Molecular predictors of response to venetoclax plus hypomethylating agent in treatment-naïve acute myeloid leukemia

Venetoclax (Ven) in combination with hypomethylating agents (HMA) is Food and Drug Administration-approved therapy for elderly/unfit acute myeloid leukemia (AML) patients. The phase 3 randomized VIALE-A study demonstrated superior efficacy and prolonged survival with Ven+HMA compared to HMA alone in previously untreated patients with AML.[1] Moreover, in the particular study, superior responses with Ven+HMA were observed across the mutational spectrum including in patients with *IDH1/2*, *FLT3*, *NPM1* and *TP53* mutations.[1] In contrast, in another study which included elderly AML patients treated with Ven-based combination therapy in the upfront setting, response was found to be favorable (complete response or compete response with incomplete count recovery [CR/CRi] >80%) with *NPM1*, *IDH1/2*, and *DNMT3A* mutations, and inferior with *TP53*, *RUNX1*, *FLT3/ITD*, and *RAS* mutations.[2] Furthermore, survival was prolonged in the presence of *NPM1* and *IDH2* mutations with 2-year overall survival (OS) of 71.8% and 79.5%, respectively.[2] A recent pooled analysis of *IDH1/2*-mutated AML patients enrolled in Ven+ azacitidine clinical trials confirmed favorable response rates and OS in the presence of *IDH1/2* mutations.[3] The aforementioned observations were based on clinical trial results and require systematic validation. Accordingly, the objective of the current study was to determine the impact of mutations on response and survival in treatment-naïve AML patients receiving Ven+HMA in routine clinical practice.

Treatment-naïve AML patients receiving Ven+HMA outside a clinical trial at the Mayo Clinic were retrospectively recruited after obtaining Institutional Review Board approval. At our institution, Ven+HMA regimen was selected by the treating physician primarily based on patient age and fitness. Cytogenetic and molecular studies were performed by conventional karyotype, and next-generation sequencing (NGS) of a 42-gene panel, respectively. Additionally, *FLT3* and *NPM1* reverse transcription polymerase chain reaction (RT-PCR) was obtained. All patients received at least one cycle of Ven+HMA, with the Ven dose adjusted based on drug interactions particularly with azole antifungal prophylaxis.[4] Azacitidine 75 mg/m^2 days 1 to 7 or decitabine 20 mg/m^2 days 1 to 5 was administered as part of the combination therapy. Bone marrow biopsy was obtained after either cycle 1 or 2 in the majority of cases based on treating physician discretion with response assessed according to the 2017 European LeukemiaNet (ELN) criteria.[5] Minimal/measurable residual disease (MRD) assessment by multiparametric flow cytometry with a sensitivity of 0.01% was performed at the time of achieving CR, or CRi in a subset of patients. Follow-up was updated in February 2022. Determinants of treatment response were assessed by Chi-square or Fisher's exact test for nominal data and Wilcoxon rank-sum test for continuous variables. OS was evaluated by the Kaplan–Meier method with differences compared by the log-rank test. Analyses were performed using JMP Pro 16.0.0 software package, SAS Institute, Cary, NC.

