Venetoclax in combination with hypomethylating agents in newly diagnosed and relapsed *CBFβ::MYH11*$^{+}$ acute myeloid leukemia

The combination of venetoclax (Ven) and a hypomethylating agent (HMA) has become the standard of care for newly diagnosed older/unfit acute myeloid leukemia (AML) patients who are ineligible for intensive chemotherapy.[1] Due to the simplicity, effectiveness, and reduced toxicity of this regimen, ongoing research is investigating its wider application both in the frontline and in the relapse setting. However, data on its effectiveness in patients with core-binding factor (CBF) AML are limited, as these patients are often excluded from most studies involving Ven.[2,3] Although CBF (+) AML is relatively sensitive to chemotherapy and less common in elderly patients aged 75 and above, totally excluding CBF (+) AML arbitrarily is not justifiable, as other favorable-risk AML, such as AML with normal cytogenetics, *NPM1* mutations without *FLT3*-internal tandem duplications (ITD), and AML with *bZIP* in-frame mutations in *CEBPA*, can also benefit from Ven + HMA treatment;[4,5] moreover, not every patient is suitable for an intensified regimen. Our previous research demonstrated that young and unfit patients with *CBFβ::MYH11*$^{+}$ AML have a greater response to Ven + HMA induction therapy compared to those with *RUNX1::RUNX1T1* AML.[6] Encouraged by these promising preliminary findings, we performed a larger case series to assess the efficacy of Ven + HMA in patients with *CBFβ::MYH11*$^{+}$ AML.

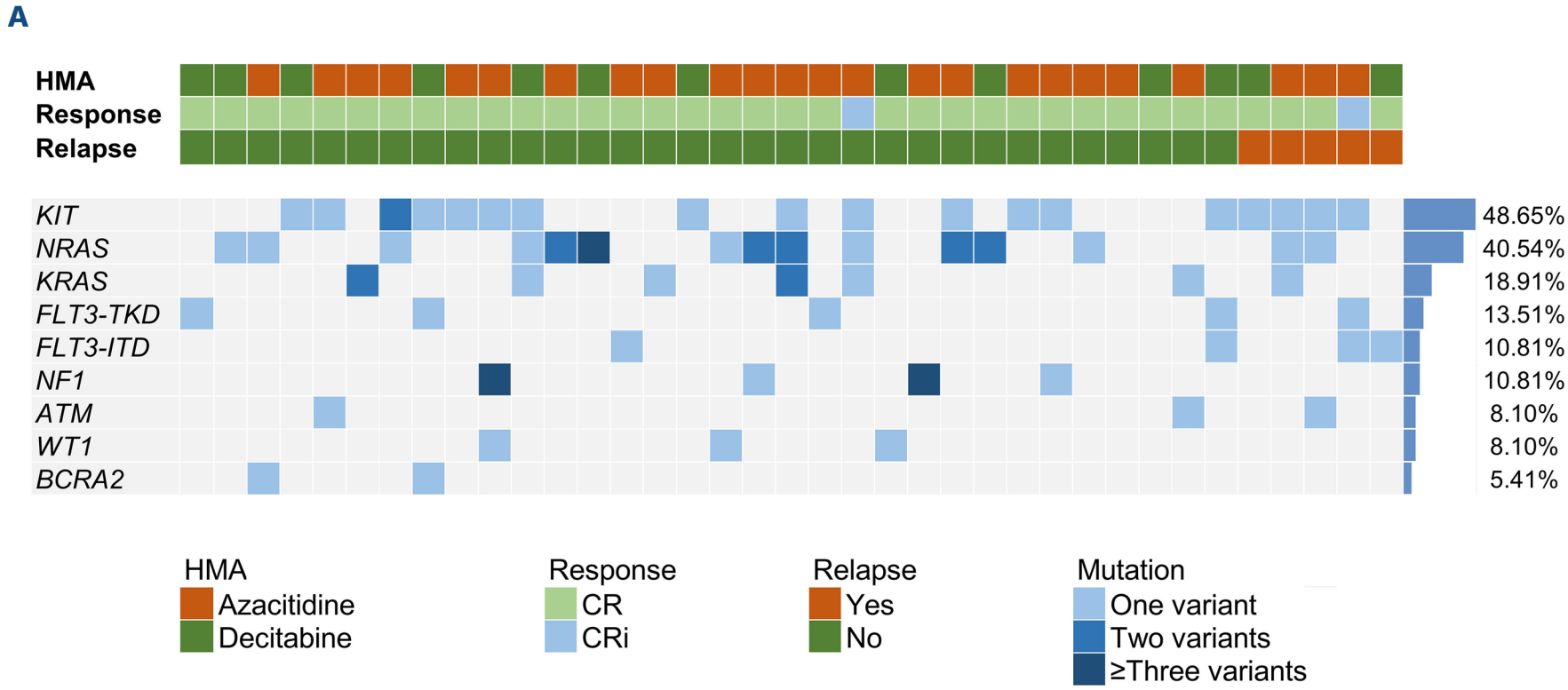
A total of 40 patients with *CBFβ::MYH11*$^{+}$ AML who received (re)induction treatment with Ven + HMA at the First Affiliated Hospital of Soochow University between January 2019 and May 2024 were included in this study. The treatment regimen was administered as described previously.[6] Mutational analysis was performed via a 172-gene next-generation sequencing (NGS) panel (*Online Supplementary Table S1*). Patients were divided into two cohorts: i) those with newly diagnosed AML and ii) those with relapsed AML. The primary objective of this study was to evaluate the overall response rate, which included complete remission (CR), CR with incomplete blood count recovery (CRi), and morphologic leukemia-free state. Bone marrow (BM) aspirates were obtained between day 21 and day 28 in cycle 1. Measurable residual disease (MRD) was monitored in BM samples using ten-color multiparameter flow cytometry (MFC) and real-time quantitative reverse transcriptase-polymerase chain reaction. The absolute copy numbers of the fusion gene transcripts were normalized to those of the *ABL* gene (expressed as copies per 10^4 copies of *ABL*). Complete molecular remission (CMR) was defined as a fusion transcript level of less than 0.01%. The choice of postinduction therapy was at the discretion of the treating physician. The probabilities of overall survival (OS) and relapse-free survival (RFS) were estimated via the Kaplan-Meier method. The data cutoff was July 31, 2024. Informed consent was obtained from all patients. The study was approved by the Research Ethics Review Committee of the First Affiliated Hospital of Soochow University.

