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Venetoclax in combination with hypomethylating agents in previously untreated patients with acute myeloid leukemia ineligible for intensive treatment: a *real-life* multicenter experience

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ABSTRACT

The addition of venetoclax to hypomethylating agents (HMA-V) improved the outcome of patients with newly diagnosed acute myeloid leukemia (AML) ineligible for intensive treatment. The aim of our study was to confirm data reported in literature, in a *real-life* multicenter experience. We retrospectively evaluated 56 naïve AML patients who received HMA-V at 8 different collaborating Hematology Units in the North-East of Italy, from September 2018 to October 2020. Patients received azacitidine or decitabine at standard dose, adding venetoclax starting from cycle 1–3. The median time-to-response was 2 cycles and composite complete remission rate (CCR) was 67.9%. Thirteen out of 38 responders (34.2%) relapsed, with a median response duration of 13.7 months. Transfusion independence (TI) was obtained in 27 (87.0%) and 28 (90.3%) out of 31 patients for red blood cells and platelets, respectively. Median OS was 12.3 months (95% CI, 8.1–16.5), and median PFS was 11.3 months (95% CI, 4.6–17.9). Cytogenetic risk was the only variable impacting on survival, while no differences were observed stratifying patients by age, bone marrow blasts, WHO classification or type of HMA. In conclusion, our *real-life* multicenter experience indicates that HMA-V treatment allows achieving good response rates in naïve AML patients, ineligible for intensive chemotherapy.

Abbreviations: AML, acute myeloid leukemia; AML-MRC, acute myeloid leukemia with myelodysplasia related changes; AML-NOS, acute myeloid leukemia not otherwise specified; CCR, composite complete remission; CR, complete remission; CRi, complete remission with incomplete hematology recovery; EFS, event-free survival; ELN, European Leukemia Net; FDA, Food and Drug Administration; G-CSF, granulocyte colony-stimulating factor; HMA, hypometylating agents; HMA-V, hypometylating agents plus venetoclax; HSCT, hematopoietic stem cell transplantation; MFC, multiparametric flow-cytometry; MRD, minimal residual disease; NGS, Next generation Sequencing; NR, non-responders; OS, overall survival; PFS, progression-free survival; PR, partial response; RD, response duration; RFS, relapse-free survival; t-AML, therapy-related acute myeloid leukemia; TI, transfusion independence; TLS, tumor lysis syndrome; TRM, treatment-related mortality; TTR, time to response; V, venetoclax; WHO, World Health Organization.

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1. Introduction

Acute myeloid leukemia (AML) is a heterogeneous group of clonal malignancies that can occur at any age even if almost 60% of cases are diagnosed among people older than 65 years [1]. Treatment of older adults remains an unsolved challenge [2,3]. In fact, AML of the elderly is often characterized by biological and genetic features that lend resistance to conventional chemotherapy; furthermore, treatment-related mortality (TRM) frequently exceeds any expected transient response in older patients, that are often affected by age-related comorbidities and poor performance status [4,5].

Hypomethylating agents (HMA) decitabine and azacitidine demonstrated a clinical benefit for elderly patients with AML in terms of hematologic improvement, reduction in transfusions and improved quality of life. However, long-term survival outcomes are unsatisfactory. Studies performed among elderly patients showed that decitabine and azacitidine (in > 30% blasts AML) may lead to a median overall survival (OS) of 7.7 and 10.4 months, respectively [6,7].

Among the novel therapies explored for AML in the last years, the BCL-2 inhibitor venetoclax (V), has played a crucial role. Preclinical studies showed induction of AML cell death in a p53-independent way, and synergistic anti-leukemia activity with HMA [8,9]. These data provided a rationale for clinical trials with the combination of hypomethylating agents plus venetoclax (HMA-V) in AML that led, in November 2018, to Food and Drug Administration (FDA) approval of this combination for the treatment of newly diagnosed AML patients aged ≥ 75 years or ineligible for intensive induction chemotherapy [10].

The aim of our study was to evaluate, in a real-life multicenter experience, the efficacy and safety of HMA-V in elderly or unfit AML patients, and to compare our results with those reported in literature.

2. Patients and methods

We retrospectively evaluated naïve AML patients who received HMA-V at 8 different collaborating Hematology Units in the North-East of Italy, from September 2018 to October 2020.

Patients were studied at diagnosis by morphology, immunophenotyping, cytogenetics and molecular genetic tests, according to the 2017 European Leukemia Net (ELN) recommendations [3]. We used collected data for classifying AML types according to the 2016 revision of World Health Organization (WHO) Classification of myeloid neoplasms and acute leukemias [11]. Patients were stratified in different risk categories by karyotype features: cytogenetic risk was evaluated according to the 2017 ELN recommendations, whereas we were not able to stratify our population by genetic profile, as mutational status of *RUNX1*, *ASXL1* and *TP53* was not available for all patients.

