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Fludarabine, busulfan, and melphalan conditioning regimen in allogeneic hematopoietic stem cell transplantation for adult patients with myeloid malignancies: A multicenter retrospective study

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Abstract

Relapse remains the main cause of treatment failure in patients with myeloid malignancies even after allogeneic hematopoietic stem cell transplantation (allo-HSCT). We observed a particularly low incidence of relapse in patients prepared with fludarabine, busulfan and melphalan in our previous study and this multicenter retrospective analysis aimed to confirm the feasibility of the regimen and to identify the potential prognostic factors. This study was performed using registry data from adults patients with myeloid malignancies who underwent their first allo-HSCT following fludarabine($\geq$100 mg/m^2), busulfan ($\geq$3.2 mg/kg) and melphalan ($\geq$100 mg/m^2) based conditioning at nine transplantation centers in China between Jan. 2020 and Mar. 2022. A total of 221 consecutive patients (AML $n = 171$, MDS-IB-1 or 2 $n = 44$,

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CMML $n = 6$) with median age of 46 were enrolled in this study. The median follow-up was 507 days for survivors. The 2-year NRM, CIR, OS and DFS were 10.6% ± 2.2%, 14.8% ± 3.3%, 79.4% ± 3.7% and 74.6% ± 3.7%, respectively. In multivariate analyses, high HCT-CI (≥3) was the only independent factor for higher NRM [hazard ratio (HR), 2.96; 95% confidence interval (CI), 1.11 to 7.90; $p = 0.030$] and ECOG score ≥2 was the only independent factor for inferior OS (HR, 2.43; 95%CI, 1.15 to 5.16; $p = 0.020$) and DFS (HR, 2.12; 95%CI, 1.13 to 4.02; $p = 0.020$). AML diagnosis and positive measurable residual disease (MRD) at transplantation were predictors for higher CIR (HR = 7.92, 95%CI 1.05-60.03, $p = 0.045$; HR = 3.64, 95%CI 1.40-9.44, $p = 0.008$; respectively), while post-transplantation cyclophosphamide based graft-versus-host disease prophylaxis was associated with lower CIR (HR = 0.24 95%CI 0.11-0.54, $p = 0.001$). The intensity of conditioning regimen did not impact CIR, NRM, DFS and OS. These results supported that double alkylating agents of busulfan and melphalan based conditioning regimens were associated with low relapse rate and acceptable NRM in adult patients with myeloid malignancies. The optimal dose remained to be confirmed by further prospective studies.

KEYWORDS
allogeneic hematopoietic stem cell transplantation, busulfan, melphalan, myeloid malignancies

1 | INTRODUCTION

Although the new targeting therapy has recently emerged, allogeneic hematopoietic stem cell transplantation (allo-HSCT) remains an important curative treatment for hematologic malignancies [1]. The pre-transplantation conditioning regimen plays a central role in allo-HSCT since it provides both tumor eradication and immunosuppressive effect for the engraftment of donor cells. The transplant conditioning intensity (TCI) is directly related to early morbidity, non-relapse mortality (NRM) and long-term disease control [2]. Fludarabine (Flu) in combination with 3–4 days standard dose (3.2 mg/kg/d) of intravenous busulfan (Bu) has become the most popular conditioning regimen in patients with myeloid malignancies, while relapse was the main obstacle for cure [3–5].

Melphalan (Mel) is an alkylating agent which is also used in allo-HSCT in acute myeloid leukemia (AML) and myelodysplasia syndrome (MDS) with potential lower incidence of relapse in reduced intensity setting [6, 7]. However, there are limited data of Mel in combination with other alkylating agents in the allo-HSCT setting for myeloid malignancies. In two previous studies, Mel (80–100 mg/m^2) was added to Flu/Bu4 as allo-HSCT conditioning in patients with AML, which showed a 2-year cumulative incidence of relapse (CIR) of 15%–20% with an NRM of 20%–25% [8, 9]. These results indicated that the combination of Bu and Mel may be feasible for the treatment of myeloid malignancies. In our previous single center phase II study (NCT04269811), a moderate intensity conditioning with Flu (150 mg/m^2), Bu (6.4 mg/kg), and Mel (100 or 140 mg/m^2) was used in patients with AML or MDS [10]. A particularly low incidence of relapse (1-year CIR of 2.1%) was observed with an NRM of 12%, and the 1-year disease-free survival

(DFS) reached as high as nearly 85%. Moreover, when we analyzed a larger cohort of 100 patients similarly treated and with longer follow-up, the 2-year CIR maintained at 5% with a similar NRM and the 2-year DFS was 82% [11].

To further confirm the efficacy and feasibility of the Flu/Bu/Mel conditioning, a multicenter retrospective study was performed based on the registry data of Dual Alkylating Conditioning Study Group of China (DACSG, China).

2 | PATIENTS AND METHODS

2.1 | Patients and eligibility criteria

This retrospective, observational study was performed using registry data from consecutive patients who underwent allo-HSCT following fludarabine, busulfan, and melphalan based conditioning at nine transplantation centers in China between January 2020 and March 2022. The last follow-up date was December 15, 2022. Informed consents were obtained from all subjects and the study protocol obtained approval from the institutional review board of DACSG, China.

The inclusion criteria were as follows: (1) adult patients (16–70 years old) with myeloid malignancies, including AML, MDS with increased blast-1 or -2 (MDS-IB-1 or MDS-IB-2) and chronic myelomonocytic leukemia (CMML); (2) patients received their first allo-HSCT from HLA matched sibling donors (MSDs), matched unrelated donors (MUDs), related haplo-identical donors (HIDs), or cord blood (CB); (3) the conditioning regimen included busulfan

(≥ 3.2 mg/kg), melphalan (≥ 100 mg/m^2), and fludarabine (≥ 100 mg/m^2). Cytarabine, etoposide, lomustine, or cyclophosphamide could be used in combination, while thiotepa and total body irradiation were excluded.

2.2 | Study endpoints and definitions

The primary endpoint of the study was DFS. Secondary endpoints included overall survival (OS), the cumulative incidence of NRM, relapse, grade II to IV acute graft-versus-host disease (aGVHD) and moderate to severe chronic GVHD (cGVHD).

