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Adding venetoclax or hypomethylating agents to induction chemotherapy as first-line treatment for adults with acute myeloid leukemia: a retrospective case-cohort study

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Abstract

Background: The response rate of traditional first-line induction chemotherapy (IC) for newly diagnosed acute myeloid leukemia needs to be improved, but it is not clear whether adding venetoclax or hypomethylating agents (HMAs) to IC will improve the response rate.

Objective: To determine whether venetoclax or HMAs could increase the response rate of IC in patients with newly diagnosed acute myeloid leukemia (AML).

Design: A retrospective, propensity score matching analysis.

Methods: Newly diagnosed AML patients at Tongji Hospital between 2021 and 2023 were included in this study. By matching cases and controls based on age, gender, baseline bone marrow blast cell proportion, type of AML, and the National Comprehensive Cancer Network (NCCN) risk stratification group, we compared the response rate (CR, CR/CRi, ORR, and MRD negative) and hematological adverse events in newly diagnosed AML treated with IC plus venetoclax or HMAs versus IC alone after one cycle of IC.

Results: The addition of venetoclax could improve CR/CRi of IC (83.8% for IC plus venetoclax vs 66.1% for IC alone, $p = 0.029$). The addition of venetoclax to IA regimen did not improve CR/CRi of IA regimen (76.9% for IA plus venetoclax vs 76.2% for IA alone, $p = 0.986$). The addition of HMAs could not only improves CR/CRi of IC (85.3%% for IC plus HMAs vs 65.4% for IC alone, $p = 0.002$) but also improves CR/CRi of IA regimen (91.3% for IA plus HMAs vs 70.0% for IA alone, $p = 0.034$). The addition of HMAs could improve CR/CRi of patients with adverse mutations (FLT3, IDH1/2, K/NRAS) after IC. The addition of venetoclax and HMAs both extended the duration of agranulocytosis and thrombocytopenia.

Conclusion: Adding HMAs might improve CR/CRi of IC including IA. Adding venetoclax might not improve CR/CRi of IA. A well-designed prospective randomized controlled study is now warranted.

Keywords: hypomethylating agents, induction chemotherapy, newly diagnosed acute myeloid leukemia, venetoclax

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Introduction

For the past 40 years, intense chemotherapy has been the main standard of induction chemotherapy (IC) for younger, fit patients with acute myeloid leukemia (AML).[1,2] Intense IC often is referred to as the "7 + 3" (7 days of cytarabine + 3 days of daunorubicin 50–60 mg/m^2; or idarubicin 10–12 mg/m^2) regimen. Response rates with standard the "7 + 3" regimen ranged from approximately 60%–80% in patients younger than 60 years old. Patients younger than 60 years old treated with an increased dose of daunorubicin did not show an advantage in the rate of complete response (CR) or the rate of overall

survival.[2] In the era of traditional chemotherapy, other IC regimens have been used for induction therapy of AML, such as HAA (homoharringtonine, aclarubicin, and cytarabine) and HAD (homoharringtonine, daunorubicin, and cytarabine).[3] These chemotherapy regimens did not achieve better response rate and lower mortality than the "7 + 3" regimen.[4] The low-dose chemotherapy regimens consisting of low-dose cytarabine and aclarubicin or homoharringtonine combined with granulocyte colony-stimulating factor (G-CSF) priming, referred to as CAG or HAG,[5,6] were proposed for elderly AML patients or younger patients with comorbidities. The low-dose chemotherapy regimens sacrificed the overall response rate (ORR) to reduce treatment-related complications. Further optimization of treatment strategies remains a priority.

The deregulated DNA methylation has been declared as a hallmark of cancer and is also highly characteristic of myeloid malignancies.[7] The DNA hypomethylating agents (HMAs) azacitidine (AZA) and decitabine (DAC), which target epigenetic changes, have evolved as epigenetic therapies for elderly myeloid malignancies.[8] But the CR was significantly lower in patients receiving AZA than in patients receiving intensive chemotherapy.[9] The B-cell lymphoma-2 (Bcl-2) protein plays an important role in the survival and persistence of AML blasts.[10] Venetoclax is a potent and selective small molecule Bcl-2 inhibitor, and venetoclax has been studied in malignancies as both monotherapy and in combination with other agents. The combination of HMAs and Bcl-2 inhibitors offered hope for prolonged survival in elderly patients with AML who cannot tolerate standard treatment.[11] However, these studies mostly focused on elderly patients who were ineligible for chemotherapy.

