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A novel intensive conditioning regimen for allogeneic hematopoietic stem cell transplantation in the treatment of relapsed/refractory acute myeloid leukemia

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We conducted a prospective study to evaluate the efficacy and safety of cladribine, cytarabine, mitoxantrone, and granulocyte colony-stimulating factor (CLAG-M) regimen combined with busulfan and cyclophosphamide (BuCy) as new intensive conditioning before allogeneic hematopoietic stem cell transplantation (allo-HSCT) in the treatment of relapsed/refractory acute myeloid leukemia (AML). 24 patients were enrolled. The median follow-up was 15.2 months (range 1.9–67.0 months). Except for one patient who died before graft infusion, the evaluable 23 patients (96%) achieved complete remission (CR). The two-year overall survival (OS) rate and leukemia-free survival (LFS) rate were 61.4% and 59.4%, respectively. The non-relapse mortality (NRM) was 9.1%. Univariate analysis revealed that the myeloid blast phase of chronic myelomonocytic leukemia (CMML), an EVI1 mutated, blood blasts ≥20% at transplant, and extramedullary disease were risk factors for LFS.

Key words: relapsed/refractory acute myeloid leukemia, allogeneic hematopoietic stem cell transplantation, intensive conditioning regimen, CLAG-M

In oncology today, the management of relapsed/refractory (R/R) acute myeloid leukemia (AML) remains one of the most challenging scenarios [1]. Due to a paucity of treatment options, the prognosis of patients with R/R AML is so poor that the median overall survival (OS) is only three to seven months and the 3-year survival rate is 10% [2]. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) remains the only feasible treatment. However, using the standard myeloablative conditioning protocols (based on total body irradiation or busulfan), the relapse incidence after transplantation is still high up to 44%, and the leukemia-free survival (LFS) is still no more than 10% at 3 years [3, 4]. It is believed that the pre-transplant leukemia load directly affects the relapse incidence after transplantation, which means the intensity of the conditioning scheme for eradicating the leukemia cells is very important. An intensive conditioning regimen may help to maximally clear residual leukemia cells and obtain better remission, thus allowing donor cell engraftment, decreasing post-transplantation relapse, and improving long-term survival. Study of Christoph Schmid using the regimen of FLAMSA (fludarabine, Amsa, and Ara-C) followed by reduced-intensity conditioning before allo-HSCT showed better survival in R/R AML patients with 2-year LFS of 40% [5]. But the toxicity is still considerable. To date, the standard intensive conditioning regimens for such patients have not yet been established, and the optimal balance between anti-leukemic activity and toxicity still needs to be defined. Focuses on the better outcome and less toxicity, further work to explore a better intensive conditioning regimen is necessary.

Cladribine, a purine analog, has been demonstrated to increase the cellular uptake of cytarabine (Ara-C) and accumulation of Ara-C triphosphate in circulating blasts by 50% to 65% [6–9]. The CLAG±Ida/M regimen, consisting of granulocyte colony-stimulating factor (G-CSF), cladribine, and cytarabine, with or without idarubicin or mitoxantrone, has been recommended as aggressive therapy for R/R AML patients by the National Comprehensive Cancer Network (NCCN) in the year 2020. Data from previous work have shown that the complete remission (CR) rate of CLAG is around 45.5% [2], and about 55.2% for CLAG-M [10]. According to these results, we developed a new intensive conditioning regimen, consisting of the CLAG-M chemotherapy and busulfan-based MAC prior to allo-HSCT in R/R AML patients. Our previous work had shown that it might be a very effective and well-tolerated regimen for these patients

[11]. To further assess the efficacy and safety, we designed a prospective study as follows.

Patients and methods

Patients. A prospective trial of 24 patients with R/R AML was conducted at the HSCT center of the Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou from December 2014 to April 2020. The research was approved by the ethical review committee of our institution and each patient signed informed consent. Patients aged between 14 and 55 years who fulfilled one of the following criteria were included in the study: 1) failure to achieve complete remission (CR) after two or more cycles of induction therapy, 2) relapse after CR, untreated or no response to the salvage chemotherapy, 3) extramedullary disease or persistence of blood leukemic blasts pre-HSCT, 4) failure to achieve CR after at least one cycle for AML secondary to myelodysplastic syndrome, or myeloid blast phase of chronic myeloid leukemia (CML) and chronic myelomonocytic leukemia (CMML). The Eastern Cooperative Oncology Group performance status score of each patient was between 0 and 2. All patients had normal serum creatinine, total bilirubin levels, and transaminase levels and did not have a serious infection. Patients with M3 subtype AML or significant dysfunctions in vital organs were excluded. The study was registered at the Chinese Clinical Trial Registry (www.chictr.org) (Identifier: ChiCTR1900025458).

