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ORIGINAL PAPER

Haematological Malignancy - Clinical

Gilteritinib-based combination therapy in adult relapsed/refractory *FLT3*-mutated acute myeloid leukaemia

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Summary

Gilteritinib, a potent FMS-like tyrosine kinase 3 (FLT3) inhibitor, was approved for relapsed/refractory (R/R) *FLT3*-mutated acute myeloid leukaemia (AML) patients but still showed limited efficacy. Here, we retrospectively analysed the efficacy and safety of different gilteritinib-based combination therapies (gilteritinib plus hypomethylating agent and venetoclax, G + HMA + VEN; gilteritinib plus HMA, G + HMA; gilteritinib plus venetoclax, G + VEN) in 33 R/R *FLT3*-mutated AML patients. The composite complete response (CRc) and modified CRc (mCRc) rates were 66.7% (12/18) and 88.9% (16/18) in patients received G + HMA + VEN, which was higher compared with that in G + HMA (CRc: 18.2%, 2/11; mCRc: 45.5%, 5/11) or G + VEN (CRc: 50.0%, 2/4; mCRc: 50.0%, 2/4). The median overall survival (OS) for G + HMA + VEN, G + HMA and G + VEN treatment was not reached, 160.0 days and 231.0 days. The median duration of remission (DOR) for G + HMA + VEN, G + HMA and G + VEN treatment was not reached, 82.0 days and 77.0 days. Four patients in the G + HMA + VEN group received alloHSCT after remission exhibited prolonged median DOR. The most common grade 3/4 adverse events were cytopenia, febrile neutropenia and pulmonary infection; there were no differences among the three groups. In conclusion, our data demonstrated promising response of G + HMA + VEN combination therapy in R/R *FLT3*-mutated AML, and it may be considered an effective therapy bridge to transplantation.

KEYWORDS
acute myeloid leukaemia, FMS-like tyrosine kinase 3 mutation, gilteritinib, relapsed/refractory

Nianci Chen and Jiajia Pan contributed equally to this work.

INTRODUCTION

The FMS-like tyrosine kinase 3 (*FLT3*) mutations, including in-frame internal tandem duplications (*ITD*) within the juxta membrane region or as missense point mutations in the tyrosine kinase domain (*TKD*), occur in approximately 30% of patients with acute myeloid leukaemia (AML).[1] Historically, *FLT3* mutations were associated with poor prognosis, reduced overall survival (OS) and higher rates of relapse.[2,3] Several FLT3 tyrosine kinase inhibitors (TKIs) have been developed for the treatment of *FLT3*-mutated AML.[4] The RATIFY trial demonstrated that the addition of midostaurin to standard induction chemotherapy improved the OS, which led to the approval of midostaurin in combination with first-line induction chemotherapy for newly diagnosed *FLT3*-mutated AML patients.[5] Moreover, other randomized studies showed midostaurin or sorafenib maintenance therapy reduced the risk of relapse and death after allogenic haematopoietic stem cell transplantation (alloHSCT) in *FLT3-ITD*-mutated AML patients.[6,7] Gilteritinib is a second-generation FLT3 tyrosine kinase inhibitor that has been shown to significantly improve the survival of relapsed/refractory (R/R) *FLT3*-mutated AML patients compared to chemotherapy.[8] Based on the results of the phase 3 ADMIRAL trial, gilteritinib is approved as monotherapy in R/R *FLT3*-mutated AML patients due to the superior OS and CR + CRh (complete remission [CR] + CR with partial haematological recovery [CRh]) rates compared to those in salvage chemotherapy.[9] However, the efficacy of FLT3 inhibitors used as monotherapy in clinical trials was limited, and few patients achieved deep or durable responses.[10] Resistance and relapse in patients who achieve CR usually occur within weeks to months due to the emergence of *FLT3* resistance mutations or activation of alternative pathways rendering the cells independent of *FLT3* signalling.[11] Therefore, new therapies to combine FLT3 inhibitors with other anti-leukaemic agents are an unmet clinical need.

To improve the treatment value of FLT3 inhibitors, studies are investigating their use in combination treatment with hypomethylating agents (HMAs) or with other targeted therapies. In a prospective clinical study, the phase 3 trial (NCT02752035) evaluated the efficacy of gilteritinib plus azacitidine versus azacitidine alone in newly diagnosed *FLT3*-mutated AML ineligible for intensive chemotherapy. The composite complete response (CRc) rates were higher in the gilteritinib plus azacitidine arm compared to that in the azacitidine arm (58.1% vs. 26.5%, $p < 0.001$).[12] Recently, preclinical data suggest potent synergy between venetoclax and FLT3 inhibitors in AML cell lines and primary patient blasts.[13–15] For mechanism, FLT3 inhibitors induced downregulation of *MCL-1*, thus enhancing the activity of venetoclax.[13] Venetoclax also re-sensitized FLT3 TKI-resistant cells to gilteritinib or sorafenib treatment, mediated through *MAPK* pathway inhibition.[14] In addition, another prospective clinical trial demonstrated that the combination of venetoclax and gilteritinib was also associated with a high modified composite complete response (mCRc) rate (CR + CRh + CR with incomplete platelet recovery [CRi] + morphological leukaemia-free state [MLFS]) and molecular remission regardless of prior FLT3 inhibitor exposure.[16]

Therefore, gilteritinib combination therapy might induce earlier, deeper remission and a more durable response in R/R *FLT3*-mutated AML. We retrospectively analysed the clinical efficacy and safety of gilteritinib combined with HMAs, venetoclax or both in R/R *FLT3*-mutated AML.

PATIENTS AND METHODS

Patients

We conducted a multicentre, retrospective analysis that included R/R *FLT3*-mutated AML (de novo) patients who received gilteritinib-based triplet therapy (gilteritinib plus HMA and venetoclax, G + HMA + VEN) or doublet therapy (gilteritinib plus HMA, G + HMA; gilteritinib plus venetoclax, G + VEN) from June 2020 to April 2023.