One hundred and three AML patients (median age 74 years, 67% male, 62% *de novo*) received upfront Ven+HMA. ELN cytogenetic risk included favorable (5%, n=5), intermediate (50%, n=52) or adverse (45%, n=46). Mutations involved *TP53* in 25 patients (25%), *TET2* in 24 (23%), *IDH1/IDH2* in 20 (19%), *RUNX1* in 19 (19%), *ASXL1* in 18 (18%), *SRSF2* in 18 (18%), *K/NRAS* in 15 (15%), *NPM1* in 13 (13%), *DNMT3A* in 13 (13%), and *FLT3-ITD* in ten (10%) patients. Sixty-four (62%) patients received decitabine and the remainder azacitidine with a median Ven dose of 200 mg (range, 50-400 mg). Fourty-seven (46%) patients experienced cycle delays/interruptions; moreover, Ven and HMA dose adjustments were instituted after cycle 1 in 89 (86%) and 29 (29%) patients, respectively. Venetoclax duration was reduced in eighty patients (range; 7 to 21 days) while azacitidine duration was reduced in ten patients (range, 3 to 5 days) and dose reduced to 50 mg/m^2 in five patients; decitabine duration reduced to 3 days in 11 patients and dose reduction to 15 mg/m^2 in five patients. Pancytopenia related to therapy was noted in 35 (34%) patients and was complicated by neutropenic fever in 22 cases (21%) secondary to gram-negative bacteremia (n=3), Staphylococcal bacteremia (n=2), *Clostridium difficile* colitis (n=2), influenza A (n=2), respiratory syncytial virus (n=1), COVID19 (n=1), coccidiomycosis (n=1), Aspergillus pneumonia (n=1), Fusarium fungemia (n=1). Renal toxicity was noted in three patients which included tumor lysis syndrome in one case. 30-day mortality was 5% (n=5). Table 1 provides information regarding patient characteristics at the time of initiation of Ven+HMA, response rates, and overall outcome.

Fourty (39%) patients achieved CR, 20 (19%) CRi, resulting in CR/CRi in 60 (58%) patients with median time to best response of 1.4 months (range, 1.0-10.4 months) and median duration of response of 6.6 months (range, 1.0-32

Table 1. Clinical characteristics at time of treatment with venetoclax and hypomethylating agent for 103 patients with treatment-naïve acute myeloid leukemia stratified by achievement of complete response or compete response with incomplete count recovery.

Variables	All patients N=103	Patients in CR/CRi N=60	Patients not in CR/CRi N=43	*P* value/ Multivariate P value
Age in years, median (range), Age > 60 years, N (%)	74 (36-92) 93 (90)	74 (36-88) 56 (93)	74 (36-88) 56 (93)	0.90 0.53
Male, N (%)	69 (67)	39 (65)	30 (70)	0.42
AML type, N (%) *De novo* Secondary or therapy-related	 64 (62) 39 (38)	 42 (70) 19 (32)	 22 (51) 20 (47)	 **0.09**/0.75
Hemoglobin, g/dL, median (range)	8.6 (4.8-14)	8.6 (4.8-14)	8.5 (5.1-12.9)	0.98
Leukocyte count x 10^9/L, median (range)	3.6 (0.1-116)	3.65 (0.1-116)	3.95 (0.5-107)	0.45
Platelet count x 10^9/L, median (range)	59.5 (7-444)	61.5 (9-444)	51 (7-239)	0.54
Circulating blasts %, median (range)	14 (0-93)	15 (0-93)	11 (0-92)	0.6
Bone marrow blasts %, median (range)	48 (2-97)	47 (2-97)	48 (2-91)	0.4
2017 ELN cytogenetic risk stratification, N (%) Favorable Intermediate Adverse	 5 (5) 52 (50) 46 (45)	 4 (7) 33 (55) 23 (38)	 1 (2) 19 (44) 23 (53)	 **0.11**/0.93
Mutations on NGS, N (%) *TP53* *ASXL1* *RUNX1* *TET2* *DNMT3A* *SRSF2* *IDH1/IDH2* *NPM1* *K/NRAS* *FLT3-ITD*	 25 (25) 18 (18) 19 (19) 24 (23) 13 (13) 18 (18) 20 (19) 13 (13) 15 (15) 10 (10)	 8 (13) 15 (25) 11 (18) 15 (25) 7 (12) 12 (20) 14 (23) 9 (15) 9 (15) 3 (5)	 17 (40) 3 (7) 8 (19) 9 (21) 6 (14) 6 (14) 6 (14) 4 (9) 6 (14) 7 (16)	 **0.002/0.01** **0.01/0.02** 0.9 0.71 0.68 0.48 0.27 0.45 0.88 **0.06/0.01**
HMA used, N (%) Azacitidine Decitabine	 39 (38) 64 (62)	 20 (33) 41 (68)	 19 (44) 23 (53)	 0.20
Final dose of venetoclax, mg, (median, range)	200 (50-400)	200 (100-400)	200 (50-400)	0.47
Cycle delays, N (%)	47 (46)	32 (53)	15 (35)	0.09
Venetoclax dose adjustment, N (%)	89 (86)	56 (93)	33 (77)	0.05
HMA dose adjustment, N (%)	29 (28)	20 (33)	9 (21)	0.23
Allogeneic transplant, N (%)	9 (9)	8 (13)	1 (2)	**0.04**

