Novel homobarringtonie-containing therapy for the treatment of patients with primary acute myeloid leukemia that are resistant to conventional therapy

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Abstract. The current study investigated the efficacy and safety of a novel treatment regime consisting of homobarringtonie, cytosine arabinoside and etoposide (HCE) for the treatment of primary acute myeloid leukemia (AML). In the present study, 141 patients diagnosed with AML were divided into the HCE (n=47) and the conventional AML therapy, consisting of idamycin combined with cytarabine (IA; n=94), treatment groups. The measured patient outcome parameters were the emission and response rates, as well as medication-induced adverse events, with a median follow-up time of 28 months. There was no significant difference in the 3-year relapse-free survival rate between the HCE and IA treatment groups. The occurrence and severity of hematological or non-hematological toxicity did not differ between the two groups. However, of the 26 patients that demonstrated a poor response to the IA treatment, 19 cases were administered the HCE treatment and 14 of these patients achieved complete remission (CR). Of the 10 patients that demonstrated a poor response to the HCE treatment, 8 patients were administered the IA treatment and 7 of these achieved CR. Therefore, HCE may be an effective treatment regimen for patients with primary AML. As there was no cross-resistance between the HCE and IA regimens, HCE may be an alternative option for patients that respond poorly to IA induction therapy.

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Abbreviations: AML, acute myeloid leukemia; DNR, daunorubicin; IDA, idarubicin; RFS, relapse free survival; OS, overall survival; HBT, homobarringtonie; CML, chronic myeloid leukemia; FAB, French-American-British; ANC, absolute neutrophil count; CR, complete remission; PR, partial remission

Key words: acute myeloid leukemia, cytarabine, etoposide, homobarringtonie, idamycin

Introduction

Acute myeloid leukemia (AML) is a group of heterogeneous malignant blood tumors, and is the most common variant of adult acute leukemia, which typically causes hematopoietic failure as a result of bone marrow infiltration (1). Daunorubicin and idarubicin (IDA) anthracycline drugs combined with cytosine arabinocide (Ara-c) in the '7+3' regimen is the established inductive chemotherapy for AML (2-4).

Previous clinical studies have focused on improving the inductive regimen for patients with AML, including increasing the dosage of Ara-c from 100-200 mg/m^2 to higher doses (5,6), increasing the dosage of daunorubicin (from 45 to 90 mg/m^2) or replacing daunorubicin with idarubicin, which may be effective for younger patients and patients with a good prognosis (7-9). A third drug may be added to the original '7+3' regimen, such as fludarabine, cladribine or etoposide, to improve the remission rate, the relapse free survival (RFS) rate and the overall survival (OS) rate following induction therapy (10,11).

Homobarringtonie (HBT; also known as homoharringtonine) is a natural plant alkaloid extracted from the endemic Chinese plant *Cephalotaxus harringtonia*, and is reported to promote cell death in a number of tumor types, depolymerize cell ribosomes and inhibit protein synthesis (12,13). HBT also prevents DNA synthesis and targets the G1/G2 phases of the cell cycle (14,15). Following the demonstration of the therapeutic efficacy of omacetaxine mepesuccinate (a type of synthetic HBT) for chronic myeloid leukemia (CML) (16,17), the United States Food and Drug Administration approved its use as a tyrosine kinase inhibitor for the treatment of resistant chronic phase or accelerated CML (18). Previous Chinese studies have added HBT as the third drug to the conventional inductive therapy for AML (19-22), and these studies demonstrated that the treatment of HBT combined with aclarubicin and Ara-c resulted in a complete remission (CR) rate of 83%. As a result, an HBT, Ara-c and aclarubicin-containing chemotherapy regimen has been recommended for the treatment of AML (19-22). However, there have been little studies focusing on the efficacy and safety of HBT treatment combined with Ara-c and etoposide as a first line therapy for AML. Therefore, the present study

evaluated the efficacy and safety of combined HBT, Ara-c and etoposide (HCE) as an induction therapy in Chinese patients with AML aged <65 years. The present cohort study compares this novel treatment regimen with the conventional idamycin combined with Ara-c '7+3' treatment (IA) regimen.

Patients and methods

Patients. Between March 2011 and April 2014, 141 patients diagnosed with AML aged 18-65 years were admitted to the Hematology Department of The First Affiliated Hospital of Medical College of Zhejiang University (Hangzhou, China). According to the bone marrow examination, cell morphology, cell immune phenotypic, cytogenetic and molecular biology examination and using the World Health Organization blood system malignant tumor diagnostic criteria from 2008 (23), all patients were diagnosed with AML and acute promyelocytic myelogenous leukemia (M3) was excluded. Patients had no dysfunction of the major organs including the heart, liver and kidney. The follow-up time ended in February 2015. The current study was certified by The First Affiliated Hospital of Medical College of Zhejiang University Clinical Research Ethics Committee. Written consent was obtained from all patients and the privacy rights of patients were maintained. Using risk stratification based on bone marrow leukemic cell genetic analysis and the molecular abnormalities guidelines from the National Comprehensive Cancer Network (2), the patients were separated into three diagnostic criteria: high risk, intermediate risk and low risk. Patients with a good prognosis were defined as having recurring cytogenetic abnormalities including inv(16) or t(16;16), t(8;21), t(15;17); a normal karyotype with nucleophosmin mutation and no Fms-like tyrosine kinase 3 (FLT-3)-internal tandem duplication (ITD) mutations, or isolated CCAAT/enhancer-binding protein α double mutants. The patients with a poor prognosis were defined as having complicated karyotypes (≥3 types of abnormal chromosome), a single chromosome karyotype of -5, 5q-, -7, 7q-, 11q12[no t(9;11)], inv(3), t(3;3), t(6;9), t(9;22) and molecular abnormalities with a normal karyotype and FLT3-ITD mutation. Patients with a moderate prognosis were defined as having a normal karyotype, or separate +8, or t(9;11), or other unmentioned cytogenetic abnormalities, as well as molecular abnormalities with inv (16), t(16;16), t(8;21) and c-KIT mutation (2). Routine interphase chromosome R banding techniques were performed for cytogenetic evaluation (24) and DNA polymerase chain reaction was used to identify molecular abnormalities (25). The French-American-British (FAB) classification system was used according to the proposals of the FAB co-operative group (26).