All included patients were treated with HMA-V since they had been considered unfit for intensive treatment, but not unfit for non-intensive chemotherapy. Treatment was given outside of a clinical trial, in accordance with the authorization of the Drug Italian Agency (AIFA) (AIFA 5% regulation). Patients were treated with azacitidine or decitabine, as a free clinician choice, at standard labeled dose, and V was added starting from cycle 1–3 with a short 1-week ramp-up from 100 mg to 400 mg daily and then continued at the dose of 400 mg daily. In patients with concomitant posaconazole anti-mycotic prophylaxis the dose of V was reduced to 100 mg daily, due to drug interaction. Dose adjustments of either V or HMA were allowed in case of toxicities, permitting both the single dose reduction as well as the modulation of schedule reducing the number of days of administration. Patients who achieved a hematological improvement continued therapy until disease progression or unacceptable toxicity. Patients underwent transfusion support and concomitant treatments with antibacterial and granulocyte colony-stimulating factor (G-CSF) as local practice.

Responses were evaluated according to the 2017 ELN recommendations [3]. Response evaluation included bone marrow morphological and, if possible, multiparametric flow-cytometry (MFC) analysis for minimal residual disease (MRD) evaluation. We defined time-to-response (TTR) as the number of cycles for achieving complete remission (CR) or CR with incomplete hematological recovery (CRi). Composite complete remission (CCR) was defined as the percentage of CR+CRi. Response duration (RD) was the time between CR/CRi and relapse. Transfusion independence (TI) was defined as ≥ 8 weeks without red blood cell and/or platelet transfusion. Safety profile was evaluated using the CTCAE criteria [12]. Finally, we evaluated overall survival (OS) and progression-free survival (PFS) of our population.

Statistical analysis was performed using the IBM SPSS Statistics version 25. The confidence intervals for CR and CCR rates were calculated at 95% level. Non-parametric tests were used to evaluate the correlation between response or relapse and patient features (age, percentage of BM blasts, cytogenetic risk groups, categories by WHO Classification, or type of HMA utilized). Survival curves were estimated according to the Kaplan-Meier product-limit method and were tested for significant differences between groups using the log-rank test. All tests were 2-sided, accepting $p < 0.05$ to indicate statistically significant differences, and confidence intervals were calculated at 95% level.

This study was approved by independent local ethic committees and conducted in accordance with the Helsinki declaration. It was registered on Clinicaltrials.gov (NCT04454580).

3. Results

3.1. Patients

Between September 2018 and October 2020, 56 previously untreated AML patients started HMA-V treatment. Biological features at onset, as well as assignment to 2016 WHO Classification categories are

Table 1
Baseline characteristics of 56 patients evaluated for the analysis. AML: acute myeloid leukemia; AML-MRC: acute myeloid leukemia with myelodysplasia related changes; AML-NOS: acute myeloid leukemia not otherwise specified; ELN: European Leukemia Net; RBC: red blood cells; t-AML: therapy- related acute myeloid leukemia; WHO: World Health Organization.

Biological features at onset	N = 56 (%)
Male/Female	29/27
Age (years), median (range)	75 (range 55–82)
55–75	30 (53.6)
> 75	26 (46.4)
Hemoglobin (g/dl), median (range)	9.4 (3.8–15.3)
Platelets (x 10^9), median (range)	38.5 (7–351)
White blood cells (x 10^9), median (range)	4.95 (0.45–109)
Bone marrow blasts (%), median (range)	55 (20–100)
20–30%	16 (28.6)
> 30%	40 (71.4)
Karyotype	
Normal	21 (37.5)
Complex	17 (30.4)
Deletions or monosomies	4 (7.1)
Other abnormalities	9 (16.1)
Not evaluable	5 (8.9)
Risk category by karyotype according to ELN recommendations	
Intermediate	28/51 (54.9)
Adverse	23/51 (45.1)
Somatic mutations	
NPM1	10/54 (18.5)
FLT3-ITD	8/54(14.8)
FLT3-TKD	3/54 (5.5)
IDH1/2	9/54 (16.6)
TP53	3/7 (42.8)
Classification category according to WHO 2016	
AML-MRC	26 (46.4)
t-AML	2 (3.6)
AML with recurrent genetic abnormalities	12 (21.4)
AML-NOS	16 (28.6)
Baseline trasfusion dependence	
RBC	32 (57.1)
Platelets	31 (55.3)

