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A new intensive conditioning regimen for allogeneic hematopoietic stem cell transplantation in patients with refractory or relapsed acute myeloid leukemia

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Abstract

To explore the efficacy, and safety of the intensive conditioning regimen consisting of cladribine, cytarabine (Ara-C), and granulocyte colony-stimulating factor (G-CSF) plus modified busulfan (Bu) combined with cytoxan (Cy) (BuCy), prior to allogeneic hematopoietic stem cell transplantation (allo-HSCT) in patients with refractory, or relapsed acute myeloid leukemia (R/R AML).

Thirty-Six R/R AML patients scheduled to receive allo-HSCT were consecutively, enrolled in this prospective study, and treated using intensive conditioning regimen consisting of CLAG plus modified BuCy. Median follow-up duration was 11.25 (range 0.5 – 21.0) months and the last follow up date was August 15, 2017.

All patients (100%) achieved white blood cell (WBC) recovery within a median time of 16.00 (13.25 – 18.00) days, and 34 of them (94%) attained platelet (PLT) recovery within a median time of 13.50 (9.25 – 19.75) days. Incidence of acute graft-versus-host disease (aGVHD) was 50.00%, with median time of 71.50 (41.00 – 401.25) days. Three patients developed Grade I; nine, Grade II; 5, Grade III; and 1, Grade IV aGVHD. The incidence of chronic GVHD (cGVHD) was 44.40%, with median time of 255.00 (120.00 – 390.00) days. Four patients developed limited cGVHD, and 12, extensive cGVHD. One-year accumulating leukemia free survival (LFS), and overall survival (OS) rates between 52.9±8.8% to 69.4±7.7%, respectively. Eighteen (50%) patients were infected with cytomegalovirus; 2 (5.6%), with Epstein-Barr virus (EBV), 7 (19.4%), with hemorrhagic cystitis; 13 (36.1%), with bacteria; and 8 (22.2%), with fungus.

Intensive conditioning regimen of CLAG plus modified BuCy for allo-HSCT may be effective and well-tolerated in R/R AML patients.

Abbreviations: aGVHD = acute graft-versus-host disease, allo-HSCT = allogeneic hematopoietic stem cell transplantation, AML = acute myeloid leukemia, ATG = anti-thymocyte globulin, Bu = busulfan, CCr = creatinine clearance rate, cGVHD = chronic graft-versus-host disease, CR = complete remission, Cy = Cytoxan, DFS = disease free survival, EBV = Epstein-Barr virus, G-CSF = granulocyte colony-stimulating factor, MAL = mixed acute leukemia, MRD = minimal residual disease, NCCN = National Comprehensive Cancer Network, OS = overall survival, PALG = Polish Adult Leukemia Group, PLT = platelet, R/R AML = refractory or relapsed acute myeloid leukemia, TBI = total body irradiation, Tregs = regulatory T cells, WBC = white blood cell.

Keywords: allogeneic hematopoietic stem cell transplantation (allo-HSCT), CLAG, intensive conditioning regimen, modified BuCy, refractory or relapsed acute myeloid leukemia (R/R AML)

1. Introduction

Acute myeloid leukemia (AML) is a hematological malignancy characterized by cell proliferation, homology, and abnormally, or occasionally, poorly differentiated cells present in the bone marrow, blood, and other tissues.[1] Although the therapeutic

prognosis of AML has been extensively improved, due to more precise risk-stratification, and individual treatment based on molecular genetics, between 10 to 40% of newly, diagnosed AML patients still fail to achieve complete remission (CR) after standard induction chemotherapy; these cases are known as primary refractory AML.[2] Moreover, relapse occurs between 50 to 70% of AML patients that have achieved CR,[3] a condition termed relapsed AML. These findings indicate that aggressive treatment is greatly, required in refractory, or relapsed AML (R/R AML) patients.[3] Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the best choice for R/R AML patients, although it is reported that the 3-year overall survival (OS) for patients who have undergone allo-HSCT is only 19%.[4] Recurrence after transplantation is a major reason for the low survival rate in R/R AML patients, and thus prevention of post-transplantation relapse is of great importance to increase the efficacy of allo-HSCT therapy in R/R AML patients.

In order to prevent post-transplantation relapse, intensive conditioning regimen is a primary treatment that eliminates the majority of residual leukemic cells.[1,5] The classical conditioning regimens in clinical application are still the myeloablative

conditioning regimens including busulfan (Bu) combined with cytoxan (Cy) (BuCy), and total body irradiation (TBI) combined with Cy (Cy/TBI). Intensive conditioning regimens derive from these common conditioning regimens with the aim to attain better therapeutic effect.[6] Intensive conditioning regimen before transplantation causes maximal decrease of residual tumors, and thus leads to better CR, prevents post-transplantation relapse, and consequently, achieves long-term survival.[6] One study has revealed that the 3-year OS rate for R/R AML patients treated with individual intensive conditioning regimen combined with preventive immunotherapy prior to allo-HSCT was 62.6% and the disease-free survival (DFS) rate was 60.2%.[7] These results indicate that intensive conditioning regimen achieves good efficacy in R/R AML patients,[8] although, standardization of intensive conditioning regimens has not been established for allo-HSCT in R/R AML patients.

Cladribine (2-chloro-2'-deoxyadenosine) is a new generation purine analogue that kills proliferative and non-proliferative cells by promoting cellular apoptosis.[9,10] In addition, it was demonstrated in vitro that cladribine can increase cellular uptake of Ara-C; Ara-C is converted intracellularly into the active metabolite Ara-C-5' triphosphate (Ara-CTP), thus exerting its cytotoxic effect on leukemic blasts.[11] The accumulated Ara-CTP concentration reaches between 50 to 65%, suggesting that cladribine treatment plus Ara-C is a potential choice for intensive induction therapy.[12] Granulocyte colony-stimulating factor (G-CSF), a cytokine with widespread applications in hematopoietic recovery, was also used in a chemotherapy regimen together with cladribine and Ara-C (CLAG); among CLAG-treated R/R AML patients, 50% achieved CR after 1-year treatment.[13] Based on these above findings, CLAG therapy has been recommended as a first-line treatment in R/R AML patients by the National Comprehensive Cancer Network (NCCN) in the year 2016.[8] However, application of CLAG as intensive conditioning regimen has not been well investigated. Based on the outstanding effect of CLAG, as an intensive chemotherapy regimen, on R/R AML patients, we hypothesized that CLAG may be a good candidate for intensive conditioning prior to allo-HSCT. Thus, our study aimed to explore the efficacy, and safety of the intensive conditioning regimen of CLAG plus modified BuCy prior to allo-HSCT in R/R AML patients.

2. Methods

2.1. Participants

In total, 36 patients with R/R AML imminent to receive allo-HSCT at the Department of Hematology, Aerospace Center Hospital between August 2015 to January 2017 were consecutively enrolled in this prospective study. Patients were included based on the following criteria: firstly, age ranged between 18 to 59 years; secondly, AML diagnosis according to classification of morphology, immunology, cytogenetics, molecular biology (MICM), and consistent with the criteria of R/R AML; thirdly, more than 10% blasts in the bone marrow according to cell morphology, and immunophenotyping analysis; fourth, patients imminent to allo-HSCT treatment. Patients with the following conditions were excluded: firstly, mixed acute leukemia (MAL), blastic crisis of chronic myelogenous leukemia (CML-BC), blastic crisis of chronic myelomonocytic leukemia (CMML-BC), or myelodysplastic syndrome-refractory anemia with excess blasts (MDS-RAEB); secondly, severe heart dysfunction with left cardiac ejection fraction (LVEF) < 60%, or severe arrhythmia;

thirdly, severe lung dysfunction; fourth, severe hepatic dysfunction with abnormal alanine aminotransferase (ALT), and total bilirubin (TBIL) up to 3 times above upper normal limit (ULN); fifth, severe kidney dysfunction with abnormal serum creatine (SCr) up to 3 times above ULN, or creatinine clearance rate (CCr) < 50 mL/minute within 24 hours; sixth severe active infection before transplantation; seventh, known allergy to drugs used in this study; eighth pregnancy, or lactation (or planed for pregnancy or lactation). This study was approved by the Ethics Committee of Aerospace Center Hospital, and was performed in line with the Declaration of Helsinki. All participants signed informed consent forms.