Treatments. The composition of the HCE treatment scheme was as follows: HBT (Hangzhou Minsheng Pharmaceutical Industry Group Company Ltd., Hangzhou, China) was administered at 4 mg/day on days 1-7 and was divided into 2 intravenous infusions for 3-4 h each; a dose of 100 mg/m^2 Ara-c (Pfizer Inc., Shanghai, China) was administered as a continuous intravenous injection on days 1-7; a dose of 70 mg/m^2 VP16 (also known as etoposide; Qilu Pharmaceutical Co., Ltd., Jinan, China) was administered on days 1-5 or 1-7 via intravenous infusion for 2-3 h. The composition of the IA

regimen was 7-9 mg/m^2 Idamycin (idarubicin; Pfizer, Inc.) by intravenous infusion on days 1-3 and 100 mg/m^2 Ara-c administered by continuous intravenous injection on days 1-7. All patients completed one of the described induction therapies. The choice of induction therapy did not follow the randomized principle. The patients were admitted into the groups with the 1:2 (HCE:IA) ratio, based on the decision of the oncologist and patients and their family's preference on treatment (including financial status) were considered. The toxicity of the therapy was evaluated according to the National Cancer Institute common toxicity criteria (27). Following induction therapy or after 3-4 weeks, peripheral blood cells were obtained, including white blood cells, and a bone marrow biopsy was performed. When patients achieved complete remission (CR), consolidation therapy was administered. Patients that achieved partial remission (PR) were treated with a further course of induction therapy. Patients with peripheral blood neutrophils that were <0.5x10^9/l received 300-450 µg/day colony stimulating factor until the peripheral white blood cell count was ≥2.0x10^9/l or neutrophils were ≥1.0x10^9/l (normal range, 2.5-7.5x10^9/l). Antibiotics, antifungal drugs and blood infusion products were administered according to the clinical treatment guidelines as supportive treatment (2).

Patients that did not achieve PR following one course or CR following two courses were administered an alternative treatment scheme (patients that did not respond to the HCE scheme were administered the IA scheme and patients that did not respond to the IA scheme were administered the HCE scheme) and this was administered following the induction therapy regimen after the patient was evaluated.

Post-remission therapy. Patients that achieved CR received further consolidation therapy, which included one course of HCE or IA treatment followed by a moderate dose of 2 g/m^2 Ara-c over 12 h on days 1, 3 and 5, 7-9 mg/m^2 IDA on days 1-3 and 70 mg/m^2 VP16 on days 1-5 for 2-3 courses. Patients that were considered to have intermediate or high risk were treated with maintenance therapy, which included the aclacinomycin/Ara-c/etoposide regimen (20 mg aclacinomycin by intravenous infusion for 3-4 h on days 1-5; 100 mg/m^2 Ara-c by continuous intravenous injection on days 1-5; 70 mg/m^2 VP16 on days 1-5 by intravenous infusion for 2-3 h) and the HBT/Ara-c regimen (4 mg/day HBT on days 1-5 divided into two intravenous infusions each for 3-4 h; 100 mg/m^2 Ara-c in a continuous intravenous injection on days 1-5) respectively, for three courses. Certain patients were treated with an autologous stem cell or an allogeneic hematopoietic stem cell transplant (Table I) during their enrollment in the present study.

Outcome parameters. The outcome parameters that were evaluated were the CR rate following one course of treatment, the three-year OS rate, the RFS rate, the duration of time a patient in CR demonstrated absolute neutrophil count (ANC) reduction (measured from the beginning of chemotherapy to the final day of a patient having an ANC of <0.5x10^9/l; normal range, 2.5-7.5x10^9/l), the minimum value of ANC during the inhibition period and the duration of time required to reach this minimum ANC value. Early mortality was considered to have occurred between the beginning of chemotherapy and 30 days after the beginning of chemotherapy. OS was determined

Table I. Characteristics of patients.

Characteristics	HCE (%) n=47	IA (%) n=94	P-value
Sex			
Male	25 (53.2)	44 (46.8)	0.475
Female	22 (46.8)	50 (53.2)	
Age, years			
Median	42	43	0.922
Range	19-64	18-65	
FAB classification			
M0	4 (8.5)	5 (5.3)	0.012[a]
M1	3 (6.4)	7 (7.4)	
M2	15 (31.9)	46 (48.9)	
M4	7 (14.9)	1 (1.1)	
M5	18 (38.3)	33 (35.1)	
M6	0	2 (2.1)	
WBC count at diagnosis			
$\geq 100 \times 10^9/l$	6 (12.8)	9 (9.6)	0.624
$50\text{-}100 \times 10^9/l$	5 (10.6)	15 (16.0)	
$<50 \times 10^9/l$	36 (76.6)	70 (74.4)	
Cytogenetic risk group			
Favorable	13 (27.6)	24 (25.5)	0.649
Intermediate	24 (51.1)	55 (58.5)	
Unfavorable	10 (21.3)	15 (16.0)	
Transplantation			
Auto	1 (2.1)	3 (3.2)	0.277
Allo	3 (6.4)	14 (14.9)	

[a]There were more M4 patients in the HCE group than in the IA group. If the M4 patients were removed, there was no significant difference between the two groups (P=0.433). HCE, homobarringtonie, cytosine arabinoside and etoposide; IA, idamycin and cytosine arabinocide; n, number of patients; FAB, French-American-British; WBC, white blood cell count.

from the beginning of treatment to the date of mortality or final follow-up. The duration of RFS was the length of time between achieving CR and recurrence, mortality or the final follow-up. Patient follow-up was performed via outpatient and inpatient medical records and by telephone communications.

