Venetoclax combined with escalating doses of homoharringtonine, low-dose cytarabine, and granulocyte colony-stimulating factor demonstrates feasibility and tolerability for remission induction in pediatric acute myeloid leukemia

Advances in supportive care and intensified treatment regimens, including hematopoietic stem cell transplantation (HSCT), have markedly improved outcomes for pediatric acute myeloid leukemia (AML). However, event-free and overall survival rates have plateaued at approximately 65% and 80%, respectively.[1,2] Relapsed disease, as well as acute and long-term toxicities, remain significant challenges.

Our AML collaborative group has prioritized developing and evaluating low-dose chemotherapy (LDC) regimens for remission induction in pediatric AML.[3] A recent randomized study demonstrated that an LDC regimen for remission induction was non-inferior to standard-dose chemotherapy.[4] Nevertheless, exposure to anthracyclines and the number of patients undergoing HSCT remain high. We are currently exploring alternatives to reduce toxicity by incorporating agents with more favorable efficacy and toxicity profiles.

Homoharringtonine (HHT), a plant-derived alkaloid (from *Cephalotaxus*) that inhibits protein synthesis by targeting ribosomes, has been widely used in treating adult and pediatric AML in China.[5-7] Preclinical studies have shown that HHT peak plasma concentrations of 3 mg/m²/day and 5 mg/m²/day exceed levels required to inhibit 50% of HL-60 leukemia cell growth.[8] Further preclinical evidence suggests that HHT and venetoclax can synergistically promote apoptosis by inhibiting the *MAPK/ERK* and the *PI3K/AKT* pathways while activating the p53 pathway.[9]

Clinically, HHT has been successfully integrated into standard-dose regimens of cytarabine and daunorubicin, demonstrating feasibility and efficacy.[7] Given the potential synergy between HHT, venetoclax, and cytarabine, we explored replacing mitoxantrone with venetoclax and HHT in the LDC regimen for remission induction. Based on relapse risk, patients received two to three additional courses of standard chemotherapy as consolidation therapy. Details of this new regimen (V-HAG) are provided in *Online Supplementary Table S1*. Patients at high risk of relapse (criteria outlined in *Online Supplementary Table S1*) were considered for HSCT. Homoharringtonine was administered in a 3×3 dose escalation design at 1 mg/m² (dose level 1), 2 mg/m² (dose level 2), and 3 mg/m² (dose level 3) daily for ten days to determine the maximum tolerated dose within the context of this regimen. The study is registered under *clinicaltrials.gov identifier ChiCTR2200064901*. This study was approved by the institutional review board of the Children's Hospital of Soochow University, and conducted in accordance with the Declaration of Helsinki. Patient data were maintained with strict confidentiality.

Between October 2022 and June 2023, 12 consecutive patients were enrolled in this phase I feasibility study, with 3 patients assigned to dose level (DL) 1, 3 patients to DL 2, and 6 patients to DL 3. The cohort included 7 males and 5 females, with a median age of 8.3 years (range, 3.3-12.7 years). The most common fusion gene identified was *RUNX1::RUNXT1*, present in 5 patients, followed by *KMT2A* rearrangements in 2 patients, with one case each of *KMT2A::MLLT4* and *KMT2A::MLLT10*. Other detected fusion genes included *CBFB::MYH11* (N=1) and *NUP98::NSD1* (N=1). The most frequently identified gene variants were *NRAS* (N=3), *CEBPA* double mutant (N=2), and *KIT* (N=3), located in exon 17 in 2 cases and exon 8 in one case. Additionally, 2 patients had *CEBPA* single mutations (non-bZip) and 2 had *KRAS* mutations (Table 1).