Relapse was defined as > 5% blasts in bone marrow and/or extramedullary relapse documented by biopsy. Only patients with successful neutrophil engraftment were evaluated for aGVHD, and cGVHD was evaluated only in patients survived more than 100 days. Patients were considered to have died of NRM if there was no evidence of disease relapse or progression before death. OS was defined as the time from transplantation to death from any cause or to the date of the last follow-up in surviving patients. DFS was calculated from transplantation to the date of disease relapse or death from any cause. Acute GVHD grading was based on 1994 consensus conference criteria [12], while cGVHD was graded on the basis of the National Institutes of Health consensus conference criteria [13].

2.3 | Statistical analyses

The patients' baseline characteristics were reported descriptively. OS and DFS were estimated using the Kaplan–Meier method and compared by log-rank tests. The CIR and NRM were calculated using a competing-risk setting: for relapse, death without relapse was the competing-risk event; and for NRM, death from relapse was the competing-risk event. The difference between cumulative incidences in the presence of a competing risk was tested using the Gray method. The Cox proportional hazards model was used for univariate and multivariate analysis for OS and DFS. As for CIR and NRM, the Gray test was used for univariate analysis, and Fine–Gray proportional hazard regression for multivariate analysis. Potential prognostic factors considered in the univariate analysis included age, sex pair of recipient and donor, diagnosis, the disease risk index (DRI) for patients undergoing allo-HSCT [14], disease status at transplantation, measurable residual disease (MRD) at transplantation, TCI [15], donor type, method of GVHD prophylaxis, performance status (the Eastern Cooperative Oncology Group, ECOG score), and hematopoietic cell transplantation comorbidity index (HCT-CI). Only variables with a p-value < 0.10 determined by the univariate analysis were considered for entry into the multivariate analysis. Statistical analyses were performed by SPSS 17.0 and R 4.0.4 software at Shanghai Clinical Research Center.

3 | RESULTS

3.1 | Patients' characteristics

A total of 221 patients were enrolled in this study. The median follow-up was 507 days (range, 259–1,077 days) for survivors. Demographic data were shown in Table 1. The median age was 46 years (range, 16–67 years) and 125 (56.6%) patients were males. One hundred and seventy-one (77.4%) patients were diagnosed as AML with 123 cases in CR1. Based on DRI for patients undergoing allo-HSCT, 21, 96, and 54 patients were stratified into the low, intermediate, and high/very high risk groups accordingly [14]. Forty-four (19.9%) patients with MDS-IB-1 or IB-2 were enrolled in the study, which including 23 patients with DRI high or very high risk. Six patients with CMML were also enrolled with only two out of them achieving complete remission (CR) or complete remission with incomplete hematological recovery (CRi) at transplantation. All CMML patients were considered as having a high risk DRI. MRD was tested by multicolor flow cytometry (MFC) based on leukemia associated immunophenotype (LAIP) for almost all patients ($n = 217$), while specific fusion genes or mutations tested by polymerase chain reaction (PCR) or next generation sequencing (NGS) were only available in 68 patients. One hundred and thirteen patients were MRD positive at transplantation, of which 15 patients were tested positive by both MFC and PCR/NGS, 19 were positive only by PCR/NGS, and 79 were positive only by MFC. The donors included 49 (22.2%) from MSDs (including 1 from a real twin brother), 10 (4.5%) from MUDs, 158 (71.5%) from HIDs, and 4 (1.8%) from CB. As for GVHD prophylaxis, 183 (82.8%) patients received post-transplantation cyclophosphamide (PTCy) based strategy, including 50-100 mg/kg cyclophosphamide in combination with calcineurin inhibitors (CNI), mycophenolate mofetil (MMF), ruxolitinib, or low-dose anti-thymocyte globulin (ATG, less than 2.5 mg/kg). Other patients received ATG-based GVHD prophylaxis, which consisted of ATG at a total dose of 5–10 mg/kg before transplant with CNI, MMF, and methotrexate in combination.

Notably, 199 (90.0%) patients received conditioning only consisted of Flu, Bu, and Mel (FBuM) in different dose combinations. To clarify the impact of dose of these alkylating agents on the transplantation outcomes, we graded the overall intensity of conditioning regimen based on the EBMT TCI score system [15]. Briefly, regimens such as Flu150/Bu2/Mel140 or Flu150/Bu3/Mel100 with TCI score of 3.5 (comparable to Flu/Bu4) were considered as standard intensity, while those with TCI score of 2.5 or 3.0 (such as Flu150/Bu2/Mel100, Flu180/Bu1/Mel100, or Flu180/Bu2/Mel100) were considered as decreased intensity, and those with TCI score of 4.0 or higher (such as Flu150/Bu3/Mel140 or Flu150/Bu4/Mel100) were regarded as increased intensity. Cytarabine, cyclophosphamide, and lomustine were used adjunct to FBuM in 22 patients and were also scored depending on the dosage according to the EBMT TCI system. The proportions of patients who received increased, standard, or decreased intensity conditioning were 13.1%, 37.1%, and 49.8%, respectively.

TABLE 1 Patient characteristics.

Characteristics	Values, n (%)
All eligible patients	221
Age, median (range) years	46 (16–67)
Gender	
Male	125 (56.6%)
Female	96 (43.4%)
Diagnosis and disease status	
AML	171 (77.4%)
CR1	123 (55.7%)
≥CR2	32 (14.5%)
NR	16 (7.2%)
MRD+ at transplantation	93 (42.1%)
MRD− at transplantation	75 (33.9%)
MRD unavailable	3 (1.4%)
DRI-low risk	21 (9.5%)
DRI-intermediate risk	96 (43.4%)
DRI-high/very high risk	54 (31.6%)
MDS	44 (19.9%)
Untreated	19 (8.6%)
CR/Cri	13 (5.9%)
NR	12 (5.4%)
DRI-intermediate risk	21 (9.5%)
DRI-high/very high risk	23 (10.4%)
CMML	6 (2.7%)
Untreated	1 (0.4%)
CR/Cri	2 (0.9%)
NR	3 (1.3%)
Donor type	
MSD (1 homogeneic)	49 (22.2%)
MUD	10 (4.5%)
HID	158 (71.5%)
CB	4 (1.8)
GVHD prophylaxis	
No prophylaxis	1 (0.5%)
PTCy based	183 (82.8%)
ATG based	37 (16.7%)
TCI score	
2.5–3.0	110 (49.8%)
3.5	82 (37.1%)
≥4.0	29 (13.1%)
Performance status	
ECOG score 0–1	181 (81.9%)
ECOG score ≥2	40 (18.1%)
HCT-CI	
0–2	205 (92.8%)
≥3	16 (7.2%)