HMA: hypomethylating agent; NGS: next-generation sequencing; ELN: European LeukemiaNet; CR: complete response; CRi: CR with incomplete count recovery.

months). MRD by multiparametric flow cytometry was assessed in a subset of patients achieving CR/CRi (n=20) and was negative in 15 (75%) of patients. The remainder of the responses included morphological leukemia-free state in five (5%), partial remission in one (1%), and stable disease in 19 (18%) patients while progressive disease was noted in four (4%) cases. Follow-up molecular studies were performed in three of eight *TP53*-mutated patients that achieved CR/CRi; *TP53* variant allele frequency pre- and post-therapy were 81%/39%, 52%/24% and 42%/6%, respectively.

In univariate analysis, presence of *ASXL1* mutation was associated with favorable response (CR/CRi 83% *vs.* 53%, *P*=0.01), while secondary AML (CR/CRi 49% *vs.* 65%, *P*=0.09), adverse karyotype (50% *vs.* 65%, *P*=0.11), presence of *TP53* (32% *vs.* 67%, *P*=0.002) and *FLT3-ITD* mutations (30% *vs.* 61%; *P*=0.06) predicted inferior response. In multivariable analysis, including the aforementioned variables of significance/borderline significance, presence of *ASXL1* mutation (83% *vs.* 53%; overall response [OR] 4.5) and absence of *TP53* (67% *vs.* 32%; OR 3.3) and *FLT3-ITD* mutations predicted favorable response (61% *vs.* 30%; OR

6.4). Moreover, in *ASXL1*-mutated patients, CR/CRi was not impacted by the presence of *TP53* mutation (100% *vs.* 81%) whereas in *ASXL1*-unmutated patients, the presence of *TP53* mutation predicted inferior response (26% *vs.* 63%; *P*=0.001). *TP53* mutations clustered with absence of *ASXL1* mutations; 23 of 85 (27%) *ASXL1*-unmutated patients harbored *TP53* mutations *versus* two of 18 (11%) of *ASXL1*-mutated patients (Table 2). *ASXL1* and *TP53* are typically mutually exclusively,[6] however presence of *TP53* mutation in two patients with *ASXL1* mutation was not detrimental to achievement of response.

Presence of *NPM1* (CR/CRi; 69% *vs.* 57%, *P*=0.41), *IDH1/2* (70% *vs.* 55%; *P*=0.23), *DNMT3A* (54% *vs.* 59%; *P*=0.73), *RUNX1* (58% *vs.* 58%; *P*=0.1), *TET2* (63% *vs.* 57%; *P*=0.63), *SRSF2* (67% *vs.* 56%; *P*=0.42) and *K/NRAS* mutations (60% *vs.* 58%, *P*=0.88) did not impact response. In addition, the likelihood of response was not impacted by prior HMA use (n=7); CR/CRi was 43% *vs.* 59% with or without prior HMA therapy, (*P*=0.40). Similar responses were noted with azacitidine and decitabine with CR/CRi rates of 51% and 64%, respectively (*P*=0.20).

Furthermore, *ASXL1*-mutated patients were compared to their unmutated counterparts and were more likely to be males (83% *vs.* 63%; *P*=0.09), *RUNX1*-mutated (33% *vs.*

Table 2. Patient characteristics at time of treatment with venetoclax and hypomethylating agent for 103 patients with treatment-naïve acute myeloid leukemia stratified by *ASXL-1* mutation status.