All patients received at least one cycle of V-HAG and were evaluated for toxicity and response to Induction I. One patient in DL1 was classified as a non-responder after Induction I, with more than 20% blasts observed in the bone marrow on day 22. This patient subsequently withdrew from the trial. The remaining 11 patients completed both induction courses and achieved remission. These patients proceeded to a median of three consolidation cycles (range, 2-4 cycles), with 5 undergoing allogeneic HSCT. As of August 1, 2024, at a median follow-up of 18 months (range, 11-19 months), all patients were alive and disease-free, including the one patient who withdrew from the protocol. Relevant demographic data are summarized in Table 1.

No dose-limiting toxicities (DLT) or deaths were observed within the first 30 days following the initiation of Induction I. The most common non-hematologic toxicities were febrile neutropenia, nausea or vomiting, lung infections, electrocardiogram (ECG) T-wave changes (inverted T-waves, flattened T-waves, and bidirectional changes), and sinus tachycardia (Table 2). Prolongation of the QT interval was not observed in any of the patients. The median duration of neutropenia (<0.5x10^9/L) and thrombocytopenia (<20x10^9/L) during Induction I was 22 days (range, 14-38 days) and 16

Table 1. Patients' characteristics and outcome.

N	Age/ sex	WBC at diagnosis, ×10⁹/L	Fusion genes and mutations	Initial# risk group	HHT dose level	BM blasts <5 % Induction I/II	MRD& Induction II Flow cytometry/ fusion genes, RT qPCR	Consolidation cycles	Allo-HSCT	Alive	OS, mth
1	12.1 y/F	50.5	*RUNX1::RUNXT1/ KIT* (exon 17 p.D816Y), *ASXL2*	IR	1 mg	No	NA	1	Yes	Yes	19§
2	11.9 y/M	7.2	Negative/ *CEBPA*-dm	LR	1 mg	Yes/Yes	<0.1%	3	No	Yes	19
3	11.9 y/F	2.6	*RUNX1::RUNXT1/ EZH2*	IR	1 mg	Yes/Yes	<0.1%/ Negative	3	No	Yes	18
4	6.7 y/F	9.9	*RUNX1::RUNXT1/ KIT* (exon 17 p.D820Y), *ASXL2*	IR	2 mg	Yes/Yes	<0.1%/ Negative	4	No	Yes	19
5	3.5 y/M	22.1	*NUP98::NSD1/ NRAS, WT1, CEBPA-sm*	HR	2 mg	Yes/Yes	<0.1%/ Negative	2	Yes	Yes	19
6	1.8 y/M	67.6	*CBFB::MYH11/ KIT* (exon 8 p.T417), *KRAS*	IR	2 mg	Yes/Yes	<0.01%/ Negative	2	Yes	Yes	19
7	4.5 y/M	13.5	Negative/ *CEBPA*-dm, *CSF3R, JAK3*	IR	3 mg	Yes/Yes	<0.1%	4	No	Yes	19
8	9.7 y/F	62.8	*KMT2A::MLLT10/ NRAS*	HR	3 mg	Yes/Yes	<0.1%/ Negative	2	Yes	Yes	17
9	7.3 y/F	3.0	*RUNX1::RUNXT1/ KRAS, ASXL2*	IR	3 mg	Yes/Yes	<0.1%/ Negative	3	No	Yes	19
10	3.9 y/M	10.5	Negative, *CEBPA*-sm, GATA2, *CCND3*	IR	3 mg	Yes/Yes	<0.1%	3	No	Yes	11
11	11.7 y/M	6.7	*RUNX1::RUNXT1/ CCND2*	IR	3 mg	Yes/Yes	<0.1%/ Negative	3	No	Yes	11
12*	11.4 y/M	12.0	*KMT2A::MLLT4 NRAS, FLT3*-TKD	HR	3 mg	Yes/Yes	<0.1%/ Negative	2	Yes	Yes	11

