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A retrospective assessment of real-world experience with venetoclax and azacitidine therapy in elderly acute myeloid leukemia

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This study aimed to examine the effect of venetoclax coupled with azacytidine in treating older adults with relapsed and refractory (R/R) acute myeloid leukemia (AML). The clinical data of 10 senior patients with AML over 65 years old who were treated with venetoclax and azacytidine, including six patients with R/R AML, were retrospectively evaluated. This study comprised seven males and three females with a median age of 71 years. Five patients had at least one relapse, and one patient did not achieve remission after four cycles of azacytidine monotherapy, considering it resistant. AML with myelodysplasia-related changes was found in four cases. One of the 10 patients died early after 1–13 cycles of venetoclax plus azacytidine treatment due to a protracted period of neutropenia and severe lung infection induced by medications. Six of the remaining nine patients, including six R/R patients, achieved a complete remission (CR) or a CR with incomplete hematologic recovery (CRi). After two cycles of therapy, one patient did not react. Neutropenia lasted an average of 10.5 (6–15) days in all patients, with the most severe cases occurring in the second and third weeks of therapy. Three patients who tested positive for the TP53 gene mutation had the following outcomes: One relapsed patient has been in progression-free remission (PFS) for the past 24 months, whereas another has been in full remission but relapsed 2 months later. Another patient experienced complete remission in myelology for 4 months, but the variable allele fraction (VAF) value steadily rose, suggesting that the illness was on the verge of progressing. IDH2 gene alterations were found in three of four patients who obtained maintained CR for more than 18 months following recurrence. Venetoclax in combination with azacytidine is a successful and well-tolerated therapy for R/R AML in the elderly. Venetoclax and azacytidine may help patients with TP53 mutations and reduce VAF. The IDH2 mutation might be a good predictor of veneclax sensitivity. A notable adverse response in the treatment phase of the regimen is severe infection induced by neutropenia. *Anti-Cancer Drugs* 34: 344–350 Copyright © 2022 The Author(s). Published by Wolters Kluwer Health, Inc.

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Introduction

Acute myeloid leukemia (AML) is one of the hematological system's malignant malignancies. In adults, it is the most frequent kind of leukemia. With the world's population continuing to age, the median age of AML onset has grown to 68 years old [1]. Because of several major treatment-related problems and poorly preserved organ function, most elderly AML patients cannot take intense therapy or the standard combination of cytarabine and anthracycline (7 + 3 regimen) [2]. As a result, this group has a low complete remission (CR) rate, a short remission duration, a high risk of early death, and a poor prognosis overall [3]. According to Appelbaum *et al.* [4], senior patients aged 66–75 years with a performance status (PS) of more than 2 points were 31% more likely to die within 30 days after receiving induction chemotherapy, and their remission rate was significantly lower than that of adult patients . From 2000 to 2016, the median overall survival (OS) of AML patients over 65 years old in the USA was projected to be 2.67 months, with a 1-year survival rate of just 21.8% in the same cohort [5].

With the advancement of tumor epigenetics, it has been shown that aberrant DNA methylation plays a key role in the onset and progression of older AML. Hypomethylation agents (HMA) have been demonstrated to offer benefits over conventional chemotherapy for elderly leukemia patients in recent trials [6]. In an older patient with AML, a phase III clinical study (DACO-016) assessed the effectiveness of HMA (decitabine) to standard therapy.

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A total of 485 patients were enrolled, with a median age of 73 years. The two groups' CR rates plus incomplete hematologic recovery (CRi) rates were 17.8% (decitabine group) and 7.8%, respectively, with median OS times of 7.7 months (decitabine group) and 5.0 months ($P = 0.108$) [7]. Low-intensity chemotherapy, such as HMA, has become the standard treatment for elderly AML patients who are not candidates for high-intensity chemotherapy in recent years [8]. However, since HMA is unable to remove leukemic stem cells, AML often relapses after medication discontinuation, necessitating long-term treatment. As a result of these flaws, new targeted medications have emerged.

