Impact of different fludarabine doses in the fludarabine-based conditioning regimen for unrelated bone marrow transplantation

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Abstract

To compare the effect of fludarabine (Flu) dose, the clinical outcomes of patients who received Flu and busulfan (FB; \( n = 1647 \)) or melphalan (Flu with melphalan (FM); \( n = 1162 \)) conditioning for unrelated bone marrow transplantation were retrospectively analyzed using Japanese nationwide registry data. In the FB group, high-dose Flu (180 mg/m\(^2\); HFB) and low-dose Flu (150/125 mg/m\(^2\); LFB) were given to 1334 and 313 patients, respectively. The three-year overall survival (OS) rates were significantly higher in the HFB group than in the LFB group (49.5% vs. 39.2%, \( p < 0.001 \)). In the HFB and LFB groups, the cumulative incidences were 30.4% and 36.6% (\( p = 0.058 \)) for three-year relapse. In the multivariate analysis for OS and relapse, Flu dose was identified as an independent prognostic factor (hazard ratio: 0.83, \( p = 0.03 \); hazard ratio: 0.80, \( p = 0.043 \)). In the FM group, high-dose Flu (180 mg/m\(^2\); HFM) and low-dose Flu (150/125 mg/m\(^2\); LFM) were given to 118 and 1044 patients, respectively. The OS and relapse did not differ significantly between the HFM and LFM groups. These findings suggest that high-dose Flu was associated with favorable outcomes in the FB group but not in the FM group.

Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is a potentially curative therapy for patients with various hematological disorders [1]. Reduced-intensity conditioning (RIC) and myeloablative reduced-toxicity conditioning (RTC) regimens have been developed to reduce early non-relapse mortality (NRM) and complications associated with myeloablative conditioning (MAC) regimens. These regimens allow for the extension of HSCT to older and unsuitable patients who are unable to tolerate MAC regimens [2–4]. Although RIC and RTC regimens may increase the risk of relapse compared to MAC regimens, the graft-versus-tumor effect mediated by graft immune cells is expected [5].

Due to a lack of prospective data comparing RIC and RTC regimens, a wide variety of conditioning regimens are used by transplant centers worldwide. To date, the most commonly used RIC regimen in the literature is the combination of fludarabine (Flu) and low to intermediate doses of busulfan or melphalan [6]. However, Flu with high doses of busulfan (12.8 mg/kg intravenously), known as a MAC regimen, is also commonly used as an RTC regimen in elderly or frail patients [7–9].

The purine analog Flu is recognized as a potent immunosuppressive agent and plays a central role in RIC and RTC regimens due to its limited non-hematologic toxicities. It inhibits lymphocyte proliferation, induces apoptosis of hematologic malignant cells and has a synergistic tumor killing effect with alkylating agents such as busulfan and melphalan [10]. However, overdosage of Flu may result in increased infections, central nervous system toxicity, and severe side effects. Although previous reports have frequently shown Flu doses of 180 mg/m\(^2\) in the Flu with busulfan (FB) regimen and 125 mg/m\(^2\) in the Flu with melphalan (FM) regimen, the Flu doses vary across reports [3, 11–21]. In addition, these reports did not evaluate the impact of different Flu doses on clinical outcome or compare Flu doses to determine the optimal dose in RIC or RTC. In this study, to compare the effect of different Flu doses in RIC or RTC, the clinical outcomes of patients who received FB or FM conditioning were retrospectively
analyzed using nationwide registration data from the Japan Society for Hematopoietic Cell Transplantation [22, 23].

Patients and Methods

Study design

This study included patients aged 16 years or older with acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), myelodysplastic syndrome (MDS), chronic myeloid leukemia (CML), or malignant lymphoma (ML) who received either FB or FM conditioning regimens and underwent unrelated donor bone marrow transplantation between 2007 and 2016. However, patients with no survival data or who had received oral busulfan were excluded from this study. Patients with renal dysfunction in the Hematopoietic Cell Transplantation Comorbidity Index were also excluded because the Flu dose needs to be adjusted due to renal function. As a result, 2766 patients were included in this study. All transplantation data in this study were obtained from the Transplant Registry Unified Management Program (TRUMP), which included clinical data on HSCT performed in Japan [22, 23]. The study was approved by the Data Management Committee of TRUMP and the Institutional Review Board of the Japanese Red Cross Kyoto Daiichi Hospital.