Variables	All patients N=103	ASXL1-mutated patients N=18	ASXL1-unmutated patients N=85	*P* value
Age in years, median (range), Age > 60 years, N (%)	74 (36-92) 93 (90)	73 (56-88) 17 (94)	74 (36-92) 76 (89)	0.50 0.49
Male, N (%)	69 (67)	15 (83)	54 (64)	0.09
AML type, N (%) *De novo* Secondary or therapy-related	 64 (62) 39 (38)	 10 (56) 8 (44)	 54 (64) 31 (36)	0.53
Hemoglobin, g/dL, median (range)	8.6 (4.8-14)	8.9 (6.7-12.3)	8.6 (4.8-14)	0.24
Leukocyte count x 10^9/L, median (range)	3.6 (0.1-116)	8 (1.1-98.7)	3.1 (0.1-116.7)	0.18
Platelet count x 10^9/L, median (range)	61 (7-444)	62 (9-444)	59 (7-239)	0.06
Circulating blasts %, median (range)	14 (0-93)	9 (0-86)	14 (0-93)	0.58
Bone marrow blasts %, median (range)	48 (2-97)	40 (4-92)	48 (2-97)	0.20
2017 ELN cytogenetic risk stratification, N (%) Favorable Intermediate Adverse	 5 (5) 52 (50) 46 (45)	 1 (6) 11 (61) 6 (33)	 4 (5) 41 (48) 40 (47)	0.56
Mutations on NGS, N (%) *TP53* *RUNX1* *TET2* *DNMT3A* *IDH1/IDH2* *NPM1* *FLT3-ITD*	 25 (25) 19 (19) 24 (23) 13 (13) 20 (19) 13 (13) 10 (10)	 2 (11) 6 (33) 6 (33) 0 (0) 2 (11) 1 (6) 2 (11)	 23 (27) 13 (15) 18 (21) 13 (15) 18 (21) 12 (14) 8 (9)	 0.13 0.09 0.28 0.02 0.30 0.31 0.83
CR/CRi, N (%)	60 (58)	15 (83)	3 (17)	**0.01**
HMA used, N (%) Azacitidine Decitabine	39 (38) 64 (62)	3 (17) 15 (83)	36 (42) 49 (58)	**0.03**
Final dose of venetoclax, mg, (median, range)	200 (50-400)	200 (100-400)	200 (50-400)	0.68
Cycle delays, N (%)	47 (46)	6 (33)	41 (48)	0.24
Venetoclax dose adjustment, N (%)	89 (88)	18 (100)	72 (85)	**0.03**
HMA dose adjustment, N (%)	29 (28)	4 (22)	25 (29)	0.51
Allogeneic transplant, N (%)	9 (9)	1 (6)	8 (9)	0.58

HMA: hypomethylating agent; AML: acute myeloid leukemia; CR: complete response; CRi: CR with incomplete count recovery; ELN: European LeukemiaNet; NGS: next generation sequencing.

