=0.34, Figure 4a), while the cumulative incidence of platelet engraftment at 6 months was 60.6% (95% CI: 51.7%–68.4%) and 63.2% (95% CI: 57.0%-68.7%), respectively (*P*=0.85, Figure 4b). In the non-TBI group, the cumulative incidence of neutrophil engraftment at 50 days was 67.9% in the BU2 group (95% CI: 47.3%–81.8%) and 63.0% in the BU4 group (95% CI: 48.7%-74.3%)(*P*=0.47, Figure 4c), while the cumulative incidence of platelet engraftment at 6 months was 42.9% (95% CI: 24.6%–60.0%) and 48.1% (95% CI: 34.4%-60.6%), respectively (*P*=0.77, Figure 4d).

The impact of the busulfan dosage was not significant regardless of TBI. Furthermore, no significant differences were noted in the engraftment rate between BU2 and BU4 regardless of TBI.

Discussion

This study revealed that a higher busulfan dose intensity (FLUBU4) prolonged DFS in CBT compared to the intermediate intensity (FLU/BU2), mainly through a reduction in relapse without an increase in NRM. A previous nationwide retrospective analysis of BU2 and BU4 in BMT/PBSCT using the Japanese registry data of patients with AML, acute lymphoblastic leukemia (ALL), and myelodysplastic syndrome (MDS) showed that BU4 was associated with a lower incidence of relapse; however, no significant differences were found in OS or DFS because of higher NRM [10]. Other studies suggested that a higher busulfan dose intensity increased NRM in BMT/PBSCT [7, 9]. In the present study on CBT, no significant increase was observed in NRM with higher doses of busulfan, which resulted in better outcomes. In comparisons with previous findings on BMT/PBSCT in Japanese patients, these results appear to be characteristic of busulfan dose intensities in CBT. The results of the subgroup analysis suggested that this impact was more prominent in non-CR patients and patients younger than 60 years. Therefore, a higher busulfan dose intensity is recommended in CBT, particularly for non-CR or younger patients. These results appear to be reasonable because sufficient cytotoxic activity by busulfan is required, particularly for non-CR patients, and the toxicity of a high busulfan dose intensity is reduced in younger patients. Furthermore, in the older than 60 years group, outcomes did not significantly differ between the BU2 and BU4 groups

and a high busulfan dose intensity was tolerated. Therefore, the amount of busulfan in CBT does not need to be reduced for elderly patients.

Regarding GVHD, in the univariate analysis in the present study, the cumulative incidence of chronic GVHD was significantly higher in the BU4 group. In the multivariate analysis, a higher busulfan dose intensity correlated with higher acute GVHD (grades II-IV) and higher chronic GVHD. In a previous nationwide study of Japanese patients with AML, ALL, and MDS in BM/PMSCT, a higher busulfan dose intensity was associated with the incidence of acute GVHD (II-IV and III- IV) [10]. These findings are consistent with the present results in that a higher dose of busulfan was associated with the incidence of GVHD, whereas the increased frequency of chronic GVHD appeared to be characteristic of CBT. In CBT, conditioning intensity reportedly correlates with the incidence of chronic GVHD [21], and the present results are in accordance with these findings. A correlation has been reported between the area under the plasma concentration versus time curve for busulfan and the incidence of acute GVHD [22]. Similar to TBI, busulfan-induced mucosal injury and inflammatory cytokines may be involved in the pathogenesis of GVHD [23]. Previous studies revealed that the incidence of GVHD in CBT was associated with a better prognosis because of a lower relapse rate [24, 25]. These effects of GVHD have been attributed to graft-versus-

leukemia (GVL) [26, 27, 28], and GVHD caused by a higher busulfan dose intensity may lead to longer DFS in CBT. Multivariate analysis results show that tacrolimus significantly suppresses acute GVHD, which is also consistent with previous reports. [29]

In terms of engraftment, no significant differences were found in the cumulative incidence of neutrophil and platelet engraftment between the BU2 and BU4 groups. Limited information is currently available on engraftment rates in the FLU/BU regimen [30, 31, 32, 33], and we clarified the engraftment rate in FLU/BU in this larger study. In comparisons with previous studies [30,31,33], the higher engraftment rate of the FLU/BU regimen in the present study may be due to the large number of cases treated with TBI. We performed a subgroup analysis of engraftment rates in patients treated with and without TBI, and showed that the impact of the busulfan dosage was not significant regardless of TBI.

