Mediterranean Journal of Hematology and Infectious Diseases

Case Report

The Venetoclax/Azacitidine Combination Targets the Disease Clone in Acute Myeloid Leukemia, Being Effective and Safe in a Patient with COVID-19

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Competing interests: The authors declare no conflict of Interest.

Abstract. The addition of Venetoclax (VEN) to Hypomethylating agents (HMAs) significantly improves the probability of complete remission and prolongs survival in patients with Acute Myeloid Leukemia (AML) when compared to HMA alone. However, the mutated clone composition may impact the probability of response and its duration.
Here, we describe the molecular profile of a patient with AML rapidly evolved from a previous therapy-related-Chronic MyeloMonocytic Leukemia, who achieved safely complete remission after treatment with the VEN/Azacitidine combination, even in the presence of SARS-COVID-2 infection.
The targeted NGS analysis showed that the VEN/AZA combination led to the eradication of the *FLT3-ITD* and *RUNX1* mutated clone/s primarily associated with AML evolution, and subsequently, the *SRSF2, NRAS,* and *ASXL1* mutated clone/s.
This case also underlines the importance of the sequential use of targeted NGS for disease monitoring: the deep molecular remission achieved by this patient allowed to safely guide adjustments of drug dosage and treatment intervals in the presence of neutropenia, helping to rule out disease progression.

Keywords: Venetoclax; Venetoclax/azacitidine combination; AML; t-CMML; targeted-NGS.

Citation: Cristiano A., Palmieri R., Fabiani E., Ottone T., Divona M., Savi A., Buccisano F., Maurillo L., Tarella C., Arcese W., Voso M.T. The venetoclax/azacitidine combination targets the disease clone in Acute Myeloid Leukemia, being effective and safe in a patient with COVID. Mediterr J Hematol Infect Dis 2022, 14(1): e2022041, DOI: http://dx.doi.org/10.4084/MJHID.2022.041

Published: May 1, 2022 **Received: March 3, 2022** **Accepted: April 15, 2022**

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Introduction. Hypomethylating agents (HMA) were considered, until recently, the standard of care for older patients with acute myeloid leukemia (AML) ineligible for conventional induction chemotherapy and for patients with high-risk myelodysplastic syndromes (MDS).[1,2] The overall response rate to HMA in AML is about 50%, with complete remission (CR) achieved in about 15-30% of patients.[3] Adding venetoclax (Venclyxto, VEN, ABBVie) to HMA significantly improves the probability of CR and prolongs survival in elderly patients with newly diagnosed AML compared to HMA alone.[4] However, the clone composition may impact on the probability of response and its duration.[5-6] Patients with AML and *NPM1* and/or *IDH2* mutations

have the highest probability of response to VEN/HMA combinations, but also *RUNX1* mutations appear to be associated with favorable responses. On the other hand, activating kinase mutations such as *FLT3-ITD*, *N/KRAS*, *CBL*, or *KIT* have been associated with treatment resistance.[7] The impact of the combination treatment on the dynamics of the disease clones during VEN combination treatments has been only partially described.

We monitored the molecular profile of a patient with AML evolved from a previous therapy-related chronic myelomonocytic leukemia (t-CMML) during the treatment with a venetoclax/azacitidine (VEN/AZA) combination. The improved quality of clinical response to VEN/HMA versus HMA alone prompted us to reflect on the possible role of the VEN/AZA combination on the molecular response at both clonal and subclonal levels.

Case Report. A 74-year-old man with a previous history of prostate adenocarcinoma, originally diagnosed in 2000, relapsed in 2016, and treated with radiotherapy, was referred to our Hematology clinic in August 2020 because of anemia and persistent monocytosis.

At the first hematology consultation, the patient was in good general condition, complaining of fatigue and dizziness. Complete blood counts showed mild anemia (hemoglobin: 9.5 g/dl) and monocytosis (2.1×10^9/L), with normal platelet and white blood cell (WBC) counts. Bone marrow (BM) aspirate revealed multilineage dysplasia in more than 50% of the cells, a 20-30% infiltrate of monocytic/promonocytic cells, and 3% myeloid blasts (CD34+, CD117+, CD13+, CD33+, CD38+, HLA-DR+), consistent with the diagnosis of a t-CMML.

Conventional cytogenetic analysis revealed normal karyotype, and fluorescent *in-situ* hybridization was negative for chromosomal abnormalities involving chromosomes 5, 7, 8, and 20. The CMML CPSS score was intermediate.[8]

The patient was then started on human recombinant erythropoietin. However, after only two months of therapy, he was admitted to our Hematology ward because of fever and leukocytosis (WBC: 46.9×10^9/L), with blasts in the peripheral blood and increased lactate dehydrogenase levels (LDH: 628 UI/L). The BM aspirate displayed 60% monoblasts, confirmed both by morphology and flow cytometry (CD34-/CD117-/HLA-DR-/CD64+/CD11b+/CD13+/CD33+/CD45+), indicating AML evolution. At the time of admission, a simultaneous SARS-CoV-2 infection was diagnosed.

