Discover Oncology

Research

Efficacy and safety of mitoxantrone hydrochloride liposome-containing regimens in treating refractory/relapsed acute myeloid leukemia

Ni Lu[1,2] · Li Liu[1,2] · Yigeng Cao[1,2] · Rongli Zhang[1,2] · Weihua Zhai[1,2] · Xin Chen[1,2] · Qiaoling Ma[1,2] · Donglin Yang[1,2] · Aiming Pang[1,2] · Jialin Wei[1,2] · Yi He[1,2] · Sizhou Feng[1,2] · Mingzhe Han[1,2] · Erlie Jiang[1,2]

Received: 27 November 2024 / Accepted: 28 April 2025
Published online: 12 May 2025

Abstract

Background Despite the poor prognosis for patients with relapsed/refractory (R/R) acute myeloid leukemia (AML), an optimal treatment strategy remains undefined. Mitoxantrone (MIT) is widely used to treat R/R AML.

Methods This prospective, single-center, open-label study assessed the efficacy and toxicity of mitoxantrone hydrochloride liposome (Lipo-MIT)-containing regimen therapy, intensified by adding cytarabine (Ara-C), cyclophosphamide (CTX) or other agents. The primary endpoint was composite complete remission (CRc), including complete remission (CR), complete remission with incomplete count recovery (CRi), and morphologic leukemia-free state (MLFS). The secondary endpoints included overall response rate (ORR), event-free survival (EFS), overall survival (OS), and safety.

Results We enrolled 20 patients (median, 38.50 years; range, 20.00–53.00 years) and treated them with a Lipo-MIT-containing regimen from April 29, 2022, to July 11, 2023. Twelve patients (60.00%) achieved CRc after one course of induction therapy, of which MAC (Lipo-MIT, Ara-C, CTX)-based regimen was the most commonly used (12/20, 60.00%) with a CRc rate of 66.67% (8/12). Additionally, 13 patients relapsed after allogeneic stem cell transplantation (allo-HSCT) with a CRc of 69.20% (9/13). The median follow-up time was 6.64 months, with a median OS of 9.99 months (range, 1.64–19.61; 95% confidence interval [CI], 2.05–17.92). Moreover, 95% patients experienced grade 3/4 hematologic treatment-related adverse events (TRAEs), including anemia (60.0%), thrombocytopenia (60.0%), leukopenia (65.0%), and neutropenia (55.0%). All patients experienced nonhematologic TRAEs, with 14 patients showing grade 3/4 toxicity.

Conclusion The Lipo-MIT-based regimens demonstrate preliminary efficacy in R/R AML, particularly in those who relapsed after allo-HSCT, though hematologic toxicity warrants careful monitoring.

Clinical Trial Registration: Clinicaltrials.gov Identifier: NCT04645199 in November 27, 2020.

Keywords Acute myeloid leukemia · Relapsed/refractory acute myeloid leukemia · Mitoxantrone hydrochloride liposome · Allogeneic hematopoietic stem cell transplantation · Composite complete remission

Ni Lu and Li Liu contributed equally as first authors.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12672-025-02526-y.

✉ Erlie Jiang, doctor_eljiang@163.com | [1]State Key Laboratory of Experimental Hematology, National Clinical Research Center for Blood Diseases, Haihe Laboratory of Cell Ecosystem, Hematopoietic Stem Cell Transplantation Center, Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin 300020, China. [2]Tianjin Institutes of Health Science, Tianjin 300020, China.

1 Introduction

Relapse/refractory (R/R) scenarios pose significant challenges in managing acute myeloid leukemia (AML). For patients with R/R AML, salvage therapies are expected to be one of the most effective methods. Despite progress with targeted treatments [1, 2], strategizing an ideal regimen for patients with R/R AML, particularly those without targetable mutations, remains a complex endeavor. For patients without actionable mutations, salvage chemotherapy before allogeneic stem cell transplantation (allo-HSCT) is commonly performed, utilizing regimens, such as FLAG-IDA (fludarabine, cytarabine (Ara-C), granulocyte colony-stimulating factor [G-CSF], and idarubicin), CLAG-M (cladribine, Ara-C, mitoxantrone [MIT], and G-CSF), MEC (MIT, Ara-C, and etoposide) [3], and HAA (high-dose Ara-C and aclacinomycin) [4]. However, these approaches often yielded unsatisfactory outcomes, with complete remission (CR) rates ranging from 40 to 60%, and unexpected toxicities. Furthermore, patients who relapsed after transplantation have a poor prognosis [5] with no specific management strategy. Therefore, innovative combinations of drugs that can improve the rate of achieving remission and act as a bridge to allo-HSCT in R/R AML should be identified while minimizing excessive toxicity.

MIT is an anthracycline drug mainly used to treat multiple hematologic malignancies, including AML, as induction and consolidation therapy, but it is limited by its cardiotoxicity and myelosuppressive characteristics [6]. Prior studies demonstrated that mitoxantrone-based regimens achieve comparable CR rates to other anthracyclines (e.g., idarubicin/daunorubicin) in AML induction, with potential advantages in reducing relapse risk [7]. Since introducing liposomes more than 50 years ago, they have become a cornerstone in drug delivery, significantly mitigating the off-target toxicity of various drugs, facilitating extended blood circulation, and advantageous drug distribution [8, 9]. Wang et al. demonstrated that mitoxantrone hydrochloride liposome (Lipo-MIT) and MIT showed comparable clinical benefits in treating breast cancer, whereas Lipo-MIT presented a lower toxicity profile [10]. Additionally, Lipo-MIT also showed a favorable efficacy and manageable safety profile in non-Hodgkin lymphoma [11]. However, its efficacy in treating R/R AML remains to be further explored. Considering that drug combinations can achieve better efficacy than single agents, new combinations incorporating agents with different mechanisms should be developed. Therefore, the flexible utilization of Lipo-MIT combined with other established drugs may yield synergistic effects in treating R/R AML.

Our prospective study explores the efficacy and safety of a Lipo-MIT-containing regimen for patients with R/R AML. Regimens in this study combined Lipo-MIT with conventional drugs, such as cytarabine and cyclophosphamide, as well as novel therapeutic agents, including venetoclax, gilteritinib, and selinexor based on different disease characteristics.