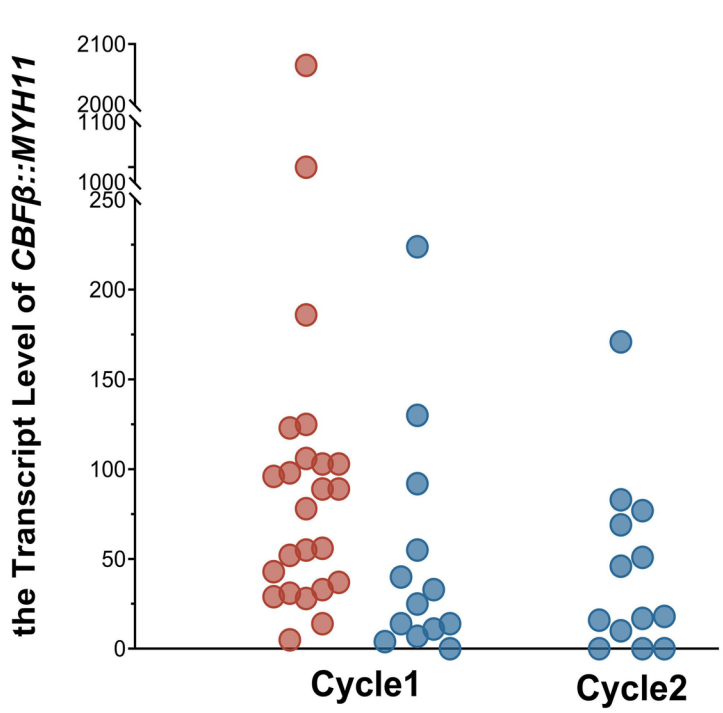
Thirty-seven patients with newly diagnosed *CBFβ::MYH11*$^{+}$ AML were treated. The baseline characteristics are shown in Table 1. The median age was 49 years (range, 15-79 years), and 23 patients were males. Fourteen fit patients received Ven + HMA because of the wishes of either the patients or their treating physicians. Thirty-three (89.2%) patients had tyrosine kinase gene mutations (including *KIT*, *N/KRAS*, and *FLT3*) (Figure 1A). Four patients discontinued Ven administration due to infections. The median duration of Ven treatment was 25 days (range, 15-28). After