(Continues)

TABLE 1 (Continued)

Characteristics	Values, n (%)
Infused cell number	
MNC median (range)	$9.7 (3.4–28.5) \times 10^8$/kg
CD34+ median (range)	$7.5 (1.5–22.8) \times 10^6$/kg

Abbreviations: AML, Acute myeloid leukemia; ATG, anti-thymoglobuline; CB, cord blood; CMML, chronic myelomonocytic leukemia; CR, complete remission; CRi, complete remission with incomplete hematology recovery; DRI, disease risk index; GVHD, graft-versus-host disease; HID, haploidentical donor; MSD, matched sibling donor; MUD, matched unrelated donor; MDS, myelodysplastic syndrome; MRD, measurable residual disease; MNC, mononuclear cell; PTCy, post-transplantation cyclophosphamide; TCI, transplant conditioning intensity;.
NR, non-remission; ECOG PS score, the Eastern Cooperative Oncology Group performance status score; HCT-CI, the hematopoietic cell transplantation comorbidity index.

3.2 | Engraftment, chimerism, and graft-versus-host disease

Except four cases of CB transplantation, all the other patients received mobilized peripheral blood stem cells (PBSC) as graft with a median number of mononuclear cells and CD34+ cells infused of 9.1×10^8/kg (range, $3.4–28.6 \times 10^8$/kg) and 7.5×10^6/kg (range, $1.5–22.3 \times 10^6$/kg), respectively. Three patients who died before day14 were unevaluable for engraftment and primary engraft failure was documented in other two patients. Neutrophil engraftment occurred in 216/218 (99.1%) evaluable patients at a median of 13 days (range, 10–22 days). Besides, five patients died before platelet engraftment and delayed platelet reconstitution (recovery at more than 60 days post-transplantation) was documented in another five patients. The incidence of platelet reconstitution within 60 days was 208/218 (95.4%) at a median of 14 days (range, 9–51days). Chimerism analysis on day 28 showed that all engrafted patients achieved full donor chimerism (≥95%), while no donor signal could be detected in those two patients with primary graft failure.

The 100-day cumulative incidence of all grade aGVHD and II–IV aGVHD were 24.4 ± 2.9% and 14.5 ± 2.4%, respectively (Figure 1A,B). While the cumulative incidence of all cGVHD and moderate/severe cGVHD at 2 years were 30.2% ± 3.2% and 9.3% ± 2.0%, respectively (Figure 1C,D).

3.3 | Clinical outcomes

At last follow-up, NRM was documented in 22 patients and the causes were listed in Table 2. The most common reasons of NRM were infection related events. The 100-day and 2-year NRM were 5.4% ± 1.5% and 10.6% ± 2.2%, respectively (Figure 2A). Twenty-three patients experienced relapse and 11 of them were still alive at last follow-up, making the 2-year CIR at 14.8% ± 3.3% (Figure 2B). The 2-year OS and DFS were 79.4% ± 3.7% and 74.6% ± 3.7%, respectively (Figure 2C,D).
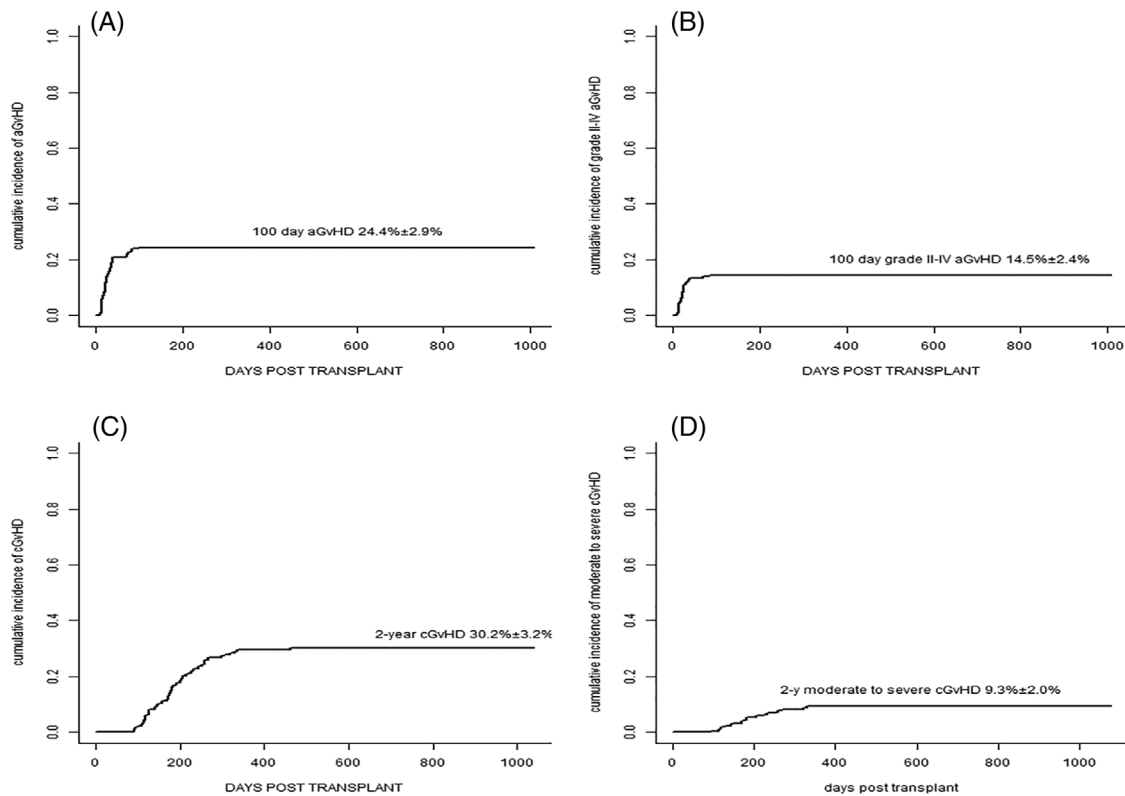
FIGURE 1 Acute and chronic GVHD after transplantation. (A) The cumulative incidence of all grade aGVHD, (B) the cumulative incidence of II–IV aGVHD, (C) the cumulative incidence of all cGVHD, and (D) the cumulative incidence of moderate/severe cGVHD. GVHD, Graft-versus-host disease.