Molecular analysis for recurrent fusion genes (*BCR/ABL1*, *RUNX1/RUNX1T1*, *DEK/NUP214*, *CBFbeta/MYH11*) and common AML mutations (*NPM1*, *FLT3*, *IDH1*, *IDH2*) identified an *FLT3-ITD* mutation, with an allelic ratio (AR) of 0.22 by capillary electrophoresis (CE).

Given the concomitant SARS-CoV-2 infection and the t-AML, the patient was considered unfit for standard induction therapy and received a standard dose of Azacitidine (75 mg/m^2 for seven days, every day 28-day cycle) in combination with venetoclax (400 mg once daily, 28-day cycles).

Targeted Next Generation Sequencing (t-NGS) was performed on DNA samples extracted from BM mononuclear cells (MNC) at t-CMML diagnosis, at the time of AML progression, and during treatment (at 6 and 12 months) (**Figure 1**). We used the MYeloid Solution panel (MYS_1) by Sophia GENETICS (Saint Sulpice, Switzerland) to screen for somatic mutations in 30 genes frequently mutated in myeloid malignancies, using a variant allelic frequency (VAF) cut-off of 1%.

Somatic variants in 3 genes were detectable at t-CMML diagnosis, including *SRSF2* p.Pro95His (P95H, VAF: 46%), *NRAS* p.Gly12Ser (G12S, VAF: 37%), and *ASXL1* p.Glu635Arg_*fs**15 (E635R _*fs**15, VAF: 12%). These last variants are associated with a high probability of rapid CMML progression.[9]

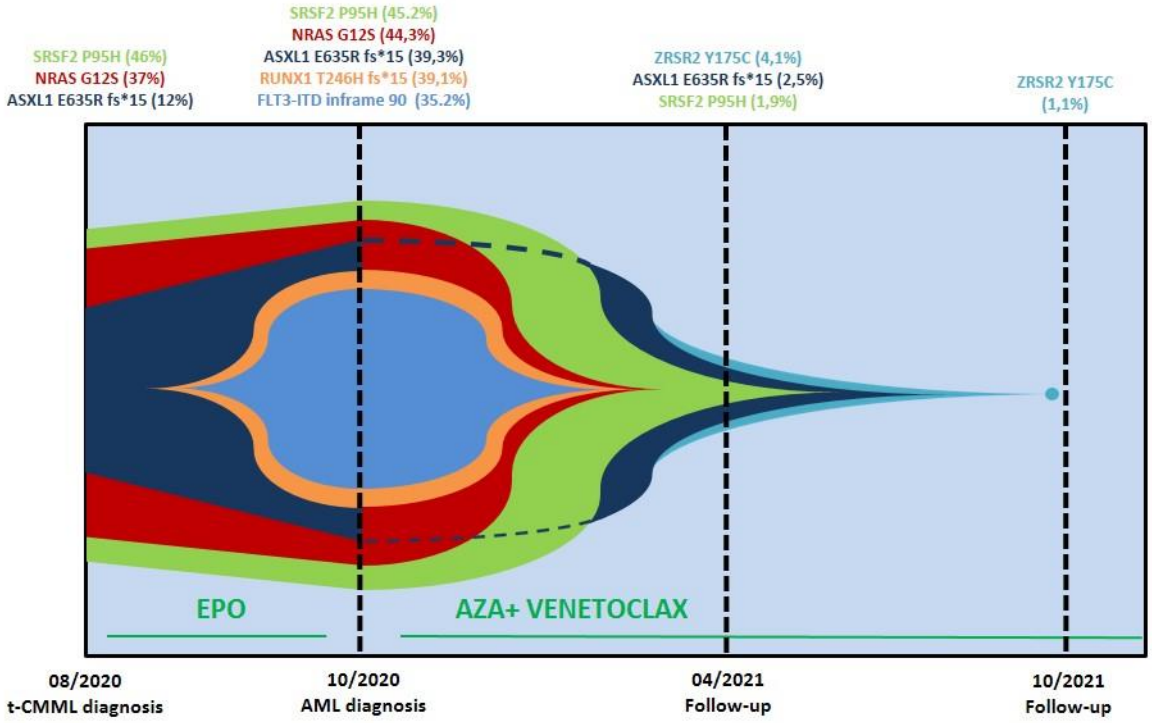
Leukemic evolution was associated with the acquisition of additional mutations, including the *FLT3-ITD* mutation (inframe_90, VAF=35.2%, also detected by CE), and a *RUNX1* p.Thr246Hisfs*15 variant (T246Hfs*15, VAF=39.1%), consistent with a high-risk AML profile, according to ELN 2017 stratification.[10]

After two cycles of VEN/AZA treatment, hematologic CR was achieved, residual disease assessment showed a decrease in subclonal levels for *FLT3-ITD* mutation on both DNA and RNA (AR <0.05), whereas it became negative after six cycles. However, MRD monitoring by flow cytometry (FC) was impaired due to the monocytic phenotype of AML blasts and the impossibility of defining the leukemia-associated immunophenotype.