Although the "7 + 3" regimen has been the standard of treatment for younger, fit patients with AML in the past 40 years, it has been challenged by the emergence of new drugs. Venetoclax plus intensive chemotherapy with fludarabine, cytarabine, G-CSF, and idarubicin (FLAG-IDA) in patients with newly diagnosed AML achieved an overall response rate of 97% with a CR of 69%.[12] In a prospective clinical trial (NCT03214562) analyzing the efficacy of venetoclax plus FLAG-IDA, 73% of treatment-naïve AML patients achieved CR.[13] Venetoclax plus the "7 + 3" regimen as first-line treatment for adults with AML achieved the composite CR of 91% after one cycle of treatment in single-arm trial.[14] The "7 + 3" regimen combined with HMAs has an advantage over historical controls in terms of CR.[15,16] However, the regimens of HMAs or venetoclax combined with intensive chemotherapy were based more on single-arm clinical studies than controlled studies.

We retrospectively investigated the clinical data of newly diagnosed AML between 2021 and 2023. By case-cohort study, we compared the treatment outcomes of venetoclax or HMAs combined with the "7 + 3" regimen versus the "7 + 3" regimen in a cohort of patients with newly diagnosed AML. We aimed to determine whether venetoclax or HMAs could increase the response rate of IC in patients with newly diagnosed AML.

Methods

Study design and study population

This was a retrospective case-cohort study of venetoclax and HMAs in the treatment of newly diagnosed AML. We analyzed data from patients with newly diagnosed AML at Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China between January 1, 2021, and January 1, 2023. Patients with acute promyelocytic leukemia were excluded from the analysis. AML patients included primary AML and secondary AML patients with a history of myelodysplastic syndrome (MDS) or chronic myelomonocytic leukemia.

According to the clinical practice of our center bone marrow aspirate smears with bone marrow biopsies were performed on every patient at the time of presentation at our hospital. Bone marrow aspirate smears were evaluated by Wright-Giemsa stain followed by cytochemical analysis for myeloperoxidase and α-naphthyl butyrate esterase. Eight-color flow cytometry immunophenotypic analysis was performed on bone marrow aspirate specimens. The response was assessed according to the criteria of the National Comprehensive Cancer Network (NCCN) about 1 month after initial IC treatment. Briefly, complete response (CR) was defined by the presence of less than 5% blasts in the bone marrow with the recovery of peripheral blood counts (neutrophil $\geqslant 1.0 \times 10^9$/L and platelet $\geqslant 100 \times 10^9$/L). CRi was defined by CR with incomplete blood count recovery (blast

cells in bone marrow <5%, neutrophil $<1.0\times10^9$/L, or platelet $<100\times10^9$/L). Partial remission (PR) was defined by the presence of 5%–25% blasts and more than 50% lower than before treatment in the bone marrow. The overall response rate (ORR) in our study included CR, CRi, and PR. Risk stratification by biological disease factors is performed by routine cytogenetic analysis, multiplex reverse transcription polymerase chain reaction,[17] and Sanger sequencing (including FMS-like tyrosine kinase 3 (FLT3), kinase insert domain receptor (KIT), CCAAT enhancer binding protein A gene (CEBPA), DNA methyltransferase 3 A (DNMT3A), nucleophosmin 1 (NMP1), isocitrate dehydrogenase 1 (IDH1), and isocitrate dehydrogenase 2 (IDH2)). Eighty-one (35.1%) patients completed Sanger sequencing and 108 (46.8%) patients completed next-generation sequencing. Measurable residual disease (MRD) was assessed by multiparametric flow cytometry testing.[18] The definition of infections included fever (temperature ⩾38.5°C), pulmonary infection reported in X-ray or CT scan, or cultures for bacteria, fungus, and tuberculosis.

Enrolled patients completed at least one IC treatment and underwent bone marrow aspiration assessment. IC regimens included the IA regimen (idarubicin 10–12 mg/m^2 on days 1–3, cytarabine 100 mg/m^2 on days 1–7), PA regimen (pirarubicin 50–60 mg/m^2 on days 1–3, cytarabine 100 mg/m^2 on days 1–7), CAG regimen (cytarabine 10 mg/m^2, subcutaneous injection, q12hr on days 1–14, aclarubicin 7 mg/m^2, qd on days 1–8, and G-CSF 200 μg/m^2, subcutaneous injection qd on days 1–14), and HAG regimen (cytarabine 10 mg/m^2, subcutaneous injection, q12hr on days 1–14, homoharringtonine 2 mg/m^2, qd on days 1–8, and G-CSF 200 μg/m^2, subcutaneous injection qd on days 1–14). In addition to chemotherapy, some patients were given venetoclax (total dose 2800–5600 mg) and/or HMAs (AZA (75 mg/m^2 d1–7) or DAC (20 mg/m^2 d1–5)). The initial dose of venetoclax was 100 mg, 200 mg the next day, with a maximum dose of 400 mg per day for 1–4 weeks (200 mg qd for 28d, 400 mg qd for 14d, or 100 mg qd for 28 combined with voriconazole). Patients treated with low-dose cytarabine (LDAC) regimens or HMAs alone were excluded. Patients with FLT3 mutations were additionally treated with sorafenib.