Table 1. Baseline characteristics and first induction responses of the study population.

Characteristic	Newly diagnosed cohort N=37	Relapsed cohort N=7
Age in years, median (range)	49 (15-79)	51 (20-79)
Sex, N (%)		
male	23 (62.2)	6 (85.7)
female	14 (37.8)	1 (14.3)
WBC level ×10^9/L, median (range)	31.45 (1.22-147.66)	2.58 (1.67-3.39)
BM blasts %, median (range)	57.5 (14-87)	10.5 (2-43.5)
Additional cytogenetic abnormalities, N (%)	12 (32.4)	3 (42.9)
Kinase mutations, N (%)		
KIT	18 (48.6)	2 (28.6)
FLT3-ITD	4 (10.8)	1 (14.3)
FLT3 D835	3 (8.1)	0 (0)
RAS	18 (48.6)	1 (14.3)
HMA type, N (%)		
Azacitidine	24 (64.9)	6 (85.7)
Decitabine	13 (35.1)	1 (14.3)
Hematologic response, N (%)		
CR/CRi	37 (100)	4 (57.1)
MLFS	0 (0)	0 (0)
PR	0 (0)	1 (14.3)
No response	0 (0)	2 (28.6)

WBC: white blood cell; BM: bone marrow; HMA: hypomethylating agent; CR: complete remission; ITD: internal tandem duplication; CRi: complete remission with incomplete blood count recovery; MLFS: morphologic leukemia-free state; PR: partial remission.