summarized in Table 1. The median age was 75 years (range 55–82), and 40 patients (71.4%) had a bone marrow blast count > 30%. Among 51 patients with evaluable cytogenetic profile, no *core-binding-factor (CBF)* AMLs were found, 37.5% of patients had normal and 30.4% had complex karyotype (21 and 17 patients, respectively). Two patients resulted chromosome 5 deletion carriers, while other 2 had monosomal karyotype for chromosome 7. Among other abnormalities of significance, we found 1 patient with the *BCR-ABL1* fusion gene, and 1 patient positive for a *KMT2A* rearrangement. When stratified by risk according to cytogenetic profile, we had no favourable risk patients, while intermediate and adverse groups were well distributed (54.9% vs 45.1%, respectively). We had limited data about mutational profile, and only 8 patients underwent Next Generation Sequencing (NGS) analysis. According to 2016 WHO Classification, AML with myelodysplasia related changes (AML-MRC) was the most representative category in our population (26/56 patients, 46.4%), and this diagnosis was based on cytogenetic criteria in 20/26 (76.9%) patients. We grouped this category with therapy-related AML (t-AML), in order to evaluate the impact of their common features on response and survival to our treatment. Transfusion-dependency data were available only for 31 patients at onset.

3.2. Treatment outcome

3.2.1. Response

All patients received HMA-V treatment. Fourty-three patients (76.8%) were treated with azacitidine, while 13 (23.2%) received decitabine (Table 2). The median number of cycles was 7 (range 1–29), and 14 patients (25.0%) received ≥ 12 cycles of therapy. At the time of data analysis 21 patients (37.5%) were on-therapy.

CR and CRi were achieved in 30 and 8 patients, respectively 53.6% (95% CI, 39.7–67.0) and 14.3%, with a CCR rate of 67.9% (95% CI, 54.0–79.7) and a median time to CR/CRi of 2 cycles (range 1–6). No differences in terms of response were observed in our population when stratified by age (55–75 vs >75 years), bone marrow blast count (20–30% vs >30%), cytogenetic risk groups, categories by WHO Classification, or type of HMA utilized (Table 3). Four patients obtained a partial remission (PR) as the best response, within the 5th cycle; 2 of them relapsed, and all died for infectious causes. Among the 14 non-responders (NR), bone marrow blast count was higher (>30%) in 9 patients (64.3%), and 7 (50.0%) were classified at onset as AML-MRC or t-AML.

Among the 38 CR/CRi patients, 13 (34.2%) relapsed, and the median RD was 13.7 months (95% CI, 10.8–16.7 months). Although not statistically significant, we observed that only 6 out of 21 (28.6%) responders with intermediate-risk cytogenetic relapsed, whereas half of patients (7/14, 50.0%) with adverse-risk karyotype lost their response (p = 0.19). We also observed a higher relapse rate in AML-MRC + t-AML group, when compared to other Classification categories of AMLs, with percentages of 50.0% and 20.0%, respectively (9/18 vs 4/20 patients, p = 0.052, Table 3),

Regarding mutational status, 5/8 *FLT3*-ITD positive patients obtained CR/CRi within the 4th cycle, and no one of them relapsed: median RD was 10.8 months, considering one early death for a major thrombocytopenia-related intracranial hemorrhage during 2nd cycle. Two out of 4 *TP53* mutated patients achieved CR, but 1 relapsed after 9.9 months and 1 died in remission for a COVID-related pneumonia after the 8th cycle of therapy.

We were not able to perform an analysis about MRD results in CR/CRi patients, due to partial collected data in a retrospective real-life experience.

Among 31 patients with known transfusion-dependence at onset, overall TI was obtained in 27 (87.0%) and 28 (90.3%) patients for red blood cells and platelets, respectively.

Table 2
Response rates and time-to-response (TTR) by type of hypometylating agent (HMA). CCR: composite complete response; CR: complete remission; CRi: complete remission with incomplete hematological recovery; NR: no responders; PR: partial response; V: venetoclax.

	Azacitidine + V	Decitabine + V	HMA+V
Quality of response, n. pts (%)	**N = 43**	**N = 13**	**N = 56**
CR	21 (48.8)	9 (69.2)	30 (53.6)
CRi	8 (18.6)	0 (0)	8 (14.3)
CR + CRi (CCR)	*29 (67.4)*	*9 (69.2)*	*38 (67.9)*
PR	2 (4.7)	2 (15.4)	4 (7.1)
NR	12 (27.9)	2 (15.4)	14 (25)
Number of cycles, median (range)	7 (1–24)	9 (1–29)	7 (1–29)
Number of cycles for CR+CRi, median (range)	2 (1–6)	3 (2–5)	2 (1–6)

3.2.2. Toxicities

During treatment, 15 patients (26.8%) presented pneumonia, 10 (17.8%) developed a sepsis, 7 (12.5%) had febrile neutropenia, and 2 (3.6%) had major hemorrhages. Gastro-intestinal symptoms were reported in 10 patients (17.8%), mostly represented by diarrhea or nausea of any grade. Neutropenia and thrombocytopenia gr. 3–4 were observed in 16 (28.5%), and 8 (14.3%) out of 56 patients, respectively.