2 Methods

2.1 Study design and patients

This prospective, single-center, open-label study enrolled patients with R/R AML aged 18–60 years old. Relapse AML was defined as the reappearance of leukemia cells in peripheral blood or bone marrow blasts > 5% after achieving CR/CRi (excluding reasons, such as bone marrow regeneration after consolidation therapy) or extramedullary infiltration of leukemia cells. Refractory AML was defined as patients whose standard treatment was ineffective after two cycles of initial therapy; relapse within 12 months after achieving CR/CRi despite consolidation therapy; failure to respond to conventional chemotherapy after relapsing 12 months post-initial remission; multiple recurrences, and persistent extramedullary leukemia. Inclusion criteria required patients to have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2, adequate baseline renal and hepatic function, and a left ventricular ejection fraction ≥ 50%. Patients were ineligible if they had uncontrolled infections or received systemic antineoplastic therapy or radiotherapy within 7 days before the initiation of the study treatment, except for hydroxyurea or 6-mercaptopurine.

The Ethics Committee of Blood Diseases Hospital, Chinese Academy of Medical Sciences (IIT2021011-EC-1) approved the research protocol. This study follows the Declaration of Helsinki. All patients provided written and oral informed consent. This study was registered with clinicaltrials.gov (Identifier, NCT04645199).

2.2 Procedures

All patients received the Lipo-MIT-containing regimen intravenously. The recommended dose of Lipo-MIT ranged from 20 to 24 mg/m^2 on day 1 of each cycle based on the drug instructions and guideline consensus. In addition, in the most commonly used MAC regimen (Lipo-MIT, Ara-C, and CTX), Ara-C was administered at a dose of 100 mg/m^2/day on days 1–7, while CTX was administered at a dose of 750 mg/m^2 on day 1. Furthermore, the dosages of additional medications, determined based on the patient's disease characteristics, were aligned with those in traditional salvage treatment regimens. Patients who underwent allo-HSCT were provided the option of donor lymphocyte infusion (DLI) after relapse as combination therapy. Patients were administered one treatment cycle, with supportive care provided based on institutional guidelines.

2.3 Outcomes

The primary endpoint was composite complete remission (CRc), including CR, complete remission with incomplete count recovery (CRi), and morphologic leukemia-free state (MLFS). The secondary endpoints included overall response rate (ORR), event-free survival (EFS), overall survival (OS) and safety. Response was assessed through bone marrow aspirate/biopsy after treatment completion. The response were defined based on the 2022 European Leukemia Net (ELN) recommendations [12]. Using the Common Terminology Criteria for Adverse Events (CTCAE, version 5.0), physicians recorded and graded treatment-related adverse events (TRAEs).

2.4 Statistical analysis

Data were analyzed using SPSS statistical software version 28.0.1.1 (IBM Corp). Patient demographics and disease characteristics were summarized using descriptive statistics. The primary endpoint, CRc, was calculated for the intent-to-treat population. For the primary efficacy analysis, an unstratified chi-squared test was used to compare the CR rates. OS and EFS were secondary endpoints and estimated using the Kaplan–Meier method with two-sided 95% confidence intervals (CIs). P-values were calculated using the log-rank test. The swimming plot was generated using R statistical software version 4.3.2 (R Project for Statistical Computing).

3 Results

3.1 Patient characteristics

From April 29, 2022, to July 11, 2023, 20 patients with R/R AML, with a median age of 38.50 (range, 20.00–53.00) years and 10 (50%) women were included and treated with Lipo-MIT-containing regimen. Table 1 shows the demographic and baseline characteristics. Among the patients, 3 (15%) experienced their first relapse, whereas 17 (85%) were deemed refractory, with 8 (40%) having prior remissions lasting < 12 months, and 4 (20%) patients experiencing multiple relapses. Of the 20 patients with AML, two had a documented history of myelodysplastic syndrome. Additionally, 13 patients had previously allo-HSCT. Before the last allo-HSCT, all patients achieved remission, and the pre-HSCT minimal residual disease (MRD) status was negative and positive in nine and four patients, respectively. At initial diagnosis, the specific mutations were isocitrate dehydrogenase 1 (IDH1) mutant in two patients, fms-like tyrosine kinase 3-internal tandem duplication (FLT3-ITD) in two patients, additional sex combs-like 1 (ASXL1) in one patient, and runt-related transcription factor 1 (RUNX1) in one patient. Based on the 2022 ELN criteria, the risk classification at initial diagnosis was favorable, intermediate, adverse and unknown in 1, 10, 8, and 1 patients, respectively. Additionally, four patients had extramedullary infiltration without central nervous system involvement.

3.2 Treatment

Among 20 patients, 13 patients who previously received allo-HSCT were treated with Lipo-MIT with a dosage of 19.90 mg/m^2 on day 1. The remaining 7 patients had either relapsed or were refractory following chemotherapy, and were treated with Lipo-MIT at a dosage of 20.00 mg/m^2 on day 1 (Fig. 1).

Table 1 Patient characteristics and disease history

Characteristics		N = 20
Age	Median (Min, Max)	38.50 (20.00,53.00)
Sex, no. (%)		
	Male	10 (50.00)
	Female	10 (50.00)
ECOG, no. (%)		
	0	6 (30.00)
	1	9 (45.00)
	2	5 (25.00)
Characteristics at the start of therapy, no. (%)		
	Relapse	3 (15.00)
	Refractory	17 (85.00)
	Primary refractory	5 (25.00)
	First relapse < 12 m	8 (40.00)
	Relapse more than twice	4 (20.00)
Previous transplantations, no. (%)		
	Yes	13 (65.00)
Extramedullary infiltration, no. (%)		
	Yes	4 (20.00)
Baseline mutations*		
	Mutation	5 (25.00)
	IDH1	2 (10.00)
	FLT3-ITD	2 (10.00)
	ASXL1	1 (5.00)
	RUNX1	1 (5.00)
	None	15 (75.00)
Primary/secondary AML		
	Primary	18 (90.00)
	Secondary[†]	2 (10.00)
Prior treatments	Venetoclax	16 (80.00)
	Intensive chemotherapy	20 (100.00)
	7 + 3	18 (90.00)
	MD/HD + Ara-C	15 (75.00)
	HAG/CAG	5 (25.00)
	FLAG	2 (10.00)
	Others	5 (25.00)
	Hypomethylating agent	14 (70.00)
Prior treatment lines	Median (range)	5 (2.00,14.00)

AML Acute myeloid leukemia, *ECOG* Eastern Cooperative Oncology Group, *7 + 3* cytarabine plus anthracycline, *MD/HD + Ara-C* middle/high-dose cytarabine, *HAG/CAG*, homoharringtonine plus cytarabine and granulocyte colony-stimulating factor/cytaarabine plus aclarubicin and granulocyte colony-stimulating factor, *FLAG*, fludarabine plus cytarabine and granulocyte colony-stimulating factor

[†]Secondary AML evolving from myelodysplastic syndrome

*Baseline Mutations: only WHO 2016 prognostic stratification genes were originally displayed