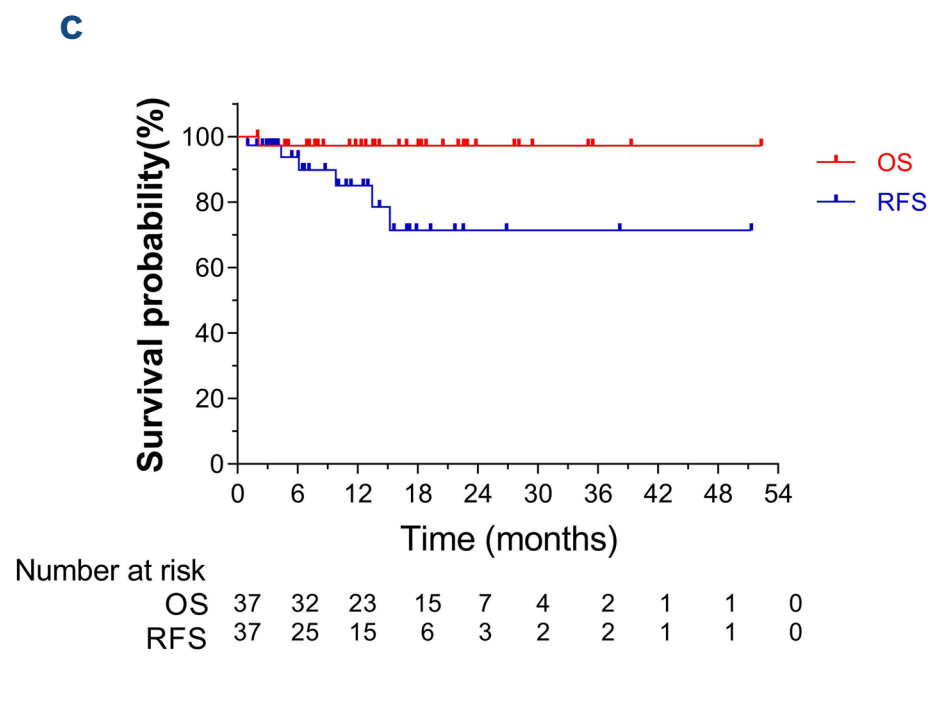


Figure 1. Clinical characteristics and outcome in 40 patients in this study. (A) Mutational landscapes (only genes with a frequency of 5% or higher are shown). (B) Real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) measurable residual disease (MRD) for *CBFβ::MYH11* after cycle 1 and cycle 2. (C) Kaplan-Meier estimate of overall survival (OS) and relapse-free survival (RFS) for the newly diagnosed cohort, RFS censored at allogeneic hematopoietic stem cell transplantation. HMA: hypomethylating agent.

the first course of induction therapy, 35 patients (94.6%) achieved CR and two patients (5.4%) achieved CRi, for a composite CR/CRi rate of 100%, with 33 (89.2%) patients achieving MRD negativity by MFC (defined as <0.1%). The median transcript level of *CBFβ::MYH11* at the end of cycle 1 was 55 copies (range, 0-2,065). The post-induction therapy was varied according to the treating physician's discretion. Except for one 79-year-old patient, all patients switched to intermediate- or high-dose cytarabine (ID/HD Ara-C) consolidation therapy, but 13 patients received a second course of Ven + HMA before transition. The median transcript level of the 13 patients after cycle 2 Ven + HMA was 18 copies (range, 0-171). CMR was achieved in three patients within two cycles of Ven + HMA (1 after the first cycle and 2 after the second cycle) (Figure 1B). The median duration of follow-up in the frontline cohort was 14 months (range, 2-52 months), and the 2-year probability of OS was 97.2% (95% confidence interval [CI]: 92-100) (Figure 1C). Overall, five patients experienced relapse after a median of 11 months (range, 6-16 months) (1 isolated central nervous system, 4 systemic relapses). All five patients had persistent detectable molecular MRD in the consolidation period. One patient died of infection during consolidation with HD Ara-C. Twelve patients proceeded to allogeneic

hematopoietic stem cell transplantation (allo-HSCT) due to persistent molecular MRD (N=10) or relapse (N=2) after a median of 5 months (range, 3-19 months). The 2-year probability of RFS censored at allo-HSCT was 71.3% (95% CI: 53.2-95.7) (Figure 1C). Patients with molecular MRD above the median after the first cycle of Ven + HMA showed a trend towards inferior 2-year PFS (*Online Supplementary Figure S1*). The disposition of the 37 frontline patients is shown in the Figure 2.

Seven patients with relapsed *CBFβ::MYH11*$^{+}$ AML were treated, including four systemic relapsed patients from the aforementioned cohort during follow-up period. The median age was 51 years (range, 20-79 years), and six patients were males. Targeted bulk NGS analysis in six of the seven relapsing patients revealed clonal changes in 66.7% (N=4). The primary emergent mutations at the time of relapse in four patients were *FLT3*-ITD, *PHF6*/*SETBP1*, *ASXL1*, and *KRAS*, respectively (*Online Supplementary Figure S2*). All patients received Ven + HMA as the first salvage therapy. Three of the four patients with prior treatment using Ven + HMA, and one of the three patients without prior Ven treatment, achieved CR/CRi after one cycle of reinduction therapy. All four patients achieved negative MFC-MRD, with the transcript levels of *CBFβ::MYH11* being 0, 92, 105, and 2,697 copies, respectively. Two patients without apparent clonal changes both achieved remission; two patients with new *FLT3*-ITD and *ASXL1* mutations also achieved CR2, whereas two patients with new *PHF6/SETBP1* and *KRAS* mutations did not respond. The patient who did not undergo mutation testing at the time of relapse also did not respond. Flow cytometry suggested that the phenotypic profile of the non-responsive patients did not shift to a