Conditioning regimens. All patients received the CLAG-M chemotherapy plus the classic BuCy as an intensive conditioning regimen before allo-HSCT. This regimen consisted of 5 mg/m^2/day cladribine and 2 g/m^2/day cytarabine for 5 consecutive days (days –15 to –11), mitoxantrone 10 mg/day from days –15 to –13, and G-CSF 300 μg/day from days –16 to –11 (CLAG-M regimen), and, after a 3-day rest, busulfan 3.2 mg/kg/day from days –7 to –4, cyclophosphamide 60 mg/kg/day from days –3 to –2 (BuCy regimen). Day 0 was defined as the day of donor cell infusion.

Prophylaxis of graft-versus-host disease. For graft-versus-host disease (GVHD) prophylaxis, patients were given cyclosporine A (CSA), a short course of methotrexate (MTX), and anti-thymocyte globulin (ATG). CSA was administered intravenously from day –1, with a target plasma concentration of 200–300 ng/ml. MTX was given intravenously at doses of 15 mg/m^2 on day +1, 10 mg/m^2 on days +3 and +6. ATG was given at a low dose (3 mg/kg total dose) in the case of an HLA-identical sibling donor, or a standard dose (ATG 10 mg/kg total dose or ATG-Fresenius 20 mg/kg total dose) in the case of an unrelated or mismatched related donor. Mycophenolate mofetil was also added at 500 mg every 12 h from days +1 to +15 and tapered to a dose of 500 mg daily from days +16 to +30 for unrelated or mismatched related donors. CSA was tapered after transplantation according to minimal residual disease (MRD) and GVHD.

Evaluation. At days +28, patients were assessed for hematopoietic reconstitution, disease response, and chimerism. CR was defined as less than 5% blasts in the bone marrow without evidence of dysplasia, and more than 1,500 neutrophils/UL in peripheral blood. Bone marrow samples were analyzed at days +28, +56, +84 and 6, 9, 12, 18, 24, 36, 48, 60 months after transplantation for a test of MRD and chimerism. The primary endpoint was the leukemia response rate (CR rate). Secondary endpoints included the 2-year overall survival (OS) after transplantation, leukemia-free survival (LFS), relapse rate, non-relapse rate (NRM), regimen-related toxicity, incidences of acute and chronic GVHD. Regimen-related toxicity was graded as described [12].

Statistical methods. We assume that the primary endpoint, the CR rate at +28 days, increases from 50–75%. Then a total of 25 patients would be needed to provide the trial with 80% power, at a one-sided alpha level of 0.05, to show the superiority of our conditioning regimens. OS and LFS were calculated using the Kaplan-Meier method. The univariate Cox regression analysis and multivariate Cox regression analysis were used to finding the risk factors for LFS. A p-value <0.05 was considered significant.

Results

Patients' characteristics. Patients' characteristics are listed in Tables 1 and 2. The median age of the AML patients was 32 years (range, 13–49). With the approval of the research steering committee, one patient younger than 14 years was allowed to be enrolled in the trial. 18 (75%) patients had a *de novo* AML, other patients had a secondary AML (two secondary to myelodysplastic syndrome, four were myeloid blast phase of CML or CMML). According to the NCCN guideline, the risk stratification by genetics was poor in 10 (41.7%) patients and intermediate in 12 (50%) patients. Two patients had a favorable cytogenetics t(8, 22) (q22; q22) but one of them relapsed after auto-HSCT and the other one was a primary induction failure. At HSCT, 14 (58%) patients were in primary induction failure (PIF), while 8 (33%) were in the first relapse. 14 (58%) patients had more than 20% blasts in the bone marrow at transplant, whereas 12 (50%) patients had peripheral blood blasts. For the three patients who had <5% bone marrow blasts, they had persistent circulating blasts after CR or relapse. And one of them was the patient who relapsed after allo-HSCT, another one had a very short CR duration of two months. For the ten patients who were in first or second relapse, the duration of the first CR was <6 months in 6 (60%) patients. The median pretransplant chemotherapy cycles were 3 (1–13). Only one patient received only one chemotherapy before transplantation because this patient was in the blast crisis of chronic myelomonocytic leukemia and had received several courses of decitabine before the blast crisis. Eight (33%) donors were HLA-identical siblings, thirteen donors (54%) were mismatch-related donors, and the remaining three were

unrelated. 16 (67%) patients received peripheral blood stem cells (PBSC) as the stem cell source, others received both bone marrow stem cells and PBSC.