Clinical outcomes and definitions

The primary endpoint of this study was overall survival (OS) after allo-HSCT. The secondary endpoints included relapse, NRM, acute graft-versus-host disease (GVHD), and chronic GVHD. OS was defined as the time from HSCT to death from any cause or the last follow-up. NRM was defined as death without relapse. Acute and chronic GVHD were graded according to previously published criteria [24, 25]. Chronic GVHD was evaluated in patients who survived more than 100 days without relapse. Standard risk was defined as the first or second complete remission of acute leukemia, the first or second chronic phase of CML, MDS, and the first or second complete remission of ML. In contrast, high risk was defined as any other malignancy status.

Statistical analysis

Fisher's exact test was used to compare differences in the distribution of clinical characteristics. The OS probabilities were calculated using the Kaplan–Meier method and compared using the log-rank test. The cumulative incidences of NRM, relapse, and GVHD were evaluated using Gray's method. In both univariate and multivariate analyses, the Cox proportional hazards model was used to assess the effect of confounding variables on OS. For NRM, relapse, and GVHD, Fine and Gray's proportional hazards model was used. In the competing risk models for NRM, relapse was defined as a competing risk, while in the competing risk models for relapse; NRM was defined as a competing risk. In the univariate analysis, factors with two-sided p-values of less than 0.10 were included in the multivariate analysis. A backward stepwise selection method was used, and only statistically significant variables were retained in the final model. A two-tailed p-value of less than 0.05 was considered statistically significant. The following
variables were included in these analyses: patient age at transplant (age ≥ 50 vs. age < 50), patient sex (male vs. female), disease type (AML vs. ALL vs. CML vs. MDS vs. ML), disease risk (low vs. high risk), Eastern Cooperative Oncology Group Performance Status (PS, 0–1 vs. 2–4), GVHD prophylaxis (cyclosporine-based vs. tacrolimus-based), use of total body irradiation (yes vs. no), use of in vivo T-cell depletion (yes vs. no), ABO compatibility (match vs. minor mismatch vs. major mismatch vs. major minor mismatch), sex mismatch (female to male vs. others), human leukocyte antigen (HLA) mismatch (none vs. one allele vs. more than one allele), Flu dose (180 mg/m² vs. 150 or 125 mg/m²), and busulfan or melphalan dose of the conditioning regimen (busulfan: 6.4 mg/kg vs. 12.8 g/kg, melphalan: 80 mg/m² vs. 140 mg/m²). Statistical analyses were performed using the EZR software package (Saitama Medical Center, Jichi Medical University), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria, Version 3.4.1) [26].

Results

Patient characteristics

The characteristics of patients receiving FB and FM conditioning regimens are summarized in Tables 1 and 2. In the FB group (n = 1647), high-dose Flu (180 mg/m²) with busulfan (HFB) and low-dose Flu (150/125 mg/m²) with busulfan (LFB) were given to 1334 and 313 patients, respectively (Table 1). The median age at transplantation was 59 years (range, 16–73) in the HFB group and 61 years (range, 16–75) in the LFB group. The LFB group had more patients with poor PS than the HFB group (12.5% vs. 7.0%, p < 0.01). The proportion of patients receiving 12.8 mg/kg of busulfan was significantly lower in the HFB group (54.4%) than in the LFB group (72.2%). In the FM group (n = 1119), high-dose Flu (180 mg/m²) with melphalan (HFM) and low-dose Flu (150/125 mg/m²) with melphalan (LFM) were given to 107 and 1012 patients, respectively (Table 2). The median age at transplantation was 52 years (range, 18–68) in the HFM group and 58 years (range, 17–72) in the LFM group. The HFM group had more patients with high disease risk than the LFM group (57.0% vs. 40.8%, p < 0.01). The proportion of patients receiving 140 mg/m² melphalan was significantly lower in the HFM group (57.0%) than in the LFM group (73.6%). When comparing the FB and FM groups, there was a difference in the ratio of patients receiving 180 mg/m² Flu and 150/125 mg/m² Flu.

