Homoharringtonine overcomes the negative impact of genetic patterns on venetoclax plus azacitidine regimen in relapsed/refractory acute myeloid leukemia: a multi-center, cohort study

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Abstract

Background The response of venetoclax (VEN)-based therapy is closely associated with genetic patterns and combining regimens in acute myeloid leukemia (AML). Whether other agents added to VEN plus hypomethylating agents (HMA) regimen could overcome the negative impact of genetic patterns on response remains unclear.

Methods A multi-center, cohort study of the response and the genetic patterns of response of VEN plus AZA and HHT (VAH) versus VEN plus AZA (VA) regimens as salvage treatment in the patients with RR-AML was performed. Patients were enrolled from four studies from October 2018 to December 2022 at nine medical centers in China. The endpoints in this study were to evaluate the rate of composite complete remission (CRc), measurable residual disease (MRD), event-free survival (EFS), overall survival (OS) and relapse between VAH and VA groups.

Results A total of 321 patients were analyzed, including 150 females and 171 males, with a median age at 46 (IQR, 35–61) years. There were 172 patients in the VAH and 149 in the VA group. VAH significantly improved CRc rate (66.3% vs. 44.3%, P < 0.001) and prolonged OS (median OS, not reach vs. 14.3 months, P = 0.004), to compared with VA. VAH significantly overcame the negative impact of FLT3-ITD/TKD, N/KRAS, TET2, DNMT3A mutations, and t(8;21)/AML1-ETO, as well as non-adverse ELN risk, also apparently in adverse ELN risk or complex karyotype, on the response of VA regimen.

Conclusion The impact of genetic patterns on the response presented diversely in different VEN-based regimens. HHT added to VA regimen might improve the response and overcome the negative impact of part genetic patterns in RR-AML.

Background

Overexpression of BCL-2 is closely associated with the development and progression of acute myeloid leukemia (AML)\(^1\)–\(^3\). BCL-2 inhibitor venetoclax (VEN) has been shown high efficacy and safety in AML\(^4\)–\(^6\), and it in combination with hypomethylating agents (HMA) have been suggested for the frontline treatment of unfit AML, also a choice of the salvage treatment of relapsed/refractory (RR)-AML\(^7\). A growing number of studies reveals the impact of genetic patterns on the response of VEN-based regimens\(^8\)–\(^13\), but these come from VEN combined with HMA (VEN-HMA) regimens. Recently, studies with small cases reported that other agents added to VEN-HMA regimens might overcome the negative impact of genetic patterns and improve the outcome\(^14\)–\(^17\). Comparison of the results of VEN-HMA in our real world study\(^13\) with VEN plus azacitidine (AZA) and homoharringtonine (HHT) (VAH) in the prospective single arm study\(^14\) in the salvage therapy of RR-AML showed that VAH might improve the rate of composite complete remission (CRc) (70.8% vs. 43.3%) and 1-year overall survival (OS) (61.5% vs. 46.9%) to compare with VEN-HMA, also overcome the negative impact of part genetic patterns, such as FLT3-ITD mutation, on response. But these cross-trial comparisons need caution, and a large scale clinical study is needed. Therefore, in this study, we enrolled 321 RR-AML patients with VEN-based treatment and
compared the response and the genetic patterns of response between VAH and VA (VEN plus AZA) regimens.

**Methods**

**Data source and eligibility**

This pooled analysis included patients from our four studies including two retrospective studies\(^{13,15}\), a prospective single-arm study\(^{14}\) and a randomized controlled trial (RCT) study (NCT05457361). Eligible patients were aged between 18–65 years with RR-AML and treated with at least one cycle of VA or VAH regimens. Patients with acute promyelocytic leukemia or lack of genetic data or treatment response assessment were excluded. The diagnosis and risk stratification of AML were according to the guideline of the National Comprehensive Cancer Network (NCCN)\(^{7}\) and the recommendation of European Leukemia Net (ELN) 2017\(^{18}\). Refractory AML was defined as no CRc and a reduction in bone marrow (BM) blasts of less than 60% after one cycle or no CRc after two cycles of standard induction therapy\(^{18,19}\). Relapsed AML was defined as recurrence of blasts in the peripheral blood (PB) or BM blasts \(\geq\) 5% or development of extramedullary disease after achieving CRc\(^{18,19}\).