3.3 Outcomes

The median administered dosage of Lipo-MIT was 20.00 (range, 11.17–33.75) mg/m^2/day. Table 2 show the treatment regimens of Lipo-MIT. Figure 2 shows the clinical outcomes after one treatment cycle and the CRc and ORR rates were both 60.00% (12/20). Notably, for patients after allo-HSCT, the CRc and ORR rates were both 69.23% (95% CI, 38.57–90.91%). MRD was assessed using multiparametric flow cytometry, which showed six patients who achieved MRD-negative at the

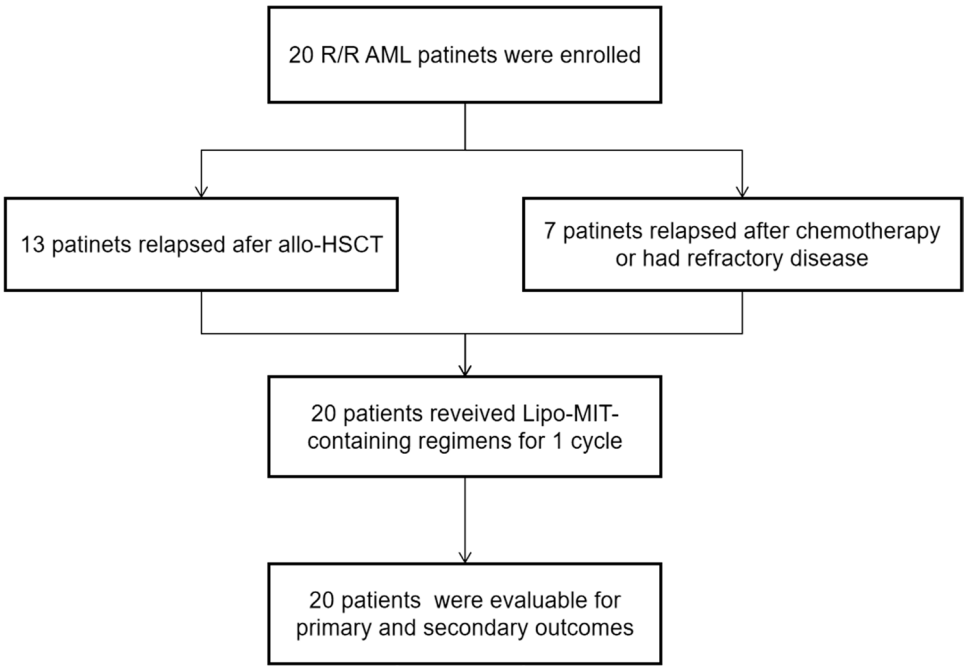

Fig. 1 Trial profile. *R/R AML* relapsed/refractory acute myeloid leukemia, *allo-HSCT* allogeneic stem cell transplantation

Table 2 Treatment regimens and proportion of patients

Treatment regimen	All (N = 20)	After allo-HSCT (N = 13)	Pre-HSCT (N = 7)	CRc rate (%)
Lipo-MIT + Ara-C + CTX	6 (30.00)	4 (30.76)	2 (28.57)	66.67
Lipo-MIT + Ara-C + CTX + selinexor	3 (15.00)	1 (7.69)	2 (28.57)	33.33
Lipo-MIT + Ara-C	2 (10.00)	2 (15.38)	0 (0.00)	50.00
Lipo-MIT + Ara-C + CTX + selinexor + venetoclax	2 (10.00)	1 (7.69)	1 (14.29)	100.00
Lipo-MIT + fludarabine + Ara-C + G-CSF	1 (5.00)	1 (7.69)	0 (0.00)	100.00
Lipo-MIT + Ara-C + CTX + venetoclax	1 (5.00)	1 (7.69)	0 (0.00)	100.00
Lipo-MIT + Ara-C + gilteritinib	1 (5.00)	0 (0.00)	1 (14.29)	100.00
Lipo-MIT + Ara-C + selinexor	1 (5.00)	1 (7.69)	0 (0.00)	0.00
Lipo-MIT + busulfan + semustine + CTX	1 (5.00)	0 (0.00)	1 (14.29)	0.00
Lipo-MIT + CTX + selinexor	1 (5.00)	1 (7.69)	0 (0.00)	0.00
Lipo-MIT + CTX + venetoclax	1 (5.00)	1 (7.69)	0 (0.00)	100.00

Lipo-MIT Mitoxantrone hydrochloride liposome, *Ara-C* cytarabine, *CTX* cyclophosphamide, *G-CSF* Granulocyte colony-stimulating factor, *allo-HSCT* allogeneic stem cell transplantation

end of therapy, with five subsequently undergoing transplantation. The median follow-up was 6.64 (range, 1.64–19.61) months. The median OS and EFS were 9.99 (range, 1.64–19.61; 95% CI, 2.05–17.92) and 2.18 (range, 0.95–11.04; 95% CI, 1.67–2.53) months, respectively. For patients relapsed after allo-HSCT, the median OS and EFS were 9.49 (range, 2.10–17.74; 95% CI, 3.44–15.54) and 3.94 (range, 1.18–6.41; 95% CI, 1.16–6.73) months, respectively. Additionally, the median OS was not reached for R/R patients after chemotherapy, but the median EFS was 1.35 (range, 0.95–11.04; 95% CI, 0.97–1.73) months. Figure 3 shows the Kaplan–Meier estimated EFS and OS for all enrolled patients.

The most common treatment regimen was MAC (Lipo-MIT, Ara-C, and CTX)-based regimen, with a CRc rate of 66.67% (8/12) and 85.71% (6/7) in general and post-allo-HSCT patients, respectively. The second most commonly used regimen was MA (Lipo-MIT and Ara-C)-based regimen, with a CRc rate of 60.00% (3/5) and 50.00% (2/4) in general and post-allo-HSCT patients, respectively. In addition to the standard therapeutic agents, DLI was administered in all 13 post-allo-HSCT patients. The swimming plot in Fig. 4 shows the treatment regimens and outcomes for all patients.