Venetoclax (VEN) is a B cell leukemia/lymphoma-2 (Bcl-2) inhibitor with a narrow therapeutic window. It has the capacity to bind directly to Bcl-2 protein, stimulate mitochondrial outer membrane permeability and caspase activation, aid in the restoration of apoptosis, preferentially kill AML cells, and boost AML cell susceptibility to chemotherapeutic treatments [9,10]. VEN has shown clinical effectiveness as a single drug for the treatment of individuals with relapsed and refractory (R/R) AML, although the response was moderate and temporary. Leukemia-free survival and OS were 2.3 and 4.7 months, respectively [11]. It has been demonstrated to be effective when coupled with azacytidine (AZA) in newly diagnosed elderly AML patients [12]. VEN and HMA have been tested in foreign clinical studies in elderly individuals with R/R leukemia [13]. In recent years, numerous novel targeted medicines for AML treatment have been licensed, whereas hypomethylating drugs (HMA) are often used as an option for older patients who cannot tolerate heavy chemotherapy [6]. There has not been a large-scale case study using VEN with AZA in the treatment of elderly R/R AML patients in China yet. We retrospectively investigated the effectiveness and side effects of AZA + VEN in the Hematology Department of Xuanwu Hospital Capital Medical University in the last 2 years to explore the safety and efficacy of this combinatorial treatment in R/R elderly AML patients.

Cases and methods

Case

AZA + VEN was used to treat ten elderly AML patients in our department from December 2018 to July 2022. Based on the FAB classification, all patients were diagnosed by morphological inspection of bone marrow cells and histochemical staining. At the start of AZA + VEN treatment, clinical and laboratory data were obtained.

Diagnostic criteria, response criteria, and survival rate

The 2017 European LeukemiaNet response criteria were used to assess response to VEN treatment [14]. The combination of CR and CRi was used to calculate the overall response rate. From the initial day of therapy until the final follow-up, the OS was computed.

Immunophenotypic analysis by flow cytometry

Before VEN + AZA therapy, heparin-anticoagulated bone marrow (2 ml) was aspirated from nine patients, and the immunophenotype was studied and assessed by flow cytometry.

Cytogenetic analysis

The chromosomes were prepared using a 24-h culture procedure using heparin-anticoagulated bone marrow (4 ml). G-banding technique was used to dye the chromosomes, and the karyotypes were evaluated under a microscope and named using the International Human Cytogenetic Naming System (ISCN2016).

Gene mutation detection

DNA was isolated from EDTA-anticoagulated bone marrow cells. Forty-two genes were identified using the AML/MDS second-generation sequencing chip (Shanghai Yuanqi Life Science and Technology Co., Ltd.) (see Table 1). The library was created using PCR amplification according to the manufacturer's instructions. The Illumina sequencer was used to sequence the hot regions of 42 exons. Sequencer 4.7 software was used to examine the sequencing findings.

Treatment regimen

AZA 75 mg/m^2/day, subcutaneous injection at 2–3 locations for 7 days; VEN: 100 mg on the first day; 200 mg on the second day; 400 mg from the third day to the 28th day to complete the 28-day treatment period. The program was supposed to start 30 min after each meal. If a CYP3A4 inhibitor, such as fluconazole and posaconazole, was used to treat the associated fungal infection, the dose of VEN was lowered to half, or ¼, respectively [15].

Follow-up

The follow-up period ended on 1 July 2021. Phone calls or medical record inquiries were used for follow-up.

Table 1 Forty-two genes were detected by acute myeloid leukemia/myelodysplastic syndrome second-generation sequencing chip

Signal path	Mutated genes
DNA methylation regulation-related genes	*TET2*, *DNMT3A*, *IDH1*, *IDH2*
Histone regulation-related genes	*EZH2*, *ASXL1*, *PPM1D*, *RAD21*, *SMC1A*, *SMC3*
Splicing factor-related genes	*SF3B1*, *SRSF2*, *U2AF1*, *ZRSR2*
Signal transcription-related genes	*FLT3*, *CBL*, *JAK2*, *NARS*, *KRAS*, *c-kit*, *CSF3R*, *MPL*, *SH2B3*, *PDGFRA*, *NF1*
Transcription factor-related genes	*NPM1, GATA2, CEBPA, ETV6, PHF6, RUNX1, SETBP1, BCOR, BCORL1*, *STAG2*, *STAT3*, *PTPN11*
DNA repair-related genes	*TP53, WT1*
Others	*CALR*, *PIGA*, *KMT2A*