TABLE 2 NRM and causes of death.

Cause of death	Description
Infection	$n = 12$
	Blood stream infection ($n = 5$)
	Blood stream infection with primary or secondary engraft failure ($n = 2$)
	Suppurative cholangitis ($n = 1$)
	Central nervous system infection ($n = 1$)
	Pneumonia with poor graft function ($n = 2$)
	Pneumonia with poor graft function and cerebral infarction ($n = 1$)
Cerebral hemorrhage	$n = 2$
Cardiovascular event	$n = 1$
SOS /TA-TMA/MOF	$n = 2$
Acute GVHD	$n = 3$
Unknown	$n = 2$

Abbreviations: MOF, multiple organ failure; NRM, non-relapse mortality; SOS, Sinusoidal obstruction syndrome; TA-TMA, transplantation associated thrombotic microangiopathy;.

3.4 | Univariate and multivariate analyses of factors associated with transplantation outcomes

To identify factors potentially associated with transplantation outcomes, a univariate analysis was carried out. It was shown that advanced disease at transplantation, high/very high risk of DRI, poor performance status (ECOG score ≥ 2) and high HCT-CI (≥ 3) were associated with increased NRM ($p = 0.009, 0.025, 0.020,$ and $0.002,$ respectively) and inferior OS ($p = 0.034, 0.014, 0.001,$ and $0.023,$ respectively). Moreover, advanced disease at transplantation and poor performance status were also associated with inferior DFS ($p < 0.001$ and 0.003) (Table 3). However, neither MRD at transplantation nor TCI were associated with OS and DFS. In the multivariate analysis, high HCT-CI (≥ 3) was the only independent factor for higher NRM (HR, 2.96; 95% CI, 1.11–7.90; $p = 0.030$), and ECOG score ≥ 2 was the only independent factor for inferior OS (HR, 2.43; 95% CI, 1.15–5.16; $p = 0.020$) and DFS (HR, 2.12; 95% CI, 1.13–4.02; $p = 0.020$), with marginal significance of disease status at transplantation for DFS (HR, 2.19; 95% CI, 0.99–4.88; $p = 0.054$) (Table 4).

Regarding CIR, age younger than 50 years, diagnosis of AML and ATG-based GVHD prophylaxis were significantly associated with a higher relapse rate in univariate analysis. MRD positive at transplantation was associated with a higher relapse rate without reaching statistical significance ($p = 0.063$) (Table 3). In multivariate analysis,
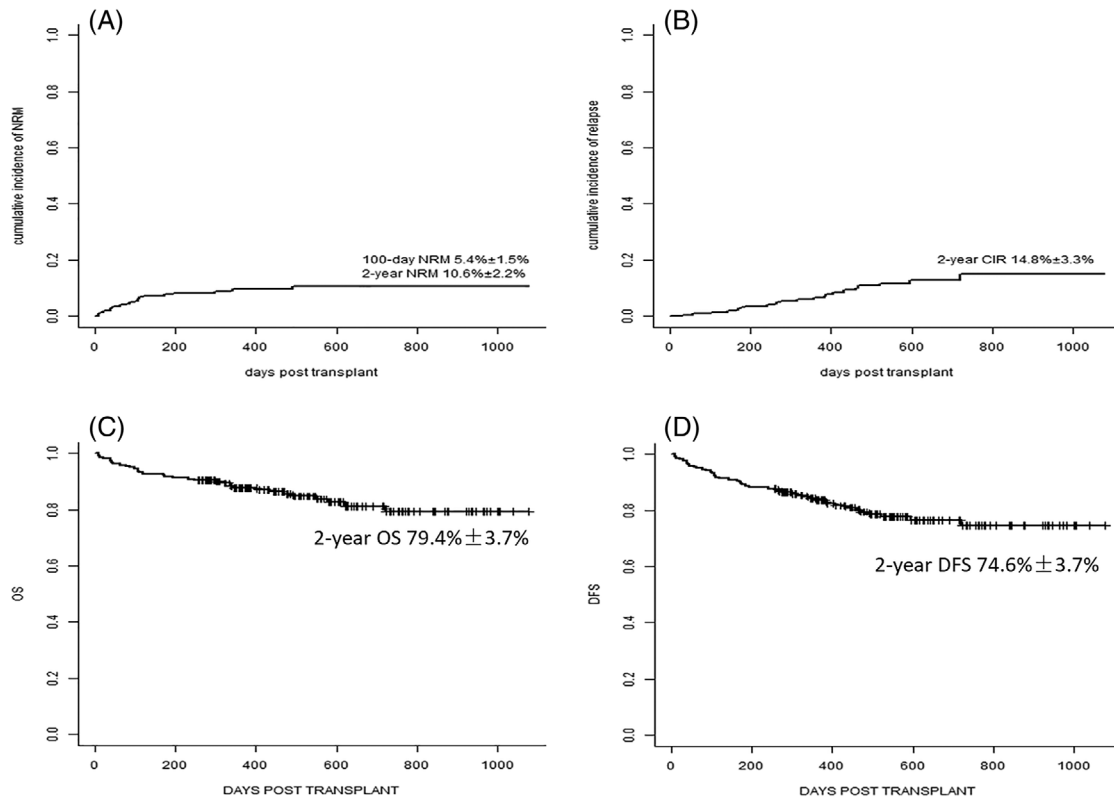
FIGURE 2 Clinical outcomes after transplantation. (A) Non-relapse mortality after transplantation, (B) the cumulative incidence of relapse after transplantation, (C) overall survival after transplantation, and (D) disease-free survival after transplantation.