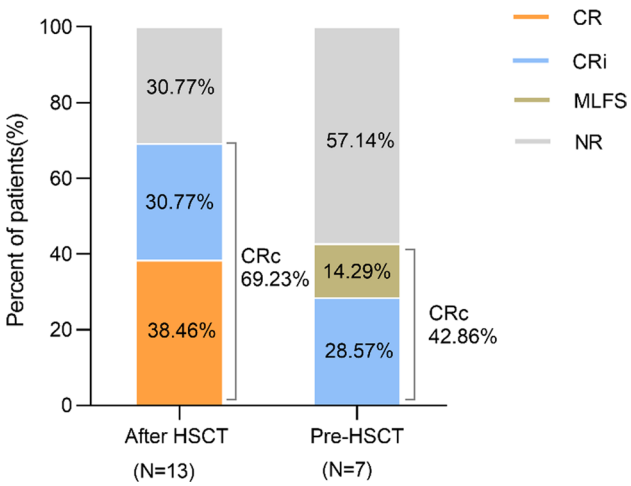

Fig. 2 Treatment response after one cycle treatment between two groups. *HSCT* hematopoietic stem cell transplantation, *CRc* composite complete remission, *CR* complete remission, *CRi* complete remission with incomplete count recovery, *MLFS* morphologic leukemia-free state, *NR* non-remission

3.4 Toxicity

Table 3 shows the TRAEs, including hematologic and non-hematologic toxicities. Of the 20 enrolled patients, 19 had grade 3/4 hematologic TRAEs, including anemia (60.0%), thrombocytopenia (60.0%), leukopenia (65.0%), and neutropenia (55.0%). Additionally, all patients had non-hematologic TRAEs, with 14 patients showing grade 3/4 toxicity. The most common non-hematologic TRAEs were respiratory system reactions, immune abnormalities and infections, and hepatic dysfunction observed in 90%, 75%, and 60% of the patients, respectively. However, most non-hematological toxicities were grade 1/2, whereas pulmonary infection (40%) and fever (15%) were grade 3/4 toxicities with an incidence of > 10%, which was potentially associated with neutropenia.

4 Discussion

This prospective study of a Lipo-MIT-containing regimen for treating R/R AML demonstrated that the regimen showed a satisfactory CRc rate and a relatively manageable safety profile. Both the CRc and ORR rates were 60.00%, though hematologic toxicity warrants careful monitoring.

Although standard salvage chemotherapy regimen is unavailable for patients with R/R AML, the Ara-C and MIT combination is widely utilized [13]. Several studies have proposed non-cross-resistant Ara-C-based combination chemotherapy regimens, such as the FLAG-IDA and CLAG-M regimens, with CR rates ranging from 30 to 60%. However, these regimens were frequently associated with severe toxicities [14–17]. In 1991, Amadori et al. reported that the MEC (MIT, etoposide, Ara-C) regimen in patients with refractory AML had a CR rate of 66% and a median OS of 36 weeks. However, all patients had severe myelosuppression, leading to fever or documented infections in 91% of cases [18]. In more recent studies, the CR rate of the MEC regimen ranged from 16 to 28% [19–21]. The combination of MIT, Ara-C and CTX, a salvage chemotherapy regimen based on the MA regimen, effectively interferes with the proliferation cycle of tumor cells and blocks DNA synthesis by adding the alkylating agent-based anti-tumor agent CTX, providing broad-spectrum anti-tumor action. Previous studies conducted by our institution identified the efficacy and safety of combining CTX with MIT and Ara-C in patients who had primary induction failure or relapse. The overall CR rate was 74.70%, with 5-year OS and disease-free survival rates of 36.70% and 43.00%, respectively. Moreover, 29.00% of patients had severe infections (grade 3 or 4) [22]. Based on previous achievements, we further updated the MIT formulation with liposome-based formulations to improve anti-leukemia efficacy and reduce toxicity. In this study, the MAC-based regimen based on Lipo-MIT was the most commonly used (12 of 20 patients), with 66.67% (8/12) patients achieving CRc. Although the outcome was suboptimal compared with the previous study, 13 of 20 patients in our study had allo-HSCT, which was associated with poor outcomes [23, 24].

Moreover, previous studies have indicated that liposomal encapsulation can significantly extend the plasma half-life of medications, leading to drug accumulation within the bone marrow [25–27]. Cortes et al. compared the use

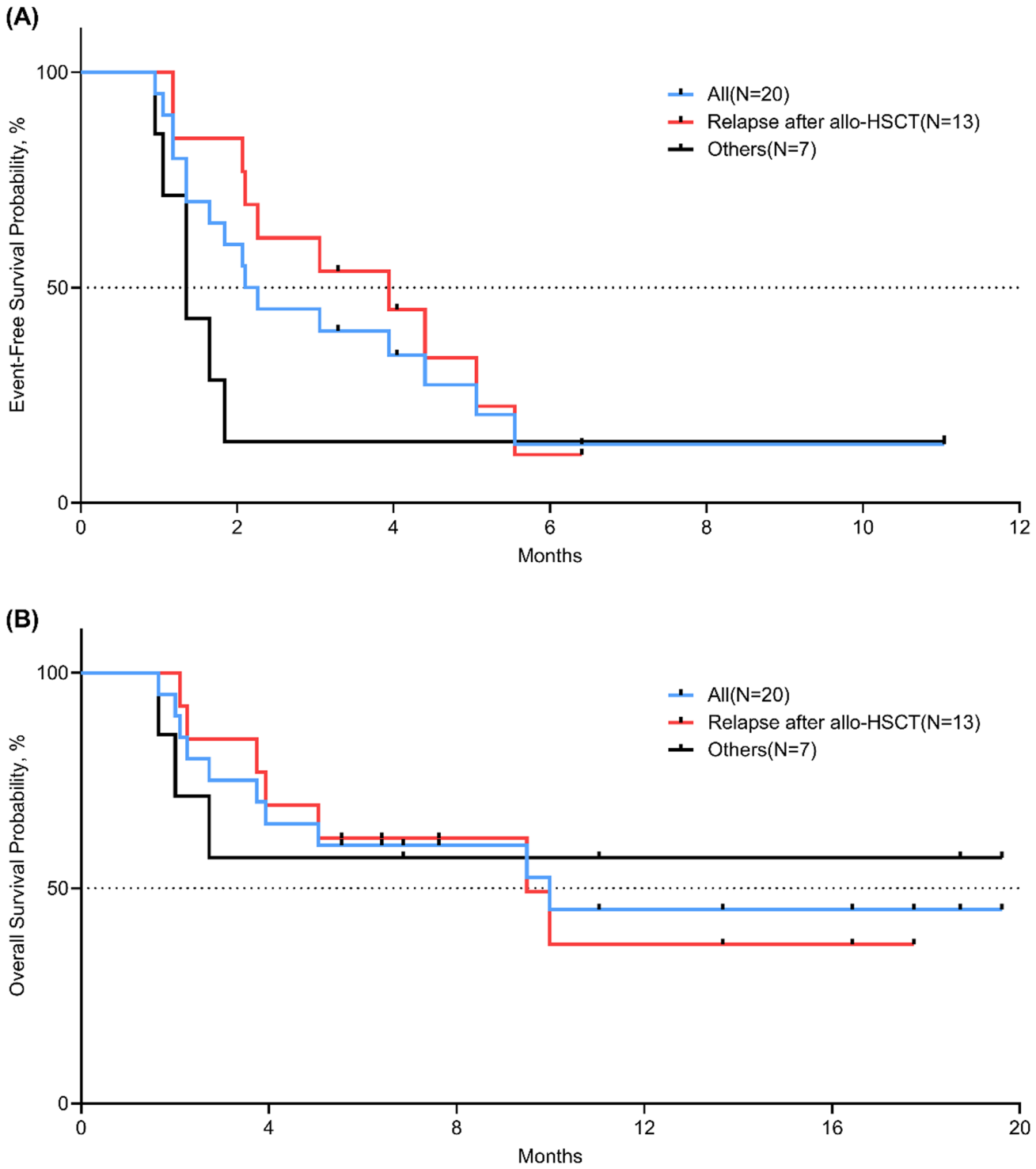

Fig. 3 Long-term outcomes for patients received Lipo-MIT-based regimens treatment. **A** The event free survival for all patients. **B** The overall survival for all patients