The study protocol was reviewed and approved by the local ethics committee review board, and written informed consents were obtained from all recipients/guardians following the Declaration of Helsinki before the initiation of the study.

**Genetic and MRD assessment**

Cytogenetic evaluation with standard metaphase karyotype and fluorescence in situ hybridization (FISH), and molecular analysis via PCR and a 167-gene panel next-generation sequencing (NGS) were performed at study enrollment\(^{13–14}\). Measurable residual disease (MRD) was monitored using flow cytometric (FCM) analysis after every cycle of therapy. A positive MRD was defined as a ratio \(\geq 0.1\%\)^{20}.

**Procedures**

According to our practical guidelines and clinical trial protocol\(^{13,14}\), patients received VEN as 100mg day 1, 200mg day 2, 400mg day 3–28; AZA at the dose of 75 mg/m\(^2\), day 1–7 in the VA regimen\(^{13}\). In the VAH regimen VEN was administered for only 14 days with dose escalation as above; AZA was commenced according to the same schedule and HHT was given at a dose of 1mg/m\(^2\), day 1-7\(^{14}\). The dose of VEN in both groups was adjusted following prescribing information recommendations if co-administered with CYP3A inhibitors. Fms related receptor tyrosine kinase 3 (FLT3) inhibitors were recommended in the patients with FLT3 mutations. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) was recommended to all the patients after the salvage therapy if there was a suitable donor available.

Response was evaluated according to the criteria of modified International Working Group (IWG) for AML\(^{14,21}\). CRc contained CR (complete remission) /CRi (CR with incomplete count recovery). CR was
defined as BM with less than 5% blasts and without extramedullary infiltration and recovery of peripheral blood cells. CRi was defined as all the criteria for CR, except for neutropenia or thrombocytopenia. Partial remission (PR) was defined as BM blasts of 5–25% and a decrease of more than 50% as compared with pre-treatment. Non-remission (NR) was defined as a failure to obtain CRc or PR. Overall response included CRc and PR.

**Statistical Analysis**

Categorical variables were compared using Pearson's $\chi^2$ test and continuous variables were compared using the Mann–Whitney $U$-test. A logistic multivariable regression model was applied for covariates showing a significant association at 0.1 levels in univariate analysis. A backward selection technique was used to build the final model for the achievement of CRc, and MRD negative. Time-to-event endpoints were evaluated by the Kaplan–Meier method, with differences between groups compared by log-rank test. OS was calculated from enrolling to death or the last follow-up. Event-free survival (EFS) was calculated from enrolling to the date of relapse or death or the last follow-up. Non-responding patients were considered as progressing on cycle 1 day 1 for EFS. Analyses were performed using SPSS 19.0 (SPSS Inc., Chicago, IL, USA) and R version 4.1.3 (R Development Core Team, Vienna, Austria), and significance was defined as a p value of < 0.05.

**Results**

**Patients characteristics**

From October 2018 to December 2022 at nine medical centers in China, a total of 379 RR-AML patients entered screening and 321 were enrolled, including 161 from two retrospective studies and 160 from two respective studies (Fig. 1). There were 150 females and 171 males, with a median age at 46 (interquartile range (IQR), 35–61) years. One hundred and twenty cases had primary refractory AML and 201 relapsed AML, including 94 relapsed after chemotherapy and 107 relapsed after allo-HSCT. One hundred and forty-nine patients were treated with VA regimen (VA group), and 172 with VAH regimen (VAH group). Seventy-seven patients harbored FLT3-ITD/TKD mutation, of whom 52 received VEN-based regimens combined with FLT3 inhibitors (47 with sorafenib, 5 with gilteritinib), and 15 with VEN-based regimens only. Of the 321 patients enrolled, a total of 265 patients had the data of cytogenetics including 229 with karyotype and 235 with FISH detection. Among these patients, 123 had a normal cytogenetics, and 142 an aberrant cytogenetics, including 21 favorable, 165 intermediate, and 79 adverse cytogenetics. A total of 112 concomitant mutations were detected, and 287 (89.4%) patients harbored at least one mutation, along with 135 (90.6%) and 152 (88.3%) in the VA and VAH groups, respectively. The mutational landscape with $\geq 1\%$ occurrence are shown in Fig. 2, in which TET2, DNMT3A, FLT3-ITD/TKD, RUNX1, and ASXL1 were the top five occurrence. As shown in Supplemental Fig. 1, analysis of pair-wise co-occurrence and mutual exclusivity were tested in the top 20 most frequently detected mutations, and showed that DNMT3A + NPM1+ (9.0%), ASXL1 + TET2+ (9.0%), NPM1 + FLT3+ (8.0%), DNMT3A + IDH1/2+ (6.8%),
DNMT3A + BCOR+ (4.9%), INPM1 + DH1/2+ (4.6%), BCOR + IDH1/2+ (3.1%) were the most frequently observed pairwise co-mutation patterns \( (n \geq 10) \). DNMT3A + FLT3 + NPM1+ (5.0%) was the most common triple mutation.

