

Cladribine with Granulocyte Colony-Stimulating Factor, Cytarabine, and Aclarubicin Regimen in Refractory/Relapsed Acute Myeloid Leukemia: A Phase II Multicenter Study

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Key Words. Cladribine • Colony-stimulating factor • Cytarabine • Aclarubicin • Acute myeloid leukemia

TRIAL INFORMATION

- **Chinese Clinical Trial Registry Number:** ChiCTR-OPC-16010166
- **Sponsor:** Sun Yat-sen University Cancer Center
- **Principal Investigator:** Yue Lu
- **IRB Approved:** Yes

LESSONS LEARNED

- Studies targeting cladribine in combination with granulocyte colony-stimulating factor, low-dose cytarabine, and aclarubicin (C-CAG) regimen in relapsed and refractory acute myeloid leukemia (R/R AML) are limited.
- The complete remission rate after two cycles of C-CAG regimen was 67.6%, and 1-year overall survival and disease-free survival rates were 59.7% and 72.9%, respectively.
- The C-CAG regimen is significantly effective against R/R AML with a low hematological toxicity and thus serves as an alternative treatment for R/R AML.

ABSTRACT

Background. The optimal salvage chemotherapy regimen for relapsed and refractory acute myeloid leukemia (R/R AML) remains uncertain. Therefore, a phase II study was conducted for the prospective evaluation of the efficacy and safety of the purine analog cladribine in combination with granulocyte colony-stimulating factor (G-CSF), low-dose cytarabine, and aclarubicin (C-CAG) regimen for patients with R/R AML.

Methods. A total of 34 patients received C-CAG regimen for salvage treatment as follows: cladribine 5 mg/m², days 1–5; G-CSF 300 µg, days 0–9; aclarubicin 10 mg, days 3–6; cytarabine 10 mg/m² every 12 hours, subcutaneously, days 3–9; 4 weeks per cycle. Patients were allowed to withdraw from the study if complete remission (CR) was not achieved after two courses of chemotherapy. If conditions were right, the patients achieving CR were recommended to receive allogeneic hematopoietic stem cell transplantation. Otherwise,

they were treated for a total of six cycles unless disease progression or unacceptable side effects were observed or they withdrew their consent.

Results. All patients received at least two cycles of C-CAG regimen chemotherapy. After two cycles of C-CAG, 23 patients (67.6%) achieved CR, and 5 patients had partial remission (14.7%). At a median follow-up of 15 months (range, 3–38 months), the 1-year overall survival (OS) and disease-free survival (DFS) rates were 59.7% (95% confidence interval [CI], 42.6%–76.8%) and 72.9% (95% CI, 54.3%–91.5%), respectively. The most common adverse effect was myelosuppression. Nonhematological toxicities were mild, and no treatment-related deaths occurred.

Conclusion. Preliminary data indicate that the C-CAG regimen chemotherapy is significantly effective against R/R AML

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with a high remission rate and a low hematological toxicity. Thus, it may serve as an alternative treatment for R/R AML. *The Oncologist* 2020;25:e1663–e1670

DISCUSSION

Most patients with acute myeloid leukemia (AML) suffer relapse or primary refractory (R/R) disease. And there is no standard rescue chemotherapy regimen for R/R AML yet. A regimen consisting of granulocyte colony-stimulating factor, low-dose cytarabine, and aclarubicin (CAG) has been widely used in patients with R/R AML; however, half of the patients are still unable to achieve CR through this regimen. It has been proved that cladribine is an attractive drug in AML because of its significant synergy with other chemotherapeutic agents and favorable toxicity profile. However, there is no report of the treatment of cladribine in combination with CAG regimen in R/R AML yet.

In this phase II, single-arm study, we aimed to evaluate the efficacy and safety of a modified CAG regimen containing cladribine in relapsed and refractory AML. The treatment scheme is shown in Figure 1. To the best of our knowledge, this work is the first prospective clinical study to explore the treatment of relapsed and refractory AML with cladribine combined with the CAG regimen.