of liposomal Ara-C combined with daunorubicin to standard chemotherapy dosages in older patients with newly diagnosed secondary AML. The liposomal formulation showed significantly prolonged survival and a comparable safety profile to conventional therapy [28], which was also reported by Franco et al. who noted that although adriamycin is commonly used in cancer therapy, its efficacy is hindered by dose-dependent cardiotoxicity. However, encapsulating adriamycin in liposomes mitigates the cardiac risk without compromising its anti-tumor activity [29]

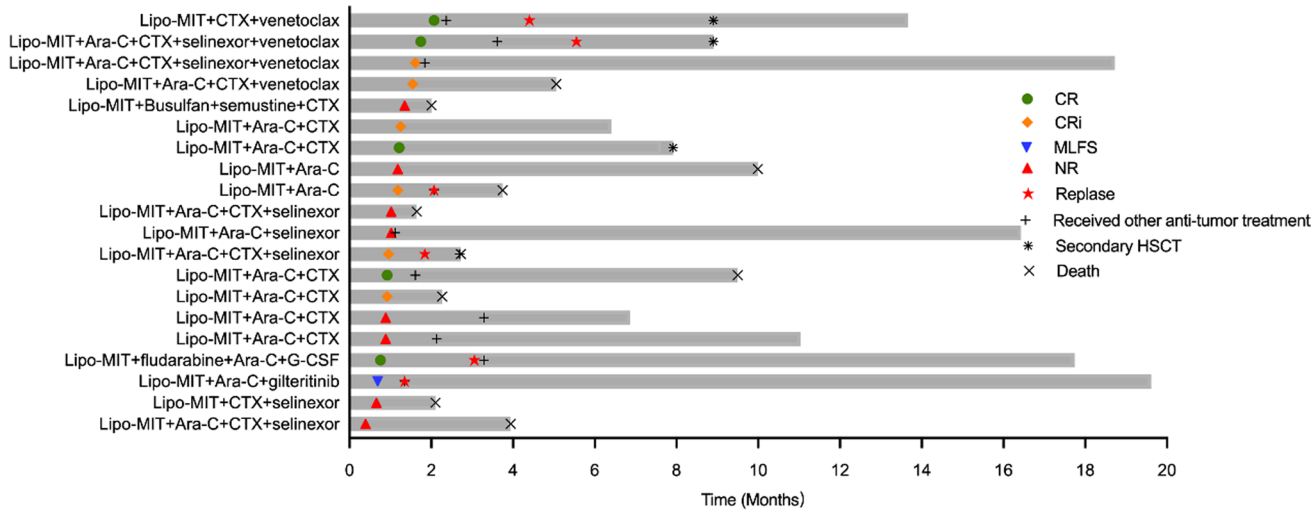

Fig. 4 Treatment regimens and outcomes for all patients. *Lipo-MIT* mitoxantrone hydrochloride liposome, *CTX* cyclophosphamide, *Ara-C* cytarabine, *G-CSF* granulocyte colony-stimulating factor, *CR* complete remission, *CRi* complete remission with incomplete count recovery, *MLFS* morphologic leukemia-free state, *NR* non-remission

and ensures stability in circulation and targeted delivery to tumor vasculature, minimizing adriamycin release in plasma and healthy tissues, thus reducing cardiotoxicity. Though lacking direct controls cohort, our observed 60% CRc rate and none cardiotoxicity incidence (vs. 25–50% ORR and 9 patients occurred cardiotoxicity in conventional mitoxantrone studies) suggest potential therapeutic advantages worthy of systematic evaluation [30–32]. Our Lipo-MIT was prepared using hydrogenated soy phosphatidylcholine, cholesterol, and pegylated lipid and showed high tissue permeability and biological activity. Therefore, Lipo-MIT retains mitoxantrone's anti-leukemic activity.

The common profiles observed here were consistent with previously reported, without unexpected safety signals [33, 34]. Notably, hematologic events reported in our study were lower than those mitoxantrone-containing regimens, such as anemia (60% vs. 76.2%), thrombocytopenia (60% vs. 99%), and neutropenia (55% vs. 76%), which could resume with dose modification and symptomatic treatment, without endangering patients' health [35, 36]. However, the Lipo-MIT-based regimen demonstrates manageable hematologic toxicity profiles comparable to conventional anthracycline-containing regimens, though close monitoring remains essential given the high-risk patient population. Besides, most non-hematological toxicities were grade 1/2, whereas pulmonary infection (40%) and fever (15%) were grade 3/4 toxicities, possibly consideration was neutropenia and COVID-19 infection. In summary, our study demonstrated the acceptable safety of Lipo-MIT-containing regimens, although its toxicity spectrum requires further evaluation in controlled settings.

Furthermore, despite allo-HSCT being the most effective consolidation therapy to prevent relapse, relapse still occurs in 45–55% of adverse-risk patients with AML post-allo-HSCT. The outcomes after post-transplant relapse are poor, with a 2-year OS rate ranging 14–25% and a limited number of long-term remissions [5]. Surprisingly, our patients who had pre-allo-HSCT showed higher CRc rates (69.23%) compared with pre-transplant patients (42.86%), with a median OS of 9.49 (range, 2.10–17.74; 95% CI, 3.44–15.54) months. This may be due to all patients in the post-transplantation group receiving combination therapy with DLI. Previous studies have emphasized the importance of DLI and its potential synergy when combined with other therapies, such as intensive chemotherapy, azacitidine, or targeted therapy [37]. Interestingly, a retrospective study from the European Society for Blood and Marrow Transplantation registry showed that survival was not significantly different between patients with post-transplant relapse who underwent DLI or received a second transplantation, highlighting the anti-leukemia efficacy of DLI [38]. Therefore, patients who had post-transplant may benefit from salvage therapy by using the Lipo-MIT regimen combined with sequential DLI.

