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RESEARCH ARTICLE

Homoharringtonine is a safe and effective substitute for anthracyclines in children younger than 2 years old with acute myeloid leukemia

Xiaoxiao Chen*, Yanjing Tang*, Jing Chen, Ru Chen, Longjun Gu, Huiliang Xue, Ci Pan, Jingyan Tang (✉), Shuhong Shen (✉)

Key Laboratory of Pediatric Hematology and Oncology Ministry of Health, Department of Hematology and Oncology, Pediatric Translational Medicine Institute, Shanghai Children's Medical Center, Shanghai Jiao Tong University School of Medicine, Shanghai 200127, China

Abstract **Homoharringtonine (HHT), a plant alkaloid from *Cephalotaxus harringtonia*, exhibits a unique anticancer mechanism and has been widely used in China to treat patients with acute myeloid leukemia (AML) since the 1970s. Trial SCMC-AML-2009 presented herein was a randomized clinical study designed based on our previous findings that pediatric AML patients younger than two years old may benefit from HHT-containing chemotherapy regimens. Patients randomized to arm A were treated with a standard chemotherapy regimen comprising mainly of anthracyclines and cytarabine (Ara-C), whereas patients in arm B were treated with HHT-containing regimens in which anthracyclines in all but the initial induction therapy were replaced by HHT. From February 2009 to November 2015, 59 patients less than 2 years old with *de novo* AML (other than acute promyelocytic leukemia) were recruited. A total of 42 patients achieved a morphologic complete remission (CR) after the first course, with similar rates in both arms (70.6% vs.72.0%). At the end of the follow-up period, 40 patients remained in CR and 5 patients underwent hematopoietic stem cell transplantation in CR, which could not be considered as events but censors. The 5-year event-free survival (EFS) was 60.2% ± 9.6% for arm A and 88.0% ± 6.5% for arm B ($P = 0.024$). Patients in arm B experienced shorter durations of leukopenia, neutropenia, and thrombocytopenia and had a lower risk of infection during consolidation chemotherapy with high-dosage Ara-C. Consequently, the homoharringtonine-based regimen achieved excellent EFS and alleviated hematologic toxicity for children aged younger than 2 years with *de novo* AML compared with the anthracycline-based regimen.**

Keywords homoharringtonine; acute myeloid leukemia; pediatrics

Introduction

Acute myeloid leukemia (AML) accounts for approximately 20%–25% of pediatric leukemia cases, and the survival rates among these patients have remained modest for decades [1–3]. Standard chemotherapy usually includes anthracyclines, such as daunorubicin (DNR), mitoxantrone (MTZ), or idarubicin (IDA). Overall survival (OS) with the use of these regimens is approximately 70%, with an event-free survival (EFS) of 55% [2]. However, relapse occurs in about 30% of patients [2], and severe infections due to myelosuppression contribute to mortality.

Homoharringtonine (HHT), a plant alkaloid derived from the trees of the genus *Cephalotaxus*, shows antitumor properties and has been widely used in China to treat acute and chronic myeloid leukemia (CML) since the 1970s [4]. It inhibits protein translation by preventing the initial elongation step in protein synthesis [4,5], thereby generally decreasing the synthetic efficiency of all proteins, especially cell survival-related proteins with short half-lives [5]. HHT primarily affects cells in the G_1 and G_2 phases, and reportedly exhibits significant synergistic effects with cytarabine (Ara-C) [6,7]. In 2012, the US FDA approved omacetaxine, a semisynthetic form of HHT, to treat CML refractory to TKIs for its potential as a broad-spectrum protein tyrosine kinase inhibitor (TKI) [5,8].

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Correspondence: Jingyan Tang, tangjingyan@scmc.com.cn;
Shuhong Shen, shenshuhong@scmc.com.cn

*These authors contributed equally to this article.

Notably, HHT shows relatively mild non-hematologic toxicity [9].

A clinical trial of the HHT-containing regimen, XH-AML-99, was conducted. This trial started in June 1998. In the cohort of 171 newly diagnosed AML patients (median age: 7.58 years, range 0.5–16.5 years), we achieved a 5-year EFS rate of 52.75% [10]. Notably, we found an excellent EFS among 12 children aged 0–2 years old (83.3%) compared with the EFS of 50.3% in older children ($P = 0.025$). These data indicated that HHT may be more effective in younger children with AML. Therefore, in February 2009, we began the randomized trial to compare HHT with anthracyclines with a focus on children younger than 2 years old.

Patients and methods

Study design and participants

The study was a randomized trial initiated in February 2009 at the Shanghai Children's Medical Center (SCMC), which had to be terminated unfortunately due to the unavailability of HHT. Inclusion criteria were newly diagnosed *de novo* AML other than acute promyelocytic leukemia (APL), age less than two years, no previous chemotherapy, normal cardiac function, and adequate liver and renal function at diagnosis. Patients with AML secondary to myelodysplastic syndrome (MDS), aplastic anemia (AA), Down syndrome, congenital immunodeficiency, or organ transplantation were excluded. For all included patients, written informed consent was provided by their parents or guardians. This study was approved by the ethics committee of SCMC.