All data for this retrospective study can be obtained through the electronic medical record system of our hospital, including (1) characteristics of the patients, such as age, gender, diagnosis, and percentage of bone marrow blast cells; (2) NCCN risk stratification group (NCCN Clinical Practice Guidelines in Oncology AML Version 1.2023) and the molecular mutation of patients; (3) response rates and MRD; (4) the time to neutrophil recovery and platelet recovery (defined as the time between the start of chemotherapy and the occurrence of the neutrophils greater than 0.5×10^9/L and platelet greater than 100×10^9/L); (5) the incidence of infections after using the first dose of chemotherapy; and (6) the mortality rate of patients before the next cycle of chemotherapy.

Propensity score matching

To best analyze the effect of venetoclax on AML patients in induction therapy and reduce the bias from cohort selection, we used propensity score matching (PSM). The 1:2 PSM was performed using the nearest neighbor method with a standard caliper width of 0.02; variables matched in this process included age, gender, type of AML, bone marrow blast cell, and NCCN risk stratification. Before matching, 231 newly diagnosed AML patients were treated with IC (191 patients in non-venetoclax group and 40 patients in venetoclax group), in which 139 patients were treated with IA regimen (126 patients in non-venetoclax group and 13 patients in venetoclax group). After matching, there were 93 patients treated with venetoclax with or without IC, and 180 patients treated with HMAs with or without IC. In the cohort of patients treated with IA regimen, the final cohort included 34 patients after 1:2 PSM according to patients treated with or without venetoclax, and 96 patients after 1:2 PSM according to patients treated with or without HMAs (Figure 1).

Statistical analysis

The *t*-test and the Mann-Whitney *U* test were used to analyze continuous data of characteristics of patients with normal and skewed distributions, respectively. Categorical variables of characteristics of patients were analyzed by chi-square test. Logistic regression analysis was used to compare the response outcome between two groups. The time to neutrophil recovery and platelet recovery were analyzed using the Cox proportional-hazard modeling. PSM analyses were performed using Stata (version 17.0), and other analyses were