Table 2 Clinical characteristics of patients

Patient no.	Age (years)	Sex	WHO diagnosis	ECOG, PS scores	Blast percentage (%)	WBC ($\times 10^9$/l)	HB (g/l)	PLT ($\times 10^9$/l)	LDH level (IU/l)	Mutated gene	Cytogenetics	Complications	Respose	OS	Cytogenetic response?
1	69	F	AML-M5 (second relapse)	2	14	0.9	80	65	144	CEBPA NPM1, TET2, TP53, DNMT3A	46, XX	Hypertension, diabetes, hypothyroidism	CR3	≥16	Yes
2	71	F	AML-M2 (second relapse)	2	7.5	1.88	130	134	113	IDH2	46, XX	Coronary heart disease	CR3	≥13	Yes
3	74	M	AML-MRC	3	20.5	3.45	51	263	317	ASXL1, BCOR, RUNX1, IDH2	46, XY	None	CR1	≥15	Yes
4	82	M	AML-MRC	3	21	4.62	82	25	312	TP53, DNMT3A	46,XY, add (4) (p16), del (5) (q21), del (7) (q31), +8, del (16) (q22)	Hypertension	CRi	11	Yes
5	71	M	AML-MRC	3	20	1.83	72	2	129	RUNX, U2AF1, FLT3-ITD	46, XY	Coronary heart disease; Coronary stenting	No response	-	No
6	65	F	AML-MRC	3	37	1.3	65	298	473	IDH1, NPM1, CEBPA,DNMT3A	46, XX	Hypertension, diabetes, postcholecystectomy	Not evaluable	-	No
7	69	M	AML-MRC	2	2.5	5.38	118	159	152	TP53	45, XY,-5, add (11) (p15), add (13) (p11), add (15) (p11), −17, −18, −20, +22,	Hypertension, diabetes	CR1	7	No
8	68	M	AML-MRC (relapse)	2	18	2.04	124	81	240	RUNX1, SRSF2, IDH1, BCOR, ASXL1, SETBP1, KMT2A, TET2	47, XY, +8	Severe pulmonary infection	CR2	≥3	Yes
9	65	M	AML-MRC (relapse)	2	13	5.48	157	83	182	ASXL1, SRSF2, GATA2	46, XY	Diabetes	CR2	≥5	Yes
10	75	M	AML (relapse)	2	37	4.36	73	68	142	WT1 TET2	46, XY, t (7,11)	COPD	CR3		YES

Table 3 Adverse events

	Hematologic toxicity		Gastrointestinal reactions		
Adverse events	Granulocytopenia	Thrombcytopenia	Nausea	Vomiting	Tumor lysis syndrome
No. 1	Yes	No	Slight	No	No
No. 2	Yes	No	Slight	No	No
No. 3	Yes, skin and soft tissue infections, staphylococcemia	No	No	No	No
No. 4	Yes, pneumonia	Yes	Slight	No	No
No. 5	Yes, Pseudomonas aeruginosa	Yes, gastrointestinal bleeding	Slight	No	No
No. 6	Yes, stenotrophomonas maltophilia pneumonia	Yes, abdominal hemorrhage	Slight	No	Uncertain
No. 7	No	Yes	No	No	No
No. 8	Yes	No	Slight	No	No
No. 9	Yes, fungal pneumonia	Yes	No	No	No
No. 10	Yes, urinary tract infection	No	No	No	No

Results

Clinical data

With a median age of 71 (65–82) years and a PS score of at least 2, there were seven men and three females in the study. There were three secondary relapses, two primary relapses, and five instances of AML with myelodysplasia-related changes (AML-MRC). Nine patients received at least one cycle of AZA or combined with low-dose chemotherapy before the application of VEN; there were three cases of TP53 gene mutation, of which two patients had complex chromosome karyotype (see Table 2); one FLT3-ITD gene mutation, three DNMT3A mutation, two IDH2 mutation, one TET2 mutation, three RUNX1 mutation, and one U2AF1 mutation.

Adverse events

Six of 10 individuals reported moderate nausea but no vomiting. Granulocytopenia was seen in nine of 10 individuals throughout the first two treatment cycles, with six of them developing serious infections. Severe neutropenia and septic shock claimed one patient's life. Five out of 10 patients had thrombocytopenia, with two dying from severe hemorrhage. In nine of the patients, there was no evidence of tumor lysis syndrome (see Table 3).

Therapeutic effect

Except for case 6, who died and whose therapeutic impact could not be assessed, the effectiveness (CR + CRi) of the other nine patients was 77.8% (7/9), which occurred in about 1.5 cycles (1–3 cycles).

In case 1, the course of AML was 4 years. The risk classification was low-risk at the time of diagnosis owing to the lack of FLT3-ITD in biallelic mutant CEBPA and mutated NPM1. Standard induction therapy, medium and high doses of cytarabine consolidation, and intensive therapy were used at the time, and the mutant gene was rectified as a result of treatment. The patient relapsed for the first time a year later, this time with mutations in the TET2, TP53 [variable allele fraction (VAF) = 4.55%], and DNMT3A genes. The patient was given low-dose chemotherapy, which included another HMA and decitabine, and the patient's bone marrow returned to CR. The second remission lasted a year. In addition to the TET2 gene, TP53, DNMT3A, and NPM1 gene mutations resurfaced in the second relapse, which did not respond to AZA therapy alone after two cycles. After two rounds of AZA + VEN therapy, the third CR (CR3) was accomplished, and the NPM1 mutation was once again repaired.