2.2. Definitions of R/R AML

The refractory AML was defined as follows: firstly, patients did not achieve CR after 2 courses of induction chemotherapy with a standard protocol; secondly, patients relapsed within 6 months after first CR; thirdly, patients relapsed at 6 months, or more after first CR, and failed in the subsequent induction chemotherapy; fourth, patients relapsed more than twice; fifth, extra-medullary infiltration of leukemia. The relapsed AML was defined according to the following criteria: leukemic cells reappeared in peripheral blood, or the percentage of bone marrow blasts exceeded 10% with extra-medullary infiltration of leukemia.

2.3. Treatments

All patients were treated with intensive conditioning regimen consisting of CLAG plus modified BuCy prior to allo-HSCT, and the day of transplantation was determined as day 0 (d0). TBI was not performed in this study. The detailed conditioning regimen included: intravenous administration of cladribine 5 mg/m^2/d between d-13 to d-9, G-CSF 5 µg/kg/d between d-14 to d-9, Ara-C 2 g/m^2/d between d-13 to d-9, Bu 3.2 mg/kg/d between d-8 to d-6, cyclophosphamide 1.8 g/m^2/d between d-5 to d-4 and oral administration of Me-CCNU 250 mg/d on d-3. During allo-HSCT, patients received bone marrow plus peripheral blood stem cells. To prevent graft-versus-host disease (GVHD), cyclosporine, a short-term methotrexate, and mycophenolate mofetil were applied in both human leukocyte antigen (HLA) matched, and mismatched patients, while additional anti-thymocyte globulin (ATG) 2.5 mg/kg/d between d-5 to d-2 was used in HLA mismatched patients.

2.4. Engraftment

Engraftment of white blood cells (WBC) was considered successful when the peripheral blood leucocytes count was above 1×10^9/L for 3 consecutive days. Additionally, engraftment of platelets (PLT) was considered successful when the PLT count was higher than 20×10^9/L for 7 consecutive days without any PLT infusion.

2.5. Assessments

The primary endpoints were accumulating leukemia free survival (LFS), and accumulating OS. LFS was calculated as the time from the date of transplantation to the time of recurrence or death from any cause, and OS was calculated as the time from the date of transplantation to the time of death from any cause. The median follow-up duration was 11.25 (range 0.5 – 21.0) months and the last follow up date was August 15, 2017.

The secondary endpoints were the following: firstly, time from the date of transplantation to WBC and PLT engraftments. secondly, occurrence, time, and severity of acute GVHD (aGVHD), or chronic GVHD (cGVHD). thirdly, incidence of various infections including: cytomegalovirus (CMV), Epstein-Barr virus (EBV), hemorrhagic cystitis, bacterial, and fungal infection.

2.6. Statistics

The statistical analysis was performed by the SPSS 22.0 software (SPSS Inc, Chicago, IL) and OFFICE 2010 software (Microsoft, Redmond). Data are mainly presented as mean ± standard deviation, median (25 – 75th value), or count (%). Kaplan-Meier (K-M) curves were performed to analyze the time to WBC and PLT engraftments, GVHD occurrence, LFS and OS. The log-rank test was used to detect the difference of LFS/OS between subgroups. $P < .05$ was considered as significant.

3. Results

3.1. Baseline characteristics

The baseline characteristics of all patients were displayed in Table 1. A total of 21 female and 15 male individuals enrolled in the study with a mean age between 26.58 ± 13.55 years old. The subtypes of AML disease in these patients included M2 (n = 19, 53%), M4 (n = 5, 14%), M5 (n = 8, 22%) and M6 (n = 4, 11%). The median duration of disease was 11.50 months (range 7.00 – 21.75 months). All patients received transplantation from related donors who were between 34.97 ± 13.49 years old. Of those, 24 were female and 12 were male donors. Ten donors (28%) were identical twins (10/10 matched HLA alleles), which were

Table 1

Patients' characteristics.

Parameters	AML patients (N = 36)
Age (years)	26.58 ± 13.55
Gender (Female/Male)	21/15
Diagnosis	
M2 (n/%)	19 (53)
M4 (n/%)	5 (14)
M5 (n/%)	8 (22)
M6 (n/%)	4 (11)
Disease duration (months)	11.50 (7.00 – 21.75)
Donor	
Age (years)	34.97 ± 13.49
Gender (Female/male)	24/12
Related/unrelated (n)	36/0
HLA match	
10/10 (n/%)	10 (28)
9/10 (n/%)	2 (6)
8/10 (n/%)	1 (3)
7/10 (n/%)	1 (3)
6/10 (n/%)	2 (6)
5/10 (n/%)	20 (56)
Donor-recipient sex match	
Male-male	15 (42)
Male-female	9 (25)
Female-male	6 (17)
Female-female	6 (17)

Data was presented as mean ± standard deviation, median (25 – 75th value) or count (%). AML = acute myeloid leukemia, CML = chronic myeloid leukemia, MDS = myelodysplastic syndrome, MPN = myeloproliterative neoplasm.

Table 2

Transfusion of MNC, CD34$^+$, CD3$^+$, CD4$^+$, CD8$^+$ and CD19$^+$ cells.

Parameters	AML patients (N = 36)
MNC (10^8/kg)	8.965 (8.710 – 9.318)
CD34$^+$ (10^6/kg)	2.995 (2.320 – 4.185)
CD3$^+$ (10^6/kg)	1.650 (1.465 – 2.228)
CD4$^+$ (10^6/kg)	0.925 (0.755 – 1.245)
CD8$^+$ (10^6/kg)	0.630 (0.515 – 0.923)
CD19$^+$ (10^6/kg)	0.385 (0.240 – 0.510)

Data was presented as median (25 – 75th value).
AML = acute myeloid leukemia. MNC = median mononuclear cells.

categorized as HLA-matched donors. Additionally, two donors (6%) had 1 HLA allele mismatched (HLA-9, 9/10), 1 (3%) had 2 alleles mismatched (HLA-8, 8/10), 1 (3%) had 3 alleles mismatched (HLA-7, 7/10), 2 (6%) had 4 alleles mismatched (HLA-6, 6/10) and 20 (56%) had 5 alleles mismatched (HLA-5, 5/10). These donors were categorized as HLA-mismatched donors. Fifteen transplantations were male donors with male recipients (42%), 9 were male donors with female recipients (25%), 6 were female donors with male recipients (17%) and 6 were female donors with female recipients (17%).

3.2. Transfusion of MNC, CD34$^+$, CD3$^+$, CD4$^+$, CD8$^+$ and CD19$^+$ cells

As shown in Table 2, the median number of mononuclear cells (MNC) transfused to AML patients was 8.965×10^8/kg (8.710 – 9.318×10^8/kg), and the number of CD34$^+$, CD3$^+$, CD4$^+$, CD8$^+$ and CD19$^+$ cells was 2.995×10^6/kg (2.320 – 4.185×10^6/kg), 1.650×10^6/kg (1.465 – 2.228×10^6/kg), 0.925×10^6/kg (0.755 – 1.245×10^6/kg), 0.630×10^6/kg (0.515 – 0.923×10^6/kg) and 0.385×10^6/kg (0.240 – 0.510×10^6/kg), respectively.

3.3. Time to WBC and PLT engraftment

All patients (100%) achieved WBC engraftment and 34 out of 36 (94%) patients attained PLT engraftment. As shown in Fig. 1, the median time of WBC and PLT recovery was 16.00 (13.25 – 18.00) days, and 13.50 (9.25 – 19.75) days, respectively.

