MYELOABLATIVE BUFLU CONDITIONING IS WELL TOLERATED AND CAN ACHIEVE EVEN BETTER OUTCOMES IN HLA-MISMATCHED DONOR TRANSPLANTATION FOR INTERMEDIATE- AND HIGH-RISK AML PATIENTS IN COMPLETE REMISSION

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ABSTRACT

Hematopoietic stem cell transplantation (HSCT) is a primary treatment in acute myeloid leukaemia (AML). Achieving stable engraftment and minimizing regimen-related toxicity (RRT) significantly affects the transplant outcome. The conventional BuCy2 conditioning regimen is associated with high RRT. Alternative myeloablative regimens, such as BuFlu, are proven to be effective and well-tolerated in HLA-matched sibling transplantation. To evaluate the efficacy and toxicity of a myeloablative BuFlu conditioning regimen in HLA-mismatched donor transplantation, we retrospectively analysed 91 AML patients who underwent HSCT using a myeloablative BuFlu conditioning regimen during the period between March 2007 to June 2018. Of these patients, 56 received HLA-mismatched related donor (MMRD: n=56) transplantation and 35 received HLA-matched sibling (MRD: n=35) transplantation. All 91 patients achieved full engraftment. No patient died of RRT within 100 days after HSCT, and the 3-year incidence of non-relapse mortality was 7.9% and 9.9% for the MMRD and MRD groups, respectively (P=0.672). The cumulative incidence rates of grade II–IV acute and chronic graft versus host disease were 19.6% and 14.3% for the MMRD and MRD groups, respectively (P=0.471). The 3-year post-HSCT relapse rates were 15.3% and 33.9% for the MMRD and MRD groups, respectively (P=0.039). In the median follow-up time of 40 months (8-123 months), the 3-year overall survival (OS) rates were 80.6% (range, 75.0-86.2%) and 60.2% (range, 51.5–68.9%) (P=.030), and the 3-year disease-free survival (DFS) rates were 77.6% (range, 71.9-83.3%) and 58.5% (range, 49.9-67.1%) (P=.039) for the MMRD and MRD groups, respectively. In summary, this myeloablative BuFlu conditioning regimen resulted in high engraftment and limited toxicity in both MMRD and MRD transplantation. Furthermore, MMRD transplantation showed a lower relapse rate, which might be due to a strong graft-versus-leukaemia effect, and eventually produced better OS and DFS.

Keywords: *Fludarabine, acute myeloid leukaemia, HLA-haploidentical related donor transplantation, efficacy, toxicity.*

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Introduction

Hematopoietic stem cell transplantation is a primary treatment in AML. The conventional BuCy2 conditioning regimen is most frequently used for myeloablation. However, cyclophosphamide metabolites can result in severe toxicities and elevated non-relapse mortality. It is well established that Bu significantly increases the level of toxic CY metabolites by decreasing the level of glutathione in hepatocytes[1]. Fludarabine, a purine analogue with considerable anti-leukemic and immunosuppressive activities, can suppress the DNA ligase and primase to inhibit the repair of alkylator-induced DNA damage[2, 3]. Therefore, fludarabine has a synergistic effect with busulfan but no overlapping organ toxicities[4]. A growing body of evidence demonstrates that myeloablative BuFlu regimens are sufficiently immunosuppressive to facilitate engraftment from HLA-matched siblings, have lower transplant-related mortality than the BuCy regimen and still retain potent antileukemic activity. Also, a recent study revealed comparable regimen-related mortality (RRM) between the BuFlu regimen and the BuCy2 regimen after haploidentical HSCT[5-10]. However, myeloablative BuFlu regimens have not been thoroughly explored