Diagnosis

The initial AML diagnosis and subtype determination were based on morphology, immunotyping, cytogenetics, and molecular biology. Morphologically, patients with 30% or more blasts in a bone-marrow smear were diagnosed with AML. At diagnosis, all samples were analyzed for the presence of t(8;21)/RUNX1-RUNXT1, inv(16)/CBFb-MYH11, t(15;17)/PML-RARα, and 11q23/MLL rearrangements (MLLr) using conventional karyotyping, fluorescence *in situ* hybridization, and/or RT-PCR. Central nervous system (CNS) involvement was defined as > 5 leukocytes per μL of cerebrospinal fluid (CSF) and the presence of leukemia cells on cytospin preparations or cranial nerve involvement.

Treatment design

Fig. 1 shows the study schema of SCMC-AML-2009. All enrolled patients were randomly assigned to one of the two study arms after induction therapy. The major chemotherapeutic drugs were anthracyclines in arm A and HHT in arm B. Both regimens included 10 cycles and began with conventional induction with DAE (DNR, Ara-C, etoposide [VP16]). Subsequently, the children in arm A received MAE (MTZ, Ara-C, VP16) as consolidation; four courses of high-dosage Ara-C plus DNR, MTZ, or VP16 as intensification; and Ara-C and 6-mercaptopurine (6MP) as continuation therapy. All of the anthracyclines and 6MP

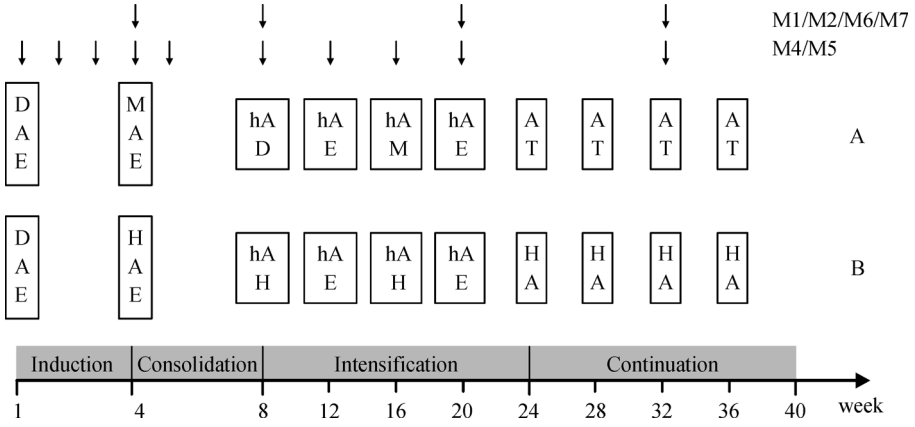

Fig. 1 Schema of the SCMC-AML-2009 trial design. The regimen comprised induction (weeks 1–4), consolidation (weeks 4–8), intensification (weeks 8–24), and continuation (weeks 24–40). DAE: daunorubicin (DNR) 40 mg/m^2, days 1–3; cytarabine (Ara-C) 100 mg/m^2 every 12 h for a total of 14 doses, days 1–7; and etoposide (VP16) 100 mg/m^2, days 5–7. MAE: mitoxantrone (MTZ) 10 mg/m^2, days 1–3, and Ara-C and VP16 administered as in DAE. hAD/hAE/hAM: Ara-C 3000 mg/m^2 every 12 h for a total of 6 doses from day 1, DNR 40 mg/m^2 or VP16 100 mg/m^2 or MTZ 10 mg/m^2, days 1–2; AT, Ara-C 75 mg/m^2 every 12 h for a total of 14 doses from day 1, 6-mercaptopurine (6MP) 75 mg/m^2 for 9 days starting from day 1. HAE: homoharringtonine (HHT) 3 mg/m^2, days 1–9, and Ara-C and VP16 as in DAE. hAH: Ara-C 3,000 mg/m^2 every 12 h for a total of 6 doses from day 1, HHT 3 mg/m^2, days 1–5. HA: HHT 3 mg/m^2, days 1–9, Ara-C 75 mg/m^2 every 12 h for a total of 14 doses from day 1. The arrows indicate intrathecal injections.

were substituted with HHT for the children in arm B. Patients with M4 or M5 underwent a total of 10 lumbar punctures and intrathecal injections to prevent central nervous system leukemia (CNS-L), whereas patients with other subtypes only underwent 4 of these procedures (Fig. 1). Patients who did not achieve complete remission (with or without incomplete blood count recovery) after induction therapy were recommended for hematopoietic stem cell transplantation (HSCT).