Efficacy evaluation criteria. The definition of CR was: Peripheral blood cell recovery, including neutrophil counts of $\geq 1.5 \times 10^9/l$ (normal range, $2.5\text{-}7.5 \times 10^9/l$), platelets of $\geq 100 \times 10^9/l$ (normal range, $100\text{-}300 \times 10^9/l$), hemoglobin of ≥ 100 g/l (normal range, 120-160 g/l) with no leukemia cells; bone marrow leukemia cells of <5%; no extramedullary invasion and mild splenic enlargement. If bone marrow leukemia cells were <5% with no extramedullary invasion, but the peripheral blood cells did not achieve complete recovery, the condition was referred to as CR with incomplete blood count recovery (CRi). The definition of PR was as follows: Neutrophil count of $\geq 1.5 \times 10^9/l$; platelet count of $\geq 50 \times 10^9/l$; no leukemia cells; bone marrow leukemia cells of 5-20%; no extramedullary invasion or enlarged lymph nodes with a diameter of <2 cm; subcostal spleen of <2 cm; subcostal liver of <5 cm; significantly improved clinical symptoms. Ineffective treatment was determined to have occurred if a patient did not achieve the

CR or PR conditions. Early mortality was defined as mortality following induction therapy and prior to efficacy evaluation.

All patients received full clinical evaluations prior to induction therapy, subsequent consolidation therapy and maintenance therapy. The clinical evaluations included peripheral blood cell counts and classification, liver and kidney function tests, an electrocardiogram and a cardiac ultrasound examination. Additionally, bone marrow aspiration smear was performed 3-4 weeks following each course of treatment to evaluate the efficacy of the treatments.

Statistical analysis. SPSS version 18.0 (SPSS, Inc., Chicago, IL, USA) was used for all statistical analyses. A Pearson, χ^2 or Fisher's exact tests were used to compare the frequency distributions between groups. An independent sample t-test was used to compare the mean between two groups and the Kaplan-Meier was used to analyze the RFS and OS of patients. P<0.05 was considered to indicate a statistically significant difference.

Results

Characteristics of the patients with AML. There was a total of 141 patients with a median age of 42 years, including

Table II. Induction therapy efficacy.

Characteristics	HCE (%) n=47	IA (%) n=94	P-value
Complete remission[a]			
Total	37 (78.7)	68 (72.3)	0.413
1st cycle CR	31 (66.0)	56 (59.6)	0.462
2nd cycle CR	6 (12.8)	12 (12.8)	
No remission	8 (17.0)	21 (22.3)	
No evaluation	2 (4.3)	5 (5.3)	
Early mortality	1 (2.1)	5 (5.3)	0.664
Patients assigned to the alternative regimen[b], n	8	19	
CR	7 (87.5)	14 (73.7)	0.633
Total CR rates, n	51 (77.3)	75 (73.5)	0.584

[a]Includes patients with complete bone marrow remission with incomplete recovery of blood count. [b]Patients that did not respond to treatment. HCE, homobarringtonie, cytosine arabinoside and etoposide; IA, idamycin and cytosine arabinocide; n, number of patients; CR, complete remission.

69 males and 72 females. All patients underwent cytogenetic examination and there were 37 (26.3%) patients with a good prognosis (low risk group), 79 patients (56.0%) with a moderate prognosis (intermediate risk group) and 25 (17.7%) patients with a poor prognosis (high risk group). In total, 47 patients were administered HCE treatment and 94 patients received IA induction therapy; the patients and the disease characteristics of the two groups are presented in Table I. Following statistical analysis, there was a significantly higher number of patients with the M4 subgroup FAB classification in the HCE treatment group compared with the number of M4 patients in the IA treatment groups (14.9 vs. 1.1%; P=0.012). There were no other significant differences between patients and disease characteristics, including age, sex, peripheral white blood cell count at initial diagnosis, risk stratification based on cytogenetic and molecular biology examination and treatment with autologous or allogeneic hematopoietic stem cell transplants.

Induction therapy efficacy. In the current study, a total of 141 patients with primary AML received 1 or 2 consecutive courses of treatment. Of these, 105 cases (74.5%) achieved CR or CRi and 87 of these cases (61.7%) obtained remission following the first chemotherapy cycle. The overall remission rate following 1 course of treatment was comparable between the HCE and IA groups (78.7 vs. 72.3 and 66.0 vs. 59.6%, respectively). A total of 7 patients did not receive therapeutic efficacy evaluation, 6 of these cases (4.3%) were as a result of early mortality (within 30 days following the start of induction therapy). A single patient (2.1%) in the HCE group succumbed to central nervous system bleeding. There were 5 cases of early mortality (5.3%) in the IA group, of which 4 were the result of central nervous system bleeding and 1 was from septic shock. There was no significant difference in the number of early mortality between the two groups. A total of 10 patients responded poorly to HCE treatment, of which 2 cases transferred to other hospitals and the remaining 8 patients were administered the IA scheme and subsequently,

7 of these patients achieved CR. Among the 21 patients that responded poorly to IA treatment, 19 were administered the HCE regimen and 14 achieved CR (Table II).