in haploidentical transplantation settings[11]. Currently, the dose of fludarabine frequently used in a myeloablative regimen varies between 120 and 180 mg/m^2. It has been indicated that this regimen has minimal extramedullary toxicity below 250 mg/m^2[6, 9, 10, 12-14]. Since 2007, we have worked to develop a myeloablative BuFlu conditioning regimen derived from Lu et al.'s modified BuCy2 regimen (including cytarabine, busulfan and cyclophosphamide)[15], replacing cyclophosphamide with fludarabine[16]. Considering HLA-haploidentical HSCT present frequently with graft failure, especially when the conditioning regimen intensity is not sufficient, we used a relatively high dose of fludarabine: 40 mg/m2 per day for 5 days. In this analysis, we evaluated the efficacy and safety of this myeloablative regimen and compared transplantation outcomes, including engraftment, RRM, acute and chronic graft versus host disease (GVHD), relapse, overall survival (OS) and disease-free survival (DFS) rates between HLA-mismatched related donor (MMRD) and HLA-matched sibling (MRD) groups.

Patients and methods

A total of 91 AML patients in CR1 or CR2 who received allogeneic HSCT from HLA-mismatched related donors (MMRD: n=56) or HLA-matched siblings (MRD: n=35) between March 2007 and June 2018 were enrolled in this study at Peking University First Hospital. All transplantations were performed by the same medical staff. All patients or their guardians were informed of the risks and consented to transplantation. The eligibility criterion was a diagnosis of acute myeloid leukaemia in CR1 or CR2. Patients were classified as low risk, intermediate risk or high risk at the time of diagnosis, according to NCCN Clinical Practice Guidelines for Acute Myeloid Leukemia, version 1.2006. HCT-CI was graded as described in Sorror's study[17]. The standard for severe obesity was revised to >30 kg/m2[18]. Patient characteristics[19] are summarized in Table 1.

Conditioning regimen and GVHD prophylaxis

The transplantation conditioning regimen consisted of cytarabine (2 g/m^2 per day) administered via i.v. on days -10 to -7, busulfan administered orally (4 mg/kg per day) or via i.v. (3.2 mg/kg per day) on days -6 to -4, and fludarabine (40 mg/m^2 per day) administered via i.v. for 5 days (total, 200 mg/m^2) from days -8 to -4. In addition, 7.5 mg/kg (n=51.91%) or 10 mg/kg (n=5, 8.9%) ATG was administered to the MMRD group via i.v. in divided doses over 4 consecutive days, specifically on days -4 to -1. In the MRD group, 12 patients (34.3%) received 5 mg/kg ATG. GVHD prophylaxis treatment consisted of cyclosporine (CsA), a short course of methotrexate (MTX) and MMF. CsA (2.5 mg/kg, twice per day) was scheduled to be given via i.v. beginning on day -6 and continued orally when tolerable, with a target serum level of 150-250 ug/L. In standard-risk patients, the CsA dosage was reduced beginning on day +60 and discontinued on day +180 if no GVHD or disease progression was documented. MMF was administrated orally at a dosage of 7.5 mg/kg twice a day, from days -10 to +30. MTX was administered via i.v. at doses of 15 mg/m^2 on day +1 and 10 mg/m^2 on days +3, +6 and +11. Acute GVHD (aGVHD) and chronic GVHD (cGVHD) were defined by standard criteria. Acute GVHD was managed with methylprednisolone at a dosage of 1-2 mg/kg/day and resumption of full-dose CsA[20, 21]. If the patient was not responsive, second-line immunosuppressive therapy such as tacrolimus and daclizumab was administrated.

Variable	MMRD HSCT (N=56)	MRD HSCT (N=35)	P
Mean age, y (range)	33.30±13.90 (6-63)	36.91±10.39 (12-54)	0.161
Patient sex			0.063
Male	29	25	
Female	27	10	
Stem cell source			0.279
Allo-PBSCT	3	5	
Allo-BMT and Allo-PBSCT	53	30	
Diagnosis			
AML			0.535
M0	0	1	
M1	6	6	
M2	20	14	
M3	1	0	
M4	13	6	
M5	12	8	
M6	4	0	
Risk status			0.931
Intermediate risk	46	29	
High risk	10	6	
Status at transplantation AML			
CR1	54	31	0.301
CR2	2	4	
Mean number of infused MNCs*10^8/kg (range)	11.65±4.63 (4.21-29.30)	10.87±2.68 (6.72-18.08)	0.317
Mean number of CD34$^+$ cells*10^6/kg (range)	4.67±2.44 (1.25-16.00)	4.39±3.32 (0.97-17.10)	0.651
HCT-CI			1.000
0	41	27	
1	14	5	
2	1	3	

Table 1: Patient characteristics.
Abbreviations: AML=acute myeloid leukaemia; CR=complete remission; MNC=mononuclear cell.