Response criteria

Following induction, the remission induction response was evaluated using bone marrow smear according to granulocyte and platelet recovery [11]. Complete remission (CR) was defined as the total disappearance of all measurable extramedullary disease, M-1 marrow status ($< 5\%$ leukemic blast cells), neutrophil count of $> 1.0 \times 10^9$/L, and platelet count of $> 100 \times 10^9$/L, independent of transfusions. Patients exhibiting incomplete blood count recovery were classified as being in complete remission with incomplete blood count recovery (CRi). Blasts of between 5%–25% were considered to indicate a partial response (PR), while blasts of $> 25\%$ were counted as no response (NR). CR criteria do not require negative results for AML–ETO or CBFb–MYH11 fusion genes. For patients with leukemia-associated aberrant immunophenotype at diagnosis, MRD monitoring was done at each bone marrow evaluation.

Myelosuppression and infection

We assessed toxicity and performance according to the modified National Cancer Institute Common Toxicity Criteria. Myelosuppression was evaluated based on the durations of leukopenia (white blood cells [WBC] $< 1.0 \times 10^9$/L), neutropenia (absolute neutrophil count [ANC] $< 0.5 \times 10^9$/L or $< 0.2 \times 10^9$/L), and thrombocytopenia (platelets [PLT] $< 50 \times 10^9$/L).

Infectious complications were defined as the observation of clinical signs and symptoms necessitating antibiotic therapy, isolation of a pathogen by physical examination, or detection of a site of infection by imaging [12,13]. Infectious episodes were categorized as fever of unknown origin (FUO), microbiologically documented bloodstream infection (BSI), or clinically documented infection [13]. Fever was recorded when a patient's temperature was > 38.5 °C once or 38 °C–38.5 °C twice within a 4-h interval. BSI was defined as fever with a positive culture for bacteria isolated from peripheral blood or from the central venous indwelling catheter [13,14]. If the bloodstream isolate was a potential skin contaminant (e.g., coagulase-negative staphylococcus), the presence of an intravascular catheter was required for the diagnosis of a bloodstream infection. The occurrence of fever with diarrhea, abdominal pain, or hematochezia was noted as an infection with gastrointestinal manifestation. Pneumonia diagnosis required clinical symptoms of lower respiratory infection accompanied by a pathological chest X-ray and/or computed tomography scan. Infection episodes involving septic shock or severe pneumonia necessitating mechanical ventilation were defined as severe infections.

Censored points and statistical analysis

December 15, 2016 was used as the reference date of analysis and the median follow-up was 2.99 years (range, 0.17–7.12 years). The date of transplantation was used as a censor point for children who underwent HSCT to exclude its impact. The disease and patient data, including survival status, were recorded in the Pediatric Oncology Networked Database (POND; www.pond4kids.org) and in a personal disease information pack.

Overall survival (OS) was calculated from the date of diagnosis to the date of death due to any cause, or to the date of last contact for survivors. EFS was calculated from the date of diagnosis to the last follow-up or first event (failure to achieve CR, relapse, second tumor, or death due to any cause—whichever occurred first). Patients who were lost to follow-up were censored at their status during their last follow-up. For patients with disease exacerbation and severe infectious complications, quitting therapy was defined as an event.

The probabilities of OS and EFS were estimated using the Kaplan–Meier method. We estimated the significance of differences among the OS and EFS using the log-rank test (Mantel–Cox). A P value of less than 0.05 was considered statistically. Computations were performed using SPSS 24.0.

Results

Between February 2009 and November 2015, 59 patients who met the entry criteria were enrolled in the study. Patients were randomized: 34 to arm A and 25 to arm B. Table 1 presents the main clinical and biological characteristics of the patient cohort. Detailed information for the cases is presented in Table S1.

After induction therapy, CR was achieved in 71.2% of the 59 patients, including 70.6% in arm A ($n = 24$) and 72.0% in arm B ($n = 18$) ($P = 0.906$). CRi was achieved in 13.5% of the total, in 8.8% of patients in arm A ($n = 3$), and 20.0% of patients in arm B ($n = 5$) ($P = 0.323$). Among the patients who did not achieve CR or CRi after the first induction, 2/7 in arm A and 0/2 in arm B remained in non-CR after the second course. A total of 3 children (all in arm A) underwent HSCT after 3–5 cycles of chemotherapy. Additionally, 2 patients (arm A) with a good response underwent HSCT because it was strongly requested by