The therapeutic effects of the treatment subgroups (HCE or IA regimen) were analyzed separately. Comparing the HCE and the IA group following induction therapy, there were no significant differences in the CR rate of patients between the sexes or age groups (<50 or >50 years).

With regard to the FAB classification, the CR rate of the M4 and M5 subgroup of patients (FAB2) was significantly increased in the HCE group (84 vs. 58.8%; P=0.038).

In the IA group, the therapeutic efficacy of induction therapy in the FAB2 (M4 and M5) subgroup was reduced compared with the FAB1 (M0, M1, M2 and M3 subgroups) group (58.8 vs. 80.0%; P=0.027).

The treatment efficacy for IA patients between the risk groups was significantly different (P=0.003), the CR was 95.8, 65.5 and 60.0% in the low, intermediate and high risk patients, respectively. In addition, the CR rates of the HCE and IA groups were not associated with the results of the peripheral white blood cell counts performed at the time of diagnosis (Table III).

Adverse reactions. The primary hematological adverse reactions that were detected include granulocyte deficiency, severe thrombocytopenia and anemia. The median duration of time of granulocyte deficiency (defined as a peripheral blood granulocyte count of $<0.5 \times 10^9$/l) in the HCE and IA groups were 17 and 15 days, respectively. The median durations of severe thrombocytopenia (defined as a peripheral blood platelet count of $<20 \times 10^9$/l) were 15 and 17 days, respectively, and there was no significant difference between the two groups. During induction therapy, a number of patients required erythrocyte and platelet suspension infusions and there was no significant difference in the quantity of blood cell suspension infusions that were required between the HCE and IA groups (Table IV).

During the induction therapy process, the primary non-hematological adverse reaction that was identified was

Table III. Induction therapy efficacies of the subgroups.

	HCE (n=47)		IA (n=94)		
Characteristics	n	CR, n (%)	n	CR, n (%)	P-value
Sex					
Male	25	20 (80.0)	44	32 (72.7)	0.500
Female	22	17 (77.3)	50	36 (72.0)	0.640
Age, years					
<50	35	26 (74.3)	65	49 (75.4)	0.904
≥50	12	11 (91.7)	29	19 (65.5)	0.128
FAB classification					
FAB1[a]	22	16 (72.7)	60	48 (80.0)	0.551
FAB2[b]	25	21 (84.0)	34	20 (58.8)	0.038
WBC at diagnosis					
<50x109/l	36	28 (77.8)	70	53 (75.7)	0.813
≥50x109/l	11	9 (81.8)	24	15 (62.5)	0.435
Risk stratification					
Low risk	13	11 (84.6)	24	23 (95.8)	0.278
Intermediate risk	24	18 (75.0)	55	36 (65.5)	0.402
High risk	10	8 (80.0)	15	9 (60.0)	0.402

[a]FAB1 refers to FAB classification as M0, M1, M2, M6 patients. [b]FAB2 refers to FAB classification as M4 and M5 patients. HCE, homobarringtonie, cytosine arabinoside and etoposide; IA, idamycin and cytosine arabinocide; n, number of patients; CR, complete remission; FAB, French-American-British; WBC, white blood cell count.

Table IV. Treatment of adverse reactions and blood cell infusion.

Adverse reactions	HCE (n=47)	IA (n=94)	P-value
Granulocytopenia phase, days (range)	17 (3->30)	15 (7->30)	0.184
Platelet deficiency phase, days (range)	15 (7->30)	17 (1->30)	0.904
Red blood cell suspension			
Infusion, units (range)	6 (0-16)	5 (0-14.5)	0.540
Platelet suspension infusion, units (range)	25 (10-95)	30.5 (0-86)	0.813
Level ≥3 infection, n (%)	18 (38.3)	45 (47.8)	0.281

HCE, homobarringtonie, cytosine arabinoside and etoposide; IA, idamycin and cytosine arabinocide; n, number of patients.

infection. There were 18 cases (38.3%) of level 3-4 infections (27) in the HCE group and 41 cases (47.8%) in the IA group, but there was no significant difference between these results. Infection occurred at numerous sites and included pulmonary infection, intestinal infection, sepsis, soft tissue infection and fever from unknown causes (1 patient from the IA group succumbed to septic shock). Another level 3-4 non-hematological adverse reaction that was identified was bleeding, and cases included bleeding from the oral cavity, nasal mucosa, digestive tract, urinary tract and vaginal bleeding. A total of 5 of the most severely affected patients succumbed to central nervous system bleeding; 1 of these cases was from the HCE group and 4 cases were from the IA group. During treatment, there were no severe heart, liver, nervous system or other vital organ injuries identified.

Medical expenses of induction therapy. During the treatment periods, all patients were hospitalized and the cost of induction therapy (the cost of the first induction therapy, excluding 6 cases of early mortality) in the HCE group was 58,358.53 Yuan (RMB) and in the IA group was 83,625.28 Yuan (RMB). Therefore, the cost of HCE induction therapy was significantly lower compared with that of the IA group (t=2.95, P=0.004).