Stem cell collection

Donor bone marrow (BM) and/or peripheral blood (PB) cells were harvested using the following mobilization protocols. Donors received 10 ug/kg/day rhG-CSF for 5-6 consecutive days before the stem cell collection. G-BM was harvested on day 0 and G-PB was harvested on days 1 and 2.

RRTs and causes of death

Regimen-related toxicities (RRTs) were scored using the Common Toxicity Criteria for Adverse Events, version 4.03, of the National Cancer Institute. Veno-occlusive disease (VOD) was diagnosed based on the presence of two of the three clinical manifestations described by Seattle criteria.

The severity of VOD was determined retrospectively by classifying the clinical outcome as mild, moderate or severe[22]. Death was categorized into two groups: relapse-related mortality and non-relapse mortality, including infectious complications, RRTs and GVHD[23].

Infection prevention and surveillance

All patients received diphenylhydantoin via i.v. to prevent busulfan-induced seizures, antifungal agents (voriconazole, 100 mg, twice daily via i.v. injection), pre-emptive anti-CMV therapy with ganciclovir (5 mg/kg/d, i.v.) and Prostaglandin E1 (0.5 ug/kg/d, i.v.) for VOD prophylaxis from the initiation of conditioning. Empiric broad-spectrum antibiotics were administered for neuropenic fever. Serum CMV and EBV-DNA levels were monitored weekly for 3 months post-transplantation.

Engraftment

Neutrophil engraftment was defined as the first of 3 consecutive days with an absolute neutrophil count>=$0.5*10^9$/l. Platelet engraftment was defined as the first of 7 consecutive days with a platelet count>=$20*10^9$/l without transfusion support. Chimerism was evaluated on days +30, +180 and +365 after HSCT by the short tandem repeat PCR (STR-PCR) or fluorescence in situ hybridization.

Follow-up

All patients were followed up with either during clinic visits or by telephone. The day of the stem cell infusion was defined as day 0.

The endpoint was June 30, 2018, or the date of death. The median follow-up time was 40 months (8-123 months). The total number of patients was 91. One patient was lost to follow-up at +6 months and other patients were lost at +8 months and +12 months. The incidence of loss to follow-up was 3%.

Donor leukocyte infusions (DLIs)

Patients who relapsed after allo-HSCT or who showed evidence of disease progression were candidates for a DLI. Pre-emptive DLIs were excluded. Eight patients in the MMRD group and 11 patients in the MRD group relapsed, and 17 of them received a DLI.

Statistical methods

Categorical variables were summarized as frequency counts and percentages, and continuous variables were summarized as means and standard deviations or medians and ranges. Differences between groups were assessed using the chi-square test or the Fisher exact test for a 2x2 table analysis, and a t-test or the Mann-Whitney U test was used for continuous variables.

Cumulative incidence, OS and DFS rates were estimated by the Kaplan-Meier method, and the log-rank test was used to analyse the differences between subgroups. All statistical analyses were performed using the SPSS19.0 software.

Results

Engraftment

In total, all 91 patients achieved hematopoietic reconstitution.

The median times to neutrophil engraftment were 12 (range, 9-29 days) and 13 days (range, 10-25 days) in the MMRD and MRD groups (P=0.385), respectively. The median times to platelet engraftment were 17.5 (range, 8–180 days) and 13.5 days (range, 6–90 days) in the MMRD and MRD groups (P=0.479), respectively.

All patients achieved full donor chimerism by day +30 post-transplantation.