Table 1 Clinical and biologic characteristics of the patients

	Arm A ($n=34$)	Arm B ($n=25$)	P
Age (year)	1.14±0.45	0.99±0.38	0.175
Gender (n)			0.022
Male	25	11	
Female	9	14	
FAB subtype (n)			0.089
M1	1	0	
M2	1	1	
M4	4	5	
M5	16	17	
M7	12	2	
Features at diagnosis			
WBC median, range ($\times 10^9$/L)	13.85 (2.20–355.4)	15.00 (2.10–160)	0.230
PLT median, range ($\times 10^9$/L)	36 (8–224)	49 (8–244)	0.632
LDH median, range (U/L)	2123 (503–9000)	2460 (689–11 250)	0.821
Fusion genes (n)			
RUNX1-RUNXT1	0	1	0.876
CBFb-MYH11	1	2	0.784
MLLr	5	4	1.0

their parents. Over the duration of follow-up, 40 children exhibited a continuous complete remission (CCR) (including 18 patients in arm A and 22 in arm B), and 2 patients in arm A that achieved CR were lost to follow-up. Unfortunately, 3 patients died of severe infections (2 in arm A, 1 in arm B). Relapse from a CR occurred in 8 children (6 in arm A and 2 in arm B), with a median time from diagnosis to relapse of 6.24 months (range, 3.73–21.13 months), and another patient with MLLr relapsed with an acute lymphocytic leukemia (ALL) phenotype. One patient in arm A with M7 (acute megakaryoblastic leukemia) developed a secondary acute monocytic leukemia after 9 months in CCR. Two of 9 cases with MLLr relapsed, one each in both arms. All 4 other cases with specific fusion genes were in CCR (Table S1).

On average, the estimated 5-year EFS was 73.7% ± 6.1%, with a median follow-up time of 2.99 years (range, 0.17–7.12 years) for this cohort of 59 cases. Within the separate study arms, the 5-year EFS was 60.2% ± 9.6% in arm A and 88.0% ± 6.5% in arm B ($P=0.024$) (Fig. 2). The estimated 5-year EFS rates of patients with M4, M5, and M7 were 76.2% ± 14.8%, 79.8% ± 7.4%, and 50.8% ± 14.4%, respectively. All 3 patients with M1 or M2 are still in CR. Clinical outcomes did not significantly differ in relation to FAB subtypes ($P=0.092$) although outcomes were generally poorer for those with M7. The distribution of M7 patients was skewed between the two study arms because we did not stratify patients based on FAB subtypes during randomization. Therefore, we compared the EFS rates of non-M7 patients between the two study arms to exclude any unfavorable impact of M7 on the EFS rate of arm A (Fig. 2). Obtaining an accurate OS rate was challenging because most of the relapsed patients did not pursue further treatment, and their parents were unwilling to be re-visited, making it difficult to obtain the patient's final status.

Myelosuppression and infection

All patients received conventional induction therapy with DAE. The durations of leukopenia and neutropenia did not differ significantly between the two arms (Fig. 3). MTZ was replaced with HHT in arm B during the consolidation phase, which led to shorter durations of leukopenia and neutropenia (WBC $< 1.0 \times 10^9$/L: 9.94 ± 5.17 days vs. 6.64 ± 5.43 days, $P=0.022$; ANC $< 0.5 \times 10^9$/L: 17.94 ± 4.92 days vs. 11.88 ± 4.10 days, $P<0.001$). The same phenomenon was observed during the intensification phase with high-dose Ara-C; HHT-treated children showed a reduced depth of myelosuppression (WBC $< 1.0 \times 10^9$/L: 9.23 ± 2.15 days vs. 7.38 ± 1.82 days, $P=0.001$; ANC $< 0.5 \times 10^9$/L: 9.75 ± 3.62 days vs. 7.47 ± 1.94 days, $P=0.006$; PLT $< 50 \times 10^9$/L: 7.41 ± 3.71 days vs. 5.08 ± 1.88 days, $P=0.006$). During the continuation phase, the conditions were opposite to those described above. Arm B which received the HA regimen showed longer durations of leukopenia, neutropenia, and thrombocytopenia than arm A. This may be explained by potential synergistic effects between HHT and cytarabine.

The 59 patients experienced a total of 332 infectious episodes (5.6 infectious episodes per patient). Of these