Case 2 was first classified as a patient with a moderate risk of death. During the consolidation phase, standard induction chemotherapy and rigorous treatment with high-dose cytarabine were employed. After 6 years of long-term remission, AZA in combination with low-dose cytarabine induction therapy was successful in attaining CR2 after the initial recurrence. The patient relapsed with IDH2 gene mutation during maintenance therapy with single medication AZA. Because an IDH2 inhibitor was not available, AZA + VEN was used instead, and after one cycle of therapy, CR3 was achieved. After two rounds, the IDH2 gene mutation was repaired.

Case 3 was diagnosed with AML-MRC due to mutations in the ASXL1, BCOR, RUNX1, and IDH2 genes. The National Comprehensive Cancer Network (NCCN) recommended AZA monotherapy at 75 mg/m^2/day as the first line of treatment. The fraction of bone marrow blast cells did not decrease after four rounds of therapy, and the patient had to continue receiving red blood cell transfusions. As a result, the patient's curative effect was invalidated, and he became a refractory case. After two rounds of therapy with AZA + VEN, CR1 was achieved, and the mutant gene BCOR was resolved. After the third therapy cycle, the mutations in the RUNX1 and IDH2 genes were restored to normal.

Following failed single-drug AZA treatment, these three patients got combination VEN therapy and obtained CR. They have been in total remission for more than 18 months and are still alive.

Case 4 included an older patient with a complicated karyotype and mutations in the 7q- and TP53 genes. Old age, a complicated karyotype, and a TP53 mutation were all

shown to be independent unfavorable prognostic markers [16]. As a result, AZA was administered in combination with VEN induction treatment. After one session of therapy, CRi was achieved, and the VAF of TP53 fell from 41.4 to 2.06%.

MDS-EB2 was first found in cases 5 and 6. The illness progressed to AML-MRC following a course of single-drug AZA therapy, and significant neutropenia developed with the inclusion of VEN. The earlier instance progressed to Pseudomonas aeruginosa, which responded well to antibiotic therapy. There was no improvement following another cycle of AZA+VEN therapy. Due to thrombocytopenia, the patient died after a protracted period of gastrointestinal bleeding. Neutropenia, Pseudomonas maltophilia infection, and carbapenem resistance emerged in case 6. The patient died of a serious lung infection on the 14th day after receiving AZA+VEN therapy.

AML-MRC with a complicated karyotype and TP53 mutation (VAF 58.1%) was identified in case 7. The VAF value of TP53 gradually increased from 6.3 to 11.8% during consolidation treatment, indicating that the case was ineffective for treatment and had a poor prognosis, despite the fact that CR1 was obtained using AZA combined with low-dose cytarabine. The VAF value of TP53 rose from 11.8 to 28% after three cycles of combination treatment with AZA+VEN. Thrombocytopenia struck more than 2 weeks after the fourth cycle of treatment, and the patient died of an abdominal hemorrhage.

When cases 8 and 9 relapsed, they were both diagnosed with AML-MRC and treated with AZA and low-dose cytarabine. CR2 was obtained after only one cycle of VEN addition. At the time of relapse, case 8 contained mutations in seven genes: RUNX1, SRSF2, IDH2, ASXL1, SETBP1, KMT2A, and TET2. The mutation genes RUNX1, IDH2, ASXL1, and SETBP1 were resolved after two treatment cycles. At the time of the second relapse, case 9 had three gene mutations: ASXL1, SRSF2, and GATA2. After two rounds of therapy, only the GATA2 mutation remained.

Case 10 was a 75-year-old man who had been diagnosed with AML-M5 with karyotype 46, XY, t (7,11), and NUP98-HOXA9 fusion genes. After induction treatment with low-dose cytarabine and azacitidine, the patient attained complete remission. During the maintenance of azacitidine monotherapy, there was a relapse. VEN was added on the basis of AZA. Complete remission of bone marrow was achieved after two cycles of therapy; however, the fusion gene remained positive after four courses of treatment.