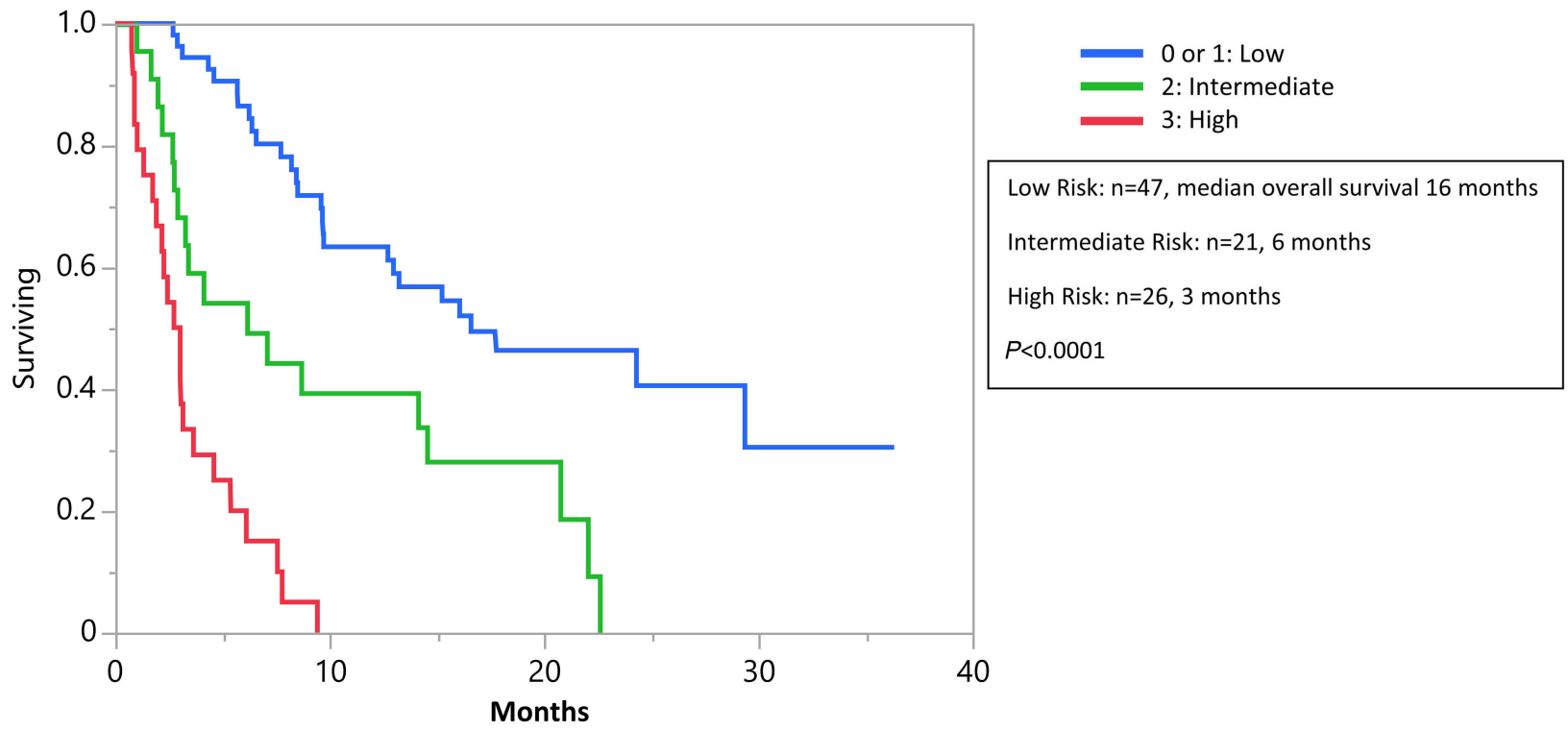

Figure 1. Survival according to risk groups. Survival of 94 treatment-naïve, non-transplanted acute myeloid leukemia (AML) patients receiving venetoclax and hypomethylating agent (Ven+HMA), stratified by hazard ration (HR)-weighted scoring system, HR in the absence of complete response or compete response with incomplete count recovery (CR/CRi), 5.9 (95% confidence interval [CI]: 3.3-10.6), *ASXL1* mutation, 2.9 (95% CI: 1.5-5.5), and adverse karyotype, 1.8 (95% CI: 1.1-3.0), allocating 2 adverse points for not achieving CR/CRi, 1 adverse point for adverse karyotype, and 1 adverse point for *ASXL1* mutation. Median overall survival stratified by low risk (0-1 points), intermediate risk (2 points) and high risk (3 points) is shown.

15%, *P*=0.09), *DNMT3A*-unmutated (100% *vs.* 85%, *P*=0.02), have received decitabine (83% *vs.* 58%; *P*=0.03) (Table 2). CR/CRi rates were conspicuously higher in *ASXL1*-mutated patients but accompanied by higher rates of MRD positivity; 60% *vs.* 13% in *ASXL1*-unmutated (*P*=0.04). Therefore, relapses after achieving CR/CRi were frequent in 11 of 15 (73%) *ASXL1*-mutated patients *versus* 16 of 45 (35%) of unmutated cases (*P*=0.02).