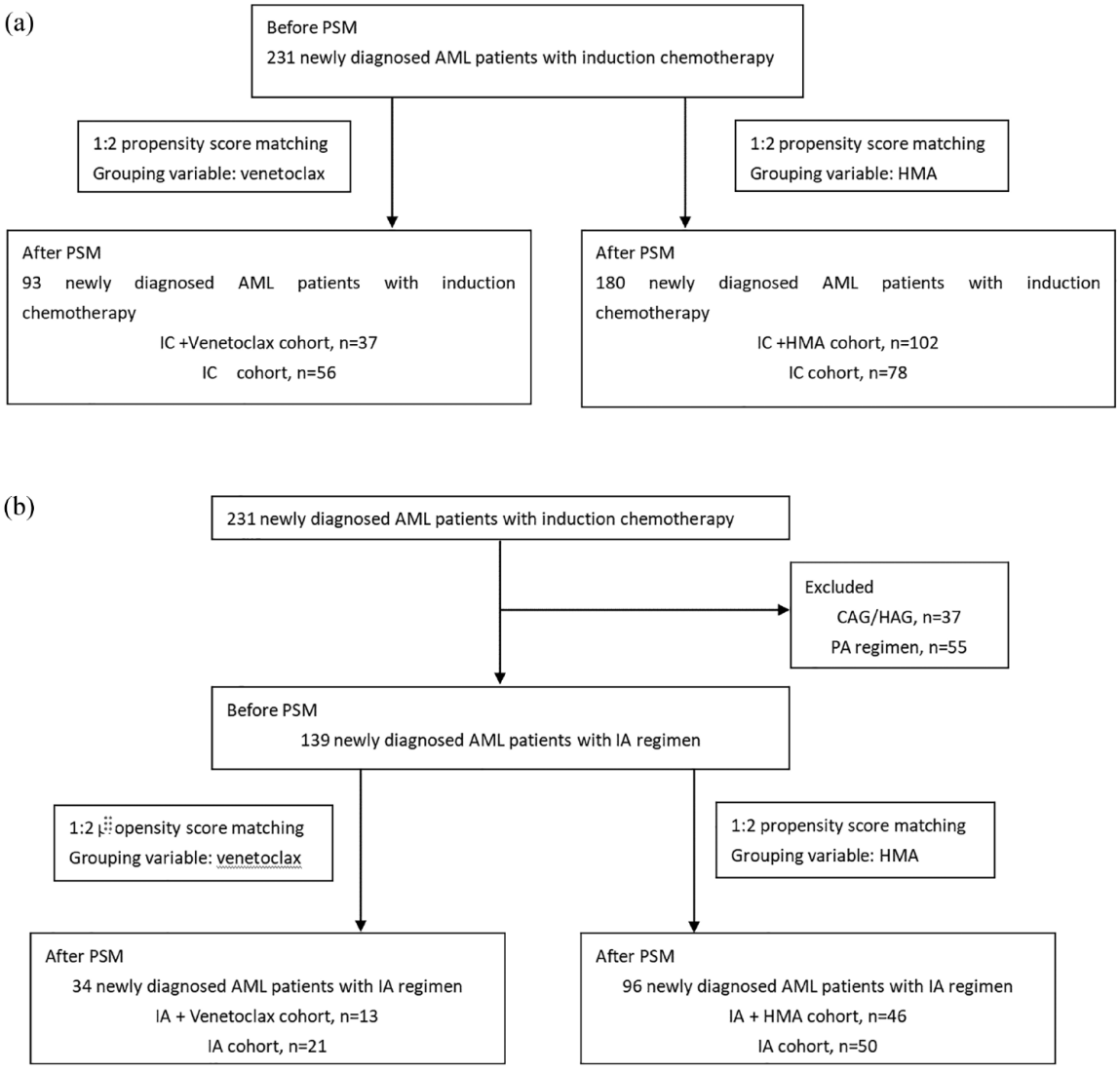

Figure 1. Flow algorithm for patient selection and analysis.
CAG, aclacinomycin, cytarabine, and G-CSF; HAG, homoharringtonine, cytarabine, and G-CSF; HMA, hypomethylating agent; IA, idarubicin combined with cytarabine; PA, pirarubicin combined with cytarabine; PSM, propensity score matching; Ven, venetoclax.

performed using IBM SPSS Statistics software (version 25.0, IBM Corporation, Armonk, NY, USA). The recorded p-values were two-sided and values of <0.05 were considered to indicate a statistically significant difference.

Results

Analysis of the therapeutic response of venetoclax or HMAs combined with IC

Analysis of the therapeutic response of venetoclax combined with IC. Ninety-three eligible cases were enrolled for the study by matching cases and controls based on age, gender, type of AML, bone marrow blast cell, and NCCN risk stratification with the use of venetoclax acting as a grouping variable (Figure 1). Patients treated with venetoclax were more likely to receive a CAG/HAG regimen (Table 1). There was a significant difference in CR/CRi between the two groups (83.8% of IC with venetoclax vs 66.1% of IC without venetoclax, $p=0.029$; Table 1), although there were no significant differences in CR, ORR, and MRD negative between the two groups. The median time to neutrophil recovery in IC with venetoclax cohort was significantly

Table 1. Analysis of therapeutic response of VEN or HMAs combined with induction chemotherapy.

Basline characteristics and therapeutic effect	With VEN, *N* = 37	Without VEN, *N* = 56	*p*	With HMAs, *N* = 102	Without HMAs, *N* = 78	*p*
Gender			0.672			1.000
Male	19 (51.4%)	25 (44.6%)		49 (48.0%)	37 (47.4%)	
Female	18 (48.6%)	31 (55.4%)		53 (52.0%)	41 (52.6%)	
Age (years)	51.4 (20–81)	48.6 (17–76)	0.357	52.5 (17–74)	45.5 (14–74)	0.653
Type of AML			0.475			1.000
Primary	32 (86.5%)	52 (92.9%)		101 (99.0%)	77 (98.7%)	
Secondary	5 (13.5%)	4 (7.1%)		1 (1.0%)	1 (1.3%)	
Baseline bone marrow blasts (%)	57.00	76.30	0.373	67.80	66.25	0.412
Risk stratification			0.841			0.288
Favorable	7 (18.9%)	14 (25.0%)		32 (31.4%)	32 (41.0%)	
Intermediate	22 (59.5%)	31 (55.4%)		53 (52.0%)	38 (48.7%)	
Adverse*	8 (21.6%)	11 (19.6%)		17 (16.6%)	8 (10.3%)	
FLT3	6 (19.4%)	11 (21.6%)		12 (13.8%)	18 (23.7%)	
TP53	2 (6.5%)	2 (3.9%)		2 (2.3%)	2 (2.6%)	
ASXL1	3 (9.7%)	3 (5.9%)		6 (6.9%)	1 (1.3%)	
DNMT3A	3 (9.7%)	4 (7.8%)		11 (12.6%)	12 (15.8%)	
TET2	2 (6.5%)	5 (9.8%)		6 (6.9%)	4 (5.3%)	
Combination						
IA	13 (35.1%)	34 (60.7%)	0.020	46 (45.1%)	67 (85.9%)	<0.001
CAG/HAG	12 (32.4%)	7 (12.5%)	0.034	32 (31.4%)	3 (3.8%)	<0.001
Therapeutic response						
CR	19 (51.4%)	29 (51.8%)	0.713	63(61.8%)	43 (55.1%)	0.196
CR/CRi	31 (83.8%)	37 (66.1%)	0.029	87 (85.3%)	51 (65.4%)	0.002
ORR	32 (86.5%)	46 (82.1%)	0.660	94 (92.2%)	63 (80.8%)	0.034
MRD negative[$]	27 (84.4%)	25 (80.6%)	0.741	67 (85.9%)	38 (79.2%)	0.190
Infection	8 (27.6%)	1(2.1%)	0.421	57 (55.9%)	44 (56.4%)	0.764
Mortality[‡]	8 (27.6%)	1 (2.1%)	0.008	6 (7.2%)	5 (7.7%)	0.304
Time to neutrophil recovery (days)	23.0 (14–48)	21.0 (0–32)	0.039	22.0 (0–59)	21.0 (0–52)	0.388
Time to platelet recovery (days)	31.5 (13–57)	27.0 (18–45)	0.534	28.0 (13–59)	27.0 (16–57)	0.773

