A lower-intensity, venetoclax-containing protocol is effective in adults with newly diagnosed mixed-phenotype acute leukemia

Mixed-phenotype acute leukemia (MPAL) is a rare type of leukemia characterized by the expression of markers of more than one lineage, including B-lymphoid, T-lymphoid, and myeloid lineages.[1] Patients with MPAL have dismal outcomes, and no optimal treatment strategy has yet been established. Available study data indicate that allogeneic hematopoietic stem cell transplantation (alloHSCT) may improve patient prognosis.[2,3] However, traditional intensive chemotherapy relies on non-specific cytotoxic drugs, which may lead to multiorgan toxicity and treatment discontinuation, potentially making patients ineligible for transplantation. In recent years, lower-intensity regimens of venetoclax and azacitidine (ven-aza) have been widely adopted for treating acute myeloid leukemia (AML) across diverse patient populations, including elderly patients, those who are unfit for intensive chemotherapy, and some younger patients. These regimens are well tolerated and effective, with reported response rates of 60-80%.[4,5] There is also growing evidence to support the therapeutic potential of ven-aza for patients with acute lymphoblastic leukemia (ALL).[6] Furthermore, blinatumomab has emerged as a promising treatment for B-cell ALL and has demonstrated favorable efficacy and safety.[7] The toxicity profiles and efficacy of these lower-intensity regimens in MPAL are still not clear. A limited number of case reports suggest potential efficacy[8-10] but more evidence is needed. Here, we report the efficacy and toxicity of a lower-intensity, venetoclax-containing protocol as induction therapy for patients with newly diagnosed MPAL.

This study included a total of 16 patients with newly diagnosed MPAL who were treated at the First Affiliated Hospital of Soochow University between July 2021 and October 2023. The patients were retrospectively recruited following approval from the institutional ethics committee (approval number: 2024749). All enrolled patients received a lower-intensity, venetoclax-containing induction regimen, with oral administration of 100 mg venetoclax on day 1, 200 mg on day 2, and 400 mg on days 3 to 28, and azacitidine 75 mg/m^2 administered subcutaneously on days 1 to 7. The dosage of venetoclax was modified according to the prescribing information when it was co-administered with strong or moderate CYP3A inhibitors. Tyrosine kinase inhibitors (TKI) were administered orally to patients with *BCR::ABL1* fusion. Blinatumomab was recommended for patients with B-lineage MPAL. For patients at a high risk of febrile neutropenia or severe, persistent neutropenia, antimicrobial prophylaxis comprising oral fluoroquinolones and posaconazole was administered. Central nervous system (CNS) prophylaxis was performed via lumbar puncture with the intrathecal administration of methotrexate, cytarabine, and dexamethasone. Treatment efficacy was evaluated after the first induction cycle according to European Leukemia Network (ELN) guidelines.[11] Measurable residual disease (MRD) was assessed through multiparameter flow cytometry, with MRD negativity defined as a level less than 1×10^{-4}. For patients who were *BCR*::*ABL1* fusion-positive (*BCR*::*ABL1*$^+$), *BCR*::*ABL1* transcripts were monitored through quantitative-polymerase chain reaction (qPCR).

Median age of the 16 patients was 45 years (range 18-60 years). Six patients had MPAL *BCR*::*ABL1* fusion, 5 patients had MPAL B/myeloid, 4 patients had MPAL T/myeloid, and one patient had MPAL B/T. Eight patients presented with significantly elevated white blood cell (WBC) counts (>30x10^9/L) at diagnosis, 5 of whom had WBC counts >100x10^9/L. These patients received cytoreduction with leukapheresis, hydroxyurea, steroids or low-dose cytarabine until the WBC counts decreased to <25x10^9/L before induction. Molecular data were available for 15 patients, with gene mutations detected in 12 (80%) patients. No patients had extramedullary involvement at diagnosis. Details of baseline patients' characteristics are presented in Table 1.

All enrolled patients achieved remission after induction therapy, including 9 (56.3%) who achieved complete remission (CR) and 7 (43.7%) who achieved complete remission with incomplete blood count recovery (CRi) (Figure 1). Eight (50%) patients achieved MRD negativity.