Prognostic factors, such as sex, age, prior HMA exposure, ELN risk stratification, bridge to allo-HSCT, and so on, were balanced between the VAH and VA groups, except higher proportion of ECOG performance \( \geq 3 \) (VA 32.9% vs. VAH 19.2%, \( P = 0.005 \)) and NPM1 mutation (VA 20.8% vs. VAH 12.2%, \( P = 0.037 \)), and less prior allo-HSCT (VA 24.8% vs. VAH 40.6%, \( P = 0.003 \)) in the VA group (Table 1 and Fig. 2).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>VA n = 149 (%)</th>
<th>VAH n = 172 (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex, M/F</strong></td>
<td>83/66</td>
<td>88/84</td>
<td>0.416</td>
</tr>
<tr>
<td><strong>Age, median (IQR), y</strong></td>
<td>51(35–62)</td>
<td>51(34.2–60)</td>
<td>0.524</td>
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<tr>
<td><strong>ECOG score ( \geq 3 )</strong></td>
<td>49(32.9)</td>
<td>33(19.2)</td>
<td>0.005</td>
</tr>
<tr>
<td><strong>De novo AML/sAML</strong></td>
<td>130/19</td>
<td>151/16</td>
<td>0.323</td>
</tr>
<tr>
<td><strong>Refractory/relapsed AML</strong></td>
<td>70/79</td>
<td>69/103</td>
<td>0.216</td>
</tr>
<tr>
<td><strong>Prior HMA</strong></td>
<td>44(29.5)</td>
<td>59(34.3)</td>
<td>0.361</td>
</tr>
<tr>
<td><strong>Prior allo-HSCT</strong></td>
<td>37(24.8)</td>
<td>70(40.6)</td>
<td>0.003</td>
</tr>
<tr>
<td><strong>ELN risk stratification (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Favorable</td>
<td>18(12.1)</td>
<td>24(14.0)</td>
<td>0.189</td>
</tr>
<tr>
<td>Intermediate</td>
<td>53(35.6)</td>
<td>45(26.2)</td>
<td></td>
</tr>
<tr>
<td>Adverse</td>
<td>78(52.3)</td>
<td>103(59.9)</td>
<td></td>
</tr>
<tr>
<td><strong>Cytogenetics (%)</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Favorable</td>
<td>8(5.4%)</td>
<td>13(7.6)</td>
<td>0.431</td>
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<tr>
<td>Intermediate</td>
<td>76(51)</td>
<td>89(51.7)</td>
<td></td>
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<tr>
<td>Adverse</td>
<td>34(22.8)</td>
<td>45(26.2)</td>
<td></td>
</tr>
<tr>
<td>Unkonwn or missing</td>
<td>31(20.8)</td>
<td>25(14.5)</td>
<td></td>
</tr>
<tr>
<td>FLT3i combination</td>
<td>23/37(62.2)</td>
<td>29/40(72.5)</td>
<td>0.333</td>
</tr>
<tr>
<td>Bridge to allo-HSCT</td>
<td>37 (24.8)</td>
<td>38 (22.1)</td>
<td>0.560</td>
</tr>
</tbody>
</table>
IQR, Interquartile range; VA, venetoclax + azacitidine; VAH, venetoclax + azacitidine + homoharringtonine; M/F, male/female; ECOG, Eastern Cooperative Oncology Group; AML, acute myeloid leukemia; sAML, secondary AML; HMA, hypomethylating agent; Allo-HSCT, allogeneic hematopoietic stem cell transplantation; ELN, European Leukemia Net; FLT3i, FLT3 inhibitor.