*Mutation data missing: six cases in cohort of patients with VEN and five cases in cohort of patients without VEN. Fifteen cases in cohort of patients with HMA and two cases in cohort of patients without HMA.
[$]Five cases and 25 cases did not assess the MRD in the cohort of patients with VEN and without VEN, respectively. Twenty-four cases and 30 cases did not assess the MRD in the cohort of patients with HMA and without HMA, respectively.
[‡]Eight cases and nine cases were lost to follow-up in the cohort of patients with VEN and without VEN, respectively. Nineteen cases and 13 cases were lost to follow-up in the cohort of patients with HMA and without HMA, respectively.
CAG, aclacinomycin, cytarabine, and G-CSF; HAG, homoharringtonine, cytarabine, and G-CSF; HMA, hypomethylating agent; IA, idarubicin combined with cytarabine; MRD, measurable residual disease; ORR, overall response rate; VEN, venetoclax.

Table 2. Response of venetoclax or HMAs combined with induction chemotherapy of patients with different gene mutations.

Mutation gene	With venetoclax* (*N* = 37)	Without venetoclax* (*N* = 56)	*p*	With HMA$ (*N* = 102)	Without HMA$ (*N* = 78)	*p*
FLT3	6 (19.4%)	11 (21.6%)		12 (13.8%)	18 (23.7%)	
CR	3	4	0.644	4	9	<0.001
CR/CRi	4	4	1.000	11	10	<0.001
TP53	2 (6.5%)	2 (3.9%)		2 (2.3%)	2 (2.6%)	
CR	2	1	1.000	2	1	0.333
CR/CRi	2	1	1.000	2	2	
IDH1/2	7 (22.6%)	9(17.6%)		22 (25.3%)	12 (15.8%)	
CR	6	6	0.585	12	7	<0.001
CR/CRi	6	7	1.000	20	9	<0.001
K/NRAS	0 (0)	7 (13.7%)		11 (12.6%)	7 (9.2%)	
CR	0	5		7	6	<0.001
CR/CRi	0	6		9	7	<0.001

*Mutation data missing: six cases in cohort of patients with venetoclax and five cases in cohort of patients without venetoclax.
$Mutation data missing: 15 cases in cohort of patients with HMA and 2 cases in cohort of patients without HMA.
CR, complete response; HMA, hypomethylating agent.

longer than the time in IC without venetoclax cohort (23.0 days vs 21.0 days, $p = 0.039$; Table 1). However, there was no significant difference in the median time to platelet recovery between these two cohorts (31.5 days vs 27.0 days, $p = 0.534$; Table 1). The incidence of infections in venetoclax cohort had no significant difference compared with the incidence in non-venetoclax cohort ($p = 0.421$; Table 1). However, before the next cycle of chemotherapy treatment, the mortality rate in venetoclax group was 27.6%, which was significantly higher than the rate in non-venetoclax group (2.1% in non-venetoclax group, $p = 0.008$; Table 1).

The response rates of patients with FLT3 and IDH1/2 between the two cohorts had no significant differences (FLT3 mutation: CR $p = 0.644$, CR/CRi $p = 1.000$; IDH1/2 mutation: CR $p = 0.585$, CR/CRi $p = 1.000$; Table 2). There was no K/NRAS mutation patient in IC plus venetoclax cohort.