Overall survival, relapse, non-relapse mortality, and graft-versus-host disease in the Flu with busulfan regimen group

After HSCT, survivors had a median follow-up of 1151 days (range, 27–3838). The OS probabilities three years after transplantation were 49.5% (95% confidence interval [CI]: 46.5–52.4%) in the HFB group and 39.2% (95% CI: 33.3–45.0%) in the LFB group (p < 0.001; Fig. 1A). In the multivariate analysis, high-dose Flu was identified as an independent prognostic factor for OS (hazard ratio: 0.83, 95% CI: 0.70–0.98, p = 0.03; Table 3). The cumulative incidences of relapse after three years were 30.4% (95% CI: 27.9–33.1%) in the HFB group and 36.6% (95% CI: 31.0–42.2%) in the LFB group (p = 0.058; Fig. 1B). In the multivariate
analysis, high-dose Flu was associated with a lower risk of relapse (hazard ratio: 0.80, 95% CI: 0.65–0.99, p = 0.043; Table 4). The cumulative incidences of NRM after three years did not differ significantly between the HFB and LFB groups (25.1%, 95% CI: 22.6–27.7 vs. 28.1%, 95% CI: 22.9–33.5%, p = 0.24; Fig. 1C). The cumulative incidences of acute GVHD Grades II–IV on Day 100, acute GVHD Grades III–IV on Day 100, and chronic GVHD on Day 365 were 37.7% (95% CI: 35.0–40.4%), 10.3% (95% CI: 9.1–12.2%), and 36.5% (95% CI: 34.2–39.3%) in the HFB group and 35.7% (95% CI: 30.1–40.8%), 12.9% (95% CI: 9.1–16.7%), and 31.1% (95% CI: 26.0–35.8%) in the LFB group, respectively (p = 0.66, 0.27, and 0.18, respectively; Figs. 1D–F).

Relapse by disease type in the Flu with busulfan regimen group

In the FB group, subgroup analysis of relapse by disease type revealed that the cumulative incidences of relapse after three years in patients with AML were 31.6% (95% CI: 28.1–35.3%) in the HFB group and 37.5% (95% CI: 30.5–44.5%) in the LFB group (p = 0.10; Supplementary Fig. 1A). Similarly, the cumulative incidences of relapse after three years did not differ significantly among patients with MDS, ALL, and ML in the HFB (28.3% [95% CI: 23.4–33.4%], 29.9% [95% CI: 19.0–41.6%], 31.9% [95% CI: 24.8–39.1%]) and LFB (36.8% [95% CI: 25.2–48.4%], 27.1% [95% CI: 5.2–56.3%], not reached) groups (p = 0.31 [Supplementary Fig. 1B], p = 0.77 [Supplementary Fig. 1C], p = 0.96 [Supplementary Fig. 1D]).

Overall survival, relapse, non-relapse mortality, and graft-versus-host disease in the Flu with melphalan regimen group

The OS probabilities three years after transplantation were 48.3% (95% CI: 37.9–58.0%) in the HFM group and 48.8% (95% CI: 45.5–52.1%) in the LFM group (p = 0.92; Fig. 2A). The cumulative incidences of relapse after three years were 23.7% (95% CI: 15.9–32.5%) in the HFM group and 27.2% (95% CI: 24.4–30.1%) in the LFM group (p = 0.55; Fig. 2B). The cumulative incidences of NRM after three years were 31.9% (95% CI: 22.9–41.3%) in the HFM group and 30.8% (95% CI: 27.8–33.9%) in the LFM group (p = 0.67; Fig. 2C). In terms of OS, relapse, and NRM, there were no statistically significant differences between the HFM and LFM groups. The cumulative incidences of acute GVHD Grades II–IV on Day 100, acute GVHD Grades III–IV on Day 100, and chronic GVHD on Day 365 were 40.2% (95% CI: 31.0–49.2%), 9.4% (95% CI: 5.1–16.0%), and 38.9% (95% CI: 29.0–48.1%) in the HFM group and 40.2% (95% CI: 37.2–43.8%), 16.1% (95% CI: 14.1–19.0%), and 29.3% (95% CI: 27.0–32.9%) in the LFM group, respectively (p = 0.87, 0.10, and 0.09, respectively; Figs. 2D–F).