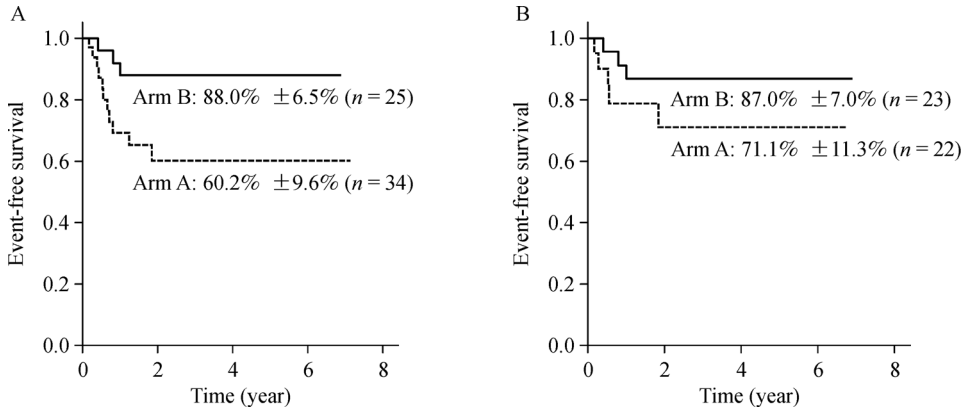

Fig. 2 The patients' estimated 5-year EFS rates. (A) The 5-year EFS rates were 60.2% ± 9.6% in arm A and 88.0% ± 6.5% in arm B ($P = 0.024$). (B) The 5-year EFS rates of patients without M7 (87.0% ± 7.0% vs. 71.1% ± 11.3%, $P = 0.222$).

episodes, 35.3% occurred during the induction and consolidation phases, whereas 43.5% occurred during the intensification phase where 83.6% of children in arm A experienced infections, whereas only 55.2% in arm B experienced these ($P < 0.001$) (Fig. 3).

Among the 332 infectious episodes, 187 (56.3%) experienced a fever of unknown origin (FUO). Microbiologically documented blood stream infections (BSI) were found during 57 episodes (17.2%) (Fig.4, and data about each arm are shown in Fig. S1). A total of 18 severe infections were observed (5.4%), which were concentrated in the first two courses ($n = 14$, 77.8%), and these rates did not significantly differ between two study arms ($P = 0.674$). Three children (5.1%; 2 in arm A and 1 in arm B) died of severe pneumonia and sepsis.

Among the 57 microbiologically documented blood stream infections, the isolates that recovered from the bloodstream included 31 Gram-positive organisms, 25 Gram-negative organisms, and 1 fungus (Table 2). The microbiology spectra did not significantly differ between two arms, either in each phase or in total.

Discussion

Cephalotaxus mainly grows in the eastern and southern regions of the Asian subcontinent, and is used as a folk medicine to treat animal tumors in southeastern China. The cytotoxic alkaloid, HHT, was first isolated from *Cephalotaxus harringtonia* (Forbes) in 1963 by Paudler and McKay, and its structure was confirmed by Powell *et al*. in 1969 [15]. Since 1977, numerous Chinese medical centers have used HHT as one of the components of combined chemotherapy regimens for AML [4], and the results indicate that HHT plus Ara-C is an effective induction regimen with tolerable toxicity compared with DNR plus Ara-C. Moreover, the HHT-based triple drug combination is reportedly effective in both newly diagnosed [6] and relapsed/refractory AML patients [16,17].

However, few studies have reported the use of HHT among children. In 1987, Tan *et al*. began a clinical trial of HHT in children with heavily treated refractory leukemia (17 with ALL and 7 with AML). Unfortunately, none of the patients achieved remission [18]. However, when Bell *et al*. assessed HHT as a potentially active drug for treating children with chemotherapy-resistant AML, they reported an 18% response rate (5/28) [19]. In 1998, Tang *et al.* tested the efficacy of an HHT-containing regimen in children with newly diagnosed AML and achieved CR in 10 of 12 patients (83%) using HHT combined with Ara-C and VP-16 as induction therapy [20].

The XH-AML-99 study with HHT-included regimen tested the first unified local protocol for pediatric AML at our hospital, and achieved a 5-year EFS of 52% [10]. Similar to other reports, the XH-AML-99 study results indicate that HHT can support long-term EFS. Moreover, we noticed a better EFS among 12 children aged < 2 years than the average. This observation prompted us to initiate the present SCMC-AML-2009 study, whose aim was to evaluate both the therapeutic and adverse effects of HHT in pediatric patients with AML, with a focus on this young subgroup. Our results show that 25 children in the HHT arm achieved a 5-year EFS of 88.0%, which was higher than the 5-year EFS of patients in the anthracyclines arm (60.2%). These findings indicate that HHT can effectively replace anthracyclines in chemotherapy regimens for pediatric AML, at least in younger children.

Unfortunately, our present study has several limitations. First, the sample size was small due to the low incidence of AML in children younger than 2 years old and a shortage of HHT. The small sample size led to skewed randomization, which was reflected by more patients achieving CRi in arm B and similar CR rates between the two arms. However, if we exclusively calculated the EFS in patients