After six cycles, *NRAS* and *RUNX1* mutations became undetectable, and the *ASXL1* and SRSF2 mutation burden steeply decreased (VAF from 39.3% to 2.5% and from 45.2% to 1.9%, respectively), while a new small *ZRSR2* p.Tyr175Cys (Y175C, VAF=4.1%) clone was acquired. Finally, after 12 treatment cycles, most gene mutations became undetectable, with the *ZRSR2* mutation burden decreasing to 1.1% (**Figure 1**).

At the time of reporting, the patient is receiving the fourteenth VEN/AZA cycle, is in CR, and in excellent general conditions. Although he underwent recurrent neutropenia, he did not show infectious complications, and the treatment schedule was adjusted to 5 days azacytidine 75 mg/sqm and 14 days venetoclax 100 mg, during cycles of 35 days.

Discussion. In this case report, we describe the evolution of the mutational profile of a patient with a t-CMML, rapidly progressing to AML, who was treated with the VEN/AZA combination, achieved hematologic CR, and undetectable somatic mutations by t-NGS.



FLT3-ITD evaluation by RT-PCR	10/2020 AML diagnosis	01/2021 Follow-up	04/2021 Follow-up	07/2021 Follow-up	10/2021 Follow-up
	Positive AR=0.22	Subclone positivity AR< 0,05	Negative	Negative	Negative

Figure 1. Clonal evolution of a t-CMML to AML, and during VEN/AZA combination treatment.

The mutation profile of t-CMML is usually characterized by a high frequency of cytogenetic abnormalities.[11] Despite the normal karyotype, our patient presented with mutations in myeloid genes commonly associated with unfavorable CMML outcome (in particular *NRAS* and *ASXL1*) and presented a rapid progression to AML (9,11). Of note, the weakening of the immune system, due to the co-occurrence of SARS-CoV-2 infection at the time of AML onset, may have contributed to disease progression. After AML onset, the patient was started on VEN/AZA combination treatment and achieved CR after two cycles. The treatment was well tolerated and confirmed the applicability of this reduced intensity regimen as induction to patients with concomitant SARS-CoV-2 infection.[12]

Treatment with Azacitidine or decitabine single agent has been shown to have a limited impact on the mutation burden in AML and MDS. Indeed, although HMA has been shown to reduce the size of mutated clones initially, they are rarely eradicated, even in patients in morphologic remission.[13-15] Furthermore, although considered disease-modifying, HMAs are not curative in AML and MDS, and mutations may persist or be acquired at disease recurrence and progression. This is also the case of *TP53* mutations, which may become undetectable following 10-day decitabine treatment, but tend to early recur and are associated with high rates of disease relapse.[15] Although speculatively, our data suggest that the addition of venetoclax to Azacitidine may have played a crucial role in eradicating leukemic clones carrying specific mutations that hardly would have been influenced by HMAs monotherapy, in line with previously published data.

After four cycles of VEN/AZA combination treatment in our patient, *FLT3-ITD*, *RUNX1* and *NRAS* mutations became undetectable, and there was a significant reduction of the *ASXL1* and *SRSF2* mutation burden.

We hypothesize that the VEN/AZA combination eradicated the *FLT3-ITD* and *RUNX1* mutated clone/s primarily associated with AML evolution and subsequently affected the underlying *SRSF2*, *NRAS*, and *ASXL1* mutated clone, which was present at the time of t-CMML diagnosis. The *ZRSR2* mutated subclone may have been initially masked by the overwhelming leukemic population and reappeared at low VAF after restoring normal hematopoiesis as part of clonal hematopoiesis of indeterminate potential (CHIP).

This case shows that the addition of venetoclax to HMA significantly improves the quality of response at

the molecular level: this is associated with prolonged disease remission, as in our patient. These data also underline the importance of the sequential use of t-NGS for disease monitoring: the deep molecular remission achieved by this patient allowed to safely guide adjustments of drug dosage and treatment intervals in the presence of neutropenia, helping to rule out disease progression.

The achievement of molecular negativity using the VEN/AZA combination will have to be confirmed on larger patient series, best by using sensitive NGS techniques. That will also help establish the role of MRD studies in this intermediate-intensity treatment of conventional chemotherapy.

Acknowledgements. This study was supported in part by AIRC 5x1000 call "Metastatic disease: the key unmet need in oncology" to MYNERVA project, #21267 (MYeloid NEoplasms Research Venture AIRC. A detailed description of the MYNERVA project is available at http://www.progettoagimm.it), by Ricerca finalizzata, code NET-2018-12365935 and PRIN grant N. 2017WXR7ZT to MTV.

Author Contributions. AC, RP, EF, AS, FB, LM, WA, CT and MTV performed research, analyzed the data, and wrote the paper; TO and MD analyzed the data. All Authors critically reviewed and approved the final version of the manuscript.

Competing Interests. MTV received honoraria from Celgene/BMS and Abbvie.

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