Adverse events were assessed in 16 patients during the induction period (*Online Supplementary Table S1*). The most common grade ≥3 treatment-related adverse events were hematologic toxicities, including neutropenia (100%; 16/16), thrombocytopenia (87.5%; 14/16), and anemia (81.3%; 13/16). The median duration for neutrophil recovery (>0.5x10^9/L) and platelet recovery (>30x10^9/L) were 21.5 and 19 days, respectively. The most common non-hematologic toxicities were fatigue (62.5%), fever (31.3%), and nausea (18.8%). No incidence of tumor lysis syndrome was reported among the patients. The most common grade ≥3 non-hematologic toxicity was infection (12.5%). No patients died during induction therapy.

Following one cycle of induction therapy, 3 patients received ven-aza-based consolidation, 11 patients received standard-dose chemotherapy consolidation, and 2 patients

received high-dose cytarabine-based consolidation. Ten patients underwent alloHSCT during the first CR, all of whom maintained continuous CR by the end of follow-up. Only one recipient of alloHSCT died due to pulmonary infection. Four patients relapsed but all achieved second CR, and the median time to relapse after remission was 3.7 months (range 0.9-5.1 months). Patient 2 received ven-aza plus flumatinib followed by alloHSCT. Patient 6 with CNS relapse received ven-aza plus ponatinib followed by alloHSCT. Patient 11 received autologous HSCT bridging to CD19 chimeric antigen receptor T-cell (CAR-T) therapy. Patient 12 had a *FLT3* mutation and received ven plus gilteritinib.

Median follow-up was 36.8 months (range 18.3-49.7 months). Median event-free survival (EFS) and overall survival (OS) were not reached. The estimated 3.5-year EFS and OS were 68.8% and 87.5%, respectively (Figure 2A, B). By the last follow-up, 2 patients had died; in one patient, death was attributed to disease relapse and in the other patient, to pulmonary infection.

Among the 6 patients with *BCR*::*ABL1*$^{+}$ MPAL, 83.3% pre-

Table 1. Characteristics of patients with mixed-phenotype acute leukemia.

Case	Age, years/ gender	ECOG-PS	WBC x10^{9}/L	MPAL type	Cytogenetics	Somatic mutations	Induction treatment
1	45/F	2	115.5	*BCR*::*ABL1* fusion	46,XX,der(9)?inv(9)(p11;q13) t(9;22)(q34;q11)der(22) t(9;22)(q34;q11)[10]	*CEBPA*	VA+TKI
2	29/F	1	267.3	*BCR*::*ABL1* fusion	46,XX,der(5)ins(5;?), t(9;22)(q34;q11)[8] /46,XX,t(9;22)(q34,q11), 19p+[2]	*RUNX1*	VA+TKI
3	25/F	1	313.8	*BCR*::*ABL1* fusion	46,XX,t(9;22)(q34;q11)[1] /46,XX[8]	-	VA+TKI
4	50/M	1	35.8	B/myeloid	46,XY,add(2)(p25)[12]/46,XY[2]	*BRAF, ARID2, IL-17R*	V+BiTE
5	48/M	1	4.8	B/T	46,XY[20]	*ASXL1, RNUX1, EZH2, NOTCH1, JAK1, JAK3, NRAS*	VA+BiTE
6	51/F	1	344.0	*BCR*::*ABL1* fusion	Low mitotic activity	-	VA+TKI
7	43/M	1	2.1	B/myeloid	46,XY,del(20)(q12;q13)[7] /46,XY[3]	*FLT3, U2AF1, WT1, TYK2,*	V+BiTE
8	45/F	2	48.8	B/myeloid	Low mitotic activity	*STAG2*	VA
9	35/M	1	2.7	T/myeloid	46,XY[20]	*CXCR4, WT1, GATA2, KMT2D*	VA
10	59/M	1	3.3	T/myeloid	45,XY,dic(21;22)(p11;p11)[10]	*CEBPA, BCORL1, BRCA2, STAT3, ERBB3, KMT2A, SETD2*	VA
11	18/F	2	25.8	B/myeloid	46,XX[20]	Missing	VA
12	60/F	2	140.3	B/myeloid	46,XX,t(10;11)(q12;q23)[9] /46,XX[1]	*FLT3, IDH1,RUNX1*	VA+BiTE
13	26/M	1	1.3	T/myeloid	46,XY[20]	*NRAS, WT1, BRAF, IDH1, ASXL2, EP300, KDM6A, SETD2, ZBTB7A*	VA
14	58/M	1	1.0	T/myeloid	46,XY,inv(16)(p13;q22)[2] /46,XY[2]	*KRAS, ATM, ARC, FAT1*	VA
15	52/F	2	13.6	*BCR*::*ABL*1 fusion	46,XX,t(9;22)(q34;q11)[10]	-	VA+TKI
16	35/M	2	36.8	*BCR*::*ABL1* fusion	46,XY,t(9;22)(q34;q11)[8] /45,idem,-7[2]	*RUNX1*	VA+TKI