Time to engraftment. Twenty-three patients were engrafted (96%) and one died during conditioning before donor cell infusion because of the electrolyte disorder. For the twenty-three patients who had received donor cell infusion, the median total nucleated cells (TNC) were 7.5 (range 5.1–12.1)$\times 10^8$/kg and the median $CD34^+$ cells were 6.4 (range 2.4–11.4)$\times 10^6$/kg. All of the 23 patients were engrafted with neutrophil recovery in a median time of 12 days (range 10–17). Three patients did not have platelet recovery after transplantation because of relapse, GVHD, or infection. The median time to platelet recovery was 13 days (range 10–70). In all patients who had platelet recovery, only one patient had recovery time beyond 20 days because of infection. Full donor chimerism was found on day +28 in all of the 23 patients who had received donor cell infusion.

Incidence of acute and chronic graft-versus-host disease. Besides the patient who died before donor cell infusion, the remaining 23 patients were included in the analysis of GVHD. Eight (35%) patients had acute GVHD, of whom four (17%) patients developed grade II–IV acute GVHD. In these four patients, one had grade III gut GVHD at day 18, one had grade IV gut GVHD at day 28. Only three (13%) patients developed chronic GVHD. Two of them had an extensive disease and one had limited chronic GVHD.

Infection and regimen-related toxicity. Septicemia was detected in nine patients. All of them developed before neutrophil recovery. Eight of them were caused by bacteria, and one was caused by *Candida tropicalis*. Seven patients developed pneumonia after neutrophil recovery between days +10 and 14 months after transplantation. The pneumonia was caused by *Aspergillus spp.* in five patients and undetermined etiology in two patients. Ten patients had cytomegalovirus (CMV) reactivation and four of them had CMV-associated hemorrhagic cystitis (grade I–III). Four patients had Epstein-Barr virus reactivation, and three patients had BK virus-associated hemorrhagic cystitis.

Regimen-related toxicity is listed in Table 3. Ten grade I–II adverse events were found in ten cases. One grade IV adverse event happened. The patient developed severe hypokalemia and acute heart failure, leading to death before donor cell infusion.

Disease response and survival. At days +28, 23 patients were alive for the evaluation and one patient died before donor cell infusion (at day –1). All 23 patients (96%) were in CR. By July 8, 2020, 15 patients were alive. Median follow-up was 15.2 months (range 1.9–67.0 months). Eight patients (34.8%) relapsed after CR at a median time of 4.7 months (range 2.6 months –13 months), of whom two patients had central nervous system (CNS) relapse three and five months after transplantation. After intrathecal chemotherapy and cranial irradiation, one of them achieved CR again. The patient is still alive without disease relapse until the last follow-up. The other one died of CNS leukemia. Except for the patient who died before donor cell infusion due to hypokalemia and heart failure, six deaths were directly due to leukemia relapse, whereas two were attributed to infection

Table 1. Patients' characteristics.

Characteristics	Study population (n=24)
Patient gender (female/male)	7/17
Patient median age, years (range)*	32 (13–49)
Donor	
Gender (female/male)	7/17
median age, years (range)	31 (17–59)
Donor-recipient sex match	
Male-male	12 (50)
Male-female	5 (21)
Female-male	5 (21)
Female-female	2 (8)
Median time from diagnosis to transplantation, months (range)	4.7 (2.3–106)
Median marrow blasts at transplantation, % (range)	23 (3–71)
Diagnosis, n (%)	
de novo AML	18 (75)
AML secondary to MDS	2 (8)
Myeloid blast phase of CML	2 (8)
Myeloid blast phase of CMML	2 (8)
Risk stratification by genetics, n (%)	
Favorable	2 (8)
Intermediate	12 (50)
Poor/Adverse	10 (41.7)
Stem cell source**, n (%)	
Bone marrow and PBSC	7 (29)
PBSC	16 (67)
Donor type, n (%)	
Matched sibling donor	8 (33)
Mismatch related donor	13 (54)
Unrelated donor	3 (13)
Cell dose, median (range)***	
TNC 10^8/Kg	7.5 (5.1–12.1)
CD34+ cells 10^6/Kg	6.4 (2.4–11.4)
Pretransplant chemotherapy cycles, median (range)	3 (1–13)
Disease status at transplant, n (%)	
PIF	14 (58)
First relapse	8 (33)
Relapse after auto-HSCT	1 (4)
Relapse after allo-HSCT	0 (0)
Second relapse	2 (8)
Relapse after auto-HSCT	0 (0)
Relapse after allo-HSCT	2 (8)