Data regarding the need for hospitalization of the whole population were not available, not allowing to perform an observational analysis and calculate the hospitalization rate. After achievement of morphologic leukemia-free state (MLFS), 28/38 patients (73.6%) needed G-CSF stimulation. Due to hematological toxicities, 14 (36.8%) and 27 (71.0%) patients required a dose reduction of HMA and V, respectively, and 21 (55.3%) had to temporarily interrupt V administration. There were no adverse events related to tumor lysis syndrome (TLS).

3.2.3. Survival

At the time of analysis, 23 out of 56 (41.1%) patients were alive. Overall, 33 patients (58.9%) died, 28 (50.0%) for leukemia-related causes, 4 (7.1%) for infectious events and 1 (1.8%) for intracranial hemorrhage. Four out of 33 (12.1%) patients died in CR. After a median follow-up of 9.9 months (range 1.2–32.5), median OS was 12.3 months (95% CI, 8.1–16.5), and median PFS was 11.3 months (95% CI, 4.6–17.9).

When stratifying patients by age, bone marrow blast count, and type of HMA, no significant differences in terms of RD, OS or PFS were observed. Although not statistically significant, patients belonging to the group of AML-MRC + t-AML had a worse prognosis, when compared to AML with *NPM1* mutated + AML-NOS where median RD and median PFS were not reached (Table 3).

Coherently with the response results described above, in our analysis cytogenetic profile had an impact on survival: 12-months OS was 60.3% versus 45.5% in intermediate-risk and adverse-risk karyotype groups, respectively (p = 0.02, Fig. 1a). Moreover, patients in CR/CRi with an intermediate-risk karyotype had a higher RD than those with adverse-risk cytogenetic (14.7 versus 9.9 months, respectively, p = 0.03).

Analyzing survival by response to treatment, we observed a median OS of 19.9 months (95%CI, 11.0–28.7), 6.36 months (95%CI, 4.8–7.8), and 2.5 months (95%CI, 1.9–3.1), in CR,PR, and NR groups, respectively; median OS of patients in CRi was not reached, with the 65% of patients alive at the end of follow up (p < 0.001, Fig. 1b).

4. Discussion

The results of this multicenter *real-life* experience indicate that HMA-V is a tolerable and significantly active front-line therapy in elderly AML patients not eligible to intensive chemotherapy. These patients often present adverse genetic/cytogenetic features that sustain mechanisms of chemo resistance; moreover, advanced age and co-morbidities lead to a higher risk of adverse events and TRM.

Table 3
Correlation between biological features and response, relapse rates and survival. AML-MRC: acute myeloid leukemia with myelodysplasia related changes; AML-NOS: acute myeloid leukemia not otherwise specified; Aza: azacitidine; BM blasts: bone marrow blasts; CR: complete remission; CRi: complete remission with incomplete hematological recovery; Dec: decitabine; PFS: progression-free survival; OS: overall survival; RD: response duration; t-AML: therapy-related acute myeloid leukemia; V: venetoclax.

	CR + CRi (%)	Relapse rate (%)	12- months PFS	12- months OS	Median PFS (months)	Median OS (months)	Median RD (months)
Age (n = 56)	38 (67.9)	13 (34.2)	49.9%	52.5%	11.3	12.3	13.7
55–75 years (30)	19 (63.3)	8 (42.1)	53.5%	52.7%	14.6	12.3	13.0
> 75 years (26)	19 (73.1)	5 (26.3)	46.2%	52.5%	10.9	12.7	n.r.
	P = 0.43	P = 0.30			P = 0.63	P = 0.71	P = 0.99
BM blasts (n = 56)	38 (67.9)	13 (34.2)	49.9%	52.5%	11.3	12.3	13.7
20–30% (16)	9 (56.2)	5 (55.5)	58.0%	43.8%	14.6	7.04	13.0
> 30% (40)	29 (72.5)	8 (27.5)	47.4%	56.3%	11.3	12.7	n.r.
	P = 0.24	P = 0.12			P = 0.36	P = 0.26	P = 0.39
Risk by Karyotype (n = 51)	35 (68.6)	13 (37.1)	65.7%	60.3%	14.6	12.7	13.7 m
Intermediate (28)	21 (75.0)	6 (28.6)	29.9%	45.5%	18.8	27.5	14.7 m
Adverse (23)	14 (60.8)	7 (50.0)			7.7	10.5	9.9 m
	P = 0.28	P = 0.19			**P = 0.02**	**P = 0.02**	**P = 0.03**
Classification by WHO 2016 (n = 56)	38 (67.9)	13 (34.2)	49.9%	52.5%	11.3	12.3	13.7
	18 (64.3)	9 (50.0)	46.6%	55.5%	11.3	12.7	13.0
AML-MRC + t-AML (28)	20 (71.4)	4 (20.0)	54.0%	49.5%	n.r.	9.1	n.r.
AML-NOS + AML with recurrent abn. (28)							
	P = 0.57	P = 0.05			P = 0.18	P = 0.61	P = 0.59
Treatment (n = 56)	38 (67.9)	13 (34.2)	49.9%	52.5%	11.3	12.3	13.7
Aza + V (43)	29 (67.4)	11 (37.9)	46.3%	49.6%	10.9	10.5	13.7
Dec + V (13)	9 (69.2)	2 (22.2)	59.8%	61.5%	n.r.	n.r.	n.r.
	P = 0.90	P = 0.38			P = 0.29	P = 0.24	P = 0.53