A: azacitidine; BiTE: blinatumomab; ECOG-PS: Eastern Cooperative Oncology Group Performance Status; F: female; M: male; MPAL: mixed-phenotype acute leukemia; TKI: tyrosine kinase inhibitors; V: venetoclax; WBC: white blood cells.

sented with WBC counts >30x10^9/L, and 66.7% had WBC counts >100x10^9/L. The expression of P190$^{bcr-abl}$ was more common than that of P210$^{bcr-abl}$. Four patients were treated with flumatinib, one with dasatinib, and one with imatinib. All 6 patients achieved a major molecular response (MMR), with a median time to MMR of 1.5 months (range 0.4-5.9 months). The 3.5-year OS and EFS rates were 100% and 66.7%, respectively. Furthermore, no significant difference in 3.5-year EFS was observed between patients with *BCR*::*ABL1*$^+$ MPAL and those with *BCR*::*ABL1* fusion-negative MPAL (*BCR*::*ABL1*$^-$ MPAL) (*P*=0.78) (*Online Supplementary Figure S1*).

Our data revealed that the lower-intensity, venetoclax-containing protocol yields promising outcomes in MPAL, with a notably higher CR rate than that reported in adult patients in previous studies (which all reported <80%).[2,12,13] Moreover, a promising long-term prognosis was observed in our study, especially in those who underwent alloHSCT during remission, with a significantly improved EFS (*P*=0.033). These results further underscore the importance of alloHSCT as a post-remission therapy in patients with MPAL.[2]

Recently, AML with mixed phenotype (AML-MP) has been the focus of studies at the Memorial Sloan Kettering Cancer Center (MSKCC). AML-MP is defined as therapy-related AML or AML with myelodysplasia-related changes that exhibit a mixed phenotype.[14] AML-MP is immunophenotypically indistinguishable from MPAL but has a worse prognosis. Among their cohort of 55 patients with AML-MP, 20 patients were treated with hypomethylating agents alone or in combination with venetoclax, but none of them achieved CR or CRi. In our cohort, 2 patients with the B/myeloid phenotype met the criteria for AML-MP (Patients 7 and 8), and both of them achieved CR after induction of venetoclax-containing treatment. However, neither of the patients had adverse cytogenetic abnormalities nor *TP53* mutations, which were frequently presented by patients in the MSKCC report. Notably, in our study, one patient received blinatumomab due to high CD19 expression on leukemic cells, which may improve the treatment efficacy and suggest the potential of targeted therapies in patients with AML-MP.

Importantly, no patients developed severe complications that delayed subsequent treatment, and no treatment-related death was observed. We hypothesized that lower-intensity induction may alleviate myelosuppression and, therefore, decrease the risk of related adverse events including severe infection or bleeding. In contrast, intensive chemotherapy is associated with more severe toxicity profiles, which may lead to treatment interruption or even early death. For example, a study of 3,728 patients with newly diagnosed AML who received intensive chemotherapy revealed 4-week mortality rates of 2%, 14%, and 50% in the low-, high-, and very high-risk groups,