Analysis of the therapeutic response of HMAs combined with IC. There were 102 cases in IC combined with HMAs cohort and 78 cases in IC cohort after PSM (Figure 1). Patients treated with HMAs were also more likely to receive CAG/HAG regimen (Table 1). The rates of CR/CRi and ORR were higher in IC plus HMAs cohort than IC cohort (CR/CRi, $p = 0.002$; ORR, $p = 0.034$; Table 1), but the duration of agranulocytosis and thrombocytopenia was not different significantly (agranulocytosis, $p = 0.388$; thrombocytopenia, $p = 0.773$; Table 1). Patients with FLT3 and IDH1/2 mutations who were treated with HMAs both showed better responses ($p < 0.001$; Table 2), but the patients with TP53 mutation treated with HMAs plus IC did not achieve better response than patients treated with IC without HMAs ($p = 0.333$; Table 2).

Analysis of the therapeutic response of venetoclax or HMAs combined with IA regimen

Analysis of the therapeutic response of venetoclax combined with IA. There were 139 newly diagnosed AML cases treated with IA regimen, of which 13 cases were combined with venetoclax (Figure 1). By PSM, there were 13 cases in

IA regimen plus venetoclax cohort and 21 cases in IA regimen without venetoclax cohort with no significant differences in gender, age, AML type, primary blasts, and risk stratification between these two cohorts. The response rates, including CR, CR/CRi, ORR, and MRD negative, all had no significant difference between two cohorts (Table 3). The median time to neutrophil recovery was 22.5 days (range = 17–35) for patients treated with IA regimen plus venetoclax, while the median time to neutrophil recovery was 21.0 days (range = 0–29) for patients treated with IA regimen alone ($p = 0.276$; Table 3). The recovery time of platelets in peripheral blood was also not different significantly (IA regimen plus venetoclax cohort vs IA regimen without venetoclax cohort: 32.0 days vs 27.0 days, $p = 0.367$; Table 3). And the incidences of infections and mortality had no significant difference (infections: $p = 0.984$, mortality: $p = 0.993$; Table 3). Two patients with FLT3 mutation in IA regimen without venetoclax group both had no response to the treatment (Table 4). The rates of CR and CR/CRi with IDH1/2 between two cohorts also had no significant difference ($p = 1.000$; Table 4).

Analysis of the therapeutic response of HMAs combined with IA. Before PSM, there were 48 patients treated with IA regimen combined with HMAs, and others treated with IA regimen without HMAs. After PSM, 46 cases and 50 cases were enrolled in IA regimen plus HMAs group and IA regimen without HMAs respectively (Figure 1). There were significant differences in CR/CRi and ORR between IA regimen with HMAs and IA regimen without HMAs (91.3% and 97.8% vs 70.0% and 78.0%, $p = 0.034$ and $p = 0.038$; Table 3), but there were no significant differences in CR and MRD negative between the two groups. The median time to neutrophil recovery and platelet recovery in IA regimen plus HMAs cohort were both slightly longer than the median time in IA regimen without HMAs cohort, but the differences were not statistically significant (neutrophil recovery: 22.0 days vs 20.0 days, $p = 0.480$; platelet recovery: 28.0 days vs 27.0 days, $p = 0.849$; Table 3). Additionally, there was no significant difference in the incidence of infections and mortality rates between two cohorts ($p = 0.119$ and $p = 1.000$ respectively; Table 3). The rates of CR and CR/CRi with FLT3, TP53, IDH1/2, and N/KRAS between two cohorts also had no significant difference (Table 4).

Discussion

AML patients without remission after the first cycle of standard IC treatment remained a challenge due to poor response. The achievement of CR during induction was indispensable and was one of the most important factors to improve prognosis.[19] The better initial IC treatment reduced or delayed the risk of subsequent relapse with better survival.[19] Therefore, it was important to achieve CR as soon as possible for a newly diagnosed AML patient. MRD status after IC treatment also played an important role in planning postinduction therapy.[20] About 15%–20% of younger patients (<61 years) are primary refractory to one cycle of standard "7 + 3" IC treatment.[1,2] Some newly diagnosed AML patients might receive low-intensity IC (CAG/HAG), rather than standard-intensity IC (IA). The low-intensity IC regimens sacrificed the ORR. Further optimization of initial IC remains a priority.