3.4. Incidence of aGVHD

Occurrence of aGVHD was 50.00% with median time of 71.50 (41.00 – 401.25) days (Fig. 2A). Three patients were grade I, 9 were grade II, 5 were grade III and 1 was grade IV aGVHD (Fig. 2B). Among patients with aGVHD, skin, and intestine involvement occurred in 13 patients, with additional skin, and liver involvement in 3 patients, skin involvement in 1 patient, and lung involvement in 1 patient. Because prevention of GVHD in HLA-matched, and HLA-mismatched patients was variable, we next compared the incidence and severity of aGVHD between the 2 subgroups. This analysis showed that there was no difference in incidence (P = .194, Fig. 2C), or severity (P = .140, Fig. 2D) of aGVHD between HLA-matched and HLA-mismatched patients.

3.5. Incidence of cGVHD

Occurrence of cGVHD was 44.40% with median time of 255.00 (120.00 – 390.00) days (Fig.3A). Four cases were classified as limited cGVHD and 12 as extensive cGVHD (Fig. 3B). Skin, and muscle involvement occurred in 6 patients; skin, liver, and lung
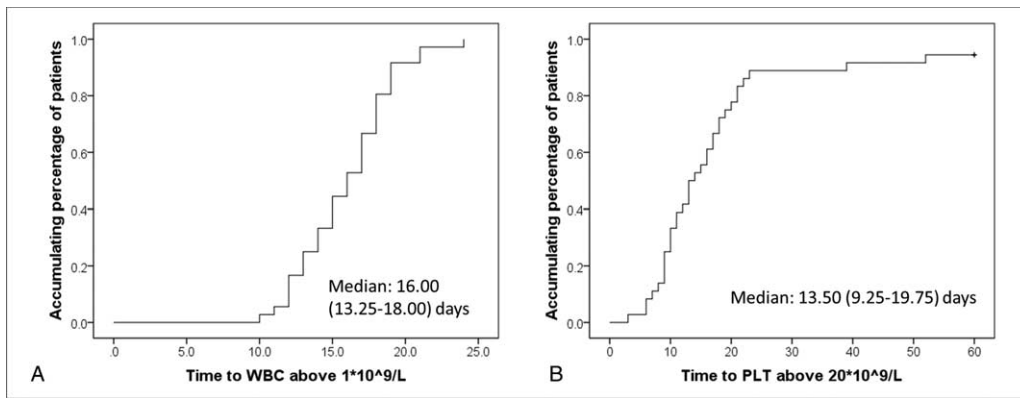
Figure 1. Time from transplantation to WBC and PLT engraftments: (A) K-M curve analysis of time from transplantation to WBC above 1×10^9/L, (B) K-M curve analysis of time from transplantation to PLT above 20×10^9/L. K-M = Kaplan-Meier curves, PLT = platelet, WBC = white blood cell.

involvement occurred in 4 patients; lung involvement occurred in 2 patients; whole body involvement occurred in 2 patients; skin, lung, and capillary leak occurred in 1 patient; and liver involvement occurred in 1 patient. We found that there was no difference in occurrence ($P = .261$, Fig. 3C), and clinical stage ($P = .349$, Fig. 3D) between the HLA-matched and HLA-mismatched groups.

3.6. Incidence of infection

Incidence of infections as well as time to occurrence, and post allo-HSCT including CMV, EBV, hemorrhagic cystitis, bacterial infection, and fungal infection were presented in Table 3. The most common infection was CMV infection, which affected 50% of patients (n = 18) with median time to occurrence of 38.50 (30.00 − 43.75) days. Bacterial infection was observed in 13
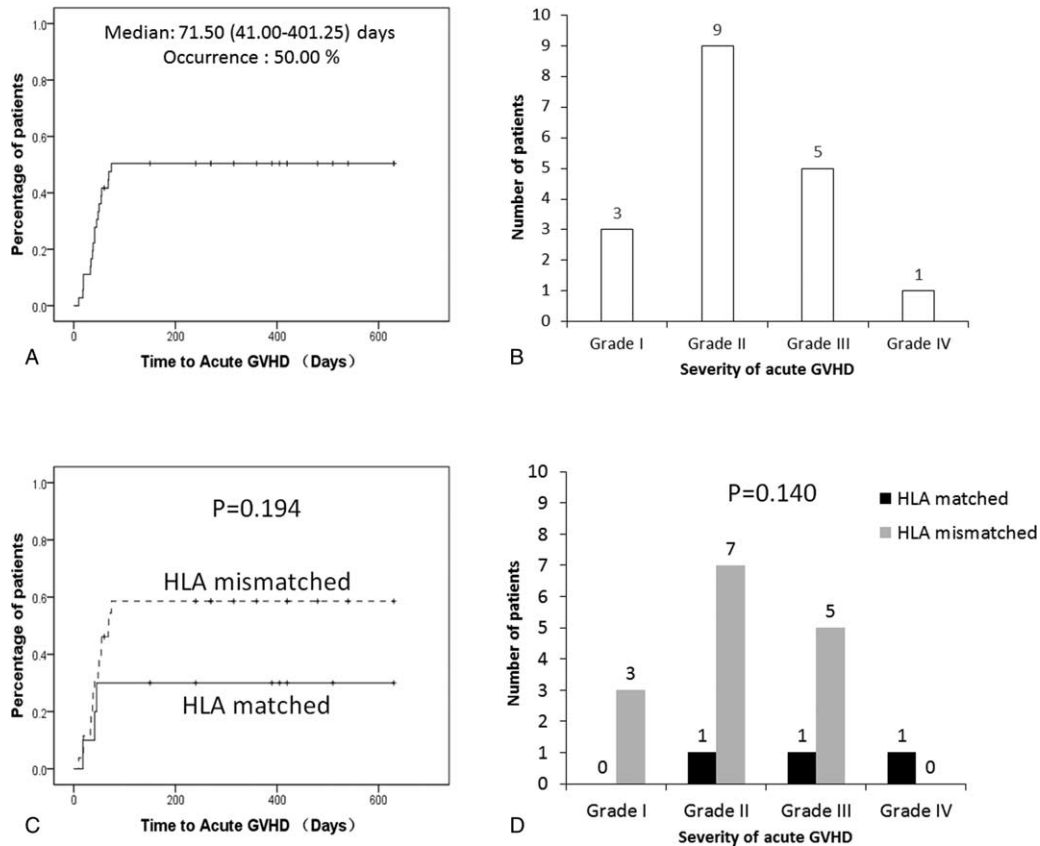