Notes: *One patient aged <14 years was included in the trial after approval of the protocol steering committee. **One patient died before donor cell infusion. ***Cell doses are indicated for the 23 patients who had received donor stem cells infusion. Abbreviations: HSCT-hematopoietic stem-cell transplantation; CR-complete remission; AML-acute myeloid leukemia; MDS-myelodysplastic syndrome; CML-Chronic myeloid leukemia; CMML-Chronic myelomonocytic leukemia; PIF-primary induction failure; PBSC-peripheral blood stem cells; TNC-total nucleated cells.

Table 2. Characteristics of the AML patients.

Patient	Diagnosis	White blood cells a diagnosis ($\times 10^9$/l)	Disease duration (months)	Molecular aberrations	Extramedullary disease	Bone marrow blasts/PB blasts at HSCT (%)	Donor match and relation
1	*De novo* M1	1.33	3.4	No	No	12.5/0.0	10/10 related
2	Myeloid blast phase of CML	354.00	6.4	BCR-ABL (+)	Yes	44.0/56.0	10/10 related
3	*De novo* M5	18.33	4.5	FLT3 wild type (+)	No	36.5/7.0	5/10 related
4	*De novo* M2	35.19	17.0	AML1/ETO (+) c-kit/D816 (+)	No	64.0/8.0	5/10 related
5	Secondary to MDS	33.72	4.0	EVI1 (+)	No	10.5/0.0	7/10 related
6	*De novo* M5	90.49	4.7	MLL-AF9 (+) EVI1 (+)	No	5.5/0.0	5/10 related
7	*De novo* M5	73.41	4.0	MLL-AF6 (+)	No	17.5/0.0	10/10 related
8	*De novo* M2	55.87	22.0	HOX11 (+) EVI1 (+)	Yes	29.5/16.0	10/10 related
9	*De novo* M5	125.75	3.0	NRAS (+) IDH1 (+)	No	21.0/0.0	9/10 related
10	*De novo* M5	3.97	6.0	TET2, EZH2, STAG2, ETV6 (+)	N0	35.5/4.0	5/10 related
11	*De novo* M2	27.21	3.5	FLT3-ITD (+)	No	16.0/0.0	9/10 related
12	*De novo* M5	0.92	2.9	TET2 (+) AML1/ETO (+)	No	40.5/6.0	5/10 related
13	*De novo* M5	29.74	5.5	FLT3-ITD (+)	N0	19.0/0.0	10/10 related
14	Secondary to MDS	87.80	3.3	RUNX1 (+)	No	24.5/0.0	10/10 related
15	*De novo* M2	66.00	9.0	FLT3-ITD, NPM1, IDH1, MPL (+)	No	66.0/5.0	10/10 related
16	*De novo* M5	120.91	2.8	MLL-AF6 (+), EVI1(+)	No	3.0/2.0	10/10 unrelated
17	Myeloid blast phase of CMML	0.51	12.5	ASXL1, BCOR (+)	N0	23.5/0.50	8/10 unrelated
18	*De novo* M5	22.15	5.3	EVI1 (+)	No	41.0/0.0	5/10 related
19	*De novo* M5	105.20	2.6	FLT3-ITD (+) low	No	7.0/0.0	5/10 related
20	Myeloid blast phase of CML	52.00	2.3	BCR-ABL (+)	No	22.5/13.0	5/10 related
21	*De novo* M2	3.72	13.0	NRAS (+)	No	3.5/1.0	10/10 unrelated
22	Myeloid blast phase of CMML	37.36	4.6	FLT3-IT, HOX11, NPM1, DNMT3A (+)	No	60.0/78.5	10/10 related
23	*De novo* M1	213.43	7.4	FLT3-ITD, NPM1, ATG2B (+)	No	71.0/68.0	6/10 related
24	*De novo* M4	18.50	106.0	RUNX1 (+)	No	3.0/1.0	8/10 related

Abbreviations: MDS-myelodysplastic syndrome; CML-Chronic myeloid leukemia; CMML-Chronic myelomonocytic leukemia

Table 3. Regimen-related toxicity.