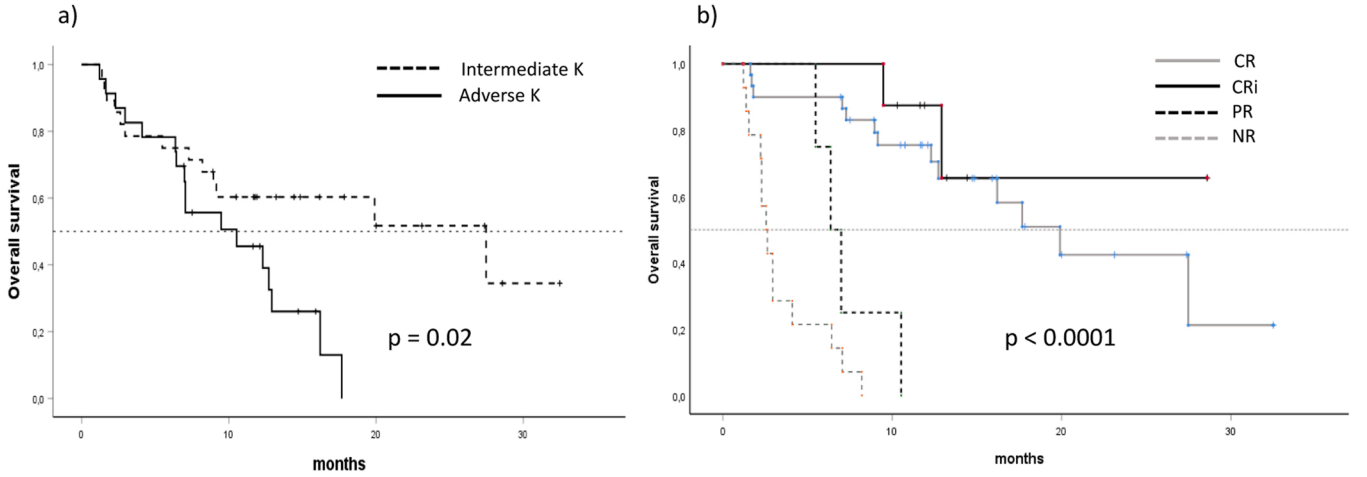

Fig. 1. Overall survival by (a) cytogenetic-risk profile and (b) type of response. CR: complete remission; CRi: complete remission with incomplete hematological recovery; NR: no responders; OS: overall survival; PR: partial response.

The biological characteristics of our population are consistent with a population of elderly patients considered unfit for intensive treatment: the median age was 75 years, and a high proportion of patients presented a cytogenetic/genetic profile in line with age and prior cytotoxic therapy [4,5]. Nevertheless, the absence of a favorable-risk karyotype group, being identified with *CBF*-AMLs, may have had a negative impact on our results, in terms of response and survival.

The median period of observation (9.9 months, range 1.2–32.5) can be considered adequate to evaluate the outcome in a patient population where the historical expected median OS is < 6 months.

Our *real-life* multicenter experience was in line with the results recently reported by other Authors. In 2018, DiNardo and colleagues reported promising results from a phase 1b study with this combination conducted in elderly, treatment-naïve, AML patients. Sixty-seven percent of patients achieved CR/CRi (73% in the V 400 mg cohort); the median OS was 17.5 months among all groups (not reached for the 400 mg V cohort) with good safety profile [13]. The phase 3 randomized, double-blind, VIALE-A study compared azacitidine + V versus azacitidine + placebo in 431 treatment-naive AML patients ineligible for intensive therapy and/or aged ≥ 75 years. Preliminary data showed a median OS of 14.7 months with V + azacitidine and 9.6 months with azacitidine alone (hazard ratio = 0.66; P < 0.001). The CCR rate was 66% and 28%, respectively (P < 0.001), with better results of azacitidine + V in all genetic subsets, including patients with adverse cytogenetic risk, secondary AML, and high-risk molecular mutations. The combination was generally well tolerated, with no increase in early mortality rate when compared to HMA alone. Moreover, benefits were also observed in terms of hematological improvement, with a proportion of patients who achieved TI significantly higher in the combination group. Due to hematological toxicities, the most of patients who received azacitidine + V (53%) needed modifications to the duration of V, and 32% also received G-CSF during remission [14,15].