The baseline characteristics of all patients in this study are summarized. The median age of the entire study population was 47 years (range, 18–72 years), and eight patients (23.5%) were ≥ 60 years. According to cytogenetic classification, the majority of the patients (28 cases, 82.4%) were classified as intermediate/high risk. Nearly half of the patients (16 cases, 47.1%) had refractory disease. The median duration of first CR for relapsed patients was 8 months. After two cycles of C-CAG, 23 patients (67.6%) achieved CR, and 5 patients had partial remission (14.7%), which was better than the rates previously reported with other regimens in RR/AML (30%–60%). At a median follow-up of 15 months (range, 3–38 months), the 1-year OS and DFS rates were 59.7% (95% CI, 42.6%–76.8%) and 72.9% (95% CI, 54.3%–91.5%), respectively. This result is of great importance because many patients with RR/AML achieved CR and would have the opportunity to receive allogeneic

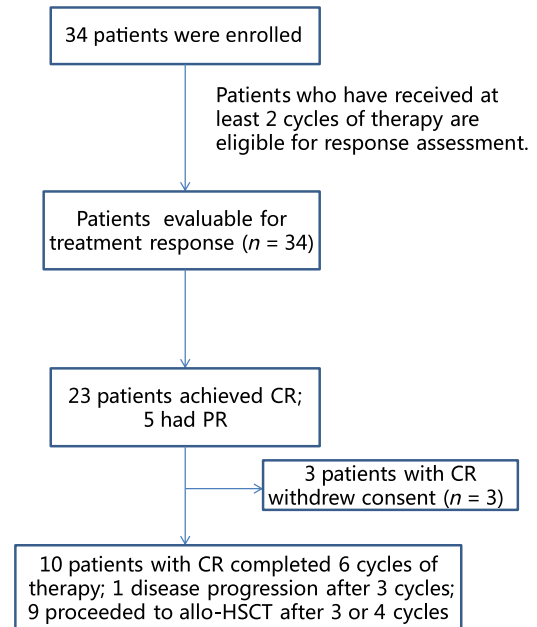


Figure 1. Treatment scheme.

Abbreviations: allo-HSCT, allogeneic hematopoietic stem cell transplantation; CR, complete response.

hematopoietic stem cell transplantation (allo-HSCT), which is the only curative treatment for RR/AML so far. The most common adverse effect was myelosuppression. The duration of hospital stay was 29 days. Nonhematological toxicities were mild, and no treatment-related deaths occurred. This result indicates that C-CAG has low toxicity and allows few patients to have dose reduction and treatment interruption due to toxicity.

The results from this phase II prospective clinical study showed that the C-CAG regimen is a promising treatment for R/R AML with a high remission rate and low hematological toxicity. The C-CAG regimen also increases the opportunity for allo-HSCT. Furthermore, promising antitumor activity of cladribine in R/R AML suggests that further investigations are warranted to confirm our findings.

TRIAL INFORMATION

Disease	Leukemia – acute – AML
Stage of Disease/Treatment	Metastatic/advanced
Prior Therapy	No designated number of regimens
Type of Study	Phase II, single arm
Primary Endpoint	Complete response rate
Secondary Endpoints	Toxicity, overall survival

Additional Details of Endpoints or Study Design

The primary endpoint, CR, was assessed according to Simon's optimal two-stage design. The CR rate of the previous salvage therapy for relapsed and refractory AML ranged from 30% to 60%. Thus, a sample size that is sufficient to reject a CR rate of 45% in support of a target CR rate of 70% was calculated. Thirteen patients were enrolled during stage 1. If seven or more complete responses were observed during stage 1, the trial would continue to enroll. Afterward, 17 patients needed to be enrolled. If 17 or more complete responses were observed by the end of stage 2, the treatment would continue into a phase III trial. Given the dropout rate of 10%, a total sample size of 34 patients was required.