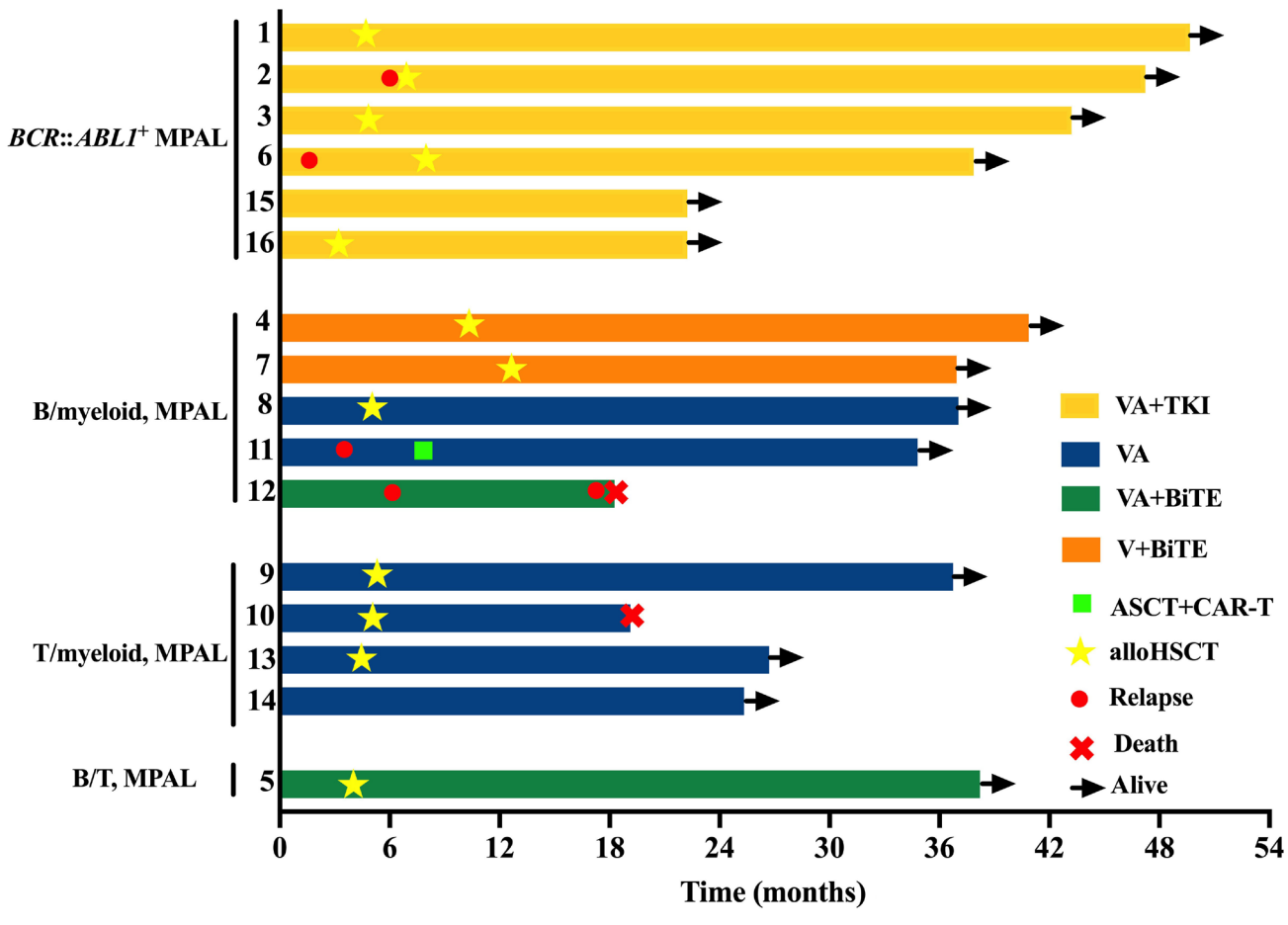

Figure 1. Swimmer plot of the dynamic response assessment. Sixteen patients (1-16) achieved remission after induction therapy, including 9 (56.3%) patients who achieved complete remission (CR) and 7 (43.7%) who achieved CR with incomplete blood count recovery (CRi). Four patients experienced relapse, but all achieved a second CR. Two mortality events were recorded: one attributed to disease relapse and one to pulmonary infection. A: azacitidine; alloHSCT: allogeneic hematopoietic stem cell transplantation; ASCT: autologous stem cell transplantation; BiTE: blinatumomab; CAR-T: chimeric antigen receptor T-cell therapy; MPAL: mixed-phenotype acute leukemia; TKI: tyrosine kinase inhibitors; V: venetoclax.

