Zhang et al. Biomarker Research (2021) 9:30
https://doi.org/10.1186/s40364-021-00288-7

Biomarker Research

LETTER TO THE EDITOR Open Access

Not *BCL2* mutation but dominant mutation conversation contributed to acquired venetoclax resistance in acute myeloid leukemia

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Abstract

Venetoclax (VEN) plus azacitidine has become the first-line therapy for elderly patients with acute myeloid leukemia (AML), and has a complete remission (CR) plus CR with incomplete recovery of hemogram rate of ≥70%. However, the 3-year survival rate of these patients is < 40% due to relapse caused by acquired VEN resistance, and this remains the greatest obstacle for the maintenance of long-term remission in VEN-sensitive patients. The underlying mechanism of acquired VEN resistance in AML remains largely unknown. Therefore, in the current study, nine AML patients with acquired VEN resistance were retrospectively analyzed. Our results showed that the known VEN resistance-associated *BCL2* mutation was not present in our cohort, indicating that, in contrast to chronic lymphocytic leukemia, this *BCL2* mutation is dispensable for acquired VEN resistance in AML. Instead, we found that reconstructed existing mutations, especially dominant mutation conversion (e.g., expanded *FLT3-ITD*), rather than newly emerged mutations (e.g., *TP53* mutation), mainly contributed to VEN resistance in AML. According to our results, the combination of precise mutational monitoring and advanced interventions with targeted therapy or chemotherapy are potential strategies to prevent and even overcome acquired VEN resistance in AML.

Keywords: Venetoclax, Acquired resistance, Acute myeloid leukemia

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To the Editor

VEN + AZA has become the first-line therapy for elderly patients with AML, and CR + CRi rates of ≥70% have been achieved [1, 2]. Despite this, the 3-year survival rate of patients who receive VEN + AZA is < 40%, mainly due to acquired VEN-R [3]. However, the underlying mechanisms of VEN-R and the status of *BCL2*^{Mut} in AML, remain largely unknown [4–6].

To address this question, we retrospectively analyzed nine elderly AML patients with acquired VEN-R at our center from July 1, 2018 until June 30, 2020 (Table 1). *BCL2*^{Mut} was detected by PCR combined with Sanger sequencing at VEN-I and VEN-R, but no VEN-R-associated *BCL2*^{Mut} was identified (Fig. 1a) [6–9]. Due to the relatively low resolution of Sanger sequencing, these samples were then submitted to TES (Novaseq platform, Illumina), in which 236 recurrently mutated genes in hematological malignancies were included. The average raw sequencing depth on target per sample was ≥1000, and a VAF ≥1% was considered significant. As VEN-R-associated *BCL2*^{Mut} was consistently negative, *BCL2*^{Mut} was considered dispensable for acquired VEN-R in AML.

Table 1 Basic characteristics of patients with acquired VEN-R AML in our cohort

Characteristics	Value
Patients (N)	9
Male/Female (N)	5/4
Age (year)	73 (68–78)
De novo/Secondary (N)	8/1
FAB: M0/M1/M4/M5 (N)	2/1/3/3
Karyotype: normal/abnormal (N)	4/5
Bone marrow blast at venetoclax initiation (%)	62 (23–92)
Molecular feature at venetoclax initiation (N)	
AML1-ETO	1
NPM1 mutation	3
FLT3-ITD	4
DNMT3A mutation	4
TP53 mutation	1
ASXL1 mutation	2
RUNX1 mutation	2
Bone marrow blast at venetoclax resistance (%)	10.5 (6–74)
Molecular feature at venetoclax resistance (N)	
AML1-ETO	1
NPM1 mutation	2
FLT3-ITD	3
DNMT3A mutation	3
TP53 mutation	2
ASXL1 mutation	2
RUNX1 mutation	1
Cycles from venetoclax initiation to resistance (N)	3 (3–15)

Regarding the difference in the mutational landscape between VEN-I and VEN-R (Supplementary Table 1), the spectrum was skewed in 7/9 patients: 3/7 exhibited a reduction in mutated genes, 1/7 exhibited an increase, and 3/7 showed a reduction in some mutated genes and an increase in others (Fig. 1b). As *TP53* mutation has been demonstrated to confer AML VEN-R [10], newly emerged *TP53* mutation definitely contributed to VEN-R as shown in Pt #8 (Fig. 1c). However, newly emerged mutations in the remaining three patients had relatively low VAFs compared to the dominant mutations, which indicated that these mutations existed in sub-clones and played a minor role in acquired VEN-R.