Survival rates of patients. All patients that received 1 or 2 courses of induction therapy and those that achieved complete remission were enrolled onto consolidation therapy and follow-up. The follow-up period ended in February 2015 and the median follow-up time was 28 months. There were 37 cases in the HCE group that achieved CR following
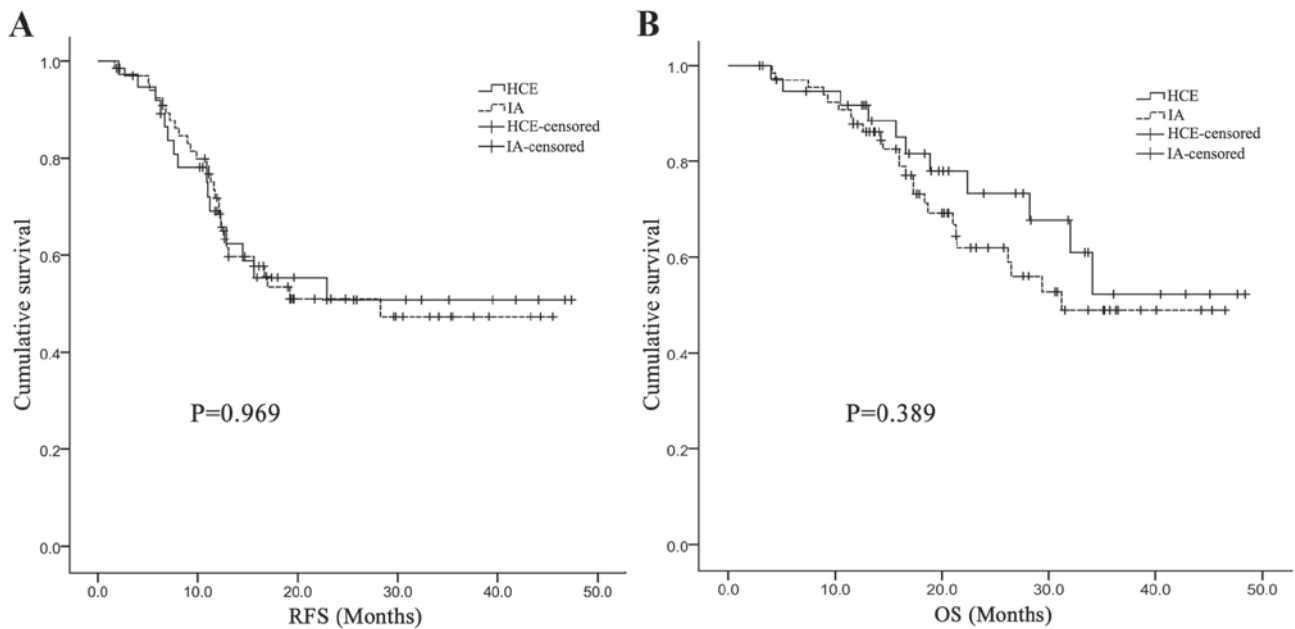
Figure 1. (A) RFS and (B) OS of patients with acute myeloid leukemia in the HCE and IA treatment groups. RFS, relapse-free survival; OS, overall survival; HCE, homobarringtonie, cytosine arabinoside and etoposide; IA, idamycin and cytosine arabinocide.

induction therapy with an overall 50.8% 3-year RFS, including 2 cases that received allogeneic hematopoietic stem cell transplantation with long-term disease-free survival, 1 case that was lost to follow-up and 16 cases that relapsed during follow-up. There were 68 cases in the IA group that achieved CR following induction therapy with an overall 47.3% 3-year RFS, including 1 case that received autologous transplantation, 11 cases that received allogeneic transplantation (10 cases with long-term disease-free survival, 1 case of mortality due to disease progression), 3 cases in which follow-up was not completed and 30 cases that relapsed during follow-up. There was no significant difference in RFS between the HCE and IA groups (P=0.969; Fig. 1A).

At the completion of follow-up, there were 47 patients in the HCE group (3-year OS rate was 52.2%) and of these 11 succumbed to the disease, 9 from disease progression and 2 cases (4.3%) from drug toxicity or other causes (1 from sepsis; 1 for indeterminate reasons). There were 25 cases of mortality in the IA group (3-year OS rate was 49.0%) included 20 cases from disease progression and 5 cases (5.3%, 5/94) from toxicity (1 case of uncontrollable massive hemoptysis; 2 from intracranial hemorrhage; 1 from sepsis; 1 for other reasons). There was no significant difference in the OS rate between the HCE and IA groups (P=0.389; Fig. 1B).

According to the FAB classifications, the M4 and M5 subgroup patients (FAB2) had a reduced RFS in the HCE and in the IA group (P=0.063 and P=0.013, respectively; Fig. 2A and B), but the OS was comparable across the FAB M4 and M5 subgroups (P=0.112 and P=0.192, respectively; Fig. 2C and D). The high, intermediate and low risk groups were divided according to cytogenetic and molecular abnormalities. In the IA group, the RFS and OS of the risk groups were significantly different (P=0.001, P=0.011, respectively; Fig. 3). By contrast, in the HCE group, the RFS and OS were comparable between the risk subgroups (P=0.482, P=0.358,

respectively; Fig. 3). Additionally, in the HCE and IA groups the RFS and OS of patients from various subgroups (including age, FAB classification, white blood cell count at diagnosis and cytogenetic and molecular risk stratification) were not significantly different (Table V).