Investigator's Analysis

Active and should be pursued further

DRUG INFORMATION**Drug 1**

Generic/Working Name	Cladribine
Drug Type	Small molecule
Drug Class	Antimetabolite
Dose	5 mg/m ²
Route	IV
Schedule of Administration	Days 1–5

Drug 2

Generic/Working Name	Granulocyte colony-stimulating factor
Drug Type	Biological
Drug Class	Immune therapy
Dose	300 µg per flat dose
Route	IV
Schedule of Administration	Days 0–9

Drug 3

Generic/Working Name	Aclarubicin
Drug Type	Small molecule
Drug Class	Anthracycline
Dose	10 mg per flat dose
Route	IV
Schedule of Administration	Days 3–6

Drug 4

Generic/Working Name	Cytarabine
Drug Type	Small molecule
Drug Class	Antimetabolite
Dose	10 mg/m ²
Route	IV
Schedule of Administration	Every 12 hours, subcutaneously, days 3–9

PATIENT CHARACTERISTICS

Number of Patients, Male	19
Number of Patients, Female	15
Stage	Relapse or refractory
Age	Median (range): 47 years (18–72 years)
Cancer Types or Histologic Subtypes	FAB subtypes M0, 1 FAB subtypes M2, 13 FAB subtypes M4, 5 FAB subtypes M5, 15

PRIMARY ASSESSMENT METHOD

Number of Patients Screened	34
Number of Patients Enrolled	34
Number of Patients Evaluable for Toxicity	34

Number of Patients Evaluated for Efficacy	34
Response Assessment CR	<i>n</i> = 23 (67.6%)
Response Assessment PR	<i>n</i> = 5 (14.7%)
Response Assessment SD	<i>n</i> = 6 (17.6%)
(Median) Duration Assessments OS	19 months, CI: 18.4–28.6

ADVERSE EVENTS							
All Cycles							
Name	NC/NA	1	2	3	4	5	All grades
Neutrophil count decreased	0%	0%	0%	71%	29%	0%	100%
Platelet count decreased	0%	0%	0%	79%	21%	0%	100%
Mucositis oral	0%	94%	0%	6%	0%	0%	100%
Infections and infestations - Infections	0%	59%	35%	3%	3%	0%	100%
Diarrhea	0%	76%	21%	3%	0%	0%	100%
Vomiting	0%	82%	15%	3%	0%	0%	100%

Adverse Events Legend

Adverse events in all patients.

Abbreviation: NC/NA, no change from baseline/no adverse event.

ASSESSMENT, ANALYSIS, AND DISCUSSION**Completion**

Study completed

Investigator's Assessment

Active and should be pursued further

Although many advances have been made in the treatment of acute myeloid leukemia (AML) in recent years, the prognosis for relapsed and refractory (R/R) AML remains poor owing to chemotherapy resistance and recurrence in patients [1, 2]. The CAG regimen, comprising low-dose cytarabine, aclarubicin, and granulocyte colony-stimulating factor (G-CSF), has been widely used in patients with myelodysplastic syndrome and AML in China [3]. CAG therapy produces a complete response (CR) rate of 40%–60% in R/R AML, providing a reasonable treatment option [4]. However, half of patients are still unable to achieve CR through this regimen. Therefore, the CAG regimen must be modified so that its CR rate improves and survival time is prolonged. In the current study, cladribine has been added to CAG to form C-CAG for patients with R/R AML. CR rate, disease-free survival (DFS), and overall survival (OS) were analyzed, and a CR rate of 66.7% for the entire cohort was achieved, which was better than the rates previously reported with other regimens in R/R AML (30%–60%). In addition, the C-CAG regimen produced a 1-year OS of 59.7% (Fig. 2A) and DFS of 72.9% (Fig. 2B). This result indicates that the high CR rate of C-CAG regimen in treating patients with R/R AML would provide more opportunity to receive allogeneic hematopoietic stem cell transplantation (allo-HSCT), which is the only curative approach to R/R AML.