Figure 2. Occurrence and severity of aGVHD: (A) K-M curve analysis of time from transplantation to aGVHD, (B) severity of aGVHD. Most patients developed Grade II and Grade III (n = 14) aGVHD and a minority of patients developed Grade I and Grade IV (n = 4) aGVHD, (C) K-M curve analysis of aGVHD in HLA-matched and HLA-mismatched patients, (D) severity of aGVHD in HLA-matched and HLA-mismatched patients. No difference in aGVHD between HLA-matched and mismatched patients was observed. Comparison between groups was determined using log-rank test. $P < .05$ was considered significant. aGVHD = acute graft-versus-host disease, HLA = human leukocyte antigen, K-M = Kaplan-Meier curves.
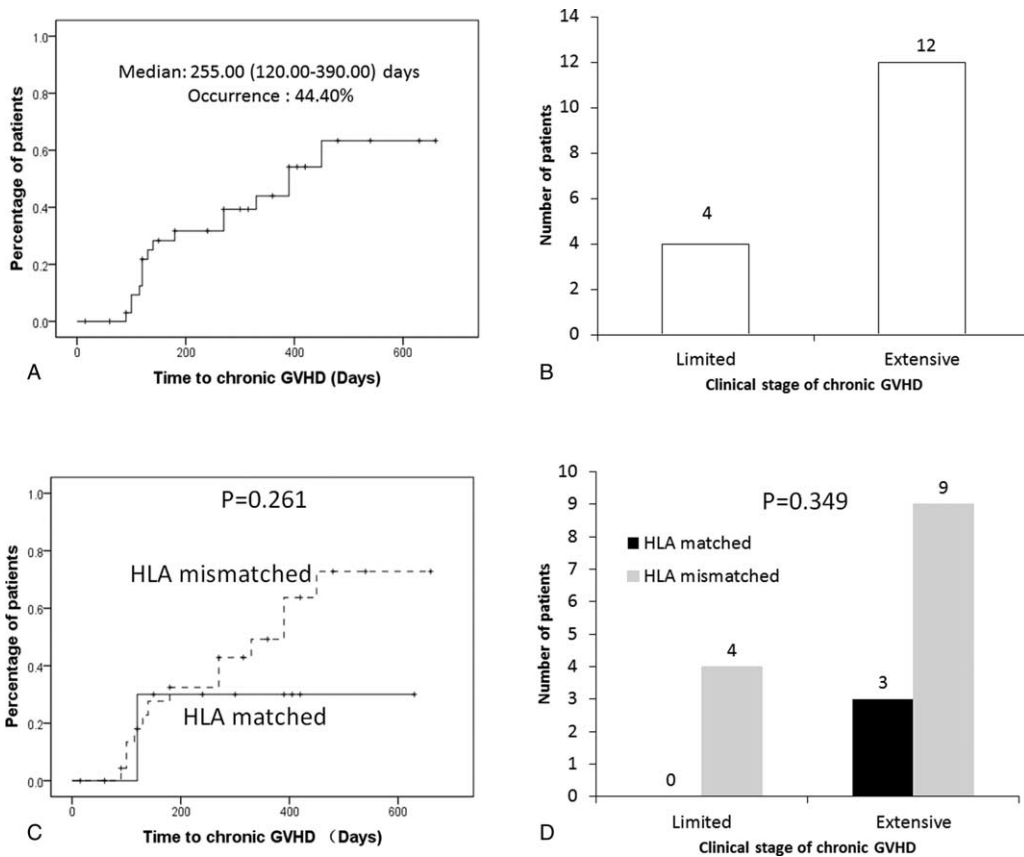
Figure 3. Occurrence and severity of cGVHD: (A) K-M curve analysis of time from transplantation to cGVHD, (B) clinical stage of cGVHD. Most patients were extensive type of cGVHD, (C) K-M curve analysis of time from transplantation to cGVHD in HLA-matched and HLA-mismatched patients, (D) clinical stage of cGVHD in HLA-matched and HLA-mismatched patients. No difference in cGVHD between HLA-matched and mismatched patients was found. Comparison between groups was determined using log-rank test. P < .05 was considered significant. cGVHD = chronic graft-versus-host disease, K-M = Kaplan-Meier curves.

Table 3

Incidence of infection.

Parameters	Number of patients (n/%)	Time to occurrence (days)
CMV	18 (50)	38.50 (30.00 – 43.75)
EBV	2 (5.6)	55.00
Hemorrhagic cystitis	7 (19.4)	36.00 (13.00 – 47.00)
Bacterial infection	13 (36.1)	50.00 (29.00 – 141.00)
Fungal infection (suspected)	8 (22.2)	24.50 (10.00 – 48.00)

Data was presented as median (25 – 75th value) or count (%).
CMV = cytomegalovirus, EBV = Epstein-Barr virus.

patients (36.1%) with median time to occurrence of 50.00 (29.00 – 141.00) days. Hemorrhagic cystitis affected 7 (19.4%) patients with median time to occurrence of 36.00 (13.00 – 47.00) days, and fungal infection was observed in 8 (22.2%) patients with median time to occurrence of 24.50 (10.00 – 48.00) days. Only 2 (5.6%) patients were infected with EBV at 55 days after allo-HSCT.

3.7. Survival profile

Mean LFS was 12.6 (95% CI: 10.1 – 15.1) months with 1-year accumulating LFS rate of $52.9 \pm 8.8\%$ (Fig. 4A). Mean OS was 14.4
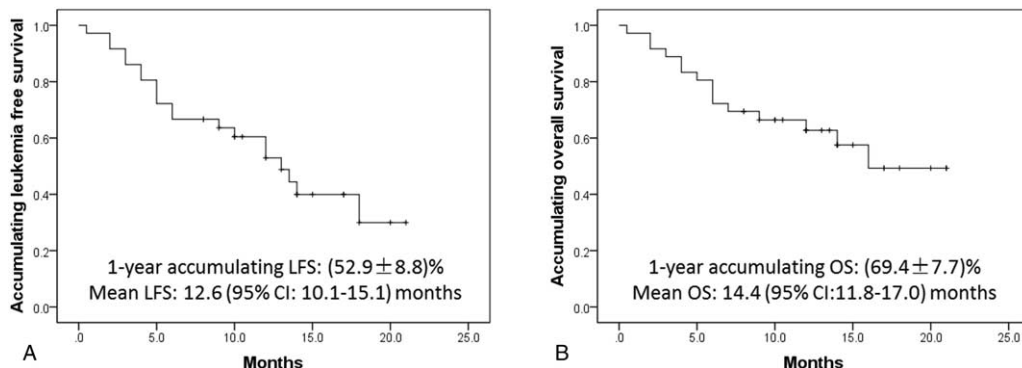

Figure 4. Accumulating LFS and OS at one year: (A) K-M curve analysis of 1-year accumulating LFS, (B) K-M curve analysis of 1-year accumulating OS. K-M = Kaplan-Meier curves, LFS = leukemia free survival, OS = overall survival.

(95% CI: 11.8 – 17.0) months with 1-year accumulating OS rate of 69.4±7.7% (Fig. 4B). Infection, GVHD, and relapse were the main causes of death: 5 patients died of infection which happened at 0.5, 2, 4, 6 and 6 months respectively; 4 patients died of GVHD; 4 patients died of relapsed leukemia; 1 patient died of infection plus GVHD.