The study included a consecutive series of patients. Eligible criteria include patients aged ≥18 years diagnosed with R/R de novo AML who failed at least one prior therapy. The *FLT3-ITD* mutation (*ITD* or *ITD/TKD*) in the bone marrow or peripheral blood was confirmed by next-generation sequencing (NGS) or PCR. Patients had an Eastern Cooperative Oncology Group performance status of 0–2, adequate liver and kidney function, and no history of advanced heart failure or long-QT syndrome. Previous exposure to venetoclax, HMA and/or FLT3 TKIs was allowed.

Cytogenetic evaluation used standard metaphase chromosome karyotype analysis (R binding). *FLT3* mutations and other associated mutations were identified utilizing NGS integrated the entire exon or hotspot regions of genes that are frequently mutated in myeloid malignancies (the gene lists of the NGS panel are shown in Table S1). The risk stratification and cytogenetic evaluation were assessed based on the 2022 European LeukemiaNet (ELN) criteria[17] and 2023 National Comprehensive Cancer Network (NCCN) guidelines.[18]

Treatment

Gilteritinib was given orally at a dose of 80 or 120 mg/day from day (d) 1 to 28. Azacitidine was administrated at a dose of 75 mg/m^2/day subcutaneously or intravenously d1–7, or decitabine was administrated at a dose of 20 mg/m^2 intravenously d1–5. Venetoclax was given by oral administration at a dose of 100 mg d1, 200 mg d2 and 400 mg d3–28. Six patients previously exposed to venetoclax were administrated at a dose of 400 mg d1–28, dose adjustments were implemented for concomitant CYP3A inhibitors

(https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/208573s009lbl.pdf).

Response and outcomes

Response was assessed at the completion of one or two cycles of treatment according to 2022 ELN criteria.[17] The CRc (CR + CRh + CRi) and modified CRc (mCRc, CRc + MLFS) rates were assessed. Minimal residual disease (MRD) was assessed using multiparameter flow cytometry (MFC-MRD) with a minimum sensitivity of 0.1%[17] or quantitative polymerase chain reaction (qPCR, Molecular-MRD, Mol-MRD). The median time to CRc or mCRc was defined as the time from the date of treatment to CRc or mCRc. OS was measured from the day of treatment until death or the last follow-up date. Duration of remission (DOR) was measured from the date of mCRc achieved until the date of relapse, death or the last follow-up date. Early death was defined as death from any cause within 30 days after treatment. Adverse events (AEs) were assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events 5.0.

Statistical analysis

We used the IBM SPSS Statistics package, version 19.0 (SPSS, Chicago, USA), to assess differences between groups. One-way ANOVA was used for the comparison of continuous variables, and the Fisher exact test was used for the comparison of categorical variables. OS and DOR were assessed as medians with 95% CI by the Kaplan–Meier method and log-rank tests. A difference of $p < 0.05$ indicated significant significance.

RESULTS

Patient characteristics

Thirty-three patients were enrolled between June 2020 and April 2023, of whom 18 patients received G + HMA + VEN treatment, 11 patients received G + HMA treatment and the other 4 patients received G + VEN treatment. The baseline characteristics of each group are shown in Table 1, and detailed information for each patient is shown in Table S2. As for relapsed patients, all patients were haematological relapse confirmed by bone marrow examination. Among patients treated with G + HMA + VEN, 12 patients were R/R and the remaining 6 patients failed to one cycle of induction therapy. All patients were R/R in the G + HMA or G + VEN group. Most of the patients had *FLT3-ITD* mutation apart from two patients gained *FLT3-ITD/TKD* dual mutations. The median age was 56.0 (ranging 27.0–88.0, G + HMA + VEN), 58.0 (range 37.0–76.0, G + HMA) and 69.0 (range 51.0–78.0, G + VEN) years at diagnosis respectively. A total of 2 (11.1%, G + HMA + VEN), 1 (9.1%, G + HMA) and 1 (25.0%, G + VEN) had an adverse cytogenetic profile according to the 2023 NCCN guidelines. A total of 8 (44.4%, G + HMA + VEN), 3 (27.3%, G + HMA) and 1 (25.0%, G + VEN) were at adverse risk according to the 2022 ELN criteria. At diagnosis, the median baseline white blood cell (WBC) count was 50.2×10^9/L (range $2.5–462.0 \times 10^9$/L, G + HMA + VEN), 41.6×10^9/L (range $2.4–207.7 \times 10^9$/L, G + HMA) and 77.8×10^9/L (range $30.1–199.0 \times 10^9$/L, G + VEN). The median percentage of bone marrow blasts was 77.0% (range 27.0–97.4%,

TABLE 1 Basic characteristics.

	G + HMA + VEN ($n = 18$)	G + HMA ($n = 11$)	G + VEN ($n = 4$)
Age, median (years)	56.0	58.0	69.0
Male/female, *N* (%)	12/6 (66.7)	5/6 (45.5)	3/1 (75.0)
Disease status, *N* (%)			
Relapsed/refractory	12 (66.7)	11 (100.0)	4 (100.0)
Induction failure	6 (33.3)	0 (0.0)	0 (0.0)
FLT3 mutation type, *N* (%)			
ITD	17 (94.4)	10 (90.9)	4 (100.0)
ITD and TKD	1 (5.6)	1 (9.1)	0 (0.0)
ELN criteria, *N* (%)			
Intermediate	10 (55.6)	8 (72.7)	3 (75.0)
Adverse	8 (44.4)	3 (27.3)	1 (25.0)
Cytogenetics, *N* (%)			
Intermediate	16 (88.9)	10 (90.9)	3 (75.0)
Adverse	2 (11.1)	1 (9.1)	1 (25.0)
Baseline counts, median			
WBC $\times 10^9$/L	50.2	41.6	77.8
ANC $\times 10^9$/L	4.3	4.0	4.1
Haemoglobin, g/dL	85.0	83.0	68.5
Platelet, $\times 10^9$/L	40.0	69.0	84.5
Peripheral blood blast (%)	62.5	60.0	41.5
Bone marrow blast (%)	77.0	75.0	83.5
Induction therapy			
Intensive therapy	12 (66.7)	9 (81.8)	1 (25.0)
Reduced intensive therapy	6 (33.3)	2 (18.2)	3 (75.0)
Previous therapy, *N* (%)			
Cycles of prior therapy, median	2	5	4
Prior exposure to TKI	6 (33.3)	5 (45.5)	1 (25.0)
Prior exposure to HMA	10 (55.6)	7 (63.6)	3 (75.0)
Prior exposure to VEN	14 (77.8)	10 (90.9)	3 (75.0)
Prior HSCT	1 (5.6)	1 (9.1)	0 (0.0)
HMA received, *N* (%)			
Azacitidine	15 (83.3)	11 (100.0)	/
Decitabine	3 (16.7)	0 (0.0)	/