**Treatment response**

Treatment response is shown in Fig. 3. After two courses of salvage therapy, totally 73.8% (127 of 172 patients, 95% Confidence Interval (CI) 66.5–80.1%) patients acquired response, including 66.3% (95% CI 58.6–78.1%) with CRc, in the VAH group, while 58.3% (87 of 149 patients, 95% CI 50–66.3%) obtained response, including 44.3% (95% CI 36.5–52.6%) with CRc, in the VA group. Both the overall response (P=0.002) and CRc (P<0.001) were significantly higher in the VAH group than VA group. The benefit in the CRc (62.2% (95% CI 54.4–69.3%) vs. 39.5% (95% CI 31.7–47.9%), P<0.001) with VAH versus VA was also observed after the first course. Of the 179 patients achieving CRc, VAH also obtained significantly higher MRD-negative rate than VA (59.3% vs. 34.8%, P=0.002, Fig. 3B). Meanwhile, forest plot analyses revealed the superiority of VAH in acquiring CRc versus VA in subgroups, especially significant in the patients with ECOG performance < 3 (P=0.002), de novo AML (P=0.001), without HMA exposure (P<0.001), or BM blasts ≥ 30% (P<0.001) (Fig. 3C).

**Survival**

Survival outcomes are shown in Fig. 4. With a median follow-up of 23 months (95% CI, 13-32.9 months), 107 (33.3%, 95% CI 28.2–38.8%) patients died, including 22 relapses, 15 treatment-related complications, 67 primary disease progressions and 3 others, of whom 63 (42.3%, 95% CI 34.3–50.6%) died in the VA group and 44 (25.6%, 95% CI 19.3–32.8%) in the VAH group (P=0.002). Of the 183 patients obtaining CRc, including 180 with chemotherapy and 3 with allo-HSCT, 46 (26.2%, 95% CI 19.4–32.6%) patients relapsed, of whom 22 (33.3%, 95% CI 22.5–46.1%) relapsed in the VA group and 24 (21.1%, 95% CI 14.2–29.8%) in the VAH group (P=0.069). The OS and EFS of VAH group were significantly better than VA group (median OS, not reach vs. 14.3 months, log-rank, P=0.004, Fig. 4A; median EFS: 9.9 vs. 2.3 months, log-rank, P<0.001, Fig. 4C). Further analyses revealed survival benefit from VAH versus VA was significant in the patients who were not bridged to allo-HSCT (P<0.001) or harbored non-adverse cytogenetics. Among those with allo-HSCT or adverse cytogenetics, the OS was comparable between the two groups (Fig. 4D, 4F). Multivariate analysis showed that VAH and bridging to allo-HSCT were the independently protective factors, while adverse cytogenetics was the negative factors for OS and EFS (Supplemental Table 1).

**Impact of VAH versus VA on the outcome based on genetic patterns**

The impact of ELN risk and cytogenetic abnormalities on the response of VAH versus VA are shown in Figs. 2 and 3C. As compared with VA, VAH regimen significantly increased the CRc rate in the patients with non-adverse ELN risk (47.9% vs. 84.1%, P<0.001) or non-adverse cytogenetics (48.8% vs. 75.5%, P<
also a trend of increased CRc in those with adverse ELN risk (41.0% vs. 54.4%, P = 0.076, Fig. 3C). Notably, the patients with t(8;21)/AML-ETO presented low response to VA (1/10, 10.0%), but responded sensitively to VAH (9/13, 69.2%, P = 0.013).