AML diagnosis (HR, 7.92; 95% CI, 1.05–60.03; $p = 0.045$), and MRD positive at transplantation (HR, 3.64; 95% CI, 1.40–9.44; $p = 0.008$) were independent factors associated with a higher CIR, while PTCy-based GVHD prophylaxis was associated with lower incidence of relapse (HR, 0.24; 95% CI, 0.11–0.54; $p = 0.001$) (Table 4).

3.5 | Subgroup analyses

To test if conditioning intensity may influent outcomes of different populations, subgroup analyses were performed. Patients were stratified by age (< 50 and ≥50 years), diagnosis (AML and MSD/CMML), MRD at transplantation (positive and negative), and disease status at transplantation (any CR or untreated and NR). For patient ≥50 years old, the NRM tends to increase numerically with higher TCI score, but there was no statistical difference due to limited number of patients (only nine patients received conditioning with TCI ≥ 4.0). For MRD^{pos} patients, the NRM, CIR, OS, and DFS were not different in patients received decreased, standard, or increased intensity conditioning. While for MRD^{neg} patients, those who received intensified conditioning seemed to have superior DFS which just reached a statistical difference ($P = 0.05$), but there were no difference in NRM, CIR, and OS among groups (Table 5). For patients with NR at transplantation, the 1-year CIR was statistically significant different ($p = 0.03$) among patients receiving conditioning with different intensity, but notably, the patient samples were limited in these subgroups (14, 10,

and 7 patients received decreased, standard and increased intensity conditioning, respectively). All other subgroup analyses showed no statistical significance (Table S1).

4 | DISCUSSION

Intensification of the preparative regimen is one way to attempt a better control of leukemia after transplantation. Alkylating agent, such as thiotepa, has been added to modify the standard Flu/Bu conditioning (TBF regimen), showing that it was feasible in myeloid malignancies such as AML, MDS, and myelofibrosis [16–18]. As compared to the standard Flu/Bu, TBF was associated with significantly lower relapse with or without detrimental effect on NRM [16, 17]. Likewise, we previously conducted a single-center phase II trial, in which we combined 100–140 mg/m^2 Mel to Flu and Bu for patients with AML/MDS undergoing allo-HSCT. Low CIR around 5% and high DFS over 80% were achieved [10, 11]. And in this multicenter retrospective study, we confirmed the promising outcomes of FBuM conditionings with 2-year CIR of 15% and DFS of 75%.

In the setting of the TBF regimen, the respective dose of thiotepa and Bu appeared to have different importance: higher dose of thiotepa (10 mg/kg vs. 5 mg/kg) and reduced dose of busulfan (6.4 mg/kg vs.9.6 mg/kg) were associated with lower relapse and reduced NRM rates respectively [14, 15]. While it was markedly different in our study. First, all engrafted patients in our study achieved a complete donor

TABLE 3 Uni-variate analyses of factors associated with transplantation outcomes.

	2y-CIR	2y-NRM	2y-DFS	2y-OS
Age				
< 50 $n = 145$	18.8 ± 4.3%	9.7 ± 2.6%	71.5 ± 4.7%	78.3 ± 4.6%
≥50 $n = 76$	5.6 ± 3.4%	12.2 ± 3.9%	82.2 ± 4.9%	83.0 ± 5.0%
P value	0.033	0.482	0.961	0.308
Sex pair of recipient and donor				
Male–female $n = 42$	14.3 ± 3.7%	10.5 ± 2.4%	75.2 ± 4.2%	82.4 ± 3.7%
Others $n = 175$	16.9 ± 6.7%	11.6 ± 5.7%	71.5 ± 8.0%	67.1 ± 10.2%
P value	0.379	0.841	0.638	0.316
Diagnosis				
AML $n = 171$	18.3 ± 4.2%	8.4 ± 2.2%	73.3 ± 4.4%	81.0 ± 3.8%
MDS/CMML $n = 50$	3.0 ± 3.0%	17.6 ± 5.9%	79.4 ± 6.3%	74.9 ± 8.9%
P value	0.024	0.116	0.633	0.591
DRI				
Low $n = 21$	19.2 ± 10.9%	4.8 ± 4.8%	76.0 ± 11.0%	90.5 ± 6.4%
Intermediate $n = 117$	12.0 ± 3.6%	6.2 ± 2.3%	81.7 ± 4.7%	81.7 ± 4.7%
High/very high $n = 83$	17.7 ± 7.4%	18.4 ± 4.6%	64.0 ± 7.9%	72.0 ± 7.7%
P value	0.845	0.025	0.058	0.014
Disease status at transplantation				
Any CR or untreated $n = 190$	12.7 ± 3.0%	8.1 ± 2.0%	79.2 ± 3.4%	81.3 ± 3.8%
NR $n = 31$	34.0 ± 2.1%	25.6 ± 9.1%	40.5 ± 17.8%	68.6 ± 9.9%
	0.151	0.009	<0.001	0.034
MRD at transplantation				
Negative $n = 104$	8.5 ± 3.6%	9.8 ± 3.0%	81.8.0 ± 4.4%	82.7 ± 4.8%
Positive $n = 113$	19.5 ± 5.2%	11.5 ± 3.2%	69.1 ± 5.6%	77.3 ± 5.3%
	0.063	0.858	0.134	0.652
TCI				
2.5–3.0 $n = 110$	12.5 ± 4.2%	12.1 ± 3.4%	75.4 ± 5.1%	80.8 ± 4.5%
3.5 $n = 82$	19.9 ± 6.1%	10.0 ± 3.4%	70.1 ± 6.5%	75.3 ± 6.8%
≥4.0 $n = 29$	7.0 ± 4.9%	6.9 ± 4.8%	86.1 ± 6.5%	87.6 ± 6.9%
P value	0.415	0.827	0.575	0.783
Donor type				
MSD $n = 49$	16.4 ± 5.8%	8.4 ± 4.1%	77.0 ± 6.6%	87.6 ± 4.8%
MUD $n = 10$	20.0 ± 13.5%	10.0 ± 10.0%	70.0 ± 14.5%	80.0 ± 12.6%
HID $n = 149$	15.4 ± 4.5%	11.5 ± 2.7%	73.1 ± 5.0%	75.8 ± 5.1%
CB $n = 4$	NA*	NA*	NA*	NA*
P value	0.599	0.872	0.673	0.705
Method of GVHD prophylaxis				
PTCy based $n = 183$	11.2 ± 2.8%	10.5 ± 2.4%	78.3 ± 3.5%	79.9 ± 3.9%
ATG-based $n = 37$	38.8 ± 18.1%	11.2 ± 5.4%	50.0 ± 16.0%	75.7 ± 9.8%
P value	0.048	0.836	0.113	0.762
Performance status				
ECOG 0–1 $n = 181$	13.3 ± 3.7%	8.1 ± 6.6%	78.6 ± 4.0%	84.1 ± 3.8%
ECOG≥2 $n = 40$	20.9 ± 7.2%	21.6 ± 7.2%	57.5 ± 8.7%	60.3 ± 9.2%
P value	0.14	0.020	0.003	0.001