Abbreviations: HMA, hypomethylating agent; HSCT, haematopoietic stem cell transplantation; *ITD*, internal tandem duplications; *TKD*, tyrosine kinase domain; *TKI*, tyrosine kinase inhibitor; VEN, venetoclax.

G + HMA + AZA), 75.0% (range 40.0%–88.0%, G + HMA) and 83.5% (range 77.0–85.0%, G + VEN).

Due to age, complications or personal intensions, not all patients received intensive chemotherapy (IC) as induction chemotherapy. Some patients received reduced-intensive chemotherapy (RIC). There are 12 (66.7%) patients, 9 (81.8%) patients and 1 (25.0%) patient who received IC as induction chemotherapy in the G + HMA + VEN, G + HMA and G + VEN groups, and the other patients received RIC. Unfortunately, midostaurin is not yet available in China, so all patients have not received midostaurin as part of first-line therapy. The median number of prior therapies was 2 (range 2–13, G + HMA + VEN), 5 (range 3–12, G + HMA) and 4 (range 3–10, G + VEN) respectively. Most of the patients had prior exposure to venetoclax (14/18 [77.8%], G + HMA + VEN; 10/11 [90.9%], G + HMA; 3/4 [75.0%], G + VEN). As for HMA, there were 10 patients (55.6%, G + HMA + VEN), 7 patients (63.6%, G + HMA) and 3 patients (75.0%, G + VEN) had prior exposure respectively. For TKI, six patients (33.3%, G + HMA + VEN), five patients (45.5%, G + HMA) and one patient (25.0%, G + VEN) were previously exposed. There were two patients who had relapsed post alloHSCT; one patient received G + HMA + VEN treatment, and the other patient received G + HMA treatment.

Response

The response of patients was summarized in Table 2 and more detail information was shown in Figure 1. Of 33 patients across all subgroups, 16 patients (48.5%) achieved CRc and 23 patients (69.7%) achieved mCRc. In a subgroup analysis, The CRc and mCRc rate were 66.7% (12/18) and 88.9% (16/18), 18.2% (2/11) and 45.5% (5/11), 50.0% (2/4) and 50.0% (2/4) in patients who received G + HMA + VEN, G + HMA and G + VEN respectively (Figure 2). The CRc and mCRc rates were higher in the G + HMA + VEN group compared with that in the G + HMA group (CRc, $p = 0.030$; mCRc, $p = 0.033$), but there is no statistic difference between the G + HMA + VEN and G + VEN groups (CRc, $p = 0.799$; mCRc, $p = 0.244$). The median time to CRc in the G + HMA + VEN, G + HMA and G + VEN groups were 27.5 days, 70.0 days and 36.0 days respectively (Figure S1A). The median time to mCRc were 28.0 days, 63.0 days and 36.0 days respectively (Figure S1B). In addition, the higher percentage of CRc with MRD negativity was observed in the G + HMA + VEN group (MFC-MRD: 100.0%; Mol-MRD: 80.0%) compared to that in the G + HMA group (MFC-MRD: 50.0%; Mol-MRD: 0.0%) and in the G + VEN group (MFC-MRD: 100%; Mol-MRD: 50%). It is noteworthy that, one patient who relapsed after alloHSCT still obtained CR after the G + HMA + VEN treatment. However, another patient who relapsed after alloHSCT, failed to response under G + HMA therapy. Five (5/33, 15.2%) patients achieved CRc eventually underwent alloHSCT, including four patients treated with G + HMA + VEN (4/18, 22.2%) and one patient treated with G + HMA (1/11, 9.1%). The relatively more common co-mutation among the entire cohort involved *NPM1* (14/33), *DNMT3A* (9/33), *TET2* (9/33), *NRAS* (6/33), *CEBPA* (5/33), *BCOR* (4/33), *STAG2* (4/33), *IDH2* (4/33), *RUNX1* (3/33) and *SF3B1* (3/33). Of which, mutations of *NRAS* (5/6, 83.3%), *BCOR* (3/4, 75.0%) and *STAG2* (3/4, 75.0%) were relatively enriched in patients failed to achieve CRc (Figure 1).

TABLE 2 Efficacy.

	G + HMA + VEN ($n = 18$)	G + HMA ($n = 11$)	G + VEN ($n = 4$)
CR, *N* (%)	6 (33.3)	0 (0.0)	1 (25.0)
CRh, *N* (%)	1 (5.6)	0 (0.0)	0 (0.0)
CRi, *N* (%)	5 (27.8)	2 (18.2)	1 (25.0)
MLFS, *N* (%)	4 (22.2)	3 (27.3)	0 (0.0)
PR, *N* (%)	0 (0.0)	0 (0.0)	0 (0.0)
NR, *N* (%)	2 (11.1)	6 (54.5)	2 (50.0)
CRc (CR + CRh + CRi), *N* (%)	12 (66.7)	2 (18.2)	2 (50.0)
CRc with MRD−, *N* (%)			
MFC	12/12 (100.0)	1/2 (50.0)	2/2 (100.0)
qPCR	8/10 (80.0)	0/1 (0.0)	0/2 (0.0)
mCRc (CRc + MLFS), *N* (%)	16 (88.9)	5 (45.5)	2 (50.0)
Median time to CRc (days)	27.5	70.0	`36.0
Median time to mCRc (days)	28.0	63.0	`36.0
Early death, *N* (%)			
<30 days	0 (0.0)	0 (0.0)	0 (0.0)
HSCT post, *N* (%)	4 (22.2)	1 (9.1)	0 (0.0)

Abbreviations: CR, complete remission; CRc, composite complete remission; CRh, CR with partial haematological recovery; CRi, CR with incomplete blood count recovery; HSCT, haematopoietic stem cell transplantation; mCRc, modified composite complete remission; MFC, multiparameter flow cytometry; MLFS, morphological leukaemia-free state; MRD, minimal residual disease; NR, no response; PR, partial remission; qPCR, quantitative polymerase chain reaction.