#None of the patients exhibited central nervous system involvement. *Complex karyotype: 46,XY,inv(3)(q21;q26),t(4;13)(q13;q13),t(6;11)(q27;q23),t(8;16)(p11;p12)[20]. &Minimal residual disease (MRD) was measured using flow cytometry. §The patient received three doses of daunorubicin (50 mg/m² daily) and 20 doses of cytarabine (100 mg/m² every 12 hours for 10 days) before proceeding to allogeneic hematopoietic stem-cell transplantation (allo-HSCT). BM: bone marrow; F: female; HHT: homoharringtonine; HR: high-risk; IR: intermediate-risk; LR: low-risk; M: male; mth: months; NA: not available; OS: overall survival; RT qPCR: real-time polymerase chain reaction; WBC: white blood cell count; y: years.

days (range, 10-28 days), respectively (*Online Supplementary Table S2*).

Similarly, no DLT were observed among the 11 patients who received Induction II. The most frequent non-hematologic toxicities during Induction II were febrile neutropenia, nausea or vomiting, ECG T-wave changes, sinus tachycardia, and lung infections (Table 2). Importantly, no impairment in cardiac ejection function was observed during Induction II. The median duration of neutropenia and platelet recovery during Induction II was 18 days (range, 7-34 days) and 15 days (range, 0-29 days), respectively (*Online Supplementary Table S2*). The overall response rate after Induction II was 100%. Furthermore, no severe adverse events (grade 4-5) occurred during either induction phase.

The venetoclax concentration values measured using liquid chromatography-tandem mass spectrometry and peak-to-trough concentration ratios are shown in Figure 1. Venetoclax concentrations were assessed 5-7 days after treatment initiation. The trough concentration was measured 30 minutes before the next dose, while the peak concentration was measured 6 hours (hr) post administration. During Induction I, the median venetoclax peak concentration was 1,375 ng/mL, and the median trough concentration was 415 ng/mL, yielding a peak-to-trough ratio of 4.1. During Induction II, the median peak concentration increased to 1,740 ng/mL, while the trough concentration was 385 ng/mL, resulting in a peak-to-trough ratio of 4.86. These ratios were used to categorize patients into high- and low-ratio groups. No significant differences between the high- and low-ratio groups were observed in hematologic or non-hematologic toxicities. Regarding treatment response, although a higher proportion of patients in the high-ratio group achieved minimal residual disease (MRD) <1% in Induction I, this difference was not statistically significant (*Online Supplementary Table S3*).

Our study demonstrates the feasibility of integrating venetoclax and HHT into a regimen of low-dose cytarabine and G-CSF for remission induction in children with *de novo* AML. This combination was well-tolerated, and the dose-limiting toxicity of HHT was not reached. Therefore, we recommend a 3 mg/m² dose over ten days for future

Table 2. Hematologic and non-hematologic toxicities during Induction I and II.

	V-HAG©								
Induction therapy	**Level 1**			**Level 2**			**Level 3**		
	Grade 2	**Grade 3**	**Grade 4**	**Grade 2**	**Grade 3**	**Grade 4**	**Grade 2**	**Grade 3**	**Grade 4**
Induction I	**N=3**			**N=3**			**N=6**		
Hematologic toxicity									
Time to recovery in days, median (range)									
neutrophil count >0.5×10⁹/L	21.5 (21-22)			22 (14-38)			23 (16-27)		
platelet count >20×10⁹/L	12.5 (10-15)			16(14-28)			17 (10-22)		
Infection, N									
Febrile neutropenia	0	2	0	0	3	0	0	6	0
Lung or sinus infection	1	1	0	0	1	0	0	3	0
Mucositis	1	0	0	0	0	0	0	0	0
Gastrointestinal, N									
Nausea or vomiting	2	0	0	3	0	0	2	0	0
Cardiac, N									
Sinus tachycardic	0	0	0	1	0	0	2	0	0
ECG T-wave changes@	0	0	0	2	0	0	2	0	0
Other, N									
Headaches	1	0	0	0	0	0	0	0	0
Induction II	**N=2**			**N=3**			**N=6**		
Hematologic toxicity									
Time to recovery in days, median (range)									
neutrophil count >0.5×10⁹/L	8 (7-9)			22 (13-27)			22 (10-34)		
platelet count >20×10⁹/L	3 (0-6)			12 (10-21)			17 (14-29)		
Infection, N									
Febrile neutropenia	0	0	0	0	3	0	0	5	0
Lung or sinus infection	0	0	0	0	1	0	0	2	0
Mucositis	0	0	0	0	0	0	0	0	0
Gastrointestinal, N									
Nausea or vomiting	1	0	0	2	0	0	2	0	0
Cardiac, N									
Sinus tachycardic	0	0	0	1	0	0	2	0	0
ECG T-wave changes@	0	0	0	2	0	0	2	0	0