3.8. Analysis of factors affecting LFS and OS

Patients were divided into subgroups according to their various pre-transplantation characteristics, and post-transplantation evaluations such as age, disease duration, and GVHD. Subsequently, the comparison of LFS between each 2 groups was calculated using K-M curves, and log rank test (Fig. 5). As illustrated in Fig. 5I, fungal infection was correlated with worse LFS (P= .017). Additionally, patients with aGVHD showed considerably, worse LFS rate than those without aGVHD, but without significant difference (P= .067, Fig. 5J). Other subgroup analyses based on age (Fig. 5A), disease duration (Fig. 5B), HLA-matching (Fig. 5C), gender-matching (Fig. 5D), CR pre-treatment (Fig. 5E), CMV infection (Fig. 5F), hemorrhagic cystitis (Fig. 5G),
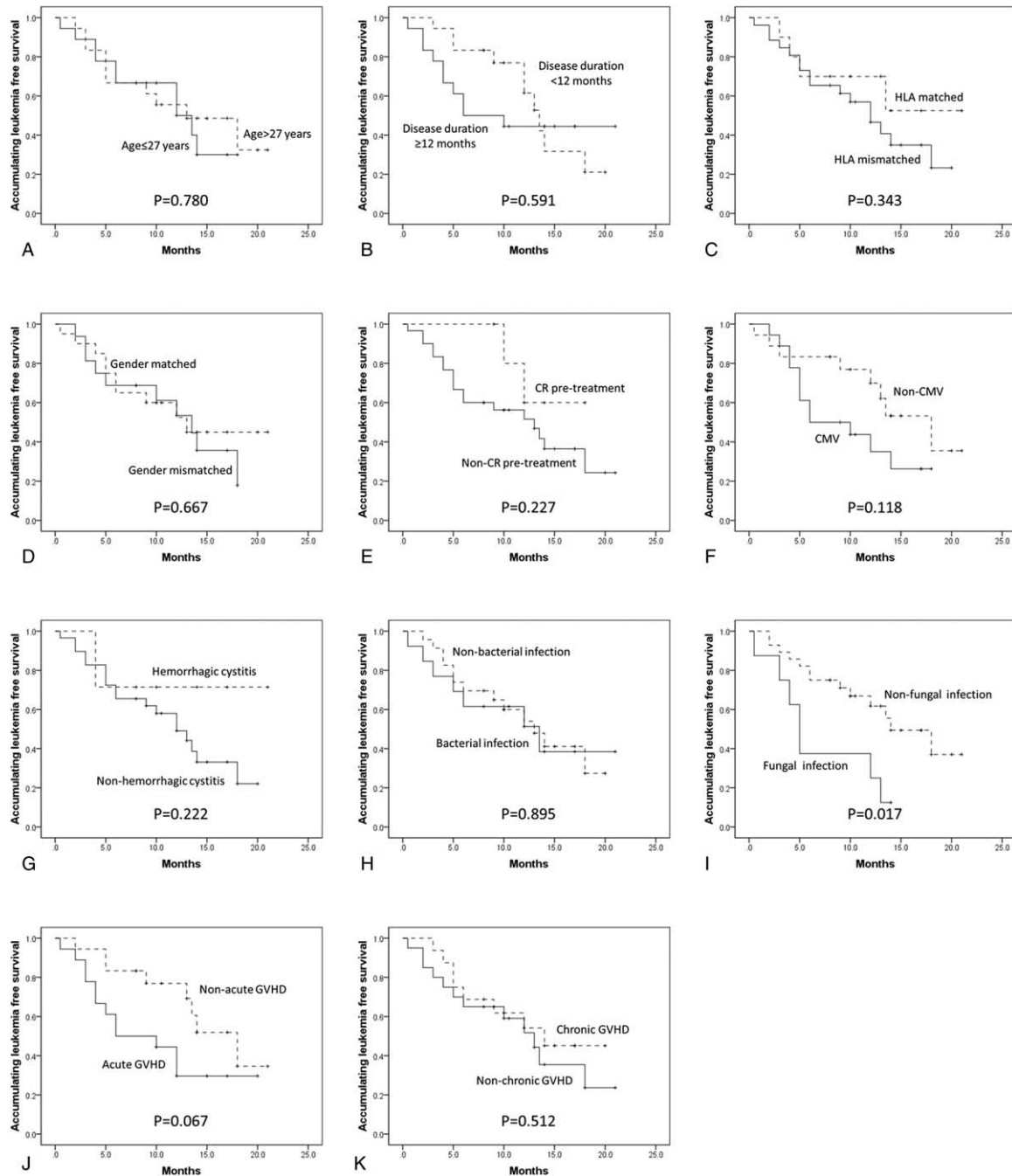

Figure 5. Accumulating LFS between groups categorized according to different characteristics. K-M curve analysis of accumulating LFS based on various factors: (A) age, (B) disease duration, (C) HLA-matching, (D) sex matching, (E). CR pre-treatment, (F) CMV infection, (G) hemorrhagic infection, (H) bacterial infection, (I) fungal infection, (J) aGVHD, (K) cGVHD. Comparison between groups was determined using log-rank test. P < .05 was considered significant. aGVHD = acute graft-versus-host disease, cGVHD = chronic graft-versus-host disease, CMV = cytomegalovirus, CR = complete remission, K-M = Kaplan-Meier curves, HLA = human leukocyte antigen, LFS = leukemia free survival, OS = overall survival.
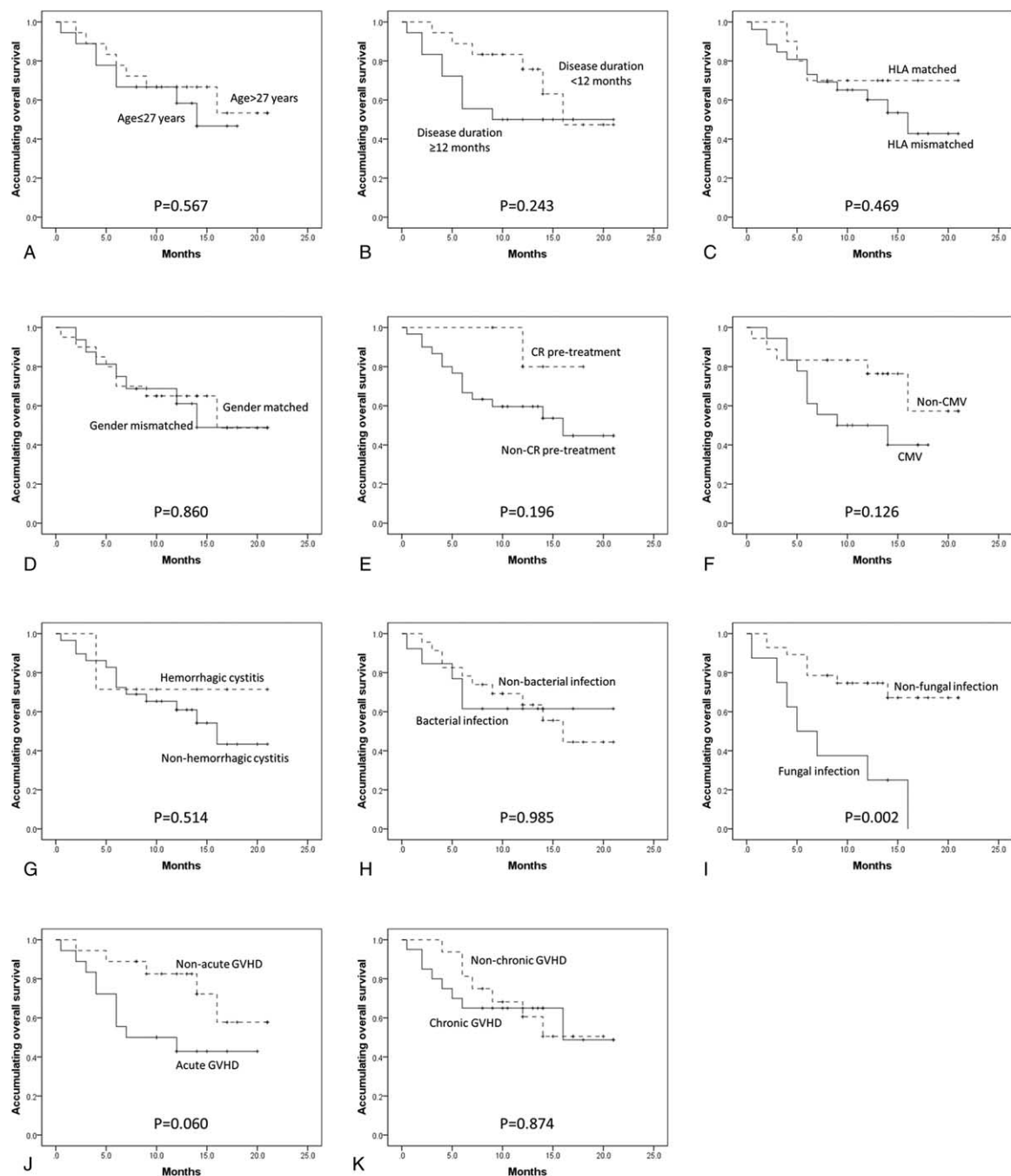
Figure 6. Accumulating OS between groups categorized according to different characteristics. K-M curve analysis of accumulating OS revealed that fungal infection (I) was correlated with unfavorable accumulating OS. Additionally, aGVHD (J) showed a trend of poor OS, but no significant difference was observed. No other factors were found associated with accumulating OS (A, B, C, D, E, F, G, H and K). Comparison between groups was determined using log-rank test. $P <$ .05 was considered significant. aGVHD = acute graft-versus-host disease , OS = overall survival, K-M = Kaplan-Meier curves.

bacterial infection (Fig. 5H), and cGVHD (Fig. 5K) showed no difference of LFS.