(Continues)

TABLE 3 (Continued)

	2y-CIR	2y-NRM	2y-DFS	2y-OS
HCT-CI				
0–2 $n = 205$	15.8 ± 3.5%	8.8 ± 2.1%	75.4 ± 3.8%	80.5 ± 3.8%
≥3 $n = 16$	0%	35.7 ± 14.4%	64.3 ± 13.6%	64.3 ± 13.6%
P value	0.2	0.002	0.129	0.023

Abbreviations: AML, Acute myeloid leukemia; ATG, anti-thymoglobuline; CMML, chronic myelomonocytic leukemia; CB, cord blood; CIR, cumulative incidence of relapse; CR, complete remission; DFS, disease-free survival DRI, disease risk index; ECOG PS score, the Eastern Cooperative Oncology Group performance status score; GVHD, graft-versus-host disease; HID, haplo-identical donor; HCT-CI, the hematopoietic cell transplantation comorbidity index; MDS, myelodysplastic syndrome; MSD, matched sibling donor; MNC, mononuclear cell; MUD, matched unrelated donor; NRM, non-relapse mortality;NR, non-remission;OS, overall survival; PTCy, post-transplantation cyclophosphamide; TCI, transplant conditioning intensity.
*NA, not applicable because of no patients survived more than 2 years at last follow-up.

TABLE 4 Multivariate analyses for factors associated with transplantation outcomes.

	HR(95% CI)	P value
CIR		
Age	0.29(0.08–1.02)	0.053
Diagnosis	7.92(1.05–60.03)	0.045
MRD at transplantation	3.64(1.40–9.44)	0.008
PTCy based GVHD prophylaxis	0.24(0.11–0.54)	0.001
NRM		
DRI	1.78(0.67–4.69)	0.244
Disease status	1.84(0.60–5.64)	0.289
ECOG	1.65(0.71–3.84)	0.239
HCT-CI	2.96(1.11–7.90)	0.030
DFS		
DRI	1.15(0.65–2.03)	0.626
Disease status	2.19(0.99–4.88)	0.054
ECOG	2.12(1.13–4.02)	0.020
OS		
DRI	1.27(0.66–2.44)	0.469
Disease status	1.63(0.64–4.12)	0.302
ECOG	2.43(1.15–5.16)	0.020
HCT-CI	1.76(0.64–4.88)	0.276

Abbreviations: DRI, disease risk index; GVHD, graft-versus-host disease; PTCy, post-transplantation cyclophosphamide; ECOG PS score, the Eastern Cooperative Oncology Group performance status score; HCT-CI, the hematopoietic cell transplantation comorbidity index; NRM, non-relapse mortality; CIR, cumulative incidence of relapse; OS, overall survival; DFS, disease-free survival; MRD, measurable residual disease.

T-cell chimerism at day 28 whatever the TCI score, suggesting the myeloablative feature of the FBuM regimen. Second, the intensity of the FBuM regimen in terms of TCI was not associated with transplantation outcomes such as CIR, NRM, OS, or DFS. Even in the subgroup analysis, we only found some inexact correlations: MRD[neg] patients with intensified conditioning seemed to have superior DFS (P = 0.05), the 1-year CIR was statistically significant different (p = 0.03) among

patients with NR at transplantation receiving different conditioning and the NRM tended to increase numerically with higher TCI score in patients over 50 years old, but no statistically different. There were two possible explanations for our observation. First, the limited number of patients in different TCI groups resulted in a lack of power to detect the potential significance. Second, in this retrospective study, the effect of selection bias could not be ruled out since the conditioning intensity was determined by transplantation physicians and possibly adjusted by patients' age, performance status and/or disease status before transplantation. Thus, the prospective study with sufficient number of patients is required to determine the dose impact of busulfan and/or melphalan.

Our observation was also different from the BMT CTN0901 study which demonstrated that myeloablative conditioning (MAC) in AML patients with genomic evidence of MRD at the time of allo-HSCT resulted in lower relapse and improved survival [19]. Of note, there were significant differences between BMT CTN0901 and our current study. First, the method of MRD analysis was different (mostly MFC in our retrospective analysis and genomic in BMT CTN0901). Second, the conditioning regimens used in the studies were quite different. In the BMT CTN0901 study, the conditioning used in the control group including Flu/Bu2 or Flu/Mel with TCI 1.5-2.0, which was lower than our study (TCI 2.5-3.0). These two factors might contribute to the different results between our study and BMT CTN0901.

The NRM in this study was only about 10%. However, patients enrolled in this study were relatively young (median age 46 years old) and most patients had good performance status (over 80% of patients with ECOG < 2) with few comorbidity (more than 90% patients with HCT-CI < 2). All these may contribute to the remarkably low incidence of NRM, since high HCT-CI (≥3) was the only independent factor for higher NRM, and ECOG score ≥2 was the only independent factor for inferior OS and DFS in multivariate analysis.

It was generally assumed that disease status at transplantation, including hematologic remission or not, MRD status and DRI, would significantly influence relapse rate after transplantation, or even further affect DFS or OS [14, 20–22]. Herein, MRD positive at transplantation was confirmed as an independent factor predicting higher CIR in multivariate analyses as reported in other studies [21, 22], while morphologic NR and high-risk DRI were recognized as

TABLE 5 Effects of TCI on transplantation outcomes: subgroup analyses.