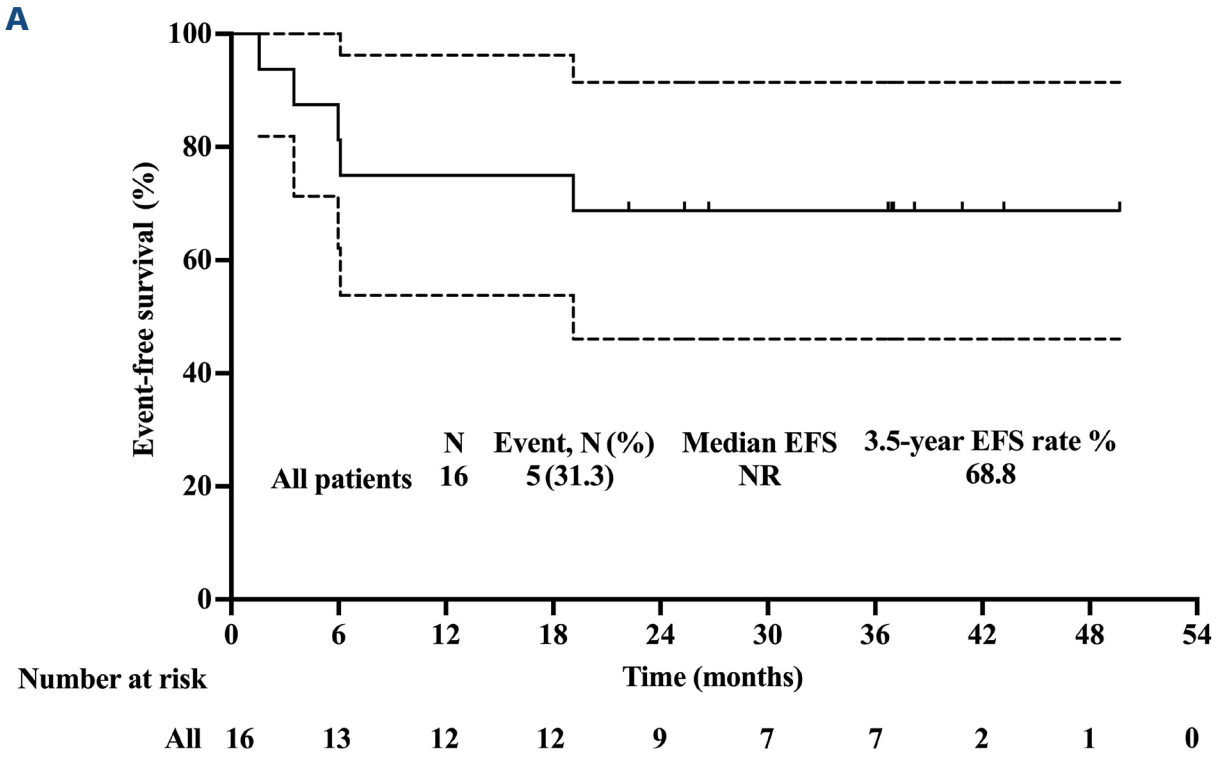

Figure 2. Survival of patients with mixed-phenotype acute leukemia. (A). Kaplan-Meier estimates of event-free survival (EFS). (B). Kaplan-Meier estimates of overall survival (OS). N: number; NR: not reached.

respectively.[15] The favorable safety and efficacy profile of the lower-intensity, venetoclax-containing protocol in our study supports a shift to lower-intensity, more targeted treatments for patients with MPAL. However, our study is limited by its retrospective nature, a lack of homogeneity among regimens, and a small sample size.

In summary, our data demonstrate that the lower-intensity, venetoclax-containing induction regimen seems to be an effective and well-tolerated treatment for patients with MPAL. This regimen enables most patients to become eligible for individualized post-induction treatment and improves long-term prognosis. Given the limitations of our study, these findings need to be validated through well-designed randomized trials and real-world data.

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Disclosures

No conflicts of interest to disclose.

Contributions

XZ, DW and JC are responsible for designing and writing the protocol; YZ and YF helped write and edit the manuscript; MX is responsible for collecting, analyzing and interpreting the data; SX, XT, HQ, MM, SC, HZ, JZ, XY and YX are responsible for patient care and data assurance. All authors gave final approval of the manuscript for publication and agreed to be accountable for all aspects of the work.

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Data-sharing statement

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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