The impact of gene mutations on the CRc are shown in Fig. 2, 3C and Supplemental Table 2. The patients with SRSF2 (85.7% vs. 80.0%, P = 0.793), IDH1/2 (73.9% vs. 71.4%, P = 0.843), CEBPA (63.2% vs. 73.9%, P = 0.453), ASXL1 (61.9% vs. 71.4%, P = 0.460), or NPM1 (61.3% vs. 81.0%, P = 0.132) mutations presented equally high response with the CRc rate of more than 60%, while those with TP53 mutation (44.4% vs. 36.4%, P = 0.714) showed relatively low response, to both VA and VAH treatment. Meanwhile, the patients with FLT3-ITD/TKD (37.8% vs. 70.0%, P = 0.005), N/KRAS (29.2% vs. 71.4%, P = 0.005), TET2 (46.3% vs. 66.7%, P = 0.044), or DNMT3A (53.1% vs. 66.7%, P = 0.010) mutations responded poor to VA, but much more sensitive to VAH. In addition, in the FLT3-ITD/TKD-positive patients, combination of FLT3 inhibitors with VEN-based regimens further improved the CRc rate as compared with those without (33/52, 63.5% vs 9/25, 36.0%, P = 0.023), significantly in the VAH group (23/29, 79.3% vs 5/11, 45.5%, P = 0.037), and apparently in the VA group (10/23, 43.5% vs 4/14, 28.6%, P = 0.365).

Based on concomitant mutations, the impact of the top 7 most frequent pairwise co-mutations and triple-mutation (DNMT3A + FLT3 + NPM1+) on response were analyzed. As shown in Supplemental Fig. 2 and Supplemental Table 2, patients with NPM1 + FLT3+ (VA 44.4% vs. VAH 75.0%) or DNMT3A + NPM1+ (VA 50.0% vs. VAH 84.6%) had poor response with VA, but responded relatively sensitive to VAH; while those with ASXL1 + TET2+, DNMT3A + IDH1/2+, DNMT3A + BCOR+, NPM1 + IDH1/2 + or BCOR + IDH1/2+ presented equally high response to both VA and VAH treatment. In the patients with DNMT3A + FLT3 + NPM1+, the CRc rate was 30.0% in the VA, while 66.7% in the VAH.

We also explored mutations in genetic pathway subgroup (Fig. 2 and Supplemental Table 2). Patients carrying mutations in active signaling (30.8%) or tumor suppressor (43.8%) presented low CRc rate, while those with chromatin modifiers mutations (22/35, 62.9%) high, with VA treatment. VAH significantly improved the response of the patients with active signaling (71.0%, P < 0.001) and methylation-related mutations (69.7% vs. 51.4% in VA, P = 0.015), also a trend of improved CRc rate in those with spliceosome (73.3% vs. 45.5, P = 0.093), transcription factors (66.2% vs. 51%, P = 0.098), or tumor suppressor mutations (65.5% vs. 43.8%, P = 0.157).

The impact of genetic patterns on the OS is shown in supplemental Fig. 3. In comparison with VA, VAH significantly improved the OS of the patients with non-adverse ELN risk or non-adverse cytogenetics but not those with adverse risk, also the patients with FLT3-ITD/TKD, DNMT3A, ASXL1 mutations. In addition, VAH tended to benefit higher OS in the patients with AML1-ETO or complex karyotype.

**Discussion**

In the present study, we explored the impact of genetic patterns on the response of Ven-based therapies in RR-AML. The results showed that HHT added to VA regimen might improve the response and overcome the negative impact of part genetic patterns.
Reported CRc rates of VEN-based therapies in patients with RR-AML vary greatly, ranging from 18–70.8%\(^1\)\(^{10−11,13−17,22}\). These responses to treatments are associated with patients’ characteristics, prior therapies, VEN-based regimens and genetic characteristics, and so on. With respect to VEN-based regimens, our study showed that VAH regimen significantly improved the CRc rate of 44.3% in the VA regimen up to 66.3% and MRD negative rate of 34.8% up to 59.3%. These results were in line with previous reports that three-drug regimens might have a superiority in acquiring CRc than two-drug regimens in RR-AML\(^1\)\(^{10−11,13−15,17}\). In the two-drug regimens, mainly as VEN-HMA, studies showed the CRc rate ranged from 12–46%\(^1\)\(^{10−11,13}\), while CRc rate was reported to range from 64.7–70.8% in the three-drug regimens\(^1\)\(^{14−15,17}\). Importantly, we also observed the superiority of VAH regimen in CRc transferred into survival benefit. These results further supported our real world\(^1\)\(^3\) and prospective study\(^1\)\(^4\) in which VAH regimen might have better response than VEN-HMA in RR-AML.