The purine analog 2-CdA increases the uptake of Ara-C and the accumulation of its active cytotoxic metabolite 5 α -triphosphate Ara-C in leukemia cells [5, 6]. This finding suggests that synergy occurs between cladribine and cytarabine. Priming with G-CSF may further increase the anti-leukemia effect of Ara-C, especially on slowly proliferating

and quiescent leukemia cells [7, 8]. Aclarubicin, the anthracene nucleus antibiotic, is a cell cycle–nonspecific anticancer drug to which most R/R AML are not resistant [9]. In the C-CAG regimen, this drug plays a role in inducing the apoptosis of blast cells by directly intercalating into DNA base pairs and preventing mRNA formation [10]. The concept of this study was based on the characteristics of the above drugs and the relationship between them. In summary, our C-CAG regimen had a lower dose of cytarabine and a different anthracycline compared with the more familiar regimen of cladribine combined with high doses of arabinoside cytosine, mitoxantrone, and G-CSF (CLAG-M). Given the significant synergistic effect between drugs and the priming of G-CSF, we were confident that we could use low-dose cytarabine, which may also reduce the side effects of treatment.

The CR rate for all patients treated with the C-CAG regimen after two courses of treatment was 67.6%, which was comparable with the rates of 58% previously reported with CLAG-M in RR/AML [11]. The baseline characteristics of patients our study (Table 1) were comparable with study of CLAG-M, and refractory or high-risk patients accounted for a large proportion according to cytogenetics [11]. Table 2 showed that in our study, patients with high-risk cytogenetic abnormality had significantly lower CR rate than low- and intermediate-risk patients. The CLAG-M regimens showed similar results with an extremely high CR rate of up to 100% for low-risk patients and a much lower CR rate for high-risk patients. In addition, the effectiveness of the C-CAG regimen was not significantly correlated with the patients' previous disease status and age (Table 2), which

may suggest that the C-CAG program is particularly suitable for older refractory patients.

Table 3 summarizes the main hematological toxicity and nonhematological side effects for patients in induction chemotherapy. Compared with the CLAG-M regimen, the median hospital stay and antibiotic use time of the C-CAG regimen were significantly shorter, and the incidence of grade 3–4 hematologic or nonhematologic adverse events was significantly lower [11]. This result indicates that C-CAG has low toxicity and is suitable for elderly patients and allows few patients to have dose reduction and treatment interruption due to toxicity.

According to cytogenetic risk stratification, the OS for high-risk patients was significantly worse than that of low/intermediate-risk patients ($p = .033$; Fig. 3A). The prognosis for patients with CR after induction chemotherapy was better than that for patients without CR ($p < .001$; Fig. 3B). The OS and DFS of patients with CR and negative minimal residual disease (MRD) after chemotherapy were significantly better than those of patients with CR and positive MRD (Fig. 4). Five high-risk patients achieved CR. Unfortunately, only one received allo-SCT for various reasons. Two patients experienced relapse and died, whereas three patients still showed no recurrence. Libura et al. reported that cladribine added to daunorubicin–cytarabine induction prolongs the survival of patients with Fms-like tyrosine kinase 3 internal tandem duplication (FLT3-ITD) positive normal karyotype AML [12]. Thus, administering chemotherapy based on cladribine to patients with FLT3-ITD⁺ is reasonable. We observed that a patient with an FLT3 mutation and normal karyotype who underwent a six-course C-CAG regimen without allo-SCT had a DFS time of

>30 months. Another high-risk patient carrying a TP53 mutation with 75% did not achieve remission after two courses of IA regimen chemotherapy. After six cycles of C-CAG chemotherapy, the duration of remission exceeded 28 months. MRD was persistently negative in both patients after chemotherapy. The recurrence rate of low/intermediate-risk patients achieving CR but without allo-SCT was as high as 60%, which was roughly equivalent to the recurrence rate of 50% for high-risk patients with CR without allo-SCT. By contrast, eight patients with low/intermediate risk who received allo-SCT in second CR (CR2) were currently all in a relapse-free state. Therefore, allo-SCT after reinduction by C-CAG is an important factor for long-term survival, whether in low/intermediate-risk or high-risk patients. Indeed, most salvage therapies are designed to minimize the burden of disease before allo-HSCT. High CR rate for C-CAG, especially in low- and intermediate-risk patients, increases the possibility of allo-SCT.