Discussion

HBT has been investigated for >40 years in Chinese studies (4,28-32). HBT has been used in China to treat acute myeloid leukemia since the 1970s (4,28-32). However, the underlying mechanistic action of HBT against tumor cells remains to be elucidated. Previous studies have indicated that HBT blocks protein synthesis by acting on the ribosomal A site in eukaryotic cells (13), particularly in the G1 and G2 phases of the cell cycle (14,15). In addition, HBT affects the expression of caspase-3 and B-cell lymphoma 2 and its downregulation promotes cell apoptosis, but may also trigger autophagosome activity (33,34). Another previous study demonstrated that HBT upregulated myosin-9 and this overexpression promoted cell cycle arrest in the S and G2/M phases and induced leukemia cell apoptosis (35).

Kantarjian *et al* (32) performed a meta-analysis of the therapeutic effects of HBT-based regimens for patients with AML. These included 21 clinical trials and 1,310 patients with AML, predominantly from China, published between 2006 and 2013 (32). The results identified that the average CR rate of the combination treatment of HBT with Ara-c and anthracyclines (daunorubicin, idarubicin, aclacinomycin and mitoxantrone) was 65.2%. However, the majority of these studies were retrospective, non-randomized, small clinical trials (32) and therefore, require validation by randomized controlled clinical trials.

A multicenter, prospective, randomized clinical trial performed by Jin *et al* (20) identified that the CR rate of

Table V. Survival rates of patients in each subgroups.

	HCE (n=37)		IA (n=68)		
	n	3-year, %	n	3-year, %	P-value
Relapse-free survival					
Age, years					
<50	26	46.7	49	48.1	0.817
≥50	11	62.3	19	47.8	0.678
FAB classification					
FAB[a]	16	65.2	48	53.9	0.492
FAB2[b]	21	39.7	20	27.0	0.596
WBC at diagnosis					
<50x10^9/l	28	52.7	53	47.6	0.861
≥50x10^9/l	9	44.4	15	45.9	0.826
Risk stratification					
Low risk	11	69.3	23	67.2	0.919
Intermediate risk	18	41.5	36	46.5	0.736
High risk	8	45.0	9	0	0.130
Overall survival					
Age, years					
<50	26	50.9	49	55.6	0.556
≥50	11	66.3	19	27.6	0.566
FAB classification					
FAB1[a]	16	80.0	48	48.9	0.191
FAB2[b]	21	29.6	20	47.1	0.619
WBC at diagnosis					
<50x10^9/l	28	58.6	53	49.7	0.435
≥50x10^9/l	9	41.5	15	46.6	0.576
Risk stratification					
Low risk	11	79.5	23	68.1	0.838
Intermediate risk	18	44.7	36	47.8	0.333
High risk	8	29.2	9	14.8	0.237

[a]FAB1 refers to FAB classification as M0, M1, M2 and M6 patients. [b]FAB2 refers to FAB classification as M4 and M5 patients. HCE, homobarringtonie, cytosine arabinoside and etoposide; IA, idamycin and cytosine arabinocide; n, number of patients; WBC, white blood cell count; FAB, French-American-British.

HBT/Ara-c/daunorubicin induction therapy was 67% and the 3-year event free survival (EFS) rate was 32.7%, which was higher compared with the Ara-c/daunorubicin regimen (61 and 23.1%, respectively), but this result was not statistically significantly different. The CR rate of the HBT/Ara-c/aclarubicin regimen was 73% and had a 3-year EFS of 35.4%, which was significantly increased compared with that of the Ara-c/daunorubicin regimen (20).

The application of an HCE scheme for myeloid leukemia has not been the focus of many studies and the current study demonstrates that treatment with the HCE scheme (HBT combined with Ara-c and etoposide) produced similar effects compared with the HBT/Ara-c/aclarubicin regime (CR, 78.7%; 3-year EFS, 50.8%). The CR rate of the two consecutive HCE treatment courses was 78.7 and 66.0% for a single treatment course, which is increased in comparison with a CR of 72.3 and 59.6%, following 2 courses or a single course of the IA regimen. The early mortality rate was lower in the HCE treated group compared with the IA treated group, but this difference was not statistically significant. By contrast, younger patients treated with the classical '7+3' daunorubicin/Ara-c regime had an overall CR rate of 60-70%. However, increasing the dosage of anthracycline or Ara-c, or adding fludarabine, cladribine or etoposide into the classical '7+3' framework produced a CR rate of 75%, but the early mortality rate was increased (5-11).