Our study has several limitations. First, a small sample size and single-arm design without a control group for comparison posed a challenge to decipher the benefits of combination treatment. Second, the limited number of patients resulted in a wide 95% CI, and we were unable to obtain long-term survival data for patients who achieved CRc due to the relatively short follow-up period. While these were inherent to this exploratory study, we will actively expand the cohort further and planning controlled comparative trials for validation. Finally, although patients who relapsed after allo-HSCT might benefit from Lipo-MIT-containing regimens combined with DLI, the underlying mechanism for this phenomenon remains unclear.

Table 3 Adverse events after one cycle of treatment

Adverse events, N (%)	Grade, N = 20					
	G1	G2	G3	G4	G3-4	All
Hematological toxicity	**0 (0.00)**	**0 (0.00)**	**6 (30.00)**	**8 (40.00)**	**14 (70.00)**	**14 (70.00)**
Anemia	0 (0.00)	1 (5.00)	4 (20.00)	8 (40.00)	12 (60.00)	13 (65.00)
Thrombocytopenia	0 (0.00)	0 (0.00)	1 (5.00)	11 (55.00)	12 (60.00)	12 (60.00)
Leukopenia	0 (0.00)	0 (0.00)	0 (0.00)	13 (65.00)	13 (65.00)	13 (65.00)
Neutropenia	0 (0.00)	0 (0.00)	0 (0.00)	11 (55.00)	11 (55.00)	11 (55.00)
Febrile neutropenia	0 (0.00)	0 (0.00)	0 (0.00)	6 (30.00)	6 (30.00)	6 (30.00)
Non-hematological toxicity	**2 (10.00)**	**4 (20.00)**	**13 (65.00)**	**1 (5.00)**	**14 (70.00)**	**20 (100.00)**
Gastrointestinal reactions	**9 (45.00)**	**2 (10.00)**	**0 (0.00)**	**0 (0.00)**	**0 (0.00)**	**11 (55.00)**
Nausea	9 (45.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	9 (45.00)
Vomiting	4 (20.00)	1 (5.00)	0 (0.00)	0 (0.00)	0 (0.00)	5 (25.00)
Diarrhea	2 (10.00)	1 (5.00)	0 (0.00)	0 (0.00)	0 (0.00)	3 (15.00)
Abdominal pain	2 (10.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	2 (10.00)
Flatulence	1 (5.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (5.00)
Respiratory system reactions	**11 (55.00)**	**3 (15.00)**	**4 (20.00)**	**0 (0.00)**	**4 (20.00)**	**18 (90.00)**
Fever	12 (60.00)	3 (15.00)	3 (15.00)	0 (0.00)	3 (15.00)	18 (90.00)
Productive cough	2 (10.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	2 (10.00)
Cough	0 (0.00)	2 (10.00)	0 (0.00)	0 (0.00)	0 (0.00)	2 (10.00)
Shiver	2 (10.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	2 (10.00)
Epistaxis	0 (0.00)	1 (5.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (5.00)
Nasal congestion	1 (5.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (5.00)
Chilly	1 (5.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (5.00)
Sore throat	1 (5.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (5.00)
Pneumonia	0 (0.00)	0 (0.00)	1 (5.00)	0 (0.00)	1 (5.00)	1 (5.00)
Respiratory alkalosis	0 (0.00)	0 (0.00)	1 (5.00)	0 (0.00)	1 (5.00)	1 (5.00)
Chest distress	1 (5.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (5.00)
Hypoxemia	0 (0.00)	1 (5.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (5.00)
Type I respiratory failure	0 (0.00)	0 (0.00)	1 (5.00)	0 (0.00)	1 (5.00)	1 (5.00)
Skin and mucosal reactions	**3 (15.00)**	**1 (5.00)**	**1 (5.00)**	**0 (0.00)**	**1 (5.00)**	**5 (25.00)**
Rash	2 (10.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	2 (10.00)
Oral mucositis	1 (5.00)	1 (5.00)	1 (5.00)	0 (0.00)	1 (5.00)	3 (15.00)
Toothache	2 (10.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	2 (10.00)
Hepatic dysfunction	**9 (45.00)**	**1 (5.00)**	**1 (5.00)**	**1 (5.00)**	**2 (10.00)**	**12 (60.00)**
Hypoalbuminemia	8 (40.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	8 (40.00)
Elevated transaminases	6 (30.00)	1 (5.00)	1 (5.00)	1 (5.00)	2 (10.00)	9 (45.00)
Elevated blood bilirubin	1 (5.00)	1 (5.00)	0 (0.00)	0 (0.00)	0 (0.00)	2 (10.00)
Elevated lactate dehydrogenase	2 (10.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	2 (10.00)
Immune abnormalities and infections	**0 (0.00)**	**2 (10.00)**	**13 (65.00)**	**0 (0.00)**	**13 (65.00)**	**15 (75.00)**
Pulmonary infection	0 (0.00)	0 (0.00)	8 (40.00)	0 (0.00)	8 (40.00)	8 (40.00)
Upper respiratory tract infection	0 (0.00)	3 (15.00)	0 (0.00)	0 (0.00)	0 (0.00)	3 (15.00)
Gingival infection	0 (0.00)	0 (0.00)	2 (10.00)	0 (0.00)	2 (10.00)	2 (10.00)
Acute graft-versus-host disease	0 (0.00)	1 (5.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (5.00)
Elevated C-reactive protein	1 (5.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (5.00)
Perianal infection	0 (0.00)	0 (0.00)	2 (10.00)	0 (0.00)	2 (10.00)	2 (10.00)
Bacteremia	0 (0.00)	0 (0.00)	1 (5.00)	0 (0.00)	1 (5.00)	1 (5.00)
Bloodstream infection	0 (0.00)	0 (0.00)	2 (10.00)	0 (0.00)	2 (10.00)	2 (10.00)
Oral candidiasis	0 (0.00)	1 (5.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (5.00)
Complicated soft tissue infection	0 (0.00)	0 (0.00)	1 (5.00)	0 (0.00)	1 (5.00)	1 (5.00)
Oral infection	0 (0.00)	0 (0.00)	1 (5.00)	0 (0.00)	1 (5.00)	1 (5.00)

Table 3 (continued)