Discussion

In the pathogenesis of elderly AML, DNA methylation and other epigenetic alterations play a role [3]. For newly diagnosed senior patients who are not candidates for high-intensity chemotherapy or hematopoietic stem cell transplantation, as well as R/R elderly AML patients, HMA has become the treatment of choice. The CR rate plus the CRi rate of the simple demethylation group was 28% after 1–3 courses of induction treatment, whereas the CR rate plus the CRI rate of the Bcl-2 inhibitor combined with demethylation group was 56%, according to a retrospective study conducted at MD Anderson Cancer Center [17]. These findings show that combining a Bcl-2 inhibitor with a demethylation agent is more successful than using a demethylation agent alone.

All of the patients we included were older people with fragile AML, including six R/R patients. Cases 1 and 2 were the second and third relapses, respectively. They had previously taken normal and high-dose chemotherapy regimens such as anthracycline and cytarabine, but they were unable to continue with regular chemotherapy owing to organ function issues. After taking VEN at doses of 100–400 mg/day in combination with AZA 75 mg/m^2/day, the majority of patients in this group had grade 3–4 myelosuppression. This is the same dosage as for adults. Neutropenia persisted 6–15 days throughout the first round of medication. Seven of the nine patients (77.8%) had a strong therapeutic impact, particularly all six R/R patients.

According to gene stratification, AZA+VEN was more effective in the low

- and medium-risk groups than in the high-risk group [18]. At the time of initial diagnosis, two of nine patients in our research were in the medium and low-risk categories, with progression-free remission (PFS) of 22 and 19 months, respectively. AML patients with RUNX1 and IDH2 mutations reacted effectively to AZA+VEN combos, according to gene mutation studies [19,20]. Three of the patients tested positive for IDH2 or RUNX1 mutations, which matched the results of the previous research [19]. Patients with FLT3-ITD, TP53, and N/KRAS gene mutations were more likely to have no response or relapse, according to a retrospective clinical study, whereas 21% of patients who received VEN combined with HAM rescue treatment had a response, and the median OS was longer (2.9 months vs. 1.3 months) than patients who did not receive rescue treatment [21]. Before therapy, TP53 mutations were discovered in instances 1, 4, and 7. Case 1 had a PFS of 22 months up till the follow-up period. Case 4 had an OS of 11 months, which was much longer than the median OS of elderly AML [3], but only a 2-month PFS. Case 7 died recently from an abdominal hemorrhage after a 7-month OS. After the addition of VEN, the VAF value of the TP53 mutation in instance 7 did not decrease. The addition of VEN to AZA monotherapy does not seem to enhance the prognosis of patients. Because individuals with the TP53 mutation may have a poor response to conventional treatment and have a

median OS duration of less than 9 months [22]. Case 1 experienced a 22-month continuous CR, but the remission length in the other two instances was brief, perhaps due to the complicated karyotype and high VAF value [23]. Both instances 4 and 7 showed a complicated karyotype with chromosomal deletions of –5/5q and/or –7/7q and TP53 mutations with VAF of more than 20%. Further research on TP53 VAF and prognosis is required.

Tumor lysis was not seen in any of the nine patients, which was attributed to the low fraction of primordial cells in the patients prior to therapy. However, one individual (case 6) died too soon due to a prolonged period of neutropenia, infection, and the possibility of tumor lysis. Greater peripheral blood leukocyte count, blood lactate dehydrogenase levels, and precursor cell count in bone marrow or peripheral blood were linked to higher death rates in AML patients treated with VEN [24].

There are a few flaws in our research. To begin with, the patient group that was studied was somewhat tiny. It is worth noting since there have not been many studies on AZA + VEN in the treatment of elderly R/R AML in Asians. During AZA monotherapy, 9/10 (90%) of participants in our study relapsed or progressed. When compared with AZA alone, the therapeutic impact of AZA + VEN was considerably enhanced, and the overall safety was similar with prior trials [13]. It should be emphasized, however, that severe bone marrow suppression is possible. Agranulocytosis is the most common adverse effect of combination treatment, according to our research, and it may lead to early mortality. This small group study's early findings are promising. With the introduction of VEN into the Chinese market, further study is required to improve the treatment scheme and elucidate the mechanism of action for senior R/R AML patients.

Conclusion

VEN in combination with AZA is a safe and effective treatment option for older people with R/R AML. Patients replied in 78% of cases. All five relapsed patients, in particular, received CR or CRi. VEN and AZA may benefit individuals with TP53 mutations and low VAF.

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Consent for publication: all authors agreed to publish.

Availability of data and material: all data are available. Identifying images or other personal or clinical details can be obtained from all of the participants.

Conflicts of interest

There are no conflicts of interest.

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