The deregulated DNA methylation was highly characteristic of myeloid malignancies, DNA methylation-related genes accounted for 44% of mutated genes associated with adult de novo AML.[21] The BCL-2 family of proteins consisted of pro-apoptotic and anti-apoptotic molecules, which played an important role in tumorigenesis and anti-tumor therapy. The Bcl-2 molecules, as anti-apoptotic molecules, played an important role in the survival and persistence of AML blasts.[10] The clinical benefit of adding venetoclax or HMAs into the induction therapy for older and/or unfit patients with newly diagnosed AML has been confirmed.[11]

Through the retrospective case-cohort study, we want to determine the clinical benefit of adding venetoclax or HMAs into intense IC for younger, fit patients with AML. In our study, the addition of venetoclax could improve CR/CRi of IC (83.8% for IC plus venetoclax vs 66.1% for IC alone, $p = 0.029$). The same conclusion seems to be supported by these studies from the patients treated with modified "5 + 2" IA regimen, FLAG-IDA or CLIA regimen (cladribine, cytarabine, and idarubicin) combined with venetoclax (Supplemental Table).[9,13,22,23] These studies were all single-arm interventional studies without control. The obvious difference in CR/CRi (72%–94%) among these studies might be related to the different intensity of chemotherapy. In our retrospective case-cohort study, there was a defect.

Table 3. Analysis of therapeutic response of VEN or HMAs combined with IA.

Basline characteristics and therapeutic effect	IA			IA		
	With VEN, *N* = 13	Without VEN, *N* = 21	*P*	With HMAs, *N* = 46	Without HMAs, *N* = 50	*P*
Gender			0.727			1.000
Male	4 (30.8%)	8 (38.1%)		18 (39.1%)	20 (40.0%)	
Female	9 (69.2%)	13 (61.9%)		28 (60.9%)	30 (60.0%)	
Age (years)	44.3 (20–68)	42.3 (17–65)	0.684	49.5 (18–68)	46.0 (19–60)	0.160
Type of AML			1.000			1.000
Primary	13 (100%)	21 (100%)		46 (100%)	49 (98.0%)	
Secondary	0	0		0 (0)	1 (2.0%)	
Baseline bone marrow blasts (%)	84.0	85.8	0.410	76.0	70.5	0.605
Risk stratification			0.573			0.490
Favorable	4 (30.8%)	5 (23.8%)		21 (45.7%)	23 (46.0%)	
Intermediate	7 (53.8%)	15 (71.4%)		22 (47.8%)	20 (40.0%)	
Adverse*	2 (15.4%)	1 (4.8%)		3 (6.5%)	7 (14.0%)	
FLT3	2 (15.4%)	7 (35.0%)		6 (14.0%)	14 (28.0%)	
TP53	1 (7.7%)	0 (0)		0 (0)	1 (2.0%)	
ASXL1	0 (0)	0 (0)		2 (4.7%)	1 (2.0%)	
DNMT3A	0 (0)	3 (15.0%)		4 (9.3%)	6 (12.0%)	
TET2	2 (15.4%)	2 (10.0%)		3 (9.3%)	3 (6.0%)	
Therapeutic response						
CR	7 (53.8%)	12 (57.1%)	0.712	33 (71.7%)	26 (52.0%)	0.173
CR/CRi	10 (76.9%)	16 (76.2%)	0.986	42 (91.3%)	35 (70.0%)	0.034
ORR	10 (76.9%)	18 (85.7%)	0.781	45 (97.8%)	39 (78.0%)	0.038
MRD negative[$]	10 (83.3%)	13 (93.0%)	0.995	31 (81.6%)	27 (84.4%)	0.788
Infection	8 (61.5%)	14 (66.7%)	0.984	28 (60.9%)	22 (44.0%)	0.119
Mortality[‡]	4 (33.3%)	0 (0)	0.993	2 (5.1%)	2 (4.8%)	1.000
Time to neutrophil recovery (days)	22.5 (17–35)	21 (0–29)	0.276	22.0 (11–31)	20.0 (0–52)	0.480
Time to platelet recovery (days)	32.0 (22–36)	27.0 (21–45)	0.367	28.0 (17–59)	27.0 (16–59)	0.849

*Mutation data missing: one case in cohort of patients without VEN; three cases in cohort of patients with HMAs.
[$]One case and seven cases did not assess the MRD in the cohort of patients with VEN and without VEN respectively. Seven cases and 18 cases did not assess the MRD in the cohort of patients with HMA and without HMA, respectively.
[‡]One case and four cases were lost to follow-up in the cohort of patients with VEN and without VEN respectively. 8 cases and 18 cases were lost to follow-up in the cohort of patients with HMA and without HMA, respectively.
AML, acute myeloid leukemia; CR, complete response; HMA, hypomethylating agent; IA, idarubicin plus cytarabine; MRD, measurable residual disease; ORR, overall response rate; VEN, venetoclax.