Adverse events, N (%)	Grade, N = 20					
	G1	G2	G3	G4	G3-4	All
Neurological reactions	**5 (25.00)**	**0 (0.00)**	**0 (0.00)**	**0 (0.00)**	**0 (0.00)**	**5 (25.00)**
Dizziness	1 (5.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (5.00)
Paresthesia	1 (5.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (5.00)
Dizziness	1 (5.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (5.00)
Headache	3 (15.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	3 (15.00)
Other reactions	**7 (35.00)**	**1 (5.00)**	**0 (0.00)**	**0 (0.00)**	**0 (0.00)**	**8 (40.00)**
Fatigue	3 (15.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	3 (15.00)
Hypocalcemia	1 (5.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (5.00)
Hyperglycemia	1 (5.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (5.00)
Localized edema	0 (0.00)	1 (5.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (5.00)
Weight loss	1 (5.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (5.00)
Heart failure	0 (0.00)	1 (5.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (5.00)
Dry eyes	1 (5.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (5.00)
Hypoglycemia	1 (5.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (5.00)
Pollakiuria	1 (5.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (5.00)
Any adverse events	**1 (5.00)**	**1 (5.00)**	**9 (45.00)**	**9 (45.00)**	**18 (90.00)**	**20 (100.00)**

Bold values represents the general classification of adverse events

5 Conclusion

In summary, our study provided evidence supporting the efficacy and manageable safety of the Lipo-MIT-containing regimens in treating R/R AML. This therapeutic approach shows particular promise in patients who relapsed after allo-HSCT. However, larger-scale studies should be conducted to comprehensively evaluate the overall effectiveness of this regimen and determine the optimal combination strategies for Lipo-MIT in managing R/R AML.

Acknowledgements None.

Author contributions Formal analysis and writing—original draft: N.L.; Conceptualization and visualization: L.L.; Data curation and writing—review & editing: Y.C.; Investigation: R.Z., W.Z., X.C., Q.M., D.Y., A.P., J.W., Y.H., S.F., M.H.; Funding acquisition and resources: E.J. All authors confirmed the content of the manuscript.

Funding This work was supported by funds from National Key R&D Program of China (2023YFC2508900); the CAMS Innovation Fund for Medical Sciences, CIFMS (2023-I2M-2-007); the National Natural Science Foundation of China (82070192); Haihe Laboratory of Cell Ecosystem Innovation Fund (22HHXBSS00034), and Tianjin Natural Science Foundation (23JCZXJC00220).

Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate The Ethics Committee of Blood Diseases Hospital, Chinese Academy of Medical Sciences (IIT2021011-EC-1) approved the research protocol. This study follows the Declaration of Helsinki. This study was registered with clinicaltrials.gov (Identifier, NCT04645199) in 27/11/2020. All patients provided written and oral informed consent.

Consent for publication The subjects gave written informed consent for the publication of any associated data.

Competing interests The authors declare no competing interests.

References

1. Ferrara F, Lessi F, Vitagliano O, Birkenghi E, Rossi G. Current therapeutic results and treatment options for older patients with relapsed acute myeloid leukemia. Cancers (Basel). 2019;11:224.
2. Medeiros BC. Is there a standard of care for relapsed AML? Best Pract Res Clin Haematol. 2018;31:384–6.
3. Archimbaud E, Thomas X, Leblond V, Michallet M, Fenaux P, Cordonnier C, et al. Timed sequential chemotherapy for previously treated patients with acute myeloid leukemia: long-term follow-up of the etoposide, mitoxantrone, and cytarabine-86 trial. J Clin Oncol. 1995;13:11–8.
4. Kern W, Aul C, Maschmeyer G, Kuse R, Kerkhoff A, Grote-Metke A, et al. Granulocyte colony-stimulating factor shortens duration of critical neutropenia and prolongs disease-free survival after sequential high-dose cytosine arabinoside and mitoxantrone (S-HAM) salvage therapy for refractory and relapsed acute myeloid leukemia. German AML Cooperative Group. Ann Hematol. 1998;77:115–22.
5. Loke J, Malladi R, Moss P, Craddock C. The role of allogeneic stem cell transplantation in the management of acute myeloid leukaemia: a triumph of hope and experience. Br J Haematol. 2020;188:129–46.
6. Feijen EAM, Leisenring WM, Stratton KL, Ness KK, van der Pal HJH, van Dalen EC, et al. Derivation of anthracycline and anthraquinone equivalence ratios to doxorubicin for late-onset cardiotoxicity. JAMA Oncol. 2019;5:864–71.
7. Pinar IE, Celik S, Polat MG, Karatas AF, Dogan A, Iltar U, et al. Idarubicin versus daunorubicin versus mitoxantrone for induction chemotherapy in acute myeloid leukemia: patient registration study of Turkish Society of Hematology-Acute Myeloid Leukemia Working Group. Blood. 2023;142:5838.
8. Gabizon A, Shmeeda H, Barenholz Y. Pharmacokinetics of pegylated liposomal Doxorubicin: review of animal and human studies. Clin Pharmacokinet. 2003;42:419–36.
9. Li C, Cui J, Wang C, Li Y, Zhang H, Wang J, et al. Encapsulation of mitoxantrone into pegylated SUVs enhances its antineoplastic efficacy. Eur J Pharm Biopharm. 2008;70:657–65.
10. Wang L, Cao J, Li C, Wang X, Zhao Y, Li T, et al. Efficacy and safety of mitoxantrone hydrochloride liposome injection in Chinese patients with advanced breast cancer: a randomized, open-label, active-controlled, single-center, phase II clinical trial. Invest New Drugs. 2022;40:330–9.
11. Jiao YQ, Xiong H, Chen Z, Yang L, Tao F, Sun M, et al. Clinical analysis of mitoxantrone liposome in the treatment of children with high-risk acute myeloid leukemia. Zhongguo Shi Yan Xue Ye Xue Za Zhi. 2024;32:365–9.
12. Dohner H, Wei AH, Appelbaum FR, Craddock C, DiNardo CD, Dombret H, et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood. 2022;140:1345–77.
13. Kern W, Aul C, Maschmeyer G, Schonrock-Nabulsi R, Ludwig WD, Bartholomaus A, et al. Superiority of high-dose over intermediate-dose cytosine arabinoside in the treatment of patients with high-risk acute myeloid leukemia: results of an age-adjusted prospective randomized comparison. Leukemia. 1998;12:1049–55.
14. Huhmann IM, Watzke HH, Geissler K, Gisslinger H, Jager U, Knobl P, et al. FLAG (fludarabine, cytosine arabinoside, G-CSF) for refractory and relapsed acute myeloid leukemia. Ann Hematol. 1996;73:265–71.
15. Thol F, Schlenk RF, Heuser M, Ganser A. How I treat refractory and early relapsed acute myeloid leukemia. Blood. 2015;126:319–27.
16. Halpern AB, Othus M, Huebner EM, Scott BL, Hendrie PC, Percival MM, et al. Phase I/II trial of cladribine, high-dose cytarabine, mitoxantrone, and G-CSF with dose-escalated mitoxantrone for relapsed/refractory acute myeloid leukemia and other high-grade myeloid neoplasms. Haematologica. 2019;104:e143–6.
17. Wierzbowska A, Robak T, Pluta A, Wawrzyniak E, Cebula B, Holowiecki J, 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.
18. Amadori S, Arcese W, Isacchi G, Meloni G, Petti MC, Monarca B, et al. Mitoxantrone, etoposide, and intermediate-dose cytarabine: an effective and tolerable regimen for the treatment of refractory acute myeloid leukemia. J Clin Oncol. 1991;9:1210–4.
19. Feldman EJ, Brandwein J, Stone R, Kalaycio M, Moore J, O'Connor J, et al. Phase III randomized multicenter study of a humanized anti-CD33 monoclonal antibody, lintuzumab, in combination with chemotherapy, versus chemotherapy alone in patients with refractory or first-relapsed acute myeloid leukemia. J Clin Oncol. 2005;23:4110–6.
20. Kohrt HE, Patel S, Ho M, Owen T, Pollyea DA, Majeti R, et al. Second-line mitoxantrone, etoposide, and cytarabine for acute myeloid leukemia: a single-center experience. Am J Hematol. 2010;85:877–81.
21. Greenberg PL, Lee SJ, Advani R, Tallman MS, Sikic BI, Letendre L, et al. Mitoxantrone, etoposide, and cytarabine with or without valspodar in patients with relapsed or refractory acute myeloid leukemia and high-risk myelodysplastic syndrome: a phase III trial (E2995). J Clin Oncol. 2004;22:1078–86.
22. Wei S, Mi Y, Wei H, Lin D, Liu K, Gong B, et al. Cyclophosphamide combined with mitoxantrone and cytarabine is an effective salvage regimen for patients with acute myeloid leukemia who experienced primary induction failure or relapse. Mol Clin Oncol. 2016;4:285–9.
23. Breems DA, Van Putten WL, Huijgens PC, Ossenkoppele GJ, Verhoef GE, Verdonck LF, et al. Prognostic index for adult patients with acute myeloid leukemia in first relapse. J Clin Oncol. 2005;23:1969–78.
24. Chevallier P, Labopin M, Turlure P, Prebet T, Pigneux A, Hunault M, et al. A new Leukemia Prognostic Scoring System for refractory/relapsed adult acute myelogeneous leukaemia patients: a GOELAMS study. Leukemia. 2011;25:939–44.
25. Feldman EJ, Lancet JE, Kolitz JE, Ritchie EK, Roboz GJ, List AF, et al. First-in-man study of CPX-351: a liposomal carrier containing cytarabine and daunorubicin in a fixed 5:1 molar ratio for the treatment of relapsed and refractory acute myeloid leukemia. J Clin Oncol. 2011;29:979–85.
26. Feldman EJ, Kolitz JE, Trang JM, Liboiron BD, Swenson CE, Chiarella MT, et al. Pharmacokinetics of CPX-351; a nano-scale liposomal fixed molar ratio formulation of cytarabine:daunorubicin, in patients with advanced leukemia. Leuk Res. 2012;36:1283–9.