Subgroup analysis for OS (Fig. 6), showed that fungal infection ($P = .002$, Fig. 6I) was correlated with unfavorable accumulating OS. Additionally, patients with aGVHD showed a trend of poor OS, although no significant difference was found ($P = .060$, Fig. 6J). No other difference in OS was observed between subgroups divided by age (Fig. 6A), disease duration (Fig. 6B), HLA-matching (Fig. 6C), gender-matching (Fig. 6D), CR pre-treatment (Fig. 6E), CMV infection (Fig. 6F), hemorrhagic

cystitis (Fig. 6G), bacterial infection (Fig. 6H), and cGVHD (Fig. 6K).

4. Discussion

In our study, R/R AML patients treated with intensive conditioning regimen consisting of CLAG plus modified BuCy prior to allo-HSCT achieved 100% WBC, and 94% PLT engraftments, with a median time to WBC engraftment of 16.00 (13.25 – 18.00) days, and a median time to PLT engraftment of

13.50 (9.25 – 19.75) days. In addition, the 1-year accumulating LFS rate for these patients was 52.9±8.8%, and the 1-year accumulating OS rate was 69.4±7.7%. Furthermore, infection, GVHD, and relapse post-HSCT were the main causes of death. K-M curves suggested that fungal infection correlated with worse LFS and OS, while aGVHD correlated with considerably shorter LFS and OS but without statistical significance.

Intensive conditioning regimen is performed prior to HSCT, and can benefit the transplanted R/R AML patients in the following aspects: firstly, it reduces drug-resistance of leukemic cells, through enhancing the intensity of chemoradiotherapy, decreases the tumor load within the patients' tolerant range, eliminates minimal residual disease (MRD), and reduces the risk of recurrent leukemia;[6,14] secondly, it allows more efficient immune cell reconstitution, and enhances graft-versus-leukemia effects after transplantation.[6,14]

Cladribine is a new generation purine analogue, which is activated by intracellular phosphorylation, and is subsequently, accumulated in lymphocytes, leading to leukemic cell death.[15] Cladribine kills leukemic cells by inducing DNA single-strand breaks at the stationary phase, or inhibiting DNA synthesis at the mitotic phase. Furthermore, cladribine promotes apoptosis via inhibiting DNA methyl-transferase (DNMT) activity indirectly, by enhancing DNA demethylation through sequestration of methyl groups.[9] A wealth of studies have confirmed that intravenous infusion with high doses of Ara-C is an effective salvage therapy for R/R AML patients, due to increase in Ara-C concentration in plasma, and in cerebrospinal fluid. As a result, 40% of patients can achieve remission again.[16] Interestingly, cladribine has the ability to increase cellular uptake of Ara-C up to 50 to 65% in leukemic cells, and Chow et al demonstrated that combination of cladribine, and Ara-C repressed differentiation of leukemic cells, accelerated their apoptosis, and destroyed the reconstruction of mitochondrial membranes.[17] These findings further confirmed that cladribine plus Ara-C has a strong synergistic anti-leukemia effect that results in better therapeutic outcomes in R/R AML patients. G-CSF has been widely used to mobilize stem cells of donors during transplantation, and is additionally, applied in chemotherapy to decrease the risk of GVHD through maintaining differentiation of T cells after transplantation, the detailed mechanisms were as follows: firstly, G-CSF accelerates the generation of Tr1 regulatory cells through IL-10 production. secondly, G-CSF expands regulatory antigen presenting cell (APCs) within the donor (immature myeloid precursors and plasmacytoid DCs), and after transplantation, these regulatory APCs promote the generation of classical CD4+, CD25+ and IL-10 to produce regulatory T cells (Tregs). Ultimately, the generation of IL-10, and TGF-β from Tr1, and Tregs contribute to further suppress the inflammatory effector phase of GVHD, thereby decreasing target tissue damage.[18] Taken together, these data indicate that CLAG-based regimens show good efficacy in treating R/R AML patients through multiple mechanisms.

A multi-center, open-labeled, and non-controlled phase II clinical trial conducted by the Polish Adult Leukemia Group (PALG), which assessed the efficacy of CLAG regimen in R/R AML patients, revealed that 50% of patients treated with CLAG achieved CR. Additionally, the 1-year OS rate, and DFS rate were between 42% to 29% respectively.[19] In order to further explore the effect of CLAG in R/R AML patients, PALG performed another clinical study using CLAG combined with mitoxantrone (CLAG-M) treatment in R/R AML patients. Of the CLAG-M treated patients, 58% achieved CR, while the 4-year OS rate was 14% and the 4-year DFS rate was 30%.[20] A retrospective study, which compared CLAG regimen, and a traditional treatment

including mitoxantrone, Ara-C, and etoposide (MEC) in R/R AML patients, showed that CLAG-treated patients attained higher CR (37.9% vs 23.8%), and longer median OS (7.3 months vs 4.5 months) compared to MEC-treated patients.[21] To verify the satisfactory prognosis of CLAG therapy in R/R AML patients, Park H et al conducted a study in the year 2016 which compared the efficacy and safety of CLAG treatment to another regimen based on fludarabine (FLAG) in R/R AML patients. They reported the following results: no difference in CR was observed between the 2 groups (62.7% vs 61.4%); CLAG-treated patients achieved considerably higher 2-year OS rate (29.7% vs 13.0%), and 2-year DFS rate (69.0% vs 40.2%) compared to FLAG-treated patients, although without statistical significance; further subgroup analysis revealed that CLAG-treated patients achieved prolonged OS, and DFS than FLAG-treated patients with new refractory AML, CR at first induction therapy, and good, or intermediate cytogenetic risk.[22] In Chinese population, a previous study exhibited that 78.8% of Chinese R/R AML patients who were treated with CLAG achieved CR, and median OS in their study was 419 (9 – 525) days, which was numerically, shorter than median OS of 16.0 (range 0.5 – 21.0) months in our study. Based on this evidence, CLAG has been proposed as the first-line therapy for R/R AML disease due to its outstanding effect;[23] thus, we hypothesized that CLAG may have the potential to be used in intensive conditioning regimen for allo-HSCT in R/R AML patients. Furthermore, BuCy as a classical conditioning regimen is widely, used before transplantation, which leads to maximal decrease of residual leukemic cells.[24] Thus, compared with CLAG treatment followed by allo-HSCT, intensive conditioning regimen of CLAG combining with modified BuCy in our study had more potential to prevent post-transplantation relapse, thereby achieving longer survival.

CLAG plus modified BuCy was applied in our research as intensive conditioning regimen with successful engraftment, as manifested by WBC and PLT recovery with a median time of 16.00 (13.25 – 18.00) days, and 13.50 (9.25 – 19.75) days, respectively. Consistent with the present study, we have previously, reported that patients treated with intensive conditioning regimens, including high dose of Ara-C (HDAra-C) +BuCy, Ara-C+Bu plus Fludarabine (Flu), G-CSF plus HDAra-C +BuCy, and FLAG followed by reduced-intensified conditioning (RIC) BuCy, achieved a median WBC recovery time of 12 (10 – 24) days, and median PLT recovery time of 15 (11 – 42) days.[25] In our study, 50.00% (n=18) of the patients developed aGVHD. Of those, 9 developed Grade II aGVHD, 3 Grade I aGVHD, 5 Grade III aGVHD and 1 Grade IV aGVHD, in line with previous results regarding intensive conditioning regimens for allo-HSCT.[26] However, our results that most patients developed Grade II aGVHD is at odds with a previous study that showed that most patients developed Grade III aGVHD.[26] In contrast, 44.40% (n=16) of the patients developed cGVHD, including 4 limited cases and 12 extensive cases. The incidence of cGVHD was lower in our study compared to previous ones, which is approximately 60%.[7,25] The relative shorter duration of follow up (median 11.25 [range 0.5 – 21.0] months) in our study, which may mask cGVHD that has not occurred yet, may account for these disparate results. It was noteworthy that no difference of incidence, and severity of aGVHD was observed between HLA-matched, and HLA-mismatched patients in our study, and this result might be on account of the following reasons: firstly, it is reported in early, studies that HLA disparity increases the risk of GVHD.[27–29] However, GVHD risk of HLA-mismatched patients has been reduced greatly, in the past decade due to