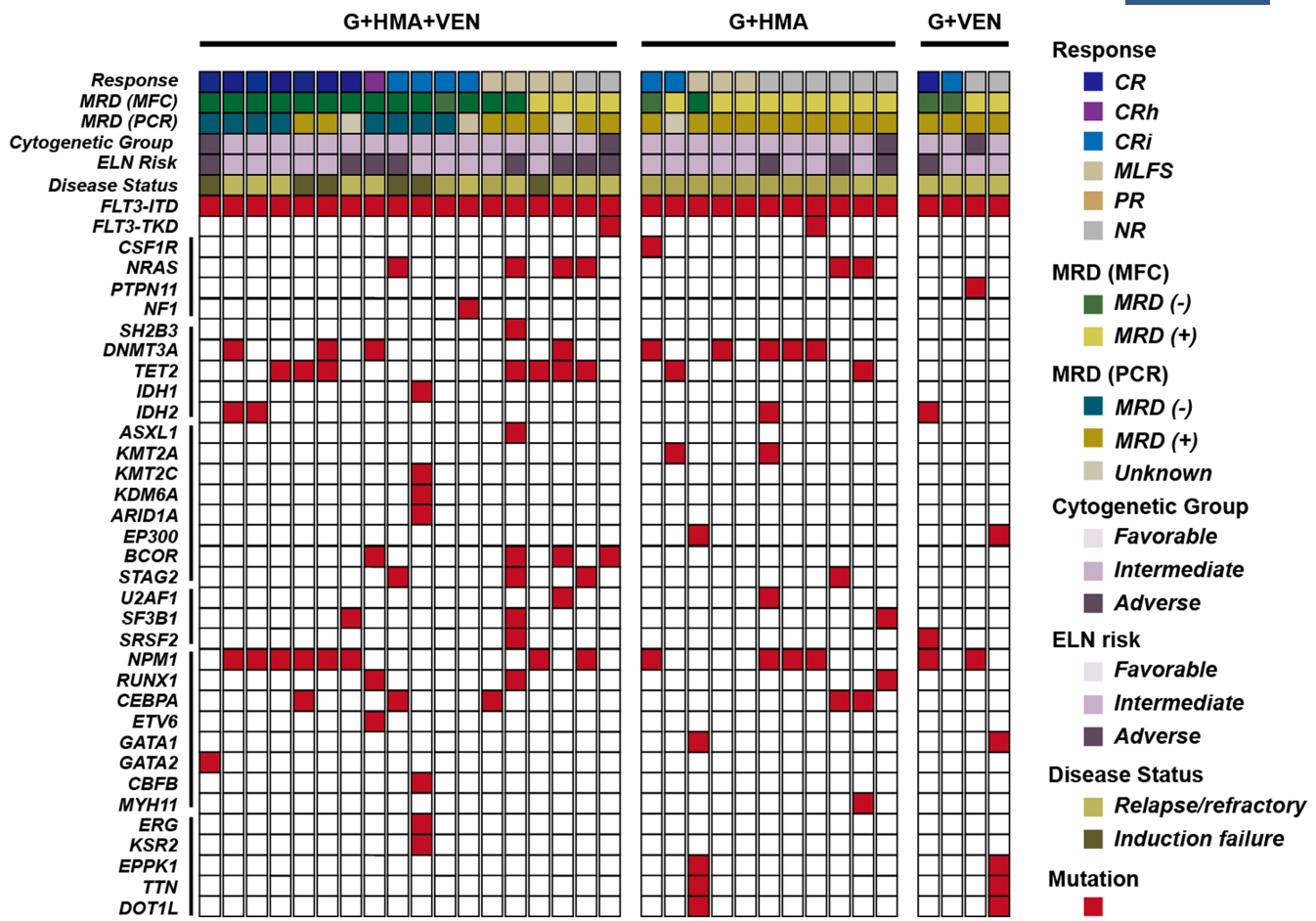

FIGURE 1 Oncoprint showing molecular landscape and responses in patients. Each column represents an individual patient. CR, complete response; CRh, CR with partial haematological recovery; CRi, CR with incomplete blood count recovery; MLFS, morphological leukaemia-free state; PR, partial response; NR, no response; ELN, European LeukemiaNet; MRD, measurable residual disease; MFC, multiparameter flow cytometry; PCR, polymerase chain reaction.

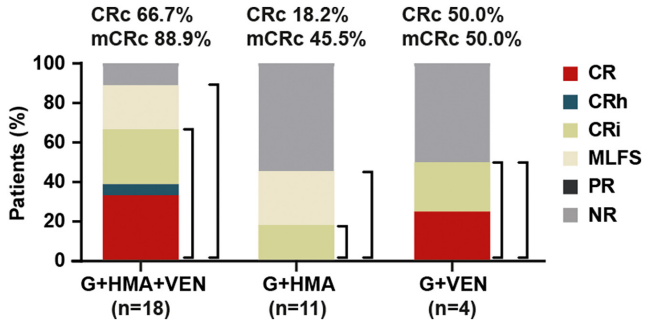

FIGURE 2 Response for all patients treated with G + HMA + VEN ($n = 18$), G + HMA ($n = 11$) or G + VEN ($n = 4$). CRc was defined as CR + CRh + CRi. CR, complete response; CRh, CR with partial haematological recovery; CRi, complete response with incomplete blood count recovery; mCRc was defined as CRc + MLFS; MLFS, morphological leukaemia-free state (G + HMA + VEN vs. G + HMA group, CRc $p = 0.030$; mCRc $p = 0.033$; G + HMA + VEN vs. G + VEN group, CRc $p = 0.799$; mCRc $p = 0.244$).