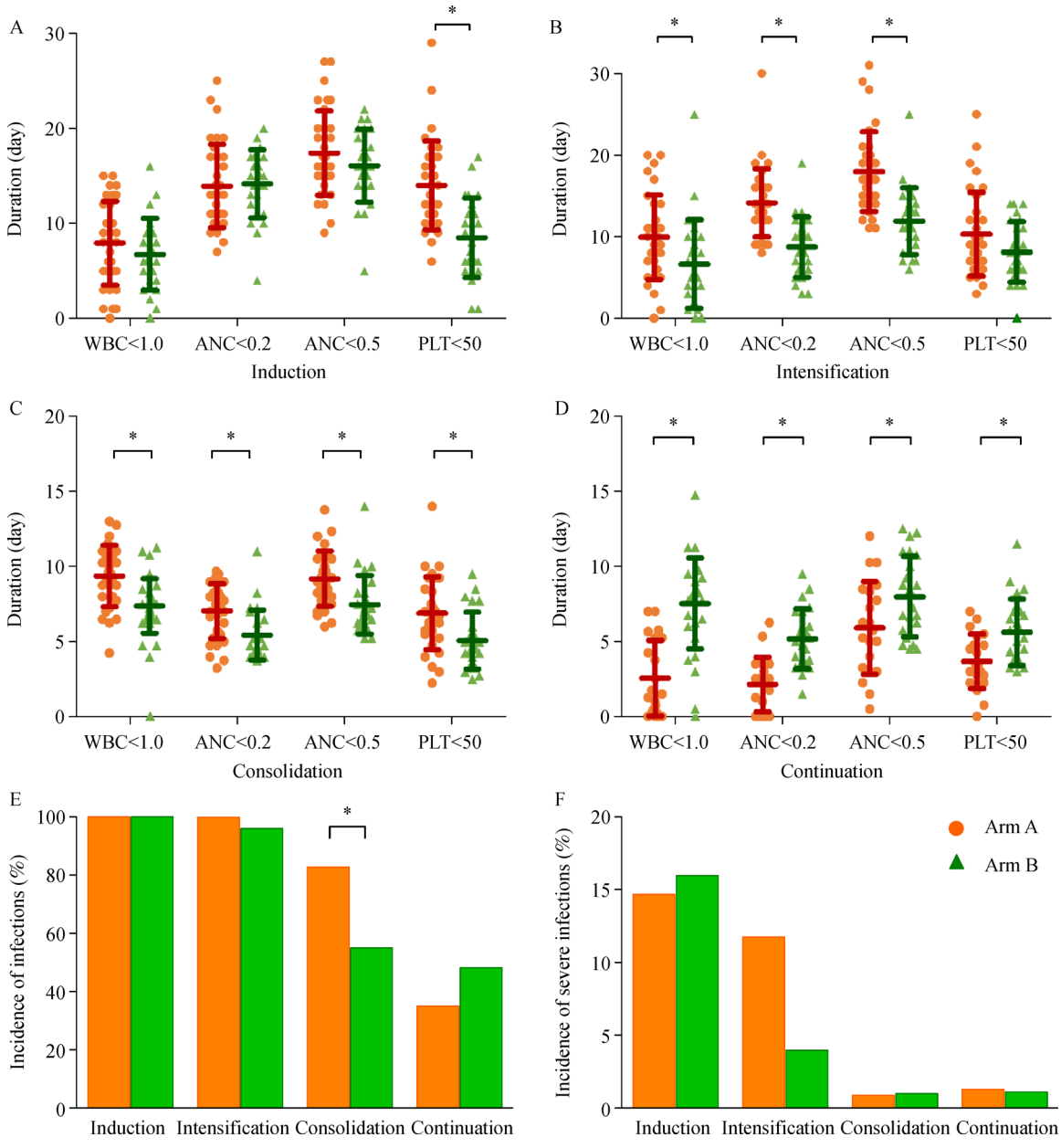

Fig. 3 Myelosuppression during the courses in the two study arms. Orange bars represent data from arm A, whereas green bars represent data from arm B (with HHT). In panels A–D, the horizontal line indicates the average, whereas the perpendicular line indicates the standard deviation. (A) During induction, the two study arms did not differ in the durations of leukopenia and neutropenia, whereas arm A showed a longer duration of thrombocytopenia (PLT $< 50 \times 10^9$/L: 14.0 ± 4.70 days vs. 8.48 ± 4.18 days, $P < 0.001$). (B) During intensification, patients in arm A showed longer durations of leukopenia and neutropenia (WBC $< 1.0 \times 10^9$/L: 9.94 ± 5.17 days vs. 6.64 ± 5.43 days, $P = 0.022$; ANC $< 0.5 \times 10^9$/L: 17.94 ± 4.92 days vs. 11.88 ± 4.10 days, $P < 0.001$). (C) During consolidation, patients in arm A showed longer durations of leukopenia, neutropenia, and thrombocytopenia (WBC $< 1.0 \times 10^9$/L: 9.23 ± 2.15 days vs. 7.38 ± 1.82 days, $P = 0.001$; ANC $< 0.5 \times 10^9$/L: 9.75 ± 3.62 days vs. 7.47 ± 1.94 days, $P = 0.006$; PLT $< 50 \times 10^9$/L: 7.41 ± 3.71 days vs. 5.08 ± 1.88 days, $P = 0.006$). (D) During continuation, arm A exhibited shorter durations of leukopenia, neutropenia, and thrombocytopenia (WBC $< 1.0 \times 10^9$/L: 2.56 ± 2.53 days vs. 7.52 ± 3.04 days, $P < 0.001$; ANC $< 0.5 \times 10^9$/L: 5.91 ± 3.10 days vs. 7.97 ± 2.69 days, $P = 0.023$; PLT $< 50 \times 10^9$/L: 3.67 ± 1.82 days vs. 5.63 ± 2.20 days, $P = 0.003$). (E) Incidences of infection in each study arm. Children treated with HHT experienced fewer infectious episodes, especially during intensification (55.2% vs. 83.6%, $P < 0.001$). (F) Incidences of severe infection in each study arm: 14.71%, 11.76%, 0.91%, and 1.30% during each period in arm A, compared with 16.0%, 4.0%, 1.04%, and 1.12% in arm B, respectively. * $P < 0.05$.