RRTs

Both groups exhibited zero RRM at 100 days and the 100-day cumulative incidence of haemorrhagic cystitis (HC) was 27% and 13.2% in the MMRD and MRD groups, respectively (P=0.102).

The most common toxicity was self-limiting mucositis and transient liver enzyme elevation.

Only one case from the MMRD group was diagnosed with mild VOD at day +13 and quickly recovered after proper supportive therapy. Data on common toxicities are shown in Table 2.

Grade II–IV	MMRD HSCT (N=56)	MRD HSCT (N=35)	P
Liver toxicity	19	10	0.594
Bilirubin	9	5	
SGPT SGOT GGT	10	5	
Mucositis	28	19	0.691
Cardiovascular	3	1	1.000
Neurological	1	0	1.000
Renal	2	1	1.000
Metabolic	2	1	1.000

Table 2: Common toxicities.

GVHD

The cumulative incidence rates of grade II–IV acute GVHD were 19.6% and 14.3% for the MMRD and MRD groups (P=0.471), respectively (Figure 1A). The 3-year cumulative incidence rates of chronic GVHD were 19.8% in the MMRD group and 34.5% in the MRD group (P=0.153) (Figure 1B).

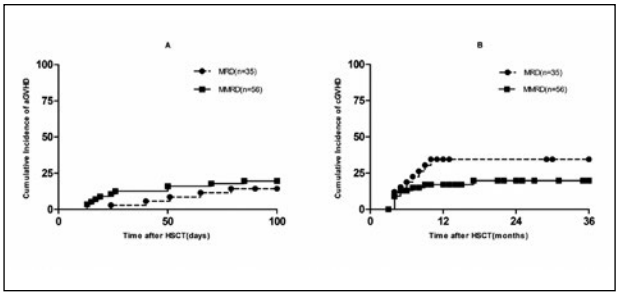

Figure 1: GVHD after transplantation. The cumulative incidence of acute GVHD grade II–IV was 19.6% versus 14.3% in the MMRD and MRD groups, respectively (P=0.471). The 3-year cumulative incidence of chronic GVHD was 19.8% in the MMRD group versus 34.5% in the MRD group (P=0.153).

Relapse and non-relapse mortality (NRM)

Nineteen patients relapsed, including 8 (14.3%) in the MMRD group and 11 (31.4%) in the MRD group. The 3-year cumulative incidence rates of relapse were 15.3% and 33.9% in the MMRD and MRD groups (P=0.039), respectively (see Figure 2A). The 3-year cumulative incidence rates of NRM were 7.9% in the MMRD group and 9.9% in the MRD group (P=0.672) (see Figure 2B).

Survival

The 3-year OS rates were 80.6%±5.6% and 60.2%±8.7% after MMRD and MRD transplantation (P=0.030), respectively (see Figure 2C).

The 3-year DFS rates were 77.6%±5.7% and 58.5%±8.6% for the MMRD and MRD groups (P=0.039), respectively (see Figure 2D).