With respect to genetic patterns of response, some studies including our own\(^8−13\) reported that the patients with IDH1/2, NPM1, RUNX1, ASXL1 or SRSF2 mutation responded sensitive, while those with FLT3-ITD/TKD, TP53, N/KRAS, SF3B1 or DNMT3A mutation or complex karyotype experienced poor response to VEN-HMA regimens. But the genetic patterns of response in three-drug regimens remains unclear, let alone other agents added to VEN-HMA could overcome the negative impact of genetic patterns. Our recent study\(^1\)\(^4\) showed that the patients with N/KRAS or MLL mutation responded poor to VAH, while FLT3-ITD/TKD or DNMT3A mutations did not significantly affect the response. In line with these reports\(^8−14\), the present large-scale study confirmed the important impact of genetic patterns on the response of VEN-based regimens, and further supported our cross-trial comparison that VAH might overcome the negative impact of genetic patterns on VEN-HMA\(^1\)\(^3,14\). In addition, the results revealed that patients with t(8;21)/AML1-ETO-positive AML might respond poor to VA; VAH might significantly overcome the negative impact of FLT3-ITD/TKD, N/KRAS, TET2, DNMT3A mutations, and t(8;21)/AML1-ETO, as well as non-adverse ELN risk, also apparently in adverse ELN risk or complex karyotype, on the response of VA. In addition, we observed that co-mutation of NPM1 with FLT3 or DNMT3A or triple-mutation of these three genes presented poor response to VA, but sensitive to VAH. Mutations in chromatin modifiers might predict good, while mutations in active signaling or tumor suppressor predict poor response to VA. VAH might improve the response of the patients carrying mutations in active signaling or methylation-related. These results might open a window for further study of the mechanism of VEN resistance. In accordance, the benefit of VAH versus VA in overcoming the negative impact of part genetic patterns was also found in the OS, presenting significantly in the patients with non-adverse ELN risk or mutations of FLT3-ITD/TKD, DNMT3A or ASXL1, and apparently in the patients with t(8;21)/AML1-ETO or complex karyotype. To be noted, in agreement with previous\(^8−11,13−14\), patients with TP53 mutation responded poorly to both VAH and VA treatment.

With respect to the synergistic mechanism of HHT with VA, several preclinical studies have demonstrated the synergy of HHT with VEN in anti-leukemia because of HHT suppressing the expression of MCL-1 and BCL-xL of AML cells\(^23−25\). Our recent study also observed that HHT enhanced the killing effect of VA in AML cells via inhibition of MCL-1 in vitro\(^1\)\(^4\). Apart from these, based on our results and other studies,
there might be other mechanisms for HHT strengthening the killing effect of VA. Firstly, in FLT3-ITD/TKD or N/KRAS mutated AML, activation of signaling pathway might account for the resistance to VA\textsuperscript{13,23}, whereas its downstream signaling proteins could be inhibited by HHT\textsuperscript{23,26−27}, thus leads to the promising outcomes of VAH in these populations. BCL-2 inhibitor combined with HHT remarkably inhibits the expression of p-FLT3 and its downstream signaling proteins, p-Stat5 and MCL-1, inducing apoptosis in AML cell lines\textsuperscript{23,27}. In addition, combination of FLT3 inhibitors could not only strengthen the anti-tumor effect of VEN\textsuperscript{28−32}, but also HHT, which was supported by the fact that FLT3-ITD mutated patients clinically benefit from HHT plus sorafenib therapy\textsuperscript{33}. Secondly, DNMT3A mutation initiates activation of mTOR pathway\textsuperscript{34}, then up-regulates the expression of MCL-1, which could be dual inhibited by HHT\textsuperscript{35−36}, that might explain the increasing response of the patients with DNMT3A mutation observed in the VAH group. Thirdly, MYC is always abnormally activated in t(8;21)/AML1-ETO-positive AML\textsuperscript{37}, which is also associated with VEN-resistance\textsuperscript{38−39}, might account for the poor response of VA in this subtype leukemia. HHT just inhibits MYC and its downstream signaling\textsuperscript{26}, and exerts promising anti-leukemia effect in mice\textsuperscript{40} and patients\textsuperscript{41}, that could explain the patients significantly benefited from VAH. However, the exact mechanism for AML1-ETO fusion gene exerting on VEN resistance and HHT overcoming the resistance needs further study. Taking together, as shown in Fig. 5, the supposed mechanism of HHT overcoming the resistance of VEN might be associated with MCL-1 suppression and signaling pathway inhibition.