In consideration of previous findings [34], engraftment rates with FLU/BU conditioning do not appear to markedly differ from those with other reduced intensity regimens, and the cumulative incidence of engraftment did not significantly differ with the dose intensity of busulfan.

In multivariate analysis, low-dose TBI increased the hazards for grade 3-4 GVHD, and was associated with lower relapse and better survival. We calculated the

interaction between TBI usage/dosage and busulfan dosage, and very weak interaction was detected (*P* for interaction 0.18) (Table S1).. Subgroup analyses for patients with low-dose TBI indicated the similar results, while the statistical models for no-TBI group were without robustness due to the small number of patients (Figure S2, Table S2). Prognostic impacts of busulfan dosage and TBI usage/dosage should be validated in the future.

There are a number of limitations that need to be addressed. This was a retrospective cohort study on patients with heterogeneous backgrounds. Although we performed a multivariate analysis and subgroup analysis, confounding factors may have influenced outcomes. Moreover, the reason for the selection of BU2 or BU4 doses remains unclear, and although we adjusted for performance status and HCT-CI in the multivariate analysis, unmeasured confounding factors related to the status of patients may have influenced the selection of busulfan doses. For example, minimal residual disease information is not included in this study. The pharmacokinetic-guided dosing of busulfan has been shown to reduce the risk of toxicity and GVHD and improve outcomes [35, 36, 37]; however, pharmacokinetic data on busulfan were not available in the present study. Therefore, further studies that include the pharmacokinetic-guided dosing of busulfan are needed.

In conclusion, the present results recommend a higher busulfan dosage in CBT, particularly for non-CR or younger AML patients. Regarding a higher dose of busulfan, the development of GVHD needs to be considered; however, its GVL effect may be associated with better outcomes. Furthermore, the dose intensity of busulfan was not associated with outcomes in patients older than 60 years, and there did not appear to be any need to reduce the amount of busulfan in CBT for elderly patients. Therefore, the dosage of busulfan needs to be increased based on the disease status.

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Conflict of interest

N.U. received honoraria from Chugai Pharmaceutical Co., Ltd, Astellas Pharma Inc, Otsuka Pharmaceutical Co., Ltd, Sumitomo Dainippon Pharma Co., Ltd, and Novartis Pharma Inc. K.N. received research funding from Kyowa Kirin Co. Y.Atsuta received honoraria from Meiji Seika Pharma Co, Ltd, JCR Pharmaceuticals Co., Ltd, Novartis Pharma KK, Kyowa Kirin Co., Ltd, AbbVie GK, and Astellas Pharma Inc. The other authors declare that they have no conflict of interest.

Author contributions

Conceptualization and design of the study: S.S. and Y.Arai, Analysis and interpretation of data: T.Kondo., S.Mizuno, K.H., S.Miyakoshi, N.U., Y.M., T.E., Y.K., K.M., K.N., S.T., N.D., M.I., K.N., T.Kawakita., J.T., T.F, T.Atsuta, and M.Y., Drafting or revising the manuscript; S.S. and Y.Arai. All authors have approved the final article.

Figure legends

Figure 1. Transplantation outcomes in a univariate analysis of BU2 and BU4 groups. Kaplan–Meier curves for overall survival (a) and disease-free survival (b). Cumulative incidence of relapse (c) and non-relapse mortality (d). *P*-values were calculated in the univariate analysis.

Figure 2. Incidence of GVHD in a univariate analysis of BU2 and BU4 groups. The cumulative incidence of grade II-IV acute GVHD (a), grade III-IV acute GVHD (b), and extensive chronic GVHD (c). *P*-values were calculated in the univariate analysis.

Figure 3. Engraftment rate in CBT between BU2 and BU4 groups. The cumulative incidence of neutrophil engraftment (a) and platelet engraftment (b). *P*-values were calculated in the univariate analysis.

Figure 4. Engraftment rates in CBT between BU2 and BU4 groups with/without TBI. The cumulative incidence of neutrophil engraftment with TBI (a) and without TBI (c) and platelet engraftment with TBI (b) and without TBI (d). *P*-values were calculated in the univariate analysis.

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