Notably, the present study identified that the HCE scheme did not demonstrate cross-resistance with the IA scheme, which is concordant with previous studies (29,30). Among the 10 patients that responded poorly in the HCE group, 8 were subsequently treated with the IA scheme and 7 cases achieved CR. Among the 26 patients that responded poorly in the IA group, 19 were then treated with the HCE scheme and 14 of these achieved CR.
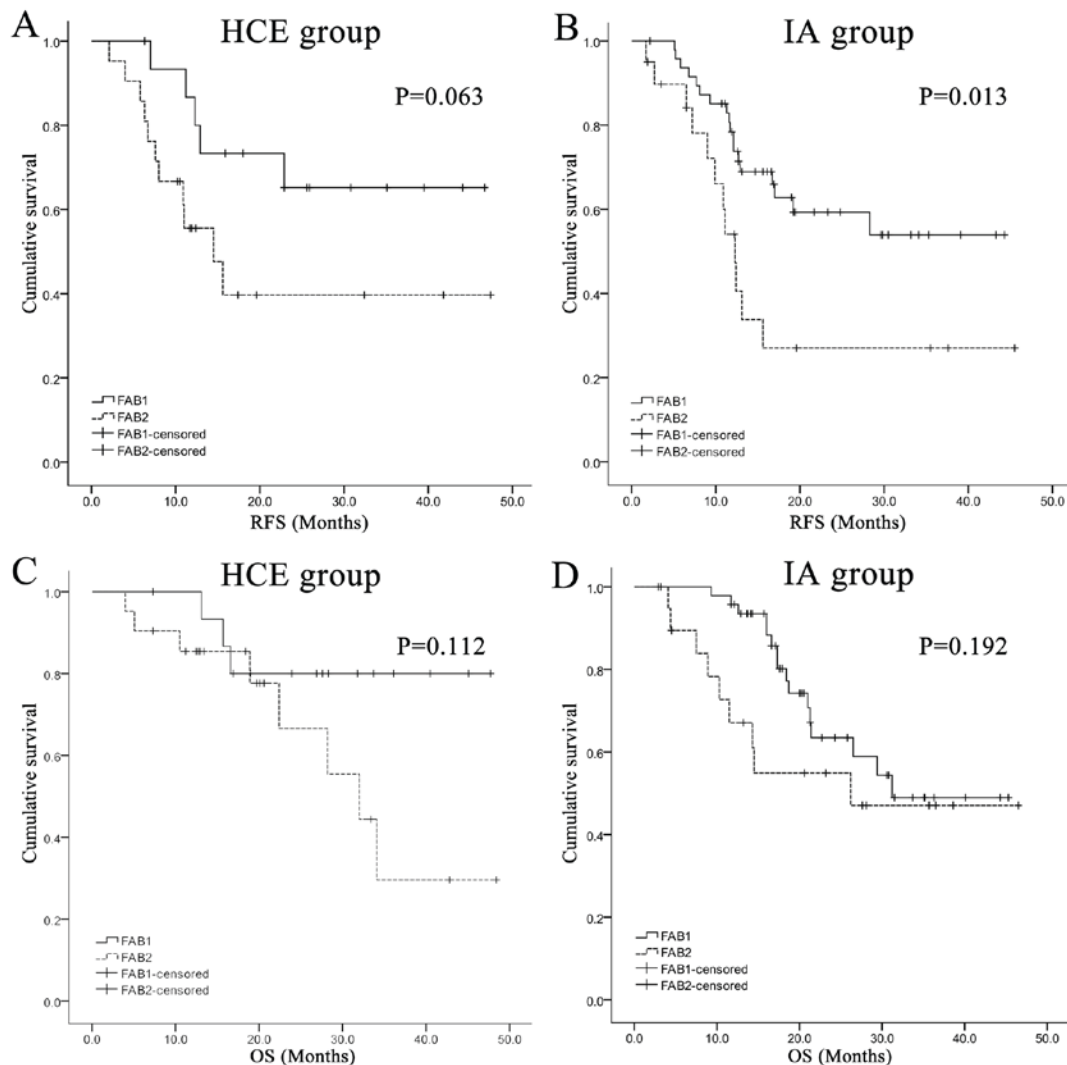
Figure 2. (A and B) RFS and (C and D) OS in patients with acute myeloid leukemia from FAB1 (M0-3) and FAB2 (M4/M5) subgroups in the HCE and IA treatment groups. FAB, French-American-British; RFS, relapse-free survival; OS, overall survival; HCE, homobarringtonie, cytosine arabinoside and etoposide; IA, idamycin and cytosine arabinocide.

When patients did not respond to HCE or IA induction therapy, they were administered the alternative treatment regimen and the resulting CR rate was >70% suggesting that the underlying mechanisms of drug resistance in the two groups may not be associated. Therefore, HCE may be an alternative option for patients that respond poorly to IA induction therapy.

From the subgroup analysis, the CR rate of the patients in the HCE regimen group of >50 years was 91.7% (11/12), which was significantly improved compared with the 74.3% CR rate of patients <50 years (26/35). However, the CR rate of patients in the IA regimen group >50 years was 65.5%, with similar values to those for HCE patients that were <50 years (CR, 75.4%). The RFS and OS rates, which were similar in patients that were <50 years in the two groups, differed in the older patients between the HCE and IA treatment groups (RFS, 62.3 vs. 47.8%; OS, 66.3 vs. 27.6%). These results contrast the meta-analysis performed by Kantarjian *et al* (32), in which the CR rate was lower in the elderly compared with that of the overall population. However, previous Chinese studies (32,36-40) have found that HBT may be administered at a rate of 4 mg/day for 5 to 7 days or at the lower dose of

1 mg/day for 14 days for elderly (>60 years) patients with AML. This previous study hypothesized that the lower CR rate in elderly patients may be due to the reduced doses of HBT that were administered (32). However, the majority of previous studies are retrospective, non-randomized or small clinical trials (32,36-40) and therefore, require further validation using a large cohort of patients in a randomized and controlled clinical trial.

The current study has demonstrated that cytogenetic and molecular abnormalities were able to affect AML induction therapy, RFS and OS, as well as being potential effective prognostic factors (41,42). In low-risk patients, the CR rates of the HCE and IA groups were 84.6 and 95.8%, respectively, and there was no significant difference between these groups. However, in the HCE group, high-risk patients experienced higher therapeutic efficacy with a CR rate of <80%, whereas the CR rate of the IA group was 60%. This result was similar to that found for patients of >50 years, but the underlying mechanisms remain to be elucidated.