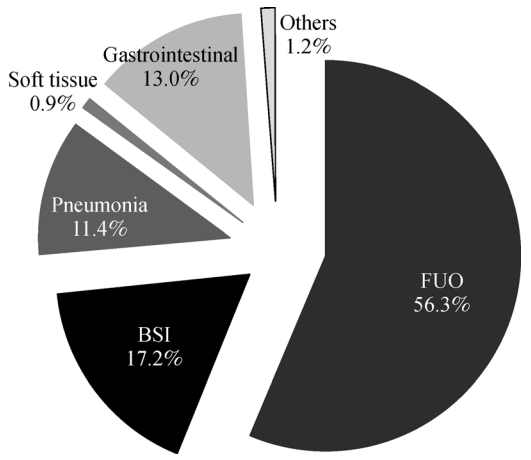

Fig. 4 Categorization of infectious episodes. A fever of unknown origin (FUO) accounted for 56.3%. Microbiologically documented bloodstream infections (BSI), pneumonia, and soft tissue infections accounted for 17.2%, 11.4%, and 0.9% of infections, respectively. Patients with gastrointestinal manifestation (13.0%) were those who had a fever with diarrhea, abdominal pain, or hematochezia. Other infections included mumps, herpes zoster, and varicella.

without HSCT who achieved CR after induction therapy, arm B (EFS: 94.4% ± 5.4%) was still superior to arm A (EFS: 61.1% ± 11.1%; $P = 0.017$) (Table S2 and Fig. 5). For those who achieved CR or CRi, the EFS in the two arms were 64.7% ± 10.3% and 91.3% ± 5.9%, respectively ($P = 0.035$) (Table S3 and Fig. 5). We can rule out the effect of the skewed randomization based on these considerations. Second, some of our patients underwent HSCT because of poor treatment response. The date of transplantation was used as a censor point for children who underwent HSCT to exclude its impact. Also, we re-analyzed the EFS in patients without HSCT, which showed similar results (59.2% ± 9.6% vs. 88% ± 6.5%, $P = 0.020$) (Table S4 and Fig. 5). Third, a skewed distribution of M7 between the two study arms was observed because we did not stratify patients based on FAB subtypes during randomization. Study arm A included 35.3% of patients with M7, whereas only 2 patients with M7 were included in arm B. The uneven distribution of M7 cases between the study arms may lead to the misinterpretation of the survival rates because children with M7 had higher relapse rates

Table 2 Distribution of Gram-negative and Gram-positive in arms

Pathogeny		A	B
Gram positive		17	11
	Staphylococci		
	S. aureus	3	0
	S. hemolyticus	1	0
	S. simulans	0	1
	S. hominis	1	0
	S. epidemidis	1	4
	S. lentus	3	1
	Streptococci		
	S. oralis	1	0
	S. mitis	4	3
	S. pneumonia	0	1
	Granulicatella adiacens	1	0
	Micrococcus luteus	1	0
	E. enterococcus	1	0
	Others*	0	1
Gram negative		14	14
	E. coli	2	2
	E. cloacae	1	0
	Klebsiella spp.	10	5
	Morganella	0	1
	Sphingomonas paucimobilis	1	0
	Burkholderia cepacia	0	2
	P. aeruginosa	0	3
	Others*	0	1
Candida parapsilosis		0	1

* Positive culture for bacteria but without detailed data about the bacteria.