In FLT3-ITD-mutated AML, FLT3-ITD induces MCL-1 expression via downstream signaling. HHT might enhance the anti-leukemia effect of VEN by directly inhibiting MCL-1 (1) and co-targeting both STAT5 (4) and PI3K/Akt pathway (5). 2. MCL-1 is also aberrantly activated by oncogenic RAS mutation. HHT might strengthen the killing effect of VEN in N/KRAS mutant AML via directly blocking MCL-1 (1). 3. Mutations in DNMT3A activates mTOR pathway via hypomethylation, and then induces the expression of c-myc and MCL-1. HHT might suppress this signaling (2) and decrease its downstream transcription of MCL-1 (3). 4. Aberrant activation of c-myc is always observed in AML-ETO positive AML. HHT might suppress MYC-mediated transcription of MCL-1 (3).

As known allo-HSCT is an important way to cure RR-AML\textsuperscript{42−44}, bridging to allo-HSCT was the independently protective factor for OS and EFS in this study. Though VAH regimen had a superiority in acquiring deeper response and better survival\textsuperscript{14−15}, among the patients with bridging to allo-HSCT, there was not significant difference in the OS between the VAH and VA groups, indicating that allo-HSCT should be recommended after salvage therapy.

There are several limitations in our study. Patients included in this study were a pooled population from 4 studies, patient selection bias might exist. Also, the follow-up time of survival was relatively short. In addition, despite the large-scale of this study, the number of patients in certain genetic pattern subgroups was still too small for a statistically significant result.
In summary, our findings highlight the significant impact of genetic patterns on the response of VEN-based therapy. HHT added to VA might improve the response and overcome the negative impact of part genetic patterns. The utility of genetic patterns with optimal VEN-based strategy might be useful for guiding future management.

**Declarations**

**Ethics approval and consent to participate**

All procedures involving human participants were performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from the patients or guardians before enrollment in the study.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

**Authors' contributions**

Dr Liu and Jin had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Yu, Zhang, S.-J. Yu, Yin were co–first authors and contributed equally to this work. Jin and Liu contributed to conceptual advice and supervision of the work, Yu, Zhang, S.-J. Yu,Yin, Jin, Liu drafting of the manuscript, Yu, Zhang, S.-J. Yu, Yin, Weng, S.-J. Yu, Xu, Du did the critical revision of the manuscript for important intellectual content. Yu, Zhang, S.-J. Yu,Yin performed the statistical analysis. Lin, Xiao, Sun, H.-Y. Zhang, X.-Q. Liang, Guo, Zhao, Dai, Fan provided the administrative, technical, or material support, Study supervision were conducted by Yu, Zhang, S.-J. Yu, Yin, Jin, Liu,Xuan, H. Liu. D. Xu, Ye, X.-J. Jiang, Shi gathering data for stat analysis. All authors interpretation of data and reviewed the manuscript.

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**Availability of data and materials**
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

Figure 1
Flow chart. VEN, venetoclax; DAC, decitabine; AZA, azacitidine; VAH, VEN plus AZA and homoharringtonine; AML, acute myeloid leukemia; allo-HSCT, allogeneic hematopoietic stem cell transplantation.

**Figure 2**

Mutational landscape and genetic patterns of response between VAH and VAGroups. Mutations were grouped according to genetic pathway. The presence of treatment regimens, ELN (European Leukemia Net) risk, clinical response (CR, complete response; CRi, CR with incomplete count recovery; MLFS, morphologic leukemia-free state; PR, partially response; NR, not response) and MRD (measurable residual disease) are shown for each case. The right side of the figure shows the CRc (composite complete remission) rate of each genetic abnormality.
Figure 3

Treatment response and subgroup analysis of VAH versus VA groups
Figure 4

Survival analysis of VAH versus VAregimens
Figure 5

Scheme of hypothetic mechanism of HHT overcoming the resistance of VEN.

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