27. Lim WS, Tardi PG, Dos Santos N, Xie X, Fan M, Liboiron BD, et al. Leukemia-selective uptake and cytotoxicity of CPX-351, a synergistic fixed-ratio cytarabine:daunorubicin formulation, in bone marrow xenografts. Leuk Res. 2010;34:1214–23.
28. Lancet JE, Uy GL, Cortes JE, Newell LF, Lin TL, Ritchie EK, et al. CPX-351 (cytarabine and daunorubicin) liposome for injection versus conventional cytarabine plus daunorubicin in older patients with newly diagnosed secondary acute myeloid leukemia. J Clin Oncol. 2018;36:2684–92.
29. Franco YL, Vaidya TR, Ait-Oudhia S. Anticancer and cardio-protective effects of liposomal doxorubicin in the treatment of breast cancer. Breast Cancer (Dove Med Press). 2018;10:131–41.
30. Im A, Amjad A, Agha M, Raptis A, Hou JZ, Farah R, et al. Mitoxantrone and etoposide for the treatment of acute myeloid leukemia patients in first relapse. Oncol Res. 2016;24:73–80.
31. Zhong S, Kurish H, Walchack R, Li H, Edwards J, Singh A, et al. Efficacy and safety of mitoxantrone, etoposide, and cytarabine for treatment of relapsed or refractory acute myeloid leukemia. Leuk Res. 2024;139: 107468.
32. Christian S, Arain S, Patel P, Khan I, Calip GS, Agrawal V, et al. A multi-institutional comparison of mitoxantrone, etoposide, and cytarabine vs high-dose cytarabine and mitoxantrone therapy for patients with relapsed or refractory acute myeloid leukemia. Am J Hematol. 2020;95:937–43.
33. Hui L, Jiyi F, Zheng B, Lianjie H, Yaqiong L, Xin W, et al. Efficacy and safety of dose-tailored mitoxantrone liposomes combined with cytarabine and venetoclax in newly diagnosed acute myeloid leukemia (non-M3) based on marrow leukemic cellularity. Blood. 2024;144:6068.
34. Wang Y, Wang W, Xu J, Ma J, Wang H, Wu B, et al. Mitoxantrone hydrochloride liposome combined with cytarabine as induction therapy for patients with newly diagnosed secondary acute myeloid leukemia. Blood. 2024;144:6074.
35. Bhatnagar B, Walker AR, Mims AS, Vasu S, Klisovic RB, Behbehani G, et al. Phase 1 study of selinexor plus mitoxantrone, etoposide, and cytarabine in acute myeloid leukemia. Am Soc Clin Oncol. 2018. https://doi.org/10.1200/JCO.2018.36.15_suppl.7048.
36. Schaich M, Parmentier S, Kramer M, Illmer T, Stolzel F, Rollig C, et al. High-dose cytarabine consolidation with or without additional amsacrine and mitoxantrone in acute myeloid leukemia: results of the prospective randomized AML2003 trial. J Clin Oncol. 2013;31:2094–102.
37. Takami A, Yano S, Yokoyama H, Kuwatsuka Y, Yamaguchi T, Kanda Y, et al. Donor lymphocyte infusion for the treatment of relapsed acute myeloid leukemia after allogeneic hematopoietic stem cell transplantation: a retrospective analysis by the Adult Acute Myeloid Leukemia Working Group of the Japan Society for Hematopoietic Cell Transplantation. Biol Blood Marrow Transplant. 2014;20:1785–90.
38. Kharfan-Dabaja MA, Labopin M, Polge E, Nishihori T, Bazarbachi A, Finke J, et al. Association of second allogeneic hematopoietic cell transplant vs donor lymphocyte infusion with overall survival in patients with acute myeloid leukemia relapse. JAMA Oncol. 2018;4:1245–53.