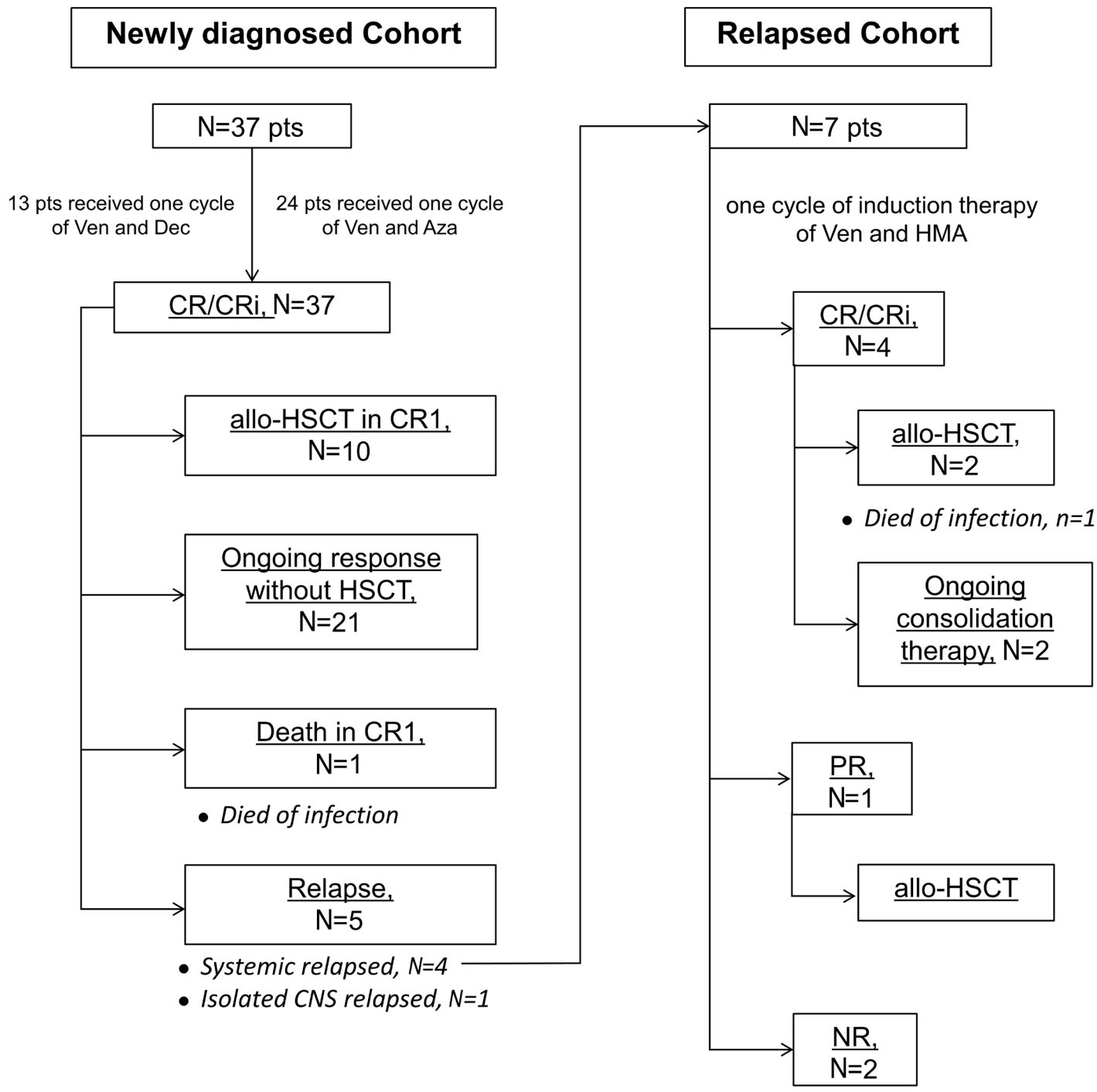


Figure 2. Patient disposition. Ven: venetoclax; Dec: decitabine; Aza: azacitidine; HMA: hypomethylating agent; CR: complete remission; CRi: CR with incomplete count recovery; allo-HSCT: allogeneic hematopoietic stem cell transplantation; CNS: central nervous system; NR: not reached; pts: patients.

monocytic pattern, which is characterized by downregulated CD117 expression and upregulated expression of CD11b and CD64. Among the responders, two patients proceeded to myeloablative allo-HSCT directly, while two patients who did not undergo allo-HSCT remained in CR2 for 2 and 3 months at the last follow-up, respectively. The disposition for the seven relapsed patients is shown in the Figure 2.