	2y-CIR	2y-NRM	2y-DFS	2y-OS
Age < 50 n = 145				
TCI 2.5–3.0 n = 67	17.6 ± 6.1%	13.5 ± 4.7%	68.9 ± 7.0%	77.2 ± 6.2%
TCI 3.5 n = 58	23.1 ± 7.9%	8.7 ± 3.7%	68.2 ± 8.1%	74.1 ± 8.6%
TCI ≥4.0 n = 20	10.0 ± 6.9%	0.0%	90.0 ± 6.7%	92.3 ± 7.4%
P value	0.72	0.27	0.42	0.42
Age ≥50 n = 76				
TCI 2.5–3.0 n = 43	2.8 ± 2.8%	9.3 ± 4.5%	87.9 ± 5.1%	87.9 ± 5.1%
TCI 3.5 n = 24	10.5 ± 7.5%	13.4 ± 7.5%	76.3 ± 9.5%	78.7 ± 10.0%
TCI ≥4.0 n = 9	0.0%	22.2 ± 14.8%	77.8 ± 13.9%	77.8 ± 13.9%
P value	0.49	0.53	0.54	0.64
MRD positive at transplantation n = 113				
TCI 2.5–3.0 n = 56	16.2 ± 6.7%	14.6 ± 5.4%	69.3 ± 7.8%	81.3 ± 5.9%
TCI 3.5 n = 46	21.3 ± 7.9%	6.6 ± 3.8%	71.5 ± 8.2%	77.6 ± 8.4%
TCI ≥4.0 n = 11	NA*	NA*	NA*	NA*
P value	0.57	0.37	0.34	0.33
MRD negative at transplantation n = 104				
TCI 2.5–3.0 n = 51	5.4 ± 3.8%	9.8 ± 4.2%	84.8 ± 5.4%	82.3 ± 6.9%
TCI 3.5 n = 35	18.3 ± 9.9%	14.6 ± 6.1%	67.1 ± 10.5%	73.4 ± 10.4%
TCI ≥4.0 n = 18	0.0%	0.0%	100%	100%
P value	0.19	0.25	0.05	0.11

Abbreviations:CIR, cumulative incidence of relapse; DFS, disease-free survival; MRD, measurable residual disease; NRM, non-relapse mortality;OS, overall survival; TCI, transplant conditioning intensity.
*NA, not applicable because of no patients survived more than 2-year at last follow-up.

unfavorable factors for NRM other than CIR. This was reasonable since those patients may be confronted with lethal non-relapse complications early after transplantation because of heavier leukemia burden and poorer performance status. These data suggested that increased intensity conditioning might not benefit patients with advanced disease. Optimization of effective leukemia debulking treatment before and/or maintenance therapy after transplant may play more important role in the setting for patients with advanced disease.

In initial studies of PTCy, the relapse rate had been reported as high as 50% [23, 24]. More recent studies showed a comparable relapse rate with PTCy and traditional CNI-based GVHD prophylaxis. Rashid et al. compared outcomes after Haplo-HSCT with PTCy-based GVHD prophylaxis (n = 336) versus MSD–HSCT with CNI-based GVHD prophylaxis (n = 869) in patients with AML in CR1, and there was no difference in the rate of relapse between the two groups (HR, 0.88; 95% CI, 0.70–1.10; p = 0.27) [25]. Moreover, in the BMT CTN 1301 trial comparing cGVHD and relapse-free survival in the setting of allo-HSCT with CD34-selected PBSC, PTCy after a bone marrow (BM) graft, or tacrolimus and methotrexate after BM graft, PTCy was associated with a trend toward lower disease relapse (HR, 0.52; 0.28–0.96; P = 0.037) in patients with AML and MDS [26]. In this study, we also demonstrated that PTCy was associated with a lower incidence of relapse, which may suggest that PTCy was a feasible GVHD prophylaxis in the setting of

dual alkylating agent conditioning regimen such as FBuM. However, because of the heterogeneity of patients' characteristics in our series, further study was warranted to confirm our findings.

In summary, we analyzed the transplantation outcomes after FBuM-based conditioning regimens in patients with myeloid malignancies and recognized prognostic factors associated with NRM, CIR, OS, and DFS. While, limited by its retrospective nature of the study, relatively small-sample size in each subgroup for comparison and the absence of control, the optimal dose combination of the FBuM regimen for suitable subpopulations could not be determined. Nevertheless, this study verified that the FBuM-based regimens were feasible for myeloid malignancies, which was associated with low relapse rate, acceptable NRM, and encouraging DFS. Randomized controlled clinical trial with well-designed grouping should be warranted to further evaluate these regimens.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author Jiong Hu (hj10709@rjh.com.cn) upon reasonable request.

ETHICS STATEMENT

The authors have confirmed ethical approval statement is not needed for this submission.

PATIENT CONSENT STATEMENT

The authors have confirmed patient consent statement is not needed for this submission.