Regarding the adverse reactions from HBT treatment, previous studies have identified that the primary toxic effects
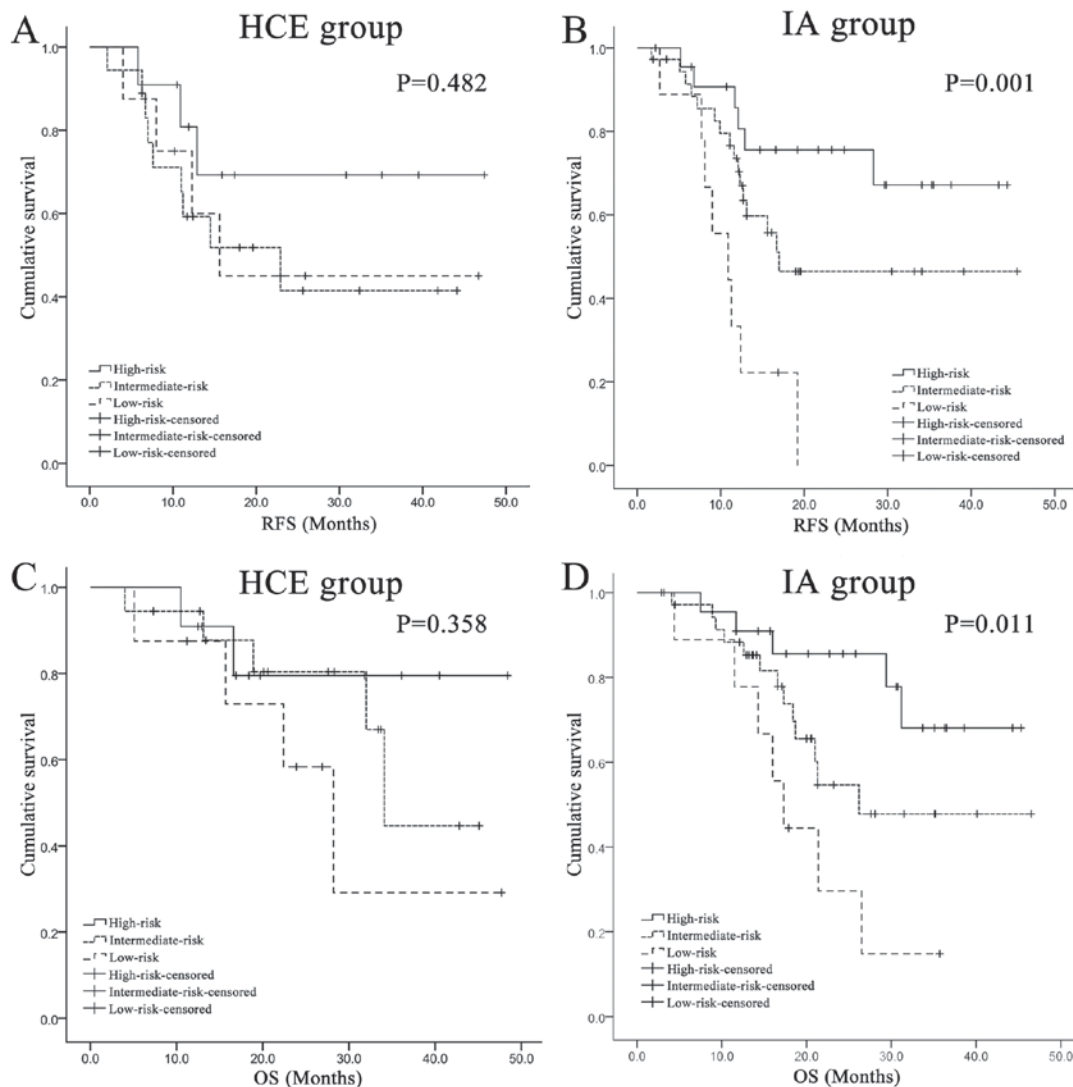
Figure 3. (A and B) RFS and (C and D) OS in patients with acute myeloid leukemia from each risk stratification group (low, intermediate and high risk), which is based on cytogenetic and molecular abnormalities of the patient samples. RFS, relapse-free survival; OS, overall survival; HCE, homobarringtonie, cytosine arabinoside and etoposide; IA, idamycin and cytosine arabinocide.

of HBT treatment are bone marrow inhibition, gastrointestinal reactions and cardiac toxicity, which frequently presents as supraventricular arrhythmias that respond to appropriate therapy (13,29,30,32). In the treatment of CML, clinicians have administered long-term treatments with a duration of 14 to 28 days, and the typical level 3-4 adverse reaction that is detected is bone marrow inhibition without obvious non-hematological toxicity (18-20). During the treatment of elderly patients with long-term low dose chemotherapy, non-hematological toxicity from HBT has not been found (32,38-41). Previous studies have identified cases of grade 4 left bundle branch block during HBT therapy, or grade 2 QT interval prolonged (42), which was not associated with the blood concentration of HBT (43). In the current study, the non-hematological toxicity adverse reactions from HBT treatment were minimal and considered acceptable, and the typical level 3-4 adverse reaction that was detected was hematological toxicity. The duration of agranulocytosis and severe thrombocytopenia in the HBT treatment group was comparable with that of the IA regimen and demonstrated no severe non-hematological toxicity.

In conclusion, the HCE regimen may be an alternative treatment option for primary AML that has no cross-resistance with the current IA treatment regimen and demonstrates similar therapeutic efficacy and toxicity to the IA scheme, which is considered to be well-tolerated by patients. However, previous clinical studies and basic mechanistic studies have yet to fully investigate this topic. The cohort size in the present study was not sufficient to present unequivocal conclusions. Therefore, further multicenter prospective clinical trials with large numbers of patients are required in order to confirm the therapeutic efficacy and safety of HBT treatment for myeloid leukemia, to validate the efficacy and survival variation among age groups, subtypes and gene characteristics and to determine the optimal drug combinations and doses.

Acknowledgements

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