the advances in prophylaxis of GVHD, and novel graft manipulation.[30] In our study, we applied ATG as an additional prophylaxis of GVHD only in HLA-mismatched patients, which decreased the risk of aGVHD in HLA-mismatched patients;[31,32] secondly, sample size of our study especially, that in HLA-matched patients (N=10, 28%) was very small, which might affect the statistical power in comparison of aGVHD incidence between HLA-matched and HLA-mismatched patients. Therefore, no difference in incidence, and severity of aGVHD was found between HLA-matched, and HLA-mismatched patients. In addition, we found that 50% of the patients were infected with CMV, 36.1% with bacteria, 22.2% with fungus, 19.4% with hemorrhagic cystitis, and 5.6% with EBV in accordance with our previous studies.[7,25]

We also discovered that patients treated with CLAG plus modified BuCy conditioning regimen prior to allo-HSCT showed satisfactory 1-year accumulating LFS and OS rates ($52.9\pm8.8\%$ and $69.4\pm7.7\%$), which were comparable with the accumulating LFS rate of $64.4\pm6.7\%$ and OS rate of $74.1\pm6.1\%$ (median follow-up duration 17.5 [2 – 34] months) in our previous research involving 4 different intensive conditioning regimens.[25] Few studies investigated the application of CLAG as the intensive conditioning regimen prior to allo-HSCT, whereas a review conducted by Craig W. Freyer et al showed that 3 previous studies reported the efficacy of CLAG-based treatment for allo-HSCT in R/R AML patients in their subgroup analysis.[33] A multicenter, open-labeled, non-comparative phase II study conducted by PALG revealed that 6 R/R AML patients, who underwent allo-HSCT after CR with CLAG treatment, achieved a median DFS of 63 weeks (ranges 8 – 122 weeks).[34] A follow-up study conducted by PALG further indicated that 7 out of 21 R/R AML patients, who had achieved CR by CLAG-M treatment, received allo-HSCT, with a median DFS of 86.5 weeks (ranges 62 – 138 weeks).[19] In another study from PLAG, which evaluated the efficacy of CLAG-M, the median OS for patients who received allo-HSCT (N=20) was 45 months with a 4-year OS rate of 38% (95% CI 7 – 68%).[20] Taken together, these studies indicated that CLAG-based salvage chemotherapy is beneficial to R/R AML patients, who are imminent to perform allo-HSCT; however, the sample sizes of the previous studies were small compared with the present study. In a previous study, we applied various intensive conditioning regimens including modified BuCy plus cyclophosphamide, HDAra-C plus Bu/Flu, FLAG plus RIC BuCy, and TBI plus Cy in 45 R/R AML patients. The 3-year OS rate for these patients was 62.6%, and the LFS rate was 60.2%; however, the sample size in each treatment group was relatively small, and CLAG conditioning regimen is not contained which might be more effective due to its multiple anti-leukemia effects.[7]

TBI is commonly used in conditioning for allo-HSCT due to the efficacy of irradiation which leads to myeloablation and immunosuppression. However, TBI is associated with occasional sequelae, such as secondary tumors, and children growth retardation, and has been recently, replaced by other regimens in several cases.[35,36] In the present study, TBI was not performed in all patients, because the provided intensive conditioning regimen of CLAG, and modified BuCy was considered to have strong effects; thus, we decided that TBI was not necessary, and its application may greatly increase toxicity.

Fludarabine, which is another purine analog, is commonly used in conditioning regimens prior to allo-HSCT in R/R AML patients. A retrospective study that applied FLAG plus modified BuCy prior to allo-HSCT in treating 10 R/R AML patients showed a median DFS of 164 days (57 – 442 days).[26] Another study, which evaluated the efficacy of reduced intensive conditioning regimen

consisting of fludarabine, Ara-C, and amsacrine (FLAMSA) in R/R AML patients, demonstrated that the 4-year OS rate for FLAMSA plus TBI treated patients was 47%, and the LFS rate was 41%, whereas the 4-year OS rate for FLAMSA plus treosulfan treated patients was 43% and the LFS rate was 40%.[37] Another cohort study conducted by Jens Marcus Chemnitz et al showed that the 1-year OS rate for R/R AML patients treated with FLAMSA plus treosulfan, cyclophosphamide, and ATG prior to allo-HSCT, was 62%, which is lower than our results (1-year OS rate of $69.4\pm7.7\%$). The disparate results may be explained by the unique effects of cladribine that induces cell apoptosis through changing the membrane potential of mitochondria, repressing DNMT activity and sequestering away methyl group donors.[38]

As to the 14 patients failed to survival in our study, 5 patients died from infection, 1 died from infection plus GVHD, 4 died from GVHD, and 4 died from disease relapse, indicating that infection, GVHD, and relapsed leukemia were major threats to short-term survival rate in patients subjected to transplantation. This inference was further confirmed by the analysis of influencing factors for survival, which showed that fungal infection was correlated with unfavorable LFS, and OS, and aGVHD was considerably, associated with shorter LFS, and OS (although no significance was discovered). In general, CLAG plus modified BuCy therapy in our research was considered to be effective due to successful WBC, and PLT engraftment, and high probability of survival, and these outcomes may be due to: firstly, cladribine, which exerts a direct cytotoxic effect on leukemic cells by interfering with cell metabolism leading to cell death;[9,15] secondly, Ara-C, which impairs DNA synthesis, and then inhibits proliferation of leukemic cells. Additionally, cladribine increases cellular uptake of Ara-C thus acting synergistically with Ara-C;[16,17] thirdly, G-CSF, which reduces incidence of GVHD, and relapse, and it enhances the anti-leukemia effects.[18]

There were still some limitations in this study: firstly, the sample size was relatively small; secondly, it was a single-arm study that lacked a control group; thirdly, follow-up duration was relatively short, thus long-term efficacy, and safety of CLAG plus modified Bucy could not be evaluated. In order to further validate the outcomes of CLAG plus modified BuCy conditioning regimen for allo-HSCT in R/R AML patients, we are now conducting a randomized, controlled study comparing the efficacy, and safety between regimens of CLAG plus modified BuCy, and modified BuCy for allo-HSCT in R/R AML patients with a 3-year observational period, which was registered on www.chictr.org.cn (Chinese Clinical Trial Registry conducted by World Health Organization) with registration number: ChiCTR-IPR-16009917.

In summary, our study indicated that intensive conditioning regimen of CLAG plus modified BuCy for allo-HSCT may be effective, and well-tolerated in R/R AML patients.

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References

[1] Dohner H, Weisdorf DJ, Bloomfield CD. Acute myeloid leukemia. N Engl J Med 2015;373:1136–52.

[2] Thol F, Schlenk RF, Heuser M, et al. How I treat refractory and early relapsed acute myeloid leukemia. Blood 2015;126:319–27.

[3] Estey E, Dohner H. Acute myeloid leukaemia. Lancet 2006;368:1894–907.

[4] Duval M, Klein JP, He W, et al. Hematopoietic stem-cell transplantation for acute leukemia in relapse or primary induction failure. J Clin Oncol 2010;28:3730–8.

[5] Koreth J, Schlenk R, Kopecky KJ, et al. Allogeneic stem cell transplantation for acute myeloid leukemia in first complete remission: systematic review and meta-analysis of prospective clinical trials. JAMA 2009;301:2349–61.