CLINICAL TRIAL REGISTRATION

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REFERENCES

1. Dohner H, Weisdorf DJ, Bloomfield CD. Acute myeloid leukemia. N Engl J Med. 2015;373(12):1136–52.
2. Vriesendorp HM. Aims of conditioning. Exp Hematol. 2003;31:844–54.
3. Ben-Barouch S, Cohen O, Vidal L, Avivi I, Ram R. Busulfan fludarabine vs. busulfan cyclophosphamide as a preparative regimen before allogeneic hematopoietic cell transplantation: systematic review and meta-analysis. Bone Marrow Transplant. 2016;51(2):232–40.
4. Patel SS, Rybicki L, Pohlman B, Bolwell B, Gerds AT, Hamilton BK, et al. Comparative effectiveness of busulfan/cyclophosphamide versus busulfan/fludarabine myeloablative conditioning for allogeneic hematopoietic cell transplantation in acute myeloid leukemia and myelodysplastic syndrome. Hematol Oncol Stem Cell Ther. 2020;13(3):160–65.
5. Rambaldi A, Grassi A, Masciulli A, Boschini C, Micò MC, Busca A, et al. Busulfan plus cyclophosphamide versus busulfan plus fludarabine as a preparative regimen for allogeneic haemopoietic stem-cell transplantation in patients with acute myeloid leukaemia: an open-label, multicentre, randomized, phase 3 trial. Lancet Oncol. 2015;16(15):1525–36.
6. Valcarcel D, Martino R, Sureda A, Canals C, Altés A, Briones J, et al. Conventional versus reduced-intensity conditioning regimen for allogeneic stem cell transplantation in patients with hematological malignancies. Eur J Haematol. 2005;74(2):144–51.
7. Duque-Afonso J, Finke J, Labopin M, Craddock C, Protheroe R, Kottaridis P, et al. Comparison of fludarabine–melphalan and fludarabine–treosulfan as conditioning prior to allogeneic hematopoietic cell transplantation—a registry study on behalf of the EBMT Acute Leukemia Working Party. Bone Marrow Transplant. 2022;57(8):1269–76.
8. Yamamoto H, Uchida N, Yuasa M, Kageyama K, Ota H, Kaji D, et al. A novel reduced-toxicity myeloablative conditioning regimen using full-dose busulfan, fludarabine, and melphalan for single cord blood transplantation provides durable engraftment and remission in nonremission myeloid malignancies. Biol Blood Marrow Transplant. 2016;22(10):1844–50.
9. Ueda T, Maeda T, Kusakabe S, Fujita J, Fukushima K, Yokota T, et al. Addition of melphalan to fludarabine/busulfan (FLU/BU4/MEL) provides survival benefit for patients with myeloid malignancy following allogeneic bone-marrow transplantation/peripheral blood stem-cell transplantation. Int J Hematol. 2019;109(2):197–205.
10. Jiang JL, Chen M, Wang LN, Wan M, Gao WH, Wang L, et al. Double alkylators based conditioning reduced the relapse rate after allogeneic peripheral blood stem cell transplantation in adult patients with myeloid malignancies: a single arm phase II study. Bone Marrow Transplant. 2022;57(5):843–45.
11. Jiang JL, Gao WH, Wang LN, Wan M, Wang L, Hu J. Low incidence of relapse with a moderate conditioning regimen of fludarabine, busulfan, and melphalan for patients with myeloid malignances: a single-center analysis of 100 patients. Transplant Cell Ther. 2023;29(8):512.
12. Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J, et al. 1994 consensus conference on acute GVHD grading. Bone Marrow Transplant. 1995;15(6):825–28.
13. Jagasia MH, Greinix HT, Arora M, Williams KM, Wolff D, Cowen EW, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. The 2014 diagnosis and staging working group report. Biol Blood Marrow Transplant. 2015;21(3):389–401.
14. Armand P, Kim HT, Logan BR, Wang Z, Alyea EP, Kalaycio ME, et al. Validation and refinement of the Disease Risk Index for allogeneic stem cell transplantation. Blood. 2014;123(23):3664–71.
15. Spyridonidis A, Labopin M, Savani BN, Niittyvuopio R, Blaise D, Craddock C, et al. Redefining and measuring transplant conditioning intensity in current era: a study in acute myeloid leukemia patients. Bone Marrow Transplant. 2020;55(6):1114–25.
16. Saraceni F, Labopin M, Hamladji RM, Mufti G, Socié G, Shimoni A, et al. Thiotepa–busulfan–fludarabine compared to busulfan–fludarabine for sibling and unrelated donor transplant in acute myeloid leukemia in first remission. Oncotarget. 2017;9(3):3379–93.
17. Sora F, Grazia CD, Chiusolo P, Raiola AM, Bregante S, Mordini N, et al. Allogeneic hemopoietic stem cell transplants in patients with acute myeloid leukemia (AML) prepared with busulfan and fludarabine (BUFLU) or thiotepa, busulfan, and fludarabine (TBF): a retrospective study. Biol Blood Marrow Transplant. 2020;26(4): 698–703.
18. El-Cheikh J, Labopin M, Al-Chami F, Bazarbachi A, Angelucci E, Santarone S, et al. Effect of the thiotepa dose in the TBF conditioning regimen in patients undergoing allogeneic stem cell transplantation for acute myeloid leukemia in complete remission: a report from the EBMT Acute Leukemia Working Party. Clin Lymphoma Myeloma Leuk. 2020;20(5):296–304.

19. Hourigan CS, Dillon LW, Gui G, Logan BR, Fei M, Ghannam J, et al. Impact of conditioning intensity of allogeneic transplantation for acute myeloid leukemia with genomic evidence of residual disease. J Clin Oncol. 2020;38:1273–83.

20. Wang Y, Liu DH, Liu KY, Xu LP, Zhang XH, Han W, et al. Impact of pretransplantation risk factors on post transplantation outcome of patients with acute myeloid leukemia in remission after haploidentical hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2013;19(2):283–90.

21. Caballero-Velázquez T, Pérez-López O, Yeguas Bermejo A, Rodríguez Arbolí E, Colado Varela E, Sempere Talens A, et al. Prognostic value of measurable residual disease in patients with AML undergoing HSCT: a multicenter study. Cancers. 2023;15(5):1609.

22. Kim HJ, Kim Y, Kang D, Kim HS, Lee JM, Kim M, et al. Prognostic value of measurable residual disease monitoring by next-generation sequencing before and after allogeneic hematopoietic cell transplantation in acute myeloid leukemia. Blood Cancer J. 2021;11(6):109.

23. Luznik L, Bolaños-Meade J, Zahurak M, Chen AR, Smith BD, Brodsky R, et al. High-dose cyclophosphamide as single-agent, short-course prophylaxis of graft-versus-host disease. Blood. 2010;115(16):3224–30.

24. Luznik L, O'Donnell PV, Symons HJ, Chen AR, Leffell MS, Zahurak M, et al. HLA-haploidentical bone marrow transplantation for hematologic malignancies using nonmyeloablative conditioning and high-dose, posttransplantation cyclophosphamide. Biol Blood Marrow Transplant. 2008;14(6):641–50.

25. Rashidi A, Hamadani M, Zhang MJ, Wang HL, Abdel-Azim H, Aljurf M, et al. Outcomes of haploidentical vs. matched sibling transplantation for acute myeloid leukemia in first complete remission. Blood Adv. 2019;3(12):1826–36.

26. Luznik L, Pasquini MC, Logan B, Soiffer RJ, Wu J, Devine SM, et al. Randomized phase III BMT CTN trial of calcineurin inhibitor-free chronic graft-versus-host disease interventions in myeloablative hematopoietic cell transplantation for hematologic malignancies. J Clin Oncol. 2021;40(4):356–68.