In conclusion, the results of our phase II prospective clinical study indicated that the C-CAG regimen shows remarkable potential as a salvage therapy for R/R AML, improves the CR rate compared with the CAG regimen, and increases the opportunity for allo-HSCT. The C-CAG regimen offers efficacy that is comparable to that of CLAG-M but has a significantly lower toxicity and is thus especially suitable for elderly patients. Further investigation in large prospective trials is warranted to confirm our findings.

DISCLOSURES

The authors indicated no financial relationships.

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FIGURES AND TABLES

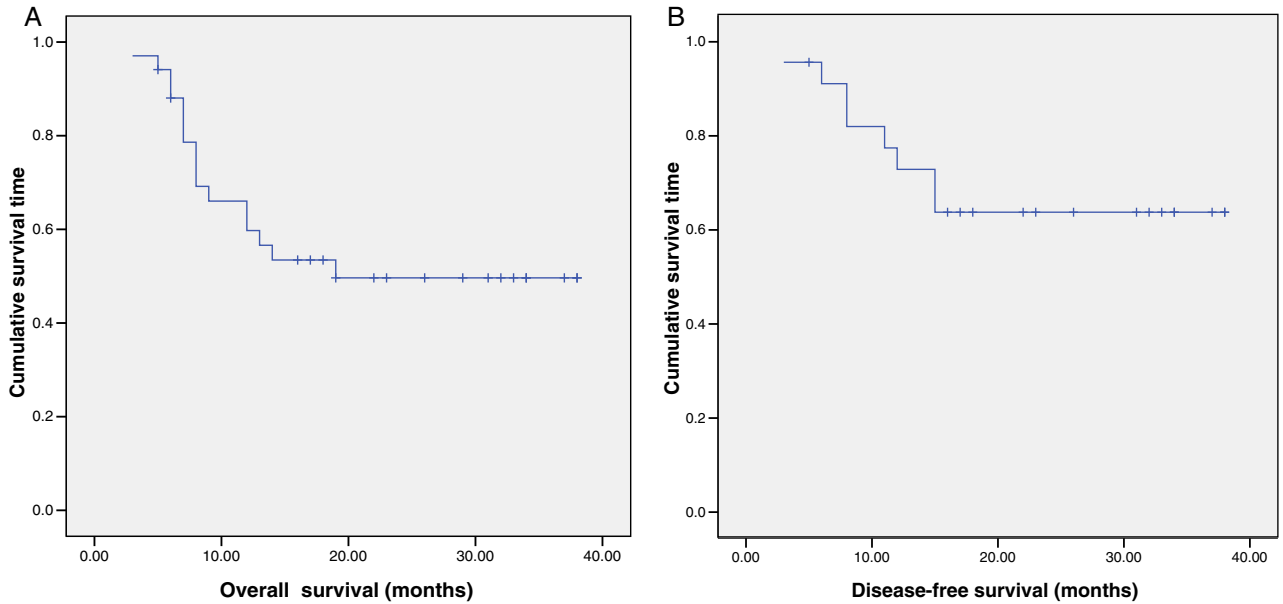


Figure 2. Kaplan–Meier curves of 34 patients with refractory/relapsed acute myeloid leukemia. **(A)** Overall survival **(B)** Disease-free survival.