©This V-HAG regimen consists of venetoclax, HHT, low-dose cytarabine, and granulocyte colony-stimulating factor (G-CSF). @During Induction I, 2 patients in the Level 2 group exhibited T-wave abnormalities, including flattened T waves in leads I and II and inverted T waves in lead III. In the Level 3 group, 2 patients also showed flattened T waves. During Induction II, all patients in the Level 2 group demonstrated flattened T waves in leads II and III. In the Level 3 group, one patient displayed bidirectional T waves in leads V2 and V3, while another presented with inverted T waves in leads V3 and V4. Importantly, none of the electrocardiograms (ECG) showed QT interval prolongation. N: number of patients.

studies. No severe complications, such as septicemia and acute cardiac toxicity, were observed. These findings are consistent with several multicenter clinical trials in China, which also reported the safety and efficacy of HHT in children with AML.[5,6]

Cardiovascular side effects of HHT, including heart rhythm abnormalities, transient hypotension, and chronic cardiotoxicity, are rare.[10] The incidence of these effects appears to be influenced by infusion duration and cumulative dosage. Patients receiving continuous HHT infusions experienced fewer cardiovascular complications than those receiving bolus injections.[11] Furthermore, patients who were administered a high cumulative dosage of HHT exhibited a higher incidence of cardiac complications than the low-dose group.[11] Our study mandated a minimum intravenous infusion time of 4 hr at a constant infusion rate (*Online Supplementary Table S1*). No abnormal left ventricular ejection fraction changes were detected on the echocar-