In our *real-life* multicenter study, the CCR rate was 67.9% (95% CI, 54.0–79.7); even considering the small number of our case series, our

results indicate interesting response rates in line with those reported by DiNardo et al. Moreover, in VIALE-A study the cytogenetic analysis led to a 2 risk-groups stratification, intermediate- and poor-karyotype, according to the 2016 National Comprehensive Cancer Network (NCCN) guidelines, and no patients were found to have a favorable profile, as well as in our experience. In fact, although in our study we referred to the 2017 ELN recommendations, the criteria for karyotype-based stratification are overlapping with those used by DiNardo et al. We observed good response rates both in intermediate- and in adverse-risk groups, with a CCR rate of 75.0% and 60.8%, respectively; again, these data are consistent with the CCR rates reported by DiNardo et al. that were 74.2% and 52.9% in intermediate- and poor-karyotype groups, respectively.

Despite the evidence of a lower median OS in our whole population than that one reported by VIALE-A study (12.3 versus 14.7 months, respectively), among our 28 patients with an intermediate cytogenetic risk, the median OS was 27.5 months (versus 20.8 months, by DiNardo), whereas among the 23 patients with an adverse cytogenetic risk, the median OS resulted 10.5 months (versus 7.6 months, by DiNardo). These data confirm that, particularly in patients with adverse cytogenetic profile, also with HMA-V that leads to initial good response rates, it is very difficult to maintain remission and increase survival. In these terms, allogenic hematopoietic stem cell transplantation (HSCT) should be considered in selected patients with adverse biological features that achieve CR/CRi and whose performance status significantly improved after treatment [16].

The VIALE-A study allowed to identify different genetic subgroups in which good response rates do not translate in better survival: in our study, the small number of patients as well as the limited available data about NGS molecular profile, did not permit to draw any correlation between *TP53, FLT3, NPM1,* and *IDH* mutational status and outcome.

The lack of data about MRD status in our population did not allow us to perform an analysis concerning the quality of response obtainable with the HMA-V combination in a *real-life* experience. Recently, Maiti et al. published the results of a trial whose objective was to investigate the correlation between MRD status and outcomes after decitabine + V in elderly or unfit AML patients. Among the 83 patients that achieved CR/CRi, 52 (54%) became MRD negative by MFC, with a median TTR of 2.0 months. This translated in a longer relapse-free survival (RFS) compared with those MRD positive (median RFS not reached versus 5.2 months, $P = 0.004$), longer event-free survival (EFS, median not reached versus 5.8 months, $P = 0.001$), and longer OS (median OS 25.1 vs 7.1 months, $P = 0.001$) [17]. These data confirm the importance of MRD status by MFC after remission to predict long-term outcome in patients treated with the HMA-V combination and suggest that MRD monitoring should be integrated in the *real-life* clinical practice.

In 2019 Winters et al. conducted a study on 33 patients treated with HMA-V in first line in a *real-life* setting, reporting CR/CRi rates similar to those of our analysis; they also tried to investigate MRD by droplet digital PCR (ddPCR) analysis, but data were limited to a too small population for drawing any conclusion [18].

Looking at the safety profile, an important data emerged from our analysis, which is in line to the same reported in VIALE-A study [15]: the most of patients required a dose reduction of V, and needed G-CSF stimulation during remission, due to hematological toxicity and concomitant medications with azoles. The interaction between V and azoles requires a dose reduction of V during the neutropenia period, but in most of patients it is difficult to maintain the standard dose of V at 400 mg, even after suspension of antimycotic prophylaxis and achievement of remission. The use of G-CSF was proportionally higher in our population than in the DiNardo's one, and this may help to justify the lower incidence of febrile neutropenia that we observed. Gastro-intestinal adverse events also appeared less frequently than those reported in literature, and it may depend on the different anti-emetics and supportive medicaments administration that may differ in every single Center clinical practice. However, the HMA-V combination should be considered as a medium-intensive treatment regimen, feasible in the *real-life* but which requires close clinical and laboratory monitoring, to optimize dose and timing of therapy and avoid toxicity.