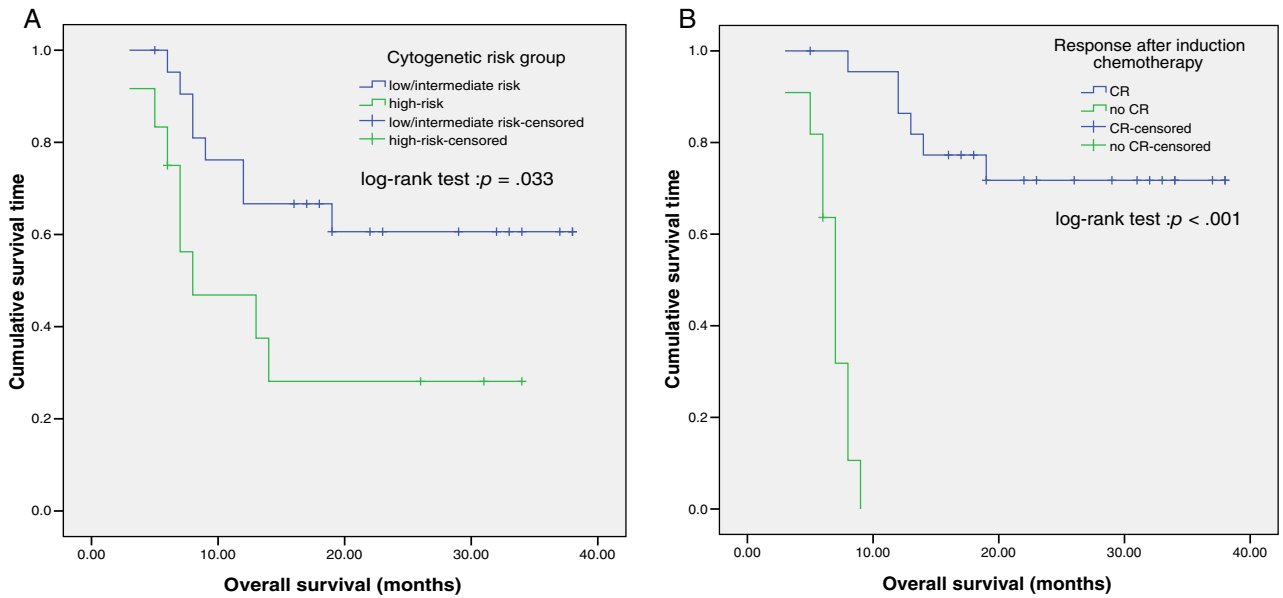


Figure 3. Kaplan–Meier curves for overall survival of patients according to cytogenetic risk group and response after induction chemotherapy. **(A):** Kaplan–Meier curves for overall survival of patients according to cytogenetic risk group (low/intermediate risk versus high-risk). **(B):** Kaplan–Meier curves for overall survival of patients according to response after induction chemotherapy (CR vs. no CR).

Abbreviation: CR, complete response.