With a median follow-up of 224.0 days for all patients received gilteritinib-based combination therapy, the median OS was 353.0 days while the median DOR was 159.0 days (Figure S2a,b). As for subgroup analysis, the median follow-up of the patients in the G + HMA + VEN, G + HMA and G + VEN was 214.0 days, 324.0 days and 224.0 days, respectively, and the median OS was not reached, 160.0 days and 231.0 days. In patients treated with G + HMA + VEN, the median OS was significantly longer compared with that in the G + HMA group, but no significant difference was observed when compared with the G + VEN groups (G + HMA + VEN vs. G + HMA, HR 0.244, 95% CI 0.073–0.813, $p = 0.012$; G + HMA + VEN vs. G + VEN, HR 0.440, 95% CI 0.055–3.525, $p = 0.484$, Figure 3A). Median DOR was not reached, 82.0 days, 77.0 days for the G + HMA + VEN, G + HMA and G + VEN subgroups respectively (G + HMA + VEN vs. G + HMA, HR 0.358, 95% CI 0.088–1.459, $p = 0.072$; G + HMA + VEN vs. G + VEN, HR 0.223, 95% CI 0.014–3.423, $p = 0.040$, Figure 3B). When censored at alloHSCT, median OS was not reached: 131.0 days and 231.0 days for the G + HMA + VEN, G + HMA and G + VEN subgroups respectively (G + HMA + VEN vs. G + HMA, HR 0.322, 95% CI 0.094–1.101, $p = 0.055$; G + HMA + VEN vs. G + VEN, HR 0.631, 95% CI 0.097–4.110, $p = 0.590$, Figure 3C). When censored at alloHSCT, median DOR was 159.0 days, 59.0 days,