5. Conclusions

Considering the exploratory nature of our study and the small number of cases, the results obtained are interesting and in line with those found in the literature. Our experience suggests that the combination of V and HMA is feasible in the *real-life*, is associated with impressive remission rates and good safety profile, representing a new treatment option for treatment-naïve elderly AML patients. The limits of this treatment strategy include the difficult in maintaining a long-term remission and improving survival rates, particularly in those patients that present adverse genetic/cytogenetic features at baseline, in which HSCT should be considered, if feasible, after achievement of remission. Moreover, even if it represents the new standard of care for elderly or unfit treatment-naïve AML patients, HMA-V combination has several toxicities to take in count at the moment of therapeutic choice.

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none.

Declaration of Competing Interest

FZ received honoraria for the participation to medical meetings and advisory board from ABBVIE. AC received honoraria as a speaker and member of advisory board from ABBVIE and JANSSEN. The other authors have no conflicts of interest to declare.

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FZ, EDB, MS, DG, CP designed the study, EDB, FZ, EL wrote the paper, MS, DG, SI, AC, AL, IT, EM, FM, ML, DL, RB, MP collected and analyzed the data, All the authors critically review the paper and approved the final manuscript.

References

[1] Institute NC. SEER cancer statistics review, 1975–2015: overview, median age at diagnosis. [Internet]. SEER. 2018. Available from: https://seer.cancer.gov/.

[2] M.S. Tallman, E.S. Wang, J.K. Altman, F.R. Appelbaum, V.R. Bhatt, D. Bixby, S. E. Coutre, M. De Lima, A.T. Fathi, M. Fiorella, J.M. Foran, A.C. Hall, M. Jacoby, J. Lancet, T.W. LeBlanc, G. Mannis, G. Marcucci, M.G. Martin, A. Mims, M. R. O'Donnell, R. Olin, D. Peker, A. Perl, D.A. Pollyea, K. Pratz, T. Prebet, F. Ravandi, P.J. Shami, R.M. Stone, S.A. Strickland, M. Wieduwilt, K.M. Gregory, OCN, L. Hammond, N. Ogba, Acute myeloid leukemia, Version 3.2019, NCCN clinical practice guidelines in oncology, J. Natl. Compr. Canc Netw. 17 (6) (2019) 721–749.

[3] H. Döhner, E. Estey, D. Grimwade, S. Amadori, F.R. Appelbaum, T. Büchner, H. Dombret, B.L. Ebert, P. Fenaux, R.A. Larson, R.L. Levine, F. Lo-Coco, T. Naoe, D. Niederwieser, G.J. Ossenkoppele, M. Sanz, J. Sierra, M.S. Tallman, H.F. Tien, A. H. Wei, B. Löwenberg, C.D. Bloomfield, Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel, Blood 129 (4) (2017) 424–447.

[4] H. Kantarjian, F. Ravandi, S. O'brien, J. Cortes, S. Faderl, G. Garcia-Manero, E. Jabbour, W. Wierda, T. Kadia, S. Pierce, J. Shan, M. Keating, E.J. Freireich, Intensive chemotherapy does not benefit most older patients (age 70 years or older) with acute myeloid leukemia, Blood 116 (22) (2010) 4422–4429.

[5] H.D. Klepin, Elderly acute myeloid leukemia: assessing risk, Curr. Hematol. Malig. Rep. 10 (2) (2015) 118–125.

[6] A.F. Cashen, G.J. Schiller, M.R. O'Donnell, J.F.Multicenter DiPersio, phase II study of decitabine for the first-line treatment of older patients with acute myeloid leukemia, J. Clin. Oncol. J. Am. Soc. Clin. Oncol. 28 (4) (2010) 556–561.

[7] H. Dombret, J.F. Seymour, A. Butrym, A. Wierzbowska, D. Selleslag, J.H. Jang, R. Kumar, J. Cavenagh, A.C. Schuh, A. Candoni, C. Récher, I. Sandhu, T. Bernal del Castillo, H.K. Al-Ali, G. Martinelli, J. Falantes, R. Noppeney, R.M. Stone, M. D. Minden, H. McIntyre, S. Songer, L.M. Lucy, C.L. Beach, H. Döhner, International phase 3 study of azacitidine vs conventional care regimens in older patients with newly diagnosed AML with >30% blasts, Blood 126 (3) (2015) 291–299.