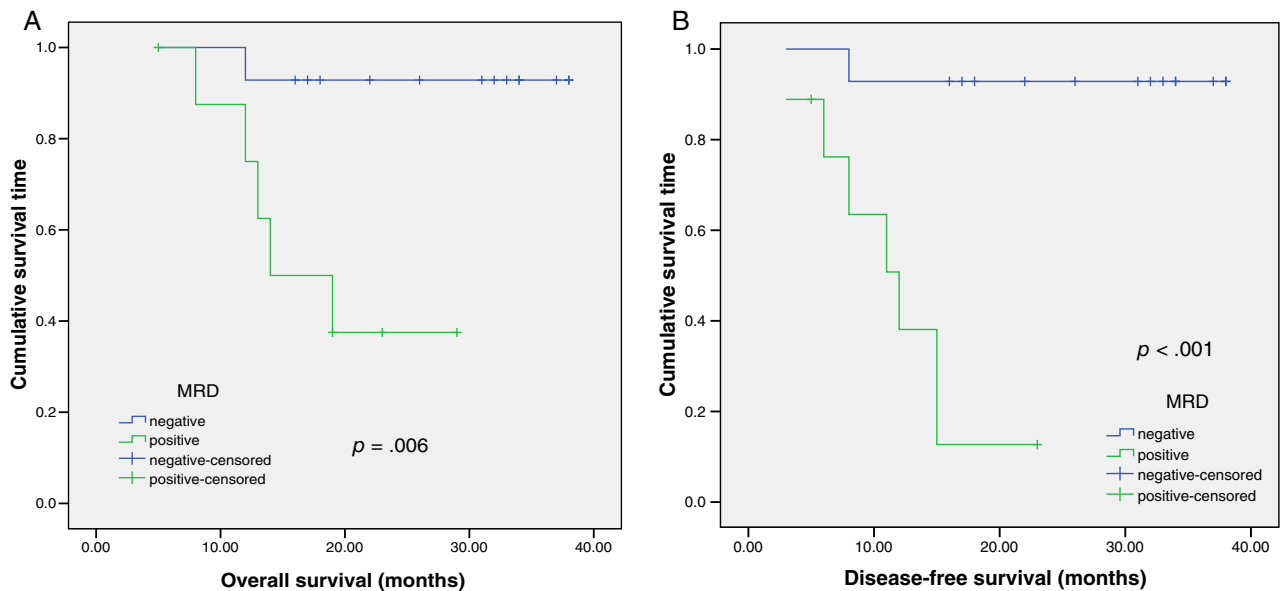


Figure 4. Kaplan–Meier curves of patients according to minimal residual disease status negative versus positive. **(A)** Overall survival **(B)** Disease-free survival.

Table 1. Patients' characteristics

Characteristics	<i>n</i>	%
Age, median (range), years	47 (18–72)	
<60	26	76.5
≥60	8	23.5
Sex		
Male	19	55.9
Female	15	44.1
FAB subtypes*		
M0	1	2.9
M2	13	38.2
M4	5	14.7
M5	15	44.2
Blood cell count before reinduction		
WBC, mean ± SD, ×10 ⁹ /L	14.2 ± 5.4	
PLT, mean ± SD, ×10 ⁹ /L	51.4 ± 9.4	
HB, mean ± SD, ×g/L	80.7 ± 5.2	
Cytogenetic risk group		
Low risk	6	17.6
Intermediate risk	16	47.1
High risk	12	35.3
Disease status		
Relapse	18	52.9
Refractory	16	47.1
Duration of first complete remission, median (range), months	8 (0–32)	
Postinduction therapy		
Allo-HSCT	9	26.5
Chemotherapy	25	73.5

*According to FAB classification system, patients were divided into four subtypes: M0, M2, M4, and M5.

Abbreviations: Allo-HSCT, allogeneic hematopoietic stem cell transplantation; FAB, French–American–British classification system; HB, hemoglobin; PLT, platelet; WBC, white blood cell.

Table 2. Responses after reinduction therapy of C-CAG–based regimens

Variable	CR, n (%)	<i>p</i> value
Total	23 (67.6)	
Age, years		.388
<60	19 (73.1)	
≥60	4 (50.0)	
Disease status		.066
Relapse	15 (83.3)	
Refractory	8 (50.0)	
Cytogenetic risk group		.031
Low risk	6 (100.0)	
Intermediate risk	12 (75.0)	
High risk	5 (41.7)	

Abbreviations: C-CAG, cladribine in combination with granulocyte colony-stimulating factor, low-dose cytarabine, and aclarubicin; CR, complete remission.

Table 3. Nonhematologic and hematologic toxicity during induction treatment, supportive care

Toxicity	<i>n</i> (%)
Nonhematological toxicity (WHO grade 3–4)	
Infections	2 (5.9)
Bleeding	0 (0.0)
Mucositis	2 (5.9)
Vomiting	1 (2.9)
Diarrhea	1 (2.9)
Hepatic failure	0 (0.0)
Cardiac failure	1 (2.9)
Renal failure	0 (0.0)
Minimal ANC × 10 ⁹ /L	
Median	0.03
Range	0–0.3
Minimal platelets × 10 ⁹ /L	
Median	5
Range	1–10
No. of days to ANC recovery >0.5 × 10 ⁹ /L	
Median	20
Range	4–42
No. of days to platelet recovery >50 × 10 ⁹ /L	
Median	27
Range	7–49
No. of days of hospital stay	
Median	29
Range	20–45
PRBC transfusions	
Median	5
Range	2–14
No. of platelet transfusions	
Median	6
Range	2–20
No. of days of IV antibiotics	
Median	15
Range	3–42

Abbreviations: ANC, absolute neutrophil count; IV, intravenous; PRBC, packed red blood cells; WHO, World Health Organization.

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