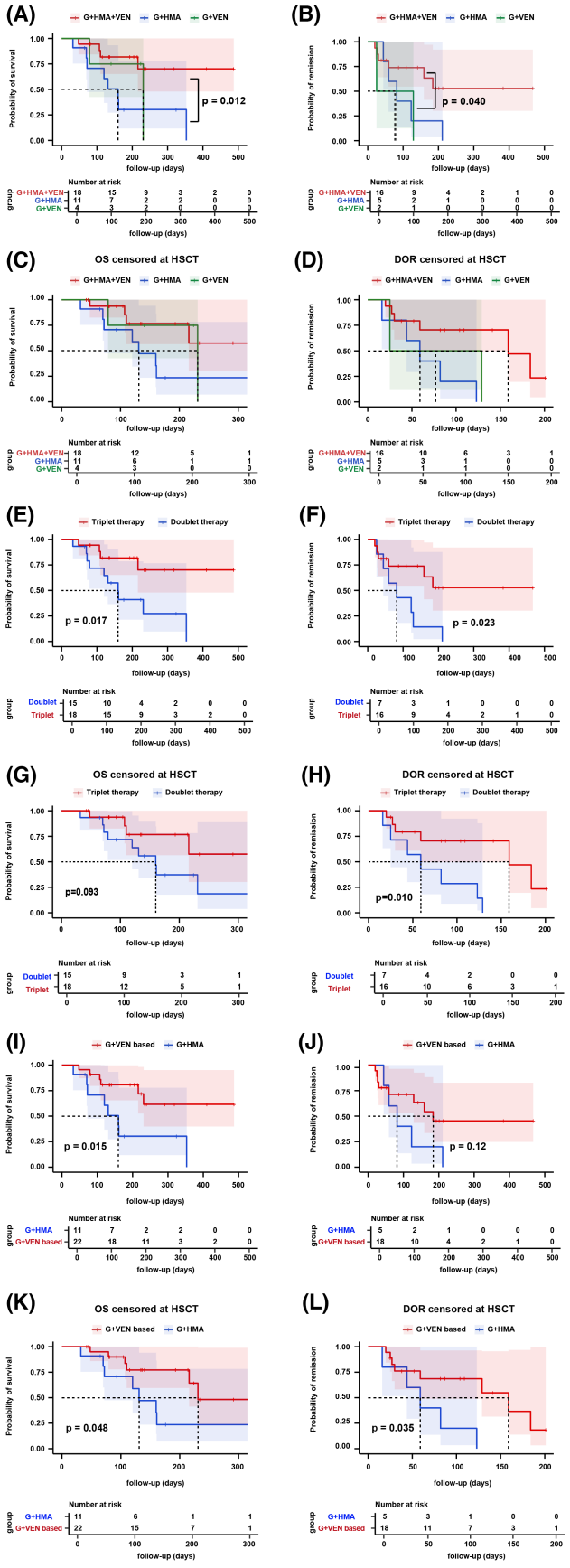

FIGURE 3 (A) Overall survival (OS) for all patients treated with G + HMA + VEN, G + HMA or G + VEN therapy (G + HMA + VEN vs. G + HMA, $p = 0.012$; G + HMA + VEN vs. G + VEN, $p = 0.328$). (B) Duration of remission (DOR) for all patients treated with G + HMA + VEN, G + HMA or G + VEN therapy (G + HMA + VEN vs. G + HMA, $p = 0.072$; G + HMA + VEN vs. G + VEN, $p = 0.040$). (C) Overall survival (OS) for all patients treated with G + HMA + VEN, G + HMA or G + VEN therapy censored at alloHSCT (G + HMA + VEN vs. G + HMA, $p = 0.055$; G + HMA + VEN vs. G + VEN, $p = 0.590$). (D) Duration of remission (DOR) for all patients treated with G + HMA + VEN, G + HMA or G + VEN therapy censored at alloHSCT (G + HMA + VEN vs. G + HMA, $p = 0.090$; G + HMA + VEN vs. G + VEN, $p = 0.162$). (E) Overall survival (OS) for all patients treated with triplet (G + HMA + VEN) or doublet (G + HMA, G + VEN) therapy (triplet vs. doublet, HR 0.271, 95% CI 0.093–0.787, $p = 0.017$). (F) Duration of remission (DOR) for all patients treated with triplet (G + HMA + VEN) or doublet (G + HMA, G + VEN) therapy (triplet vs. doublet therapy, HR 0.309, 95% CI 0.090–1.058, $p = 0.023$). (G) Overall survival (OS) for all patients treated with triplet (G + HMA + VEN) or doublet (G + HMA, G + VEN) therapy censored at alloHSCT (triplet vs. doublet, HR 0.380, 95% CI 0.128–1.130, $p = 0.093$). (H) Duration of remission (DOR) for all patients treated with triplet (G + HMA + VEN) or doublet (G + HMA, G + VEN) therapy censored at alloHSCT (triplet vs. doublet, HR 0.288, 95% CI 0.079–0.986, $p = 0.010$). (I) Overall survival (OS) for all patients treated with G + VEN-based therapy or G + HMA therapy (G + VEN-based vs. G + HMA, HR 0.293, 95% CI 0.091–0.939, $p = 0.015$). (J) Duration of remission (DOR) for all patients treated with G + VEN-based therapy or G + HMA therapy (G + VEN-based vs. G + HMA, HR 0.424, 95% CI 0.112–1.613, $p = 0.120$). (K) Overall survival (OS) for all patients treated with G + VEN-based therapy or G + HMA therapy censored at alloHSCT (G + VEN based vs. G + HMA, HR 0.317, 95% CI 0.095–1.049, $p = 0.048$). (L) Duration of remission (DOR) for all patients treated with G + VEN-based therapy or G + HMA therapy censored at alloHSCT (G + VEN based vs. G + HMA, HR 0.336, 95% CI 0.080–1.411, $p = 0.035$).

77.0 days for the G + HMA + VEN, G + HMA and G + VEN subgroups respectively (G + HMA + VEN vs. G + HMA, HR 0.285, 95% CI 0.063–1.277, $p = 0.090$; G + HMA + VEN vs. G + VEN, HR 0.277, 95% CI 0.023–3.344, $p = 0.162$, Figure 3D). These results indicated that the median OS and DOR in these three groups were similar when censored at alloHSCT. A small sample size may limit the statistical differences and conclusiveness of group analyses. Therefore, we combined groups by analysing the OS and DOR between triplet therapy and doublet therapy (G + HMA and G + VEN, Figure 3E–H), or the OS and DOR between G + VEN-based therapy (G + HMA + VEN and G + VEN) and G + HMA (Figure 3I–L). The results showed that longer DOR was observed with triplet therapy than with doublet therapy, whether censored at end-point time (triplet vs. doublet therapy, HR 0.309, 95% CI 0.090–1.058, $p = 0.023$, Figure 3F) or alloHSCT (HR 0.288, 95% CI 0.079–0.986, $p = 0.010$, Figure 3H). Similarly, G + VEN-based therapy exhibited prolonged OS when compared with the G + HMA group, whether censored at end-point time (G + VEN based vs. G + HMA, HR 0.293, 95% CI 0.091–0.939, $p = 0.015$, Figure 3I) or alloHSCT (HR 0.317, 95% CI 0.095–1.049, $p = 0.048$, Figure 3K). As for DOR, there was no significant

difference between G + VEN-based therapy and G + HMA therapy censored at the last follow-up day (HR 0.424, 95% CI 0.112–1.613, $p = 0.120$, Figure 3J), but the G + VEN-based group exhibited prolonged DOR when censored at alloHSCT (HR 0.336, 95% CI 0.080–1.411, $p = 0.035$, Figure 3L).

As we mentioned, there were four patients in the G + HMA + VEN group who received alloHSCT after remission. To detect the clinical significance of alloHSCT, we compared the median OS and DOR stratified by alloHSCT received or not in the G + HMA + VEN group. The results showed that the median OS was not reached for both the alloHSCT and non-alloHSCT groups. Even though there was no significant difference in the median OS between the alloHSCT and non-alloHSCT groups (HR = 0.229, 95% CI 0.026–2.006, $p = 0.183$, Figure 4A), the median DOR was significantly longer in patients who received alloHSCT (HR = 0.154, 95% CI 0.027–0.868, $p = 0.034$, Figure 4B).

Adverse effects

There were no grade 5 adverse effects (AEs) or tumour lysis observed across the three groups. Grade 3/4 AEs of gilteritinib combination therapy were summarized in Table 3, and no significant differences were observed among the G + HMA + VEN, G + HMA and G + VEN subgroups. More details, such as gastrointestinal (GI) AEs, impaired hepatic or renal function and electrolyte abnormalities, were shown in Table S3. As for haematological adverse effects, grade 3/4 neutropenia, anaemia and thrombocytopenia in the G + HMA + VEN, G + HMA and G + VEN groups were 94.4% vs. 100.0% vs. 50.0%; 83.3% vs. 90.9% vs. 75.0%; and 77.8% vs. 100.0% vs. 75.0% respectively. Nine patients (50.0%), six patients (54.5%) and one patient (25.0%) developed febrile neutropenia after receiving the G + HMA + VEN or G + HMA or G + VEN therapy. The most common non-haematological AE was pulmonary infection, with 10 of these patients (G + HMA + VEN: $n = 5$, 27.8%; G + HMA: $n = 4$, 36.4%; G + VEN: $n = 1$, 25.0%) developed.

DISCUSSION

Given the moderate effects of gilteritinib monotherapy in R/R *FLT3*-mutated AML patients, we explored the efficacy of gilteritinib-based combination therapies in the real world. To our knowledge, this is the first retrospective study to evaluate the different clinical responses of gilteritinib-based therapies with HMA, venetoclax, or both in R/R *FLT3*-mutated AML.