	Grade I	Grade II	Grade III	Grade IV
Cardiac toxicity		1 (4)		1 (4)
Stomatitis	2 (8)	1 (4)		
GI toxicity	4 (17)	1 (4)		
Renal toxicity	1 (4)			

Note: presented data are n (%)

and acute GVHD. The Kaplan-Meier estimates of OS and LFS at 2 years were 61.4% and 59.4% (Figures 1A, 1B). The NRM at 2 years was 9.1%. (Figure 1C).

Risk factors for post-transplant outcomes. Univariate and multivariate analysis for LFS at 2 years in the patients is shown in Table 4. Univariate cox regression analysis revealed that LFS after HSCT in CMML (myeloid blast phase) patients was worse than other patients. Patients that had an EVI1 mutated, had a worse LFS. If a patient had blood blasts ≥20% at transplant or extramedullary disease, the outcome for LFS was worse. However, in multivariate analysis, none of the factors was statistically significant in our study.

Discussion

In this paper, our study shows two main findings. First, our novel intensive conditioning combining a CLAG-M and BuCy regimen prior to allo-HSCT used in R/R AML patients has successfully achieved its goal of maintaining substantial anti-leukemia activity while achieving limited toxicity. Except for one patient who died before donor cell infusion,

Table 4. Risk factors for LFS.

	Univariate analysis			Multivariate analysis
	HR	95% CI	p-value	p-value
LFS				
Patient gender male vs. female	1.594	0.321–7.91	0.569	
Patient age >30 years old vs. ≤30 years old	0.917	0.229–3.67	0.903	
Diagnosis Myeloid blast phase of CMML vs. others	5.855	1.129–30.372	0.035	0.367
Time diagnosis/transplant ≥6 months vs. <6 months	2.25	0.555–9.131	0.256	
Status at transplant relapse vs. PIF	1.457	0.363–5.84	0.595	
WBC count at diagnosis ≥100×10^9/l vs. <100×10^9/l	2.235	0.521–9.591	0.279	
MECOM(EVI1) Yes vs. No	8.17	1.586–42.076	0.012	0.299
Blood blasts at transplant ≥20% vs. <20%	10.877	2.14–55.282	0.004	0.307
Bone marrow blasts at transplant ≥20% vs. <20%	1.542	0.367–6.472	0.554	
Donor type MRD vs. MSD or URD	0.329	0.066–1.632	0.174	
Donor gender mismatch vs. match	0.287	0.058–1.436	0.129	
Risk stratification Poor vs. others	3.299	0.663–16.420	0.145	
Extramedullary disease Yes vs. No	17.964	1.626–198.42	0.018	0.516
Acute GVHD Yes vs. No	0.719	0.144–3.581	0.687	
Chronic GVHD Yes vs. No	0.037	0–98.5115	0.412	

Abbreviations: MSD-Matched sibling donor, MRD-Mismatch related donor, URD-Unrelated donor