[6] Shimoni A, Nagler A. Optimizing the conditioning regimen for allogeneic stem-cell transplantation in acute myeloid leukemia; dose intensity is still in need. Best Pract Res Clin Haematol 2011;24:369–79.

[7] Wang JB, Wu T, Da WM, et al. Salvaged allogeneic hematopoietic stem cell transplantation for refractory/recurrent acute myeloid leukemia. Zhonghua Xue Ye Xue Za Zhi 2012;33:467–70.

[8] Liu QF, Sun J, Xu D, et al. Effects of allogeneic hematopoietic stem cell transplantation with very-high-dose conditioning regimen for refractory leukemia. Di Yi Jun Yi Da Xue Xue Bao 2004;24:1117–9.

[9] Vahdat L, Wong ET, Wile MJ, et al. Therapeutic and neurotoxic effects of 2-chlorodeoxyadenosine in adults with acute myeloid leukemia. Blood 1994;84:3429–34.

[10] Carson DA, Wasson DB, Taetle R, et al. Specific toxicity of 2-chlorodeoxyadenosine toward resting and proliferating human lymphocytes. Blood 1983;62:737–43.

[11] Ramos NR, Mo CC, Karp JE, et al. Current approaches in the treatment of relapsed and refractory acute myeloid leukemia. J Clin Med 2015;4:665–95.

[12] Robak T. Purine nucleoside analogues in the treatment of myeloid leukemias. Leuk Lymphoma 2003;44:391–409.

[13] Kolb HJ, Schattenberg A, Goldman JM, et al. Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. Blood 1995;86:2041–50.

[14] Schmid C, Schleuning M, Hentrich M, et al. High antileukemic efficacy of an intermediate intensity conditioning regimen for allogeneic stem cell transplantation in patients with high-risk acute myeloid leukemia in first complete remission. Bone Marrow Transplant 2008;41:721–7.

[15] Sigal DS, Miller HJ, Schram ED, et al. Beyond hairy cell: the activity of cladribine in other hematologic malignancies. Blood 2010;116:2884–96.

[16] Capizzi RL, Poole M, Cooper MR, et al. Treatment of poor risk acute leukemia with sequential high-dose ARA-C and asparaginase. Blood 1984;63:694–700.

[17] Chow KU, Boehrer S, Napieralski S, et al. In AML cell lines Ara-C combined with purine analogues is able to exert synergistic as well as antagonistic effects on proliferation, apoptosis and disruption of mitochondrial membrane potential. Leuk Lymphoma 2003;44:165–73.

[18] Morris ES, MacDonald KP, Hill GR. Stem cell mobilization with G-CSF analogs: a rational approach to separate GVHD and GVL? Blood 2006;107:3430–5.

[19] Wrzesien-Kus A, Robak T, Wierzbowska A, et al. A multicenter, open, noncomparative, phase II study of the combination of cladribine (2-chlorodeoxyadenosine), cytarabine, granulocyte colony-stimulating factor and mitoxantrone as induction therapy in refractory acute myeloid leukemia: a report of the Polish Adult Leukemia Group. Ann Hematol 2005;84:557–64.

[20] Wierzbowska A, Robak T, Pluta A, et al. Cladribine combined with high doses of arabinoside cytosine, mitoxantrone, and G-CSF (CLAG-M) is a highly effective salvage regimen in patients with refractory and relapsed acute myeloid leukemia of the poor risk: a final report of the Polish Adult Leukemia Group. Eur J Haematol 2008;80:115–26.

[21] Price SL, Lancet JE, George TJ, et al. Salvage chemotherapy regimens for acute myeloid leukemia: is one better? Efficacy comparison between CLAG and MEC regimens. Leuk Res 2011;35:301–4.

[22] Park H, Youk J, Kim I, et al. Comparison of cladribine- and fludarabine-based induction chemotherapy in relapsed or refractory acute myeloid leukaemia. Ann Hematol 2016;95:1777–86.

[23] Duan MH, Zhang Y, Zhang M, et al. Efficacy and safety analysis of the combination of cladribine, cytarabine, granulocyte colonystimulating factor (CLAG) regime in patients with refractory or relapsed acute myeloid leukemia. Zhonghua Xue Ye Xue Za Zhi 2016;37:571–5.

[24] Pasquini MC, Le-Rademacher J, Zhu X, et al. Intravenous Busulfan-Based Myeloablative Conditioning Regimens Prior to Hematopoietic Cell Transplantation for Hematologic Malignancies. Biol Blood Marrow Transplant 2016;22:1424–30.

[25] Wang JB, Da WM, Zhang JP, et al. Successful treatment of high risk/ refractory leukemia by tumor-ablative individualized conditioning allogeneic hematopoietic stem cell transplantation. Zhonghua Xue Ye Xue Za Zhi 2010;31:505–9.

[26] Wang ZH, Wu XX, Liu ZY, et al. Curative efficacy analysis of allo-HSCT using conditioning regimen of FLAG combined with modified BUCY for refractory and relapsed patients with hematologic malignancies. Zhongguo Shi Yan Xue Ye Xue Za Zhi 2011;19:158–62.

[27] Fujiwara H, Sconocchia G, Melenhorst J, et al. Tissue-restricted T cell alloresponses across HLA barriers: selection and identification of leukemia-restricted CTL in HLA-mismatched stimulator-responder pairs. Bone Marrow Transplant 2003;32:371–8.

[28] Anasetti C, Amos D, Beatty PG, et al. Effect of HLA compatibility on engraftment of bone marrow transplants in patients with leukemia or lymphoma. N Engl J Med 1989;320:197–204.

[29] Beatty PG, Clift RA, Mickelson EM, et al. Marrow transplantation from related donors other than HLA-identical siblings. N Engl J Med 1985;313:765–71.

[30] Fuchs EJ, Huang XJ, Miller JS. HLA-haploidentical stem cell transplantation for hematologic malignancies. Biol Blood Marrow Transplant 2010;16:S57–63.

[31] Chen SH, Li X, Huang XJ. Effect of recombinant human granulocyte colony-stimulating factor on T-lymphocyte function and the mechanism of this effect. Int J Hematol 2004;79:178–84.

[32] Jun HX, Jun CY, Yu ZX. In vivo induction of T-cell hyporesponsiveness and alteration of immunological cells of bone marrow grafts using granulocyte colony-stimulating factor. Haematologica 2004;89:1517–24.

[33] Freyer CW, Gupta N, Wetzler M, et al. Revisiting the role of cladribine in acute myeloid leukemia: an improvement on past accomplishments or more old news? Am J Hematol 2015;90:62–72.

[34] Wrzesien-Kus A, Robak T, Lech-Maranda E, et al. A multicenter, open, non-comparative, phase II study of the combination of cladribine (2-chlorodeoxyadenosine), cytarabine, and G-CSF as induction therapy in refractory acute myeloid leukemia - a report of the Polish Adult Leukemia Group (PALG). Eur J Haematol 2003;71:155–62.

[35] Choi B, Chun E, Kim M, et al. Human B cell development and antibody production in humanized NOD/SCID/IL-2Rgamma(null) (NSG) mice conditioned by busulfan. J Clin Immunol 2011;31:253–64.

[36] Gorin NC, Estey E, Jones RJ, et al. New Developments in the Therapy of Acute Myelocytic Leukemia. Hematology Am Soc Hematol Educ Program 2000;69–89.

[37] Holtick U, Herling M, Pflug N, et al. Similar outcome after allogeneic stem cell transplantation with a modified FLAMSA conditioning protocol substituting 4 Gy TBI with treosulfan in an elderly population with high-risk AML. Ann Hematol 2017;96:479–87.

[38] Chemnitz JM, von Lilienfeld-Toal M, Holtick U, et al. Intermediate intensity conditioning regimen containing FLAMSA, treosulfan, cyclophosphamide, and ATG for allogeneic stem cell transplantation in elderly patients with relapsed or high-risk acute myeloid leukemia. Ann Hematol 2012;91:47–55.