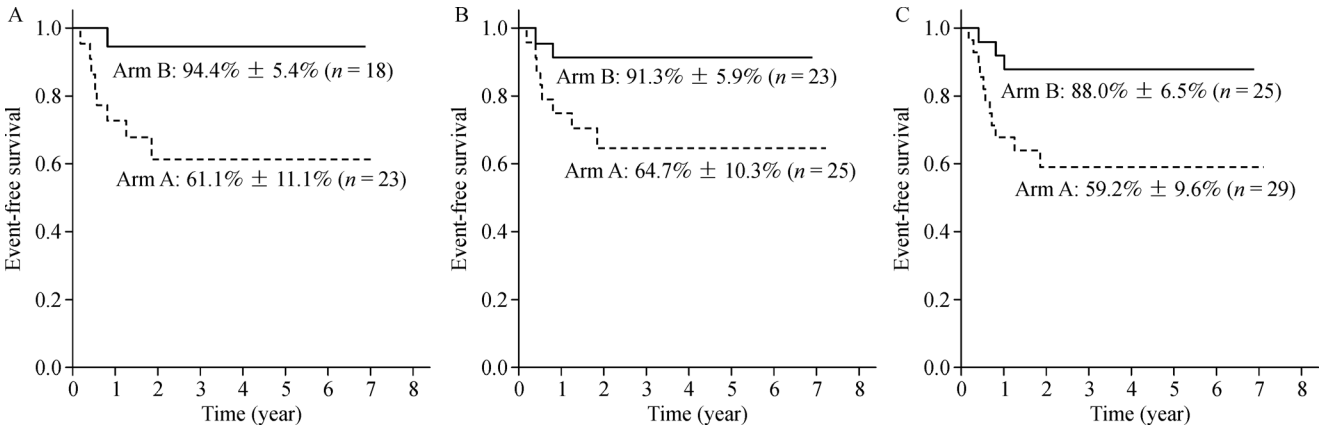

Fig. 5 The specific patients' estimated 5-year EFS rates. (A)The EFS rates of patients without HSCT who achieve CR after induction therapy were 61.1% ± 11.1% in arm A and 94.4% ± 5.4% in arm B ($P = 0.017$). (B)The EFS rates of patients without HSCT who achieve CR or CRi after induction therapy were 64.7% ± 10.3% and 91.3% ± 5.9%, respectively ($P = 0.035$). (C)The EFS of patients without HSCT (59.2% ± 9.6% vs. 88% ± 6.5%, $P = 0.020$).

and worse outcomes, which has also been reported in other studies [21–23]. The trend remained (87.0% ± 7.0% vs. 71.1% ± 11.3%) when we re-analyzed the EFS curves after excluding the M7 cases, but the *P* value was still 0.222. The difference would have been significant if we had not had a shortage of HHT and had recruited enough cases. In a parallel study including AML patients aged > 2 years at our center, relapse occurred in 7/14 cases with M7 in arm A compared with only 1/4 cases in arm B. This could indicate that the HHT-based regimen was more effective in patients with M7. In this case, an HHT-based protocol may be superior to anthracycline-based chemotherapy for AML patients younger than 2 years old, independent of the FAB subgroup.

Infectious complications are important factors that affect mortality and decrease the quality of life. Children with AML are at high risk of infections because of the intensive chemotherapy and protracted severe neutropenia [13,24]. In the present study, each patient aged < 2 years experienced an average of 5.6 infectious episodes. Gram-positive and Gram-negative organisms were at almost equal frequencies among the bloodstream infections. In this age group, a central venous catheter or implanted ports are widely used, which are reported to be closely related to BSI [25]. Children undergoing the HHT-containing protocol had shortened durations of neutropenia, which likely resulted in a lower risk of infection during intensification.

Cardiotoxicity is a well-known risk for AML patients who are at risk of early death due to cardiac-related causes that is more than five times higher (standard mortality ratio of 5.0) than the general population [26,27]. Anthracyclines have been an essential part of standard AML treatment; however, the high cumulative dose of anthracyclines is a well-known risk factor for congestive heart failure [27]. Reports from Europe and America [28–30] indicate that the late clinical or subclinical cardiotoxicity of anthracycline-based regimens varies between 1.3% and 15.3%. Substantially reducing the cumulative anthracycline dose by substituting HHT could potentially decrease the incidence of long-term cardiotoxicity among patients. HHT-related cardiotoxicity in children has not been investigated. One follow-up study of HHT treatment for APL did not reveal cardiotoxicity [31]. No child in our study exhibited symptoms of heart failure or other cardiotoxicity in either study arm during the limited follow-up period. Thus, further research is needed to evaluate the cardiotoxicity of HHT.

We conclude that a chemotherapy regimen using HHT as a backbone was more effective than the regimen based on standard anthracyclines for the treatment of *de novo* AML in children younger than 2 years old without increased hematologic toxicity. However, the need for a well-designed trial with proper stratification remains.

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Compliance with ethics guidelines

Xiaoxiao Chen, Yanjing Tang, Jing Chen, Ru Chen, Longjun Gu, Huiliang Xue, Ci Pan, Jingyan Tang, and Shuhong Shen declare that they have no conflicts of interest. All procedures were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the *Helsinki*

Declaration of 1975, as revised in 2000 (5). Informed consent was obtained from all parents.

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