Discussion

In this study, using Japanese registry data, we compared the outcomes of transplantation with different Flu doses in the FB and FM regimens. These regimens are widely used as RIC or RTC regimens for unrelated bone marrow transplantation. In this registry study, a significant difference in the proportion of patients receiving high-dose Flu was observed between the FB and FM groups. Our findings indicate that high-dose Flu was associated with favorable outcomes, particularly a lower relapse rate than low-dose
Flu, for unrelated bone marrow transplantation in the FB group. However, this association was not observed in the FM group.

Flu is a well-known, potent immunosuppressive agent that plays a central role in RIC and RTC regimens. In terms of the conditioning regimen with FB, the first retrospective study, conducted by Slavin et al., demonstrated the efficacy of Flu (180 mg/m$^2$) combined with busulfan in matched-related peripheral blood stem cell transplantation [3]. Many reports have been published since then, but Flu doses vary between 120 and 250 mg/m$^2$ [3, 12–18]. Surprisingly, there are no reports specifically addressing Flu doses or comparing the effects of different Flu doses in the setting of unrelated bone marrow transplantation. However, Geddes et al. conducted a study on the effects of high busulfan concentrations in combination with high-dose Flu (250 mg/m$^2$) and found no apparent increase in side effects [15]. In terms of the conditioning regimen with FM, Giralt et al. reported the results of initial studies using Flu (125 mg/m$^2$) in combination with melphalan for a variety of hematologic malignancies [27]. In other subsequent reports, the Flu doses in the FM regimen vary from 100 to 160 mg/m$^2$ [19–21]. Although there are many reports on different Flu doses, a Flu dose of 180 mg/m$^2$ has been more commonly used in the FB regimen, and Flu doses of 125–150 mg/m$^2$ have been used in the FM regimen, which are lower than those used in the FB regimen. In studies comparing FB to FM regimens, it is common for the FM group to receive a lower Flu dose [28–30]. Since none of the reports mention Flu doses, these dose decisions are probably influenced by the initial reports [3, 27]. In our study, as in previous reports, many cases in the FB group received a Flu dose of 180 mg/m$^2$, while many cases in the FM group received a Flu dose of 125 mg/m$^2$.

In our study, we observed a significantly lower recurrence rate in the Flu (180 mg/m$^2$) group of the FB group, but no difference was found in the FM group. However, the risk of relapse varies with the hematologic diagnosis, and this should be considered when interpreting these results. In the FB group, there was no significant difference in relapse rate based on the Flu dose across different diseases. This may be due to the small number of cases and differences in patient backgrounds. Therefore, further accumulation, and investigation of cases are necessary in the future.

Several reports have been conducted to investigate the relationship between F-Ara-A concentrations (the active component of fludarabine in the plasma) and transplantation outcomes. Flu dosing is currently based on body surface area, which can result in variable exposure to the drug. Langenhorst et al. conducted a pharmacokinetic-pharmacodynamic analysis on patients undergoing HSCT with Flu as part of a myeloablative conditioning regimen [31]. They found that event-free survival was lower in the Flu-overexposed group, owing to higher NRM associated with impaired immune reconstitution. Additionally, the risk of NRM, and graft failure was increased in the Flu-underexposed group. However, no association with relapse was found. In other reports, no significant associations were found between the degree of Flu exposure and relapse or survival in adult and pediatric HSCT [32, 33]. However, in a study of pediatric patients with hematologic diseases, maintaining adequate Flu blood concentration resulted in high disease-free survival without increasing transplant-related mortality [34]. In our study, Flu blood
concentration was not assessed, but a high Flu dose was associated with improved OS due to a lower relapse rate, with no apparent increase in NRM. While maintaining an adequate Flu blood concentration may lead to favorable outcomes, the concentration can fluctuate due to various factors such as renal function, age, and body weight. Therefore, further analysis is needed to determine individualized Flu dosing for each case in the future.