Compared with G + HMA, our data showed that G + HMA + VEN achieved a higher CRc and mCRc rate and a higher rate of CRc with MRD negativity in patients with R/R *FLT3*-mutated AML, including those who had failed multiple prior lines of therapy and those who were exposed to TKIs or HMA and venetoclax combination therapy. Consistent with the higher CRc and mCRc rates in the G + HMA + VEN group, there was a tendency towards a longer median OS in the G + HMA + VEN group than that in the G + HMA group (G + HMA + VEN vs. G + HMA, $p = 0.012$). Moreover, more patients successfully underwent alloHSCT after achieving remission in the G + HMA + VEN group ($n = 4$, 22.2%) than that in the G + HMA group ($n = 1$, 9.1%). With a median follow-up of 324.5 days, these four

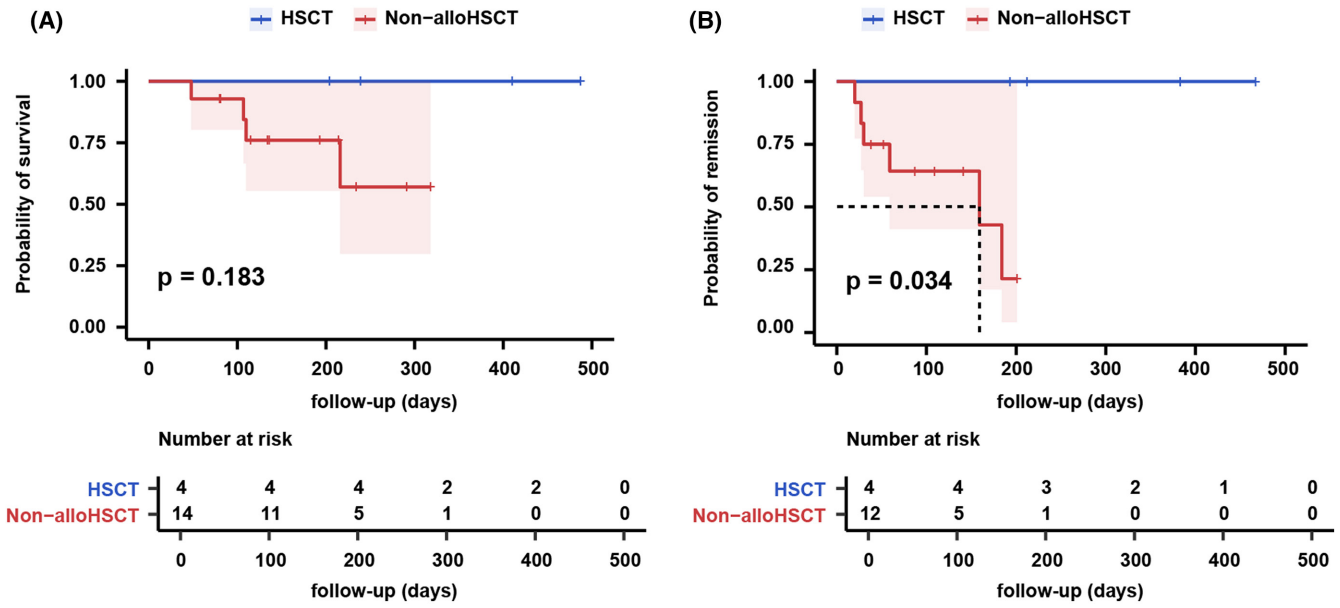

FIGURE 4 (A) Overall survival (OS) for patients who received G + HMA + VEN therapy, stratified by whether they received allogenic haematopoietic stem cell transplantation (alloHSCT) or not (HR = 0.229, 95% CI 0.026–2.006, $p = 0.183$). (B) Duration of remission (DOR) for patients who were treated with G + HMA + VEN therapy, stratified by whether they received HSCT or not (HR = 0.154, 95% CI 0.027–0.868, $p = 0.034$).

TABLE 3 Adverse effects (Grade 3/4).

	G + HMA + VEN (*n* = 18)	G + HMA (*n* = 11)	G + VEN (*n* = 4)
Haematological AEs, *N* (%)			
Neutropenia	17 (94.4)	11 (100.0)	2 (50.0)
Anaemia	15 (83.3)	10 (90.9)	3 (75.0)
Thrombocytopenia	14 (77.8)	11 (100.0)	3 (75.0)
Febrile neutropenia, *N* (%)	9 (50.0)	6 (54.5)	1 (25.0)
Infections, *N* (%)			
Pneumonia	5 (27.8)	4 (36.4)	1 (25.0)
Bacteraemia	3 (16.7)	2 (18.2)	1 (25.0)
Septicaemia	1 (5.6)	3 (27.3)	0 (0.0)
Respiratory AEs, *N* (%)			
Cough	0 (0.0)	0 (0.0)	0 (0.0)
Dyspnoea	1 (5.6)	0 (0.0)	0 (0.0)
Respiratory failure	0 (0.0)	1 (9.1)	0 (0.0)

patients maintain the status of CR with MRD negativity in the G + HMA + VEN group. Unfortunately, with a limited sample size, there was probably not sufficient power to detect a statistical variation between the G + HMA + VEN and G + VEN groups. According to the prospective clinical study,[16] mCRc rate of G + VEN therapy in R/R FLT3-mutated AML patients was 75% (CR, 18%; CR with incomplete blood count recovery, 4%; CR with incomplete platelet recovery, 18%; and MLFS, 36%). In addition, the clinical trial indicated that G + VEN therapy induced deep molecular responses, with 60% of evaluable responding patients achieving *FLT3-ITD* clearance ($<10^{-2}$) and 12% reaching undetectable levels ($<10^{-6}$). To match the same criteria as the clinical study, we also calculated the mCRc rate of gilteritinib-based combination therapy. In our retrospective study, the mCRc rate in G + HMA + VEN was 88.9% (CRc, 66.7% and MLFS, 22.2%), which was slightly higher compared to the clinical trial reported. Furthermore, based on previous studies, results have been reported using gilteritinib-based combinations in patients with R/R *FLT3*-mutated AML, with a range of CR/CRi rates between 20.0% and 62.0%.[16,19,20] Coincidentally, the highest CRc rate (62.0%) among these studies was observed after *FLT3* inhibitor plus venetoclax and decitabine triplet therapy in R/R FLT3-mutated AML.[20] These results may support the efficacy of gilteritinib combined with HMA and venetoclax for R/R *FLT3*-mutated AML. However, further trials with more patients enrolled are required to determine whether G + HMA + VEN has advantages over other gilteritinib-based combination therapies.