We next addressed the proportion of reconstructed existing mutations. Excluding Pt #9 without the molecular relapse, 6/8 patients exhibited reconstructed existing mutations, and 4/8 patients showed dominant mutational conversion (Fig. 1d). *FLT3-ITD* is the most common mutation in AML [11], but whether it affects VEN sensitivity remains controversial [1]. In Pt #3, #6, and #7, the VAF of *FLT3-ITD* increased, and it had ranged from a minor mutation at VEN-I to the most common mutation at VEN-R (Fig. 1e–g). Although *FLT3-ITD* was totally absent from Pt #5, *FLT3-ITD* still conferred VEN-R for AML in Pt #3, Pt #6, and Pt #7. In Pt #1, *IDH2*^{R140Q} and *TP53*^{L145P} mutations were the dominant mutations across the entire treatment course; however, their VAFs decreased, while those of *NF1*^{T419fs} and *PHF6*^{T300A} mutations gradually increased with AML progression. These findings indicate that minor mutations can expand and possibly contribute to VEN-R (Fig. 1h).

Although VEN-associated *BCL2*^{Mut} has been identified in CLL, it was not detected in our AML cohort. There are several possible explanations. First, there was short duration exposure to VEN in AML (AML vs. CLL [months], 5 [3-9] vs. 36[6.5–73]) [12]; second, combination therapy with AZA in AML may have eradicated the emerged *BCL2*^{Mut} at an early stage; and third, the standard dose of VEN (400 mg/qd) used in AML patients was not reached in 27% of CLL patients. Theoretically, *BCL2*^{Mut} may have mediated VEN-R in patients with AML as the duration of exposure increased, but in reality, combination therapy at a standard dose made the possibility of emerged *BCL2*^{Mut} much lower than in CLL. *BCL2*^{Mut} was still negative in our two cases with ≥1-year exposure duration. In contrast to *BCL2*^{Mut}, we found that clonal evolution, including newly emerged mutations and reconstructed existing mutations, mainly contributed to VEN-R in AML. For example, newly emerged *TP53* mutation or expanded *FLT3-ITD* could