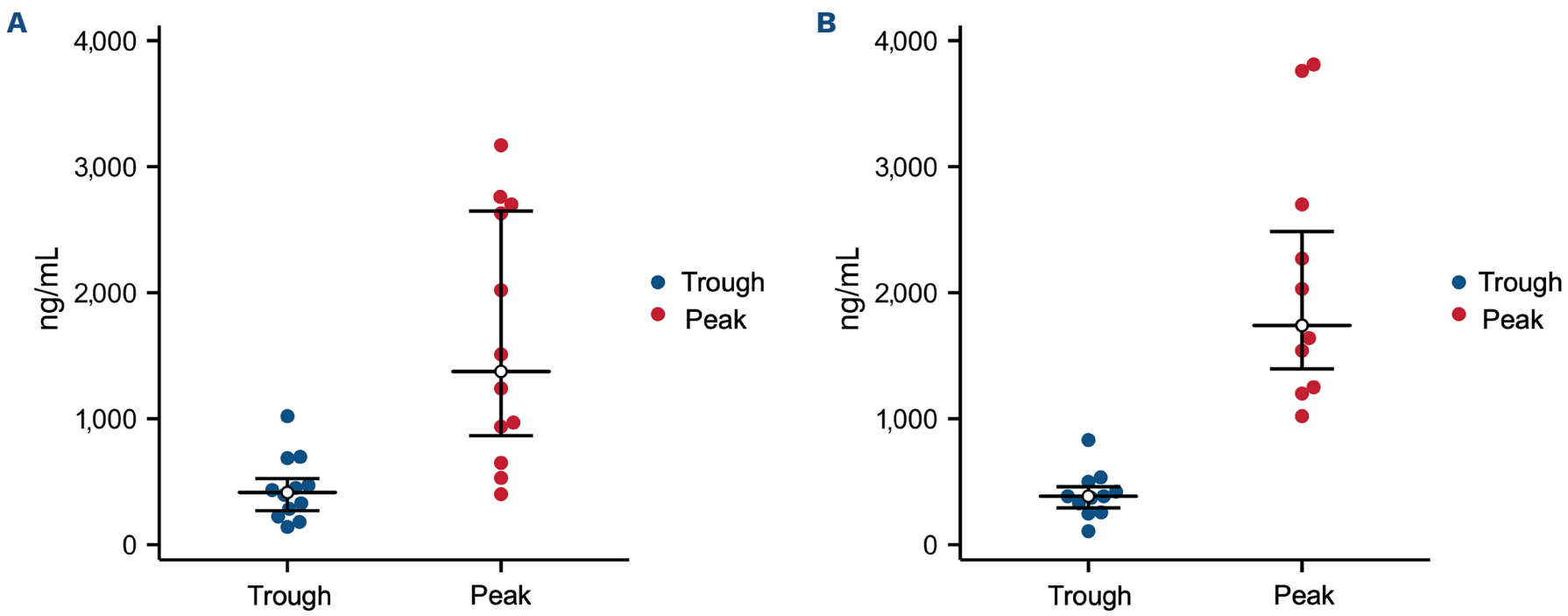

Figure 1. Venetoclax concentration in plasma during Induction therapies. (A) Venetoclax concentration in plasma during Induction I. (B) Venetoclax concentration in plasma during Induction II.

diograph throughout the treatment course. Additionally, no elevations in cardiac enzyme levels, including troponin T, were observed. These findings suggest that cumulative doses of HHT up to 30 mg/m² during remission induction are safe for pediatric patients. However, ongoing monitoring and long-term follow-up are essential to assess the potential delayed cardiac effects.

Venetoclax, a BCL-2 inhibitor, is widely used in adults with *de novo* or secondary AML, typically in combination with azacitidine or low-dose cytarabine, and has shown favorable tolerability and efficacy.[12,13] Similarly, notable responses have been observed in pediatric relapsed AML when venetoclax was combined with intensive chemotherapy.[14] A retrospective multicenter study evaluating salvage therapy with venetoclax combined with conventional chemotherapy in 31 previously treated children with AML or myelodysplastic syndromes reported an overall response rate of approximately 70% and a complete remission rate of 51%.[15] However, its use as a front-line treatment for pediatric AML remains limited.

In our protocol, venetoclax was well-tolerated at the administered doses. Although there was marked inter-patient variability in plasma concentrations, no correlation was observed between venetoclax plasma levels and toxicity. Furthermore, neutrophil and platelet recovery times showed no association with venetoclax plasma concentrations. After two induction courses, all patients, regardless of high or low venetoclax ratios, achieved negative MRD.

In conclusion, the combination of venetoclax, homoharringtonine, low-dose cytarabine, and granulocyte colony-stimulating factor represents a safe and promising minimally myelosuppressive regimen. This anthracycline-free approach for remission induction is currently under investigation in a multicenter study of children with *de novo* AML.

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Disclosures

No conflicts of interest to disclose.

Contributions

RR, SH and PX designed and directed the study, SC, GL and YH drafted the manuscript and performed all the data analysis. YW, HH, JLu and JLi are responsible for data collection. SL, FY, XW and LF helped interpret the results. JF, YY, YS and SW helped integrate all the clinical data, BL and YZ helped revise the manuscript. CC assisted with statistical data analysis. All authors read and approved the final manuscript for publication.

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Data-sharing statement

The data supporting this study's findings are available on request from the corresponding author. However, due to privacy or ethical restrictions, they are not publicly available.

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