In our study, a small portion of patients successfully underwent alloHSCT after remission. The response and survival of patients receiving alloHSCT after G + HMA + VEN chemotherapy were particularly encouraging. The results suggest that G + HMA + VEN therapy may serve as an effective therapy bridge to transplantation and prolong survival in patients with R/R *FLT3*-mutated AML. As for those R/R patients who are not eligible for transplantation, long-term maintenance of the triplet chemotherapy may also offer broader activity and better responses, according to our preliminary results. It is needed to balance the response and the potential risks of increased myelosuppression and serious infections, especially in elder patients. Optimizing the treatment by dose or duration adjustment may be needed. Due to the small sample size in each group, we combined groups by analysing the OS and DOR between triplet therapy and doublet therapy, or the OS and DOR between G + VEN-based therapy and G + HMA. The results indicated triplet therapy and G + VEN-based therapy may benefit R/R AML patients with the *FLT3-ITD* mutation. And a larger prospective study is required to further clarify the potential of the gilteritinib-based combination regimen.

Even though the favourable outcomes of G + HMA + VEN therapy, we only explored the efficacy of gilteritinib-based combination regimens in primary de novo *FLT3-ITD* patients. Many factors that may influence the response should be considered for further application. The long-term follow-up of the phase 3 ADMIRAL (NCT02421939) trial showed that, compared with salvage chemotherapy, the survival benefit of gilteritinib was maintained in the *FLT3-ITD* mutation subgroup but not in the *FLT3-TKD* subgroup.[10] With limited data, patients with only the *FLT3-TKD* mutation were not included in the study. Therefore, more patients with *FLT3-TKD* should be enrolled to further evaluate the consequences of gilteritinib-base combination therapy. As for secondary AML, two patients in the G + HMA + VEN group and one patient in the G + HMA group were identified as having no response after treatment (data not shown), which showed a trend towards an inferior response. In a previous study, prior exposure to TKI (sorafenib, quizartinib) was another factor that may also influence the response to gilteritinib-based therapy.[21,22] In our study, six patients received ≥1 prior FLT3 TKI treatment in the G + HMA + VEN group. CRc rates were 66.7% vs. 66.7% for those with or without prior FLT3 TKI exposure, and mCRc were 83.3% vs. 58.3% for those with or without prior FLT3 TKI exposure, which indicated that the combination of HMA and VEN may overcome the acquired resistance of gilteritinib and thus potentiate efficacy. Beyond the above factors, we are also interested in the relationship between co-mutations of *FLT3* and the response of gilteritinib-based therapy. With limited data, we found that the baseline *NRAS*, *BCOR* and *STAG2* gene mutations (especially *NRAS*) may be associated with treatment failure for gilteritinib-based therapy. Similar findings were observed in the study by Daver and colleagues; they showed that the pretherapy *RAS* mutations were related to primary resistance in patients treated with type I FLT3 inhibitor (gilteritinib, midostaurin and crenolanib)-based therapies, especially in patients who had pretherapy *RAS* variant allelic frequencies (VAF) >20%.[23] However, by analysing molecular profile of *FLT3*-mutated

R/R AML patients in the phase 3 ADMIRAL study, the acquisition of multiple *Ras/MAPK* pathway gene mutations at relapse, instead of at diagnosis, was related to treatment resistance to gilteritinib.[24] Perl and colleagues also found that the acquired expansion of clones containing mutations in the *RAS* pathway, primarily *NRAS* and *KRAS*, was a common and clinically important mechanism of secondary resistance to gilteritinib.[11] Therefore, it suggests RAS mutations, either pretherapy or acquired mutations, may contribute to resistance of FLT3 inhibitor-based therapies.

There were some limitations to our retrospective study. First of all, even though it was a multicentre study, our study was limited by a small sample size and a short follow-up period. The results shown are preliminary and inadequate for a final clinical decision. Second, we evaluated the response after one or two cycles of chemotherapy, because most of the patients only received one or two cycles of gilteritinib-based therapy. Other patients changed therapeutic regimens if CRc or mCRc were not achieved. Maybe the response for each group could be further improved if more cycles of such treatment were given. With the inherent flaws of retrospective analysis, selection bias as well as heterogeneous clinical backgrounds are inevitable. Nevertheless, all the patients who met the eligible criteria in consecutive series have been reported, and no patient with poor responders/early death was missing.

In summary, gilteritinib combined with HMA and venetoclax may represent a safe and favourable regimen for R/R *FLT3*-mutated AML patients. Moreover, it may serve as an effective therapy bridge to transplantation. Prospective random trials are warranted to further define the efficacy of the gilteritinib-based combination therapy.

AUTHOR CONTRIBUTIONS

Nianci Chen and Jiajia Pan designed the study, analysed the data and wrote the manuscript; Yile Zhou, Liping Mao, Yinjun Lou, Jiejing Qian, Gaixiang Xu, Juying Wei, De Zhou, Lihong Shou, Li Huang, Minchao Yan, Hui Zeng, Cuihua Fan, Gongqiang Wu and Weiying Feng collected the data and reviewed the manuscript; Hongyan Tong reviewed the manuscript; Jie Jin designed the study and reviewed the manuscript; Huafeng Wang designed the study, supervised the research, analysed the data and wrote the manuscript. All authors interpreted the data and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors have no competing interests to declare.

DATA AVAILABILITY STATEMENT

All data associated with this study are present in the paper or the supplementary materials. Supplementary information is available at *British Journal of Haematology*'s website.

ETHICS STATEMENT

This study was approved by the ethical review committees of the First Affiliated Hospital of Zhejiang University School of Medicine (IIT 20230557A). All procedures in studies involving human participants were performed in accordance with the ethical standards of the institutional research committee and with the Helsinki Declaration.

PATIENT CONSENT STATEMENT

Informed consent was obtained from all the patients.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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