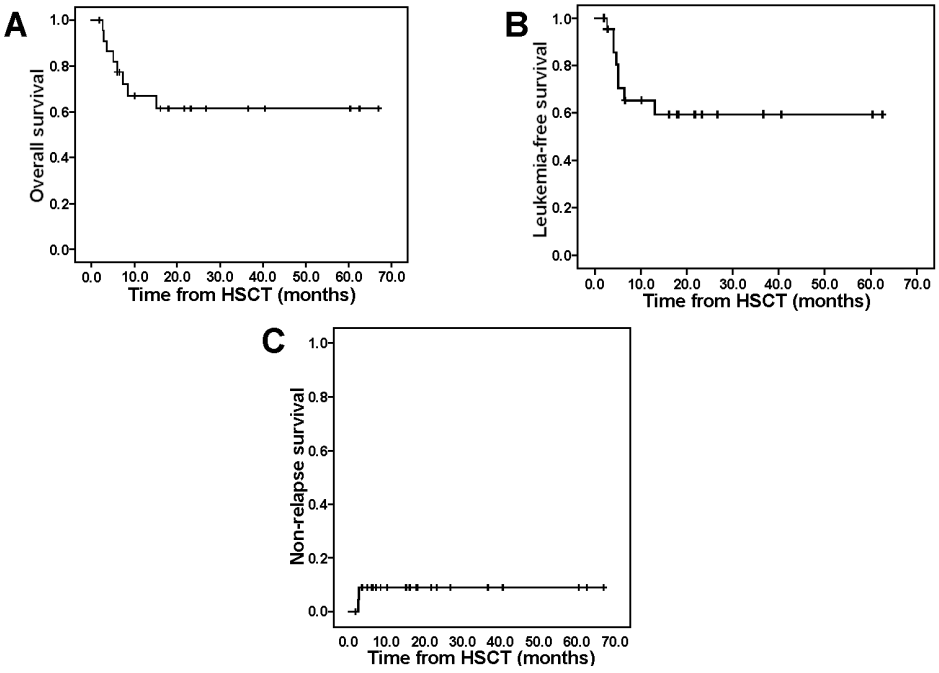

Figure 1. Outcomes after allogeneic HSCT. A) 2-year overall survival; B) 2-year leukemia-free survival; C) cumulative incidence of non-relapse mortality.

this conditioning could achieve a 100% CR rate in 23 evaluable patients who had received donor cell infusion at the time of neutrophil reconstitution and increased the OS and LFS at 2 years to 61.4% and 59.4%, respectively. The incidence of 2-year NRM was very low (9.1%), and the acute and chronic GVHD and infection were acceptable. Second, univariate cox regression analysis elicited that myeloid blast phase of CMML, a mutated EVI1, blood blasts ≥ 20% at transplant, and extramedullary disease were the risk factors for LFS post-transplant. However, in multivariate analysis, none of the factors was statistically significant.

Despite advances in treatment for AML, the prognosis for patients with R/R AML is extremely poor. Although allo-HSCT is considered to be the best treatment option for these patients, in several retrospective trials, the results have been disappointing. The long-term survival rates remain low, with a 3-year OS rate lower than 21% and 2-year OS lower than 30% [13–15]. There has been increasing interest in sequential transplantation regimens in recent years. The method is to combine the conditioning before allo-HSCT with intensive chemotherapy to minimize the burden of leukemia. Mohty et al. conducted a prospective, phase 2 trial to examine the efficacy and safety of a novel conditioning protocol, using a reduced intensity-conditioning regimen following a short course of intensive chemotherapy before allo-HSCT. Eighteen (75%) of the patients achieved CR. After a median follow-up of 24.6 months, the 1 year and 2 years OS was 54% and 38%, respectively. The LFS was 46% at 1 year and 29% at 2 years [16]. In our research, an overall CR rate of 96% was achieved, with a very low incidence of NRM (9.1%), thereby demonstrating a superior anti-leukemic efficacy and well tolerance of the protocol as compared with other studies. In addition, our protocol improved the OS and LFS at 2 years to 61.4% and 59.4%, which exhibited a remarkable effect in R/R AML patients as compared with other studies. As far as we know, our research is the first prospective clinical trial to use CLAG-M combined with BuCy as intensive conditioning before allo-HSCT in the R/R AML patients with the longest follow-up for the first patient (67 months). The intensive conditioning with CLAG-M and BuCy regimen reduced the leukemia burden to the greatest extent before allo-HSCT, which is directly related to our beneficial outcomes. Firstly, The G-CSF-containing priming regimen may obtain a better curative effect in R/R AML patients. It is generally recognized that G-CSF promoted G0 phase cells to enter the cell cycle, increased intracellular drug metabolism, and increased cytotoxicity of Ara-C and/or mitoxantrone [17, 18]. Secondly, cladribine combined with mitoxantrone and Ara-C has a strong anti-leukemia synergistic effect. Cladribine is a new generation of purine analogs. It is activated by intracellular phosphorylation and then accumulated in lymphocytes, resulting in leukemia cell death. Cladribine can kill leukemia cells through a variety of mechanisms [19]. A large number of studies have confirmed that high-dose Ara-C intravenous infusion is an effective rescue therapy for patients with R/R AML due to the increase of Ara-C concentration in plasma and cerebrospinal fluid [20, 21]. In addition, cladribine can increase the uptake of Ara-C by up to 50–65% in leukemia cells. Chow et al. have proved that the combination of cladribine and Ara-C inhibited the differentiation of leukemia cells, accelerated their apoptosis, and destroyed the reconstruction of mitochondrial membrane [21]. Another study has shown that mitoxantrone can enhance the antiproliferative activity of cladribine, both *in vivo* and *in vitro* [22].