Table 4. Response of VEN or HMAs combined with IA regimen of patients with different gene mutations.

Mutation gene	IA			IA		
	With VEN*, *N* = 13	Without VEN*, *N* = 21	*p*	With HMAs$, *N* = 46	Without HMAs$, *N* = 50	*p*
FLT3	2 (15.4%)	7 (35.0%)		6 (14.0%)	14 (28.0%)	
CR	0	3	0.500	3	7	1.000
CR/CRi	0	4	0.444	5	8	0.354
TP53	1 (7.7%)	0 (0)		0 (0)	1 (2.0%)	
CR	1	0		0	0	
CR/CRi	1	0		0	1	
IDH1/2	2 (15.4%)	3 (15.0%)		10 (23.3%)	9 (18.0%)	1.000
CR	0	1	1.000	6	6	0.582
CR/CRi	1	1	1.000	9	7	
K/NRAS	0 (0)	2 (10.0%)		6 (14.0%)	6 (12.0%)	
CR	0	2		5	6	1.000
CR/CRi	0	2		6	6	1.000

*Mutation data missing: one case in cohort of patients without VEN.
$Mutation data missing: three cases in cohort of patients with hypomethylating agents.
CR, complete response; HMA, hypomethylating agent; IA, idarubicin plus cytarabine; ORR, overall response rate; VEN, venetoclax.

There was no match in the intensity of chemotherapy between patients treated with venetoclax and patients treated without venetoclax. Patients treated with low-intensity IC (CAG/HAG) were more likely to receive venetoclax than patients treated with standard-intensity IC (IA).

To eliminate the mismatch in intensity of chemotherapy, we accomplished case-cohort study based on IA regimen. The results showed that the addition of venetoclax did not improve the CR/CRi rate of the first IA regimen induction therapy. The addition of venetoclax did not improve the rate of MRD negative. The addition of venetoclax did not increase the incidence of infection and hematological side effects. Comprehensive analysis showed that the addition of venetoclax might improve CR/CRi in low-intensity IC (CAG/HAG), rather than in standard-intensity IC (IA).

Among the matched cases, there were more intermediate/high-risk patients treated with venetoclax, although there was no significant difference in risk stratification. This reflected the idea that clinicians hoped to use venetoclax to improve the remission rate in high-risk patients. Our results showed that the addition of venetoclax did not improve the remission rate of intermediate/high-risk patients.

Similar conclusions were obtained from the retrospective case-cohort study of HMAs. The results showed that the addition of HMAs did not only improve the CR/CRi rate of the first IC but also improved the CR/CRi rate of IA regimen. However, the addition of HMAs did not improve the rate of MRD negative. The addition of HMAs could improve CR/CRi of patients with adverse mutations (FLT3, IDH1/2, and K/NRAS) after IC, but could not improve CR/CRi of patients

with adverse mutations after IA. The addition of venetoclax could not improve CR/CRi of patients with adverse mutations after IC including IA.

As a retrospective study, this study has several limitations. First, there was a small number of high-risk patients in our study so this study could not definitively show that venetoclax or HMAs could improve remission rates in high-risk patients. Second, the variety of follow-up treatments for patients in the study led to uncertainty in the assessment of long-term survival outcomes. Only one cycle follow-up prevented us from reaching a definitive conclusion about the survival benefits of the addition of venetoclax or HMAs, although the achievement of CR and MRD negative during induction was indispensable and was one of the most important factors to improve prognosis.[19] Third, with the exception of hematologic adverse events and fever, other non-hematologic adverse events were difficult to assess objectively.

In conclusion, adding venetoclax or HMAs to the conventional IC regimens resulted in a high CR/CRi, but there was no improvement in the rates of CR and MRD negative, and the extra use of venetoclax did not improve the effect of IA regimen. However, due to a small number of cases and short follow-up time in our study, a well-designed prospective randomized controlled study with long-term follow-up is now warranted.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the medical ethics committee of Tonji Hospital, Tongji Medical College, Huazhong University of Science and Technology (TJ-IRB20230749). Informed consent was not required because this study was retrospective analysis.

Consent for publication

Not applicable.

Author contributions

Fangfei Xu: Formal analysis; Investigation; Software; Supervision; Validation.

Kuangguo Zhou: Methodology; Visualization; Writing – review & editing.

Duanhao Gong: Data curation; Resources.

Wei Huang: Conceptualization; Project administration; Writing – original draft.

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Competing interests

The authors declare that there is no conflict of interest.

Availability of data and materials

For data sharing please contact the corresponding author. The data will be available upon request at any time. the study abides by STROBE guidelines.

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Supplemental material

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