[8] T. Tsao, Y. Shi, S. Kornblau, H. Lu, S. Konoplev, A. Antony, V. Ruvolo, Y.H. Qiu, N. Zhang, K.R. Coombes, M. Andreeff, K. Kojima, M. Konopleva, Concomitant inhibition of DNA methyltransferase and BCL-2 protein function synergistically

induce mitochondrial apoptosis in acute myelogenous leukemia cells, Ann. Hematol. 91 (12) (2012) 1861–1870.
[9] R. Pan, L.J. Hogdal, J.M. Benito, D. Bucci, L. Han, G. Borthakur, J. Cortes, D. J. DeAngelo, L. Debose, H. Mu, H. Döhner, V.I. Gaidzik, I. Galinsky, L.S. Golfman, T. Haferlach, K.G. Harutyunyan, J. Hu, J.D. Leverson, G. Marcucci, M. Müschen, R. Newman, E. Park, P.P. Ruvolo, V. Ruvolo, J. Ryan, S. Schindela, P. Zweidler-McKay, R.M. Stone, H. Kantarjian, M. Andreeff, M. Konopleva, A.G. Letai, Selective BCL-2 inhibition by ABT-199 causes on-target cell death in acute myeloid leukemia, Cancer Disco 4 (3) (2014) 362–375.
[10] FDA approves venetoclax in combination for AML in adults. Available from: https://www.fda.gov/drugs/fda-approves-venetoclax-combination-aml-adults.
[11] D.A. Arber, A. Orazi, R. Hasserjian, J. Thiele, M.J. Borowitz, M.M. Le Beau, C. D. Bloomfield, M. Cazzola, J.W. Vardiman, The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia, Blood 127 (20) (2016) 2391–2405.
[12] Common Terminology Criteria for Adverse Events (CTCAE) v5.0, Cancer therapy evaluation program (CTEP), Natl. Cancer Inst. (2017) (Available from), https://ctep.cancer.gov/protocoldevelopment.
[13] C.D. DiNardo, K.W. Pratz, A. Letai, B.A. Jonas, A.H. Wei, M. Thirman, M. Arellano, M.G. Frattini, H. Kantarjian, R. Popovic, B. Chyla, T. Xu, M. Dunbar, S.K. Agarwal, R. Humerickhouse, M. Mabry, J. Potluri, M. Konopleva, D.A. Pollyea, Safety and preliminary efficacy of venetoclax with decitabine or azacitidine in elderly patients with previously untreated acute myeloid leukaemia: a non-randomised, open-label, phase 1b study, Lancet Oncol. 19 (2) (2018) 216–228.
[14] C.D. DiNardo, K. Pratz, V. Pullarkat, B.A. Jonas, M. Arellano, P.S. Becker, O. Frankfurt, M. Konopleva, A.H. Wei, H.M. Kantarjian, T. Xu, W.J. Hong, B. Chyla, J. Potluri, D.A. Pollyea, A. Letai, Venetoclax combined with decitabine or azacitidine in treatment-naive, elderly patients with acute myeloid leukemia, Blood 133 (1) (2019) 7–17.
[15] C.D. DiNardo, B.A. Jonas, V. Pullarkat, M.J. Thirman, J.S. Garcia, A.H. Wei, M. Konopleva, H. Döhner, A. Letai, P. Fenaux, E. Koller, V. Havelange, B. Leber, J. Esteve, J. Wang, V. Pejsa, R. Hájek, K. Porkka, Á. Illés, D. Lavie, R.M. Lemoli, K. Yamamoto, S.S. Yoon, J.H. Jang, S.P. Yeh, M. Turgut, W.J. Hong, Y. Zhou, J. Potluri, K.W. Pratz, Azacitidine and venetoclax in previously untreated acute myeloid leukemia, N. Engl. J. Med 383 (7) (2020) 617–629.
[16] F. Ferrara, Venetoclax plus hypomethylating agents or low dose cytarabine in acute myeloid leukemia: all that glitters is gold? Blood Cancer J. 10 (1) (2020) 10.
[17] A. Maiti, C.D. DiNardo, S.A. Wang, J. Jorgensen, T.M. Kadia, N.G. Daver, N. J. Short, M. Yilmaz, N. Pemmaraju, G. Borthakur, P. Bose, G.C. Issa, A. Ferrajoli, E. J. Jabbour, N. Jain, G. Garcia-Manero, M. Ohanian, K. Takahashi, G. Montalban-Bravo, L. Masarova, J.A. Burger, P.A. Thompson, S. Verstovsek, K. Sasaki, M. Andreeff, C.R. Rausch, K.S. Montalbano, S. Pierce, W. Qiao, J. Ning, H. M. Kantarjian, M.Y. Konopleva, F. Ravandi, Prognostic value of measurable residual disease after venetoclax and decitabine in acute myeloid leukemia, Blood Adv. 5 (7) (2021) 1876–1883.
[18] A.C. Winters, J.A. Gutman, E. Purev, M. Nakic, J. Tobin, S. Chase, J. Kaiser, L. Lyle, C. Boggs, K. Halsema, J.T. Schowinsky, J. Rosser, M.D. Ewalt, B. Siegele, V. Rana, S. Schuster, D. Abbott, B.M. Stevens, C.T. Jordan, C. Smith, D.A. Pollyea, Real-world experience of venetoclax with azacitidine for untreated patients with acute myeloid leukemia, Blood Adv. 3 (20) (2019) 2911–2919.