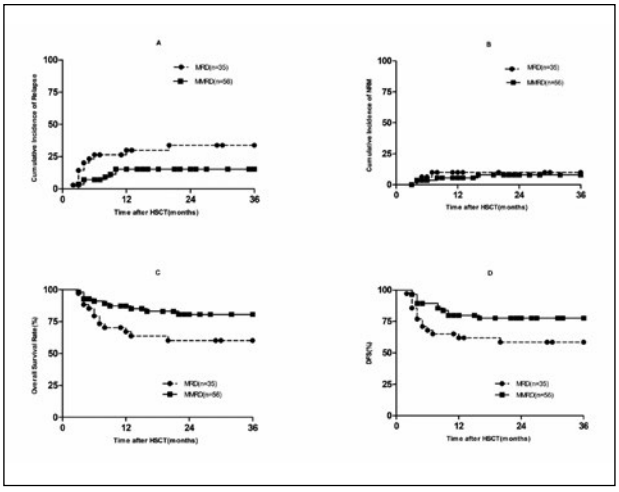

Figure 2: Cumulative incidence of relapse (A), NRM (B), OS (C), and DFS (D). The 3-year cumulative incidence of relapse was 15.3% and 33.9% in the MMRD and MRD groups, respectively (P=0.39). The 3-year cumulative incidence of NRM was 7.9% in the MMRD group versus 9.9% in the MRD group (P=0.672). The 3-year incidence of OS was 80.6%±5.6% and 60.2%±8.7% in the MMRD and MRD groups, respectively (P=0.030). The 3-year incidence of DFS was 77.6%±5.7% and 58.5%±8.6% in the MMRD and MRD groups, respectively (P=0.039).

Discussion

Allogeneic hematopoietic stem cell transplantation (HSCT) is a primary treatment for acute myeloid leukaemia (AML), but the lack of HLA-matched sibling donors has restricted its application, especially in China. However, there are numerous potential donors for haploidentical HSCT, including parents, siblings, children, cousins and unrelated donors. Due to its availability, undergoing haploidentical HSCT is far more beneficial than performing an extended search for matched unrelated donors, particularly in patients urgently in need of HSCT. Lu et al. showed that HLA-mismatched HSCT could achieve outcomes comparable to those of HLA-matched HSCT using a modified BuCy conditioning regimen[15]. However, RRTs remain an issue[16].

In a recent study, Liu and colleagues conducted a randomized comparison between BuFlu (busulfan 3.2 mg/kg and fludarabine 30 mg/m^2. for 4 days) and BuCy2 conditioning regimens for AML in CR1. The incidence of grade III–IV RRTs was significantly lower in the BuFlu group (16.7% and 0.0%, P=0.002)[9]. In addition, 133 AML/MDS patients underwent HLA-identical sibling allo-HSCT using a myeloablative BuFlu regimen (busulfan 3.2 mg/kg and fludarabine 40 mg/m2 for 4 days) and exhibited only 1.5% NRM at 100 days. Russel et al.[24] also

showed that a myeloablative BuFlu conditioning regimen (busulfan 3.2 mg/kg and fludarabine 50 mg/m2 for 4 days) in matched related donor transplantation resulted in 2% RRM at 100 days[8].

Our myeloablative BuFlu conditioning regimen also showed limited toxicities and no NRM at 100 days after allo-HSCT. The most common toxicity was self-limiting mucositis, which was probably due to the use of MTX. The 3-year cumulative incidence rate of NRM in MMRD transplantation was comparable to MRD transplantation. However, HLA-haploidentical HSCT did show a trend toward a higher incidence of HC, which might be due to the increased efficacy of immunosuppressive agents used for GVHD prophylaxis.

Furthermore, the 3-year cumulative relapse rate was significantly lower in MMRD transplantation compared to MRD transplantation (15.3% and 33.9%, respectively, P=0.039), and MMRD transplantation also produced better OS and DFS. It was proven that this myeloablative BuFlu conditioning regimen resulted in high engraftment, limited toxicities and significant chances of long-term survival while retaining a relatively low relapse rate. It can be well tolerated and can achieve even better outcomes in MMRD transplantation. Nonetheless, this study is limited due to its retrospective nature and the relatively small group size.

In conclusion, this myeloablative BuFlu conditioning regimen is a safe and efficacious regimen that produces a high engraftment rate and limited toxicities in both MMRD and MRD transplantation. Furthermore, MMRD transplantation had a lower relapse rate and better long-term survival.

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Ethics approval and consent to participate:
All participants provided their written informed consent, and this study was approved by the ethics committee of Peking University First Hospital.

Availability of data and materials:
The data used in this study are available from the corresponding author.

Abbreviations:
AML, acute myeloid leukaemia; CR, complete remission; MNC, mononuclear cell; HSCT, hematopoietic stem cell transplantation; RRT, regimen-related toxicity; MMRD, HLA-mismatched related donor; MRD, HLA-matched sibling donor; NRM, non-relapse mortality; GVHD, acute and chronic graft versus host disease; OS, overall survival; DFS, disease-free survival.

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