After a median follow-up of 6.6 months (16.5 months for alive patients; range, 0.6-36.3), 68 patients (66%) have died and nine (9%) underwent allogeneic stem cell transplant. Median OS (mOS) was 8.5 months (range, 0.6-36 months) and longer in transplanted patients (not reached *vs.* 8.4 months, *P*=0.08).

Age-adjusted survival analysis limited to 94 patients that did not receive transplant, identified CR/CRi (*P*<0.0001), *NPM1* (*P*=0.009), and *IDH1/2* mutations (*P*=0.02) as favorable, and *TP53* (*P*=0.01), *ASXL1* mutations (*P*=0.17) and adverse karyotype (*P*=0.05) as unfavorable risk factors for survival. Multivariable analysis confirmed the negative survival impact of not achieving CR/CRi, presence of *ASXL1* mutation and adverse karyotype; hazard ratio [HR] of 5.9 (95% confidence Interval [CI]: 3.3-10.6) for absence of CR/CRi, 2.9 (95% CI: 1.5-5.5) for presence of *ASXL1* mutation and 1.8 (95% CI: 1.1-3.0) adverse karyotype. Accordingly, a practical three-tiered survival model was generated by using HR-weighted risk point assignment; 2 points for absence of CR/CRi, 1 point each for *ASXL1* mutation and adverse karyotype, resulting in high (3 points, n=26; mOS, 3 months), intermediate (2 points, n=21; mOS, 6 months) and low risk (0–1 point, n=47; mOS, 16 months) categories (*P*<0.001) (Figure 1).

In the current study, the presence of *ASXL1* mutation was associated with initial favorable response to Ven+HMA; however, relapses were common resulting in a negative impact on survival due to higher rates of MRD positivity. The importance of MRD response and OS was recently highlighted in an analysis of AML patients treated with Ven+azacitidine that achieved CR/CRi; patients who achieved CR/CRi and MRD $<10^{-3}$ had longer OS than responding patients with MRD $\geq 10^{-3}$.[7] Additional predictors of inferior survival included inability to achieve CR/CRi and adverse karyotype. The association of *ASXL1* mutation and achieving CR/CRi following Ven+HMA is supported by preclinical investigations demonstrating enhanced sensitivity to Ven and azacitidine through alterations in DNA methylation and BCL-2 upregulation.[8,9] Similarly, a single institution retrospective study including relapsed/refractory AML patients treated with Ven+HMA confirmed superior responses with *ASXL1* mutation.[10] Our observations differ from those reported by DiNardo *et al.* in regard to durable remission and prolonged survival with *NPM1* and *IDH2* mutations while the association of *TP53* and *FLT3-ITD* mutations with adaptive resistance was consistent with our findings.[2] In another study on relapsed/refractory AML patients treated with Ven combination therapy, responses were superior with *NPM1* mutation and survival shortened with *TP53*, *K/NRAS* and *SF3B1* mutations.[11] The discrepant observations across studies are likely a result of differences in co-occurrence of mutations, and treatment

regimens utilized coupled with the limited number of patients studied. It is to be noted that OS in our cohort was inferior compared with the VIALE-A study[1] but akin to a real world analyses of newly diagnosed AML patients treated with Ven+ azacitidine due to enrichment with secondary AML.[1,12] Taken together, our findings which require further validation suggest an independent prognostic impact of *ASXL1* mutation in treatment-naïve AML patients receiving Ven+HMA.

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Contributions

NG, IJ and AT designed the study, collected data, performed analysis and co-wrote the paper. KM, FF, AA, HA, KHB, AM, MRL, WH, MS, MMP, AP contributed patients. All authors reviewed and approved the final draft of the manuscript.

Data-sharing statement

Please email the corresponding author.

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