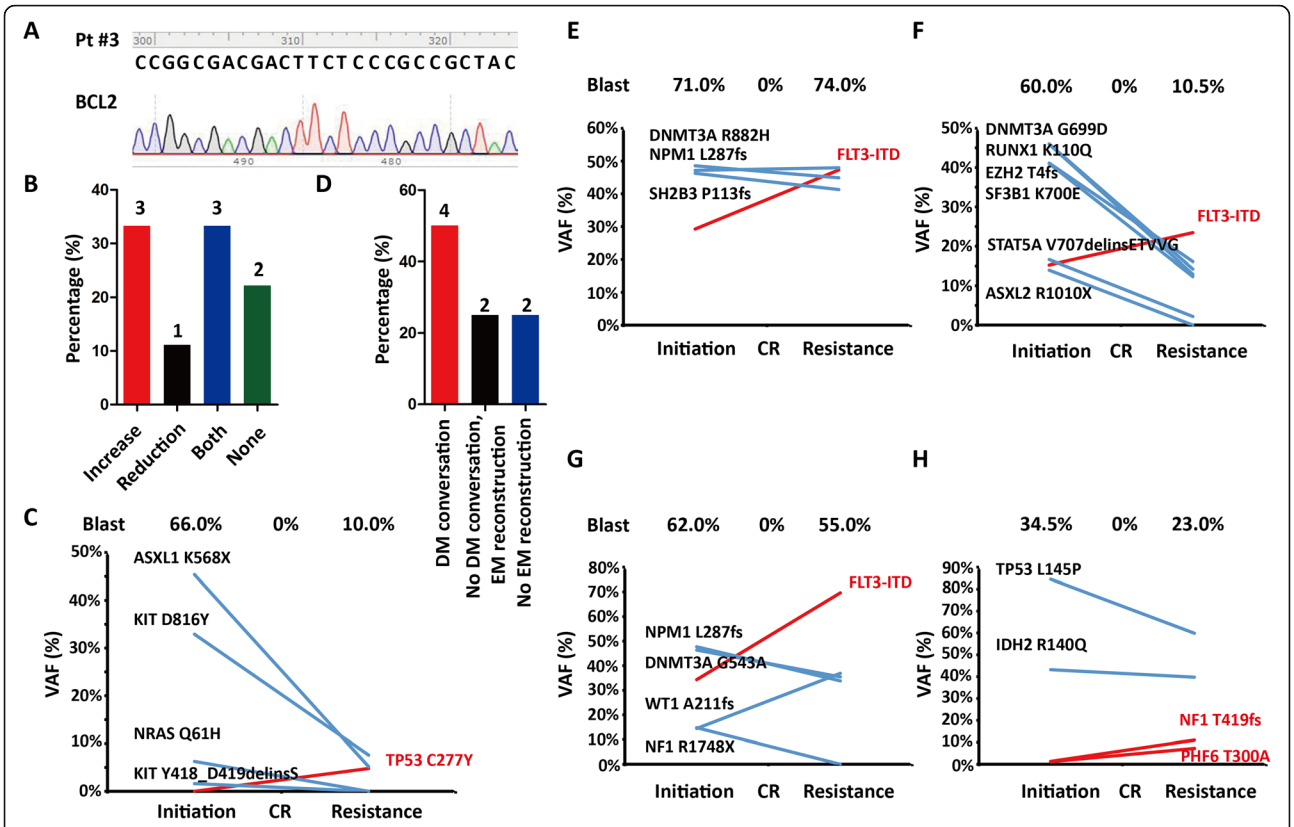

Fig. 1 Mechanism of acquired VEN-R in AML. **a** No *BCL2*^{Mut} was found in patients with acquired VEN-R AML. **b** Changes in the types of mutational genes in our AML cohort according to VEN-R. **c** The acquired *TP53* mutation played a dominant role in the relapse of Pt #8. **d** Reconstructed existing mutations (EM), especially conversed dominant mutation (DM), were important in acquired VEN-R. **e-g** Expanded *FLT3-ITD*-mediated acquired VEN-R in Pt #3 (**e**), #6 (**f**), and #7 (**g**). **h** The proportion of reconstructed existing mutations in Pt #1

mediate acquired VEN-R in AML, which was also reported by DiNardo [4]. Interestingly, acquired *TP53* mutation also mediated VEN-R in CLL independent of *BCL2*^{Mut}, and it was more common than in AML. Furthermore, reconstructed existing mutations, especially dominant mutation conversion, appear to be more important than newly emerged mutations in acquired VEN-R. More aggressive clinical strategies are required to overcome this mechanism in acquired VEN-R in AML. In our cohort, three patients with AML with expanded *FLT3-ITD*-mediated acquired VEN-R possibly benefited from dynamic monitoring of *FLT3-ITD* and early addition of an FLT3 inhibitor to prolong the response to VEN. Therefore, the combination of precise mutational monitoring and advanced interventions with targeted therapy or chemotherapy is key to preventing and overcoming acquired VEN-R in AML.

Abbreviations

AML: Acute myeloid leukemia; AZA: Azacitidine; *BCL2*^{Mut}: *BCL2* mutation; CLL: Chronic lymphocytic leukemia; CR: Complete remission; CRi: Complete remission with incomplete recovery of hemogram; PCR: Polymerase chain reaction; Pt: Patient; TES: Targeted-exome-sequencing; VAF: Variant allele frequency; VEN: Venetoclax; VEN-I: Venetoclax initiation; VEN-R: Venetoclax resistance

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40364-021-00288-7.

Additional file 1: Table S1. Differences in the mutational landscape between patients with VEN-I and VEN-R AML.

Acknowledgements

We thanked all members of the key laboratory of diagnosis and treatment in hematologic malignancies of Zhejiang and bone marrow morphology laboratory of the First Affiliated Hospital to Zhejiang University College of Medicine for technical support to diagnosis.

Authors' contributions

H.-H. Z. and X. Z. designed this study. J.-J. Q., H.-F. W., and Y. Z. collected the clinical materials. Y.-G. W. collected the samples for further sequencing. X. Z. displayed the PCR experiment. X. Z. integrated the data and wrote the manuscript. P.-X. Q., Y.-J. L., and J. J. provided advices for this work. H.-H. Z. revised this manuscript. All authors approved the manuscript. X. Z., J.-J. Q., H.-F. W., and Y.-G. W. were considered contributing equally to this work.

Funding

This study was funded by the National Natural Science Foundation of China (81800199), and the Natural Science Foundation of Zhejiang Province (LY21H080003).

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
This study was approved by the ethical review committees of the First Affiliated Hospital to Zhejiang University College of Medicine.

Consent for publication
Written informed consent was obtained from this patient.

Competing interests
The authors declare that they have no competing interests.

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Received: 5 January 2021 Accepted: 20 April 2021
Published online: 01 May 2021

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