Although 23 patients achieved CR after transplantation, 8 patients still relapsed during the follow-up, 6 of them died of relapse, which remains the main cause of death after transplantation. In univariate cox regression analysis, we found four risk factors for LFS, the myeloid blast phase of CMML, a mutated EVI1, blood blasts ≥20% at transplant, and extramedullary disease. However, in multivariate analysis, none of the factors was statistically significant. The high blood blast percentage at HSCT is considered a risk factor in R/R AML patients, which is consistent with other studies [23]. Besides, we found that all the patients with the myeloid blast phase of CMML (AML transformed from CMML) relapsed during follow-up. CMML remains a challenging malignancy to treat, the median survival time varies from several months to several years. Those with intermediate or high-risk disease have been shown to rapidly progress to AML [24]. If CMML progresses to AML, patients will face limited treatment options and on average, demonstrate a survival time of only a few months. Both of the two patients with AML transformed from CMML in our cases had high-risk diseases, according to the CPSS-Mol [24] risk stratification system. They had mutations in ASXL1 or DNMT3A, which have been shown to confer an inferior prognosis in patients with CMML. The largest transplant study on CMML patients (n=513) by the European Group for Blood and Marrow Transplantation (EGBMT) reported a 4-year OS of 33% and found the only predictor of survival was CR at the time of transplant [25]. Our patients were both NR at the time of transplant and rapidly relapsed after transplantation. This may indicate that our regimen is not effective in AML patients transformed from CMML. Ecotropic viral integration site 1 (EVI1) is an oncogenic transcription factor, which is abnormally expressed in myeloid leukemia and other several solid cancers. It is associated with short survival as well as anticancer drug resistance. To date, $EVI1^{high}$ myeloid cells have been found to be insensitive to cytarabine (Ara-c), daunorubicin (DNR), nilotinib, and adriamycin [26–29]. Besides, mutation in fms-like tyrosine kinase 3-internal tandem duplication (FLT3-ITD), is also an important risk factor in R/R AML patients, being associated with worse outcomes. Poiani et al. [30] explored the impact of cytogenetic risk on the outcomes of allo-HSCT in patients with R/R acute myeloid leukemia. They showed that compared to the favorable risk group, intermediate and adverse risk patients were associated with worse leukemia-free survival and OS

and also with a higher incidence of relapse. Peripheral blood blast was previously reported as a risk factor in R/R AML patients who underwent hematopoietic cell transplantation [31, 32]. A high blood blast percentage indicates a high leukemic burden before transplantation, which will lead to an increase in the relapse rate. The reason why multivariate analysis failed to find the difference may be due to the small sample size of our study.

Donor type is a factor that is potentially related to the outcome in other studies. Schmid et al. showed that having an HLA-identical family donor was a risk factor for leukemic death [5]. Xiao et al. showed that receiving haploidentical related donor transplantation was protective from relapse [33]. Our research showed that mismatch-related donor may be a protective factor but it was not statistically significant. Other factors such as time from diagnosis to transplant >6 months, the incidence of aGVHD or cGVHD, may also be related to the outcomes in other studies [23, 33]. In our research, all the patients who relapsed after HSCT had no cGVHD. Chronic GVHD seems to have a beneficial effect on LFS but with no statistical difference. It may be due to the relatively small number of cases in our study.

Since our study was a phase 2 trial, only a small number of patients were included, resulting in an excessive 95% confidence interval (CI) in univariate cox analysis and no statistical significance in multivariate analysis of some potential risk factors. Besides, it was a single-arm study with a relatively short follow-up duration in some of the patients. A phase 3 randomized trial is needed for further evaluation with a large number of patients and longer follow-up.

In conclusion, our results demonstrate the efficacy and safety of a novel intensive conditioning regimen with CLAG-M combined with BuCy in allo-HSCT of R/R AML patients.

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