Previous reports have indicated that the FB regimen had a higher proportion of early mixed donor chimerism than the FM regimen [35, 36]. Although our study did not assess chimerism, we believe that the reason for the significantly different relapse rate in the FB group but not in the FM group is the different timing of achieving complete chimerism in the FM and FB groups. For instance, the FM group may experience an early transition to complete chimerism, which may reduce relapse rates associated with low-dose Flu.

This retrospective cohort study has some limitations. First, the proportions of patients receiving high-dose Flu differed significantly between the FB and FM groups, with more patients receiving HFB in the FB group and more patients receiving LFM in the FM group. Therefore, a direct comparison of the results between these two groups is not possible. Second, the heterogeneous patient backgrounds, such as disease type, PS, and total body irradiation use, may have introduced statistical bias, although we attempted to adjust for this by conducting multivariate analyses. Third, depending on the dose of busulfan or melphalan, there may be interactions with different effects of the dose of Flu, which we have not been able to assess. Finally, our registry did not collect detailed information such as Flu pharmacokinetic data or chimerism. If this information had been available, it would have been possible to assess the role of different Flu doses in HSCT in more detail.

In conclusion, our retrospective data analysis revealed that high-dose Flu was associated with a favorable outcome in the FB group but not in the FM group. Prospective studies and further investigation are needed to elucidate the benefit of high-dose Flu in the setting of the FB regimen.

Declarations

Disclosures: KS has received honoraria from Takeda Pharmaceutical Co. Ltd., Sanofi and Janssen Pharmaceutical K.K., outside the submitted work. YK received honoraria from Sanofi and research grant support from Otsuka Pharmaceutical Co. Ltd., outside the submitted work. The remaining authors declare no competing financial interests associated with this paper.

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Author contributions
KK and SF designed the study. KK analyzed the data and wrote the first draft of the manuscript. SF reviewed and revised the manuscript. All the other authors contributed to data collection. All authors approved the final version of manuscript.

Competing Interests

KS has received honoraria from Takeda Pharmaceutical Co. Ltd., Sanofi and Janssen Pharmaceutical K.K., outside the submitted work. YK received honoraria from Sanofi and research grant support from Otsuka Pharmaceutical Co. Ltd. The remaining authors declare no competing financial interests associated with this paper.

References


Tables

Tables 1 to 4 are available in the Supplementary Files section.

Figures

Figure 1

Transplant outcomes of the Flu with busulfan (FB) regimen group

(A) Overall survival; (B) relapse; (C) non-relapse mortality (NRM); (D) acute graft-versus-host disease (GVHD) Grades II–IV; (E) acute GVHD Grades III–IV; and (F) chronic GVHD.

GVHD, graft-versus-host disease; LFB, low-dose fludarabine (150/125 mg/m²) and busulfan; HFB, high-dose fludarabine (180 mg/m²) and busulfan

Figure 2
Transplant outcomes of the Flu with melphalan (FM) regimen group

(A) Overall survival; (B) relapse; (C) non-relapse mortality (NRM); (D) acute graft-versus-host disease (GVHD) Grades II–IV; (E) acute GVHD Grades III–IV; and (F) chronic GVHD.

GVHD, graft-versus-host disease; LFM, low-dose fludarabine (150/125 mg/m$^2$) and melphalan; HFM, high-dose fludarabine (180 mg/m$^2$) and melphalan

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- Flu.table1.xlsx
- Flu.table2.xlsx
- Flu.table3.xlsx
- Flu.table4.xlsx
- Flu.Figure.supplementary.pptx