As expected, the treatment was well tolerated, and all side effects were temporary and reversible. Grade 3 or higher infections occurred in 28% of the patients, and febrile neutropenia occurred in 23%. For patients who achieved CR/CRi, the median time to platelet recovery (>50×10^9/L) was 24 days (range, 15-40 days). No deaths were observed in the entire cohort during the induction period.

The "7 + 3 ± gemtuzumab ozogamicin" regimen is the standard induction therapy for patients with *CBFβ::MYH11*$^+$ AML.[1] Previous studies have shown that the dosage of anthracycline drugs is critical for the rate of CR and the duration of OS.[7,8] However, the risk of early treatment-related mortality and potential cardiotoxic side effects must be considered when choosing an induction regimen. Clinical trials have confirmed that for certain types of acute leukemia, such as Philadelphia chromosome-positive acute lymphoblastic leukemia, it is entirely possible to achieve deep and safer remission with effective targeted therapies, which in turn can improve the patient OS rate.[9] Our study revealed that patients with newly diagnosed *CBFβ::MYH11*$^+$ AML can achieve a 100% CR/CRi rate using the Ven + HMA regimen, coupled with a notable reduction in MRD. Whether it could serve as an anthracycline-free alternative induction regimen in the future is currently being explored in ongoing clinical research (*clinicaltrials gov. Identifier: NCT06429098*).

In this study, all patients who achieved CR1 transitioned to consolidation therapy with either intermediate-dose/high-dose Ara-C. This approach is grounded in the understanding that the Ven + HMA regimen typically requires indefinite administration, and in most cases, it is not deemed curative.[1] No plateau in the survival curve was observed in the VIALE-A trial.[2] Moreover, data concerning the efficacy of Ven + HMA as maintenance therapy to eradicate MRD in *CBFβ::MYH11*$^+$ AML are inadequate and controversial.[10-12] In contrast, a fixed course of ID/HD Ara-C has demonstrated a long-term RFS rate of approximately 60%.[13] However, considering that patients with favorable-risk AML, such as *NPM1*-mutated AML without co-occurring *FLT3*-ITD or RAS mutations, have a median survival of 39.0 months with only Ven + HMA therapy and MRD-negative patients have an 88% 2-year treatment-free remission,[4,5] the use of Ven + HMA in patients with older age or severe comorbidities and an MRD-negative CR, warrants future investigation.

Unlike that for newly diagnosed patients, the induction CR/CRi rate for relapsed patients is relatively lower. The primary mechanisms of venetoclax resistance may include a shifted dependency on other pro-survival Bcl-2 proteins, like Mcl-1 and Bcl-XL; inactivation of the p53 protein; and mutations in active signaling genes, such as *FLT3*-ITD and *K/NRAS*.[14] Due to the limited number of cases, we did not observe a definitive association between genetic mutations and therapeutic efficacy. Meanwhile, we noticed that patients resistant to treatment did not present monocytic immunophenotypic features, which may be associated with venetoclax resistance.[15]

The major limitations of our study were that it was a retrospective analysis from a single center with a limited number of patients, the therapies administered were not uniform, and the follow-up period was relatively short. Despite these limitations, our data demonstrate that combination therapy with Ven + HMA yielded impressive responses as frontline therapy in patients with *CBFβ::MYH11*$^+$ AML, even though the impact on patients' long-term OS awaits further observation in a larger independent dataset.

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Disclosures

No conflicts of interest to disclose.

Contributions

ZYF, and XHH interpreted the data and wrote the paper. WYL, TX, YX, SLX, HYQ, XWT, YH, SNC. and ANS collected research data and

revised the manuscript. YMZ, DPW and YW designed the research, analyzed the data and edited the manuscript.

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Data-sharing statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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