Outcome of patients with acute myeloid leukemia following failure of frontline venetoclax plus hypomethylating agent therapy

Venetoclax (Ven) in combination with hypomethylating agents (HMA) is Food and Drug Administration-approved for elderly/unfit acute myeloid leukemia (AML) patients. In the phase III VIALE-A study, complete remission (CR) with or without count recovery (CRi) was achieved in 66.4% of previously untreated patients with AML receiving Ven and azacitidine. CR/CRi was superior in the presence of *IDH1/2, NPM1, FLT3,* and *TP53* mutations in the Ven and azacitidine arm compared to treatment with azacitidine alone.[1] However, disease progression or relapse was documented in 42% of patients on Ven and azacitidine therapy.[1] Similarly, in a separate study of 95 newly diagnosed AML patients treated on frontline Ven+HMA clinical trials, 41 (43%) of patients experienced relapsed or refractory disease; median overall survival after Ven+HMA failure was considerably inferior at 2.4 months, particularly in patients that did not receive salvage therapy (1.3 months).[2] On the other hand, outcomes of AML patients following failure of upfront Ven+HMA therapy outside the context of clinical trials have not been well-studied. Accordingly, in the current study, our primary objective was to describe the clinical outcomes of patients with AML following failure of frontline Ven+HMA therapy in routine clinical practice and identify clinical and molecular predictors of survival after Ven+HMA failure.

Patients with newly diagnosed AML treated with frontline Ven+HMA outside clinical trials at the Mayo Clinic between 2018 and 2020 were retrospectively recruited after Institutional Review Board approval. Follow-up was updated in November 2022. All patients received at least one cycle of either azacitidine 75 mg/m^2 days 1-7 or decitabine 20 mg/m^2 days 1-5 with Ven dose-adjusted based on azole antifungal prophylaxis.[3] Bone marrow biopsy was obtained after either cycle 1 or 2 based on treating physician discretion with response assessed according to the 2017 European Leukemia Net (ELN) criteria.[4] Treatment failure was defined as inability to achieve CR/CRi (refractory) or loss of CR/CRi (relapsed). Cytogenetic and molecular studies were performed at the time of AML diagnosis by conventional karyotype, and next-generation sequencing (42-gene panel), respectively in all patients, while a subset of patients underwent molecular testing at the time of relapsed/refractory disease. Survival was calculated from the time of treatment initiation and from onset of relapsed or refractory disease to last follow-up or death and survival curves prepared by the Kaplan-Meier method and

compared by the log-rank test. Cox proportional hazard model was used for multivariable analysis. JMP Pro 16.0.0 software package, SAS Institute, Cary, NC was used for statistical analysis.

Outcomes from initial treatment with venetoclax in combination with hypomethylating agents

Seventy-one of 103 (69%) patients with treatment-naïve AML (median age 74 years; range, 37-91; 70% males; 61% *de novo*, 23% secondary, 17% therapy-related) were either refractory (n=43, 61%) or relapsed (n=28, 39%) following Ven+HMA therapy. Initial treatment consisted of decitabine in 45 of 71 (63%) patients and the remainder received azacitidine with median Ven dose of 200 mg (range, 50-400 mg) for a median of three cycles (range, 1-16 cycles). In addition, 59 (83%) of patients received concomitant azole antifungal prophylaxis. Relapsed and refractory patients received a median of six cycles (range, 1-16 cycles) and two cycles (range, 1-19 cycles) of Ven+HMA, respectively. Median duration of CR (n=15) or CRi (n=13) was 5 months (range, 1-22 months); moreover, three of eight evaluable patients were measurable residual disease (MRD)-positive by multi-parametric flow cytometry testing. Treatment-related toxicities were noted in 51 (72%) of patients which included prolonged cytopenias (n=26), infections (n=20), major hemorrhage (n=3), and tumor lysis syndrome (n=1). Table 1 provides detailed clinical characteristics at time of initiation of Ven+HMA for AML patients that were relapsed or refractory to frontline Ven+HMA. ELN 2017 cytogenetic risk included intermediate (51%, n=36) or adverse (49%, n=35). Mutations involved *TP53* in 23 patients (32%), *ASXL1* in 12 (17%), *IDH1/2* in 11 (16%), *FLT3* in ten (14%), *N/KRAS* in nine (13%) (*NRAS*, n=5) and *NPM1* in seven (10%). All *TP53* mutations except one were classified as multi-hit based on the 2022 International Consensus classification[5], with median variant allele frequency of 44.5% (range, 6-96%). A comparison of clinical characteristics of refractory *versus* relapsed patients revealed similarities in age (median age 73 *vs.* 76 years; *P*=0.6), secondary/therapy-related AML (44% *vs.* 32%; *P*=0.5), ELN adverse cytogenetics (53% *vs.* 43%; *P*=0.4), *FLT3* (16% *vs.* 11%; *P*=0.5), *NPM1* (9% *vs.* 11%; *P*=0.8), *IDH1/2* (14% *vs.* 18%; *P*=0.7), and *N/KRAS* mutations (14% *vs.* 11%; *P*=0.7). On the other hand, patients that were refractory to Ven+HMA were more likely to harbor *TP53* mutations (40% *vs.* 21%; *P*=0.1) while *ASXL1* mutations were predominant in relapsed patients (32% *vs.* 6%; *P*=0.01). At

Table 1. Clinical characteristics at time of initiation of venetoclax plus hypomethylating agents including treatment details for 71 patients with acute myeloid leukemia relapsed or refractory to frontline therapy with venetoclax plus hypomethylating agents.

Variables	All patients N=71	Patients with relapsed AML N=28	Patients with refractory AML N=43	P
Age in years, median (range)	74 (37-91)	76 (37-88)	73 (40-91)	0.6
Male, N (%)	50 (70)	20 (71)	30 (70)	0.9
AML type, N (%)				0.5
De-novo AML	43 (61)	19 (68)	24 (55)	
Secondary AML	16 (23)	6 (21)	10 (23)	
t-AML	12 (17)	3 (11)	9 (21)	
Mutations, N (%)				
FLT3	10 (14)	3 (11)	7 (16)	0.5
TP53	23 (32)	6 (21)	17 (40)	0.1
TET2	18 (25)	9 (32)	9 (21)	0.3
ASXL1	12 (17)	9 (32)	3 (6)	<0.01
IDH1/2	11 (16)	5 (18)	6 (14)	0.7
RUNX1	13 (18)	5 (18)	8 (19)	0.9
SRSF2	12 (17)	6 (20)	6 (14)	0.4
K/NRAS	9 (13)	3 (11)	6 (14)	0.7
NPM1	7 (10)	3 (11)	4 (9)	0.8
ELN 2017 cytogenetic risk, N (%)				
Intermediate	36 (51)	16 (57)	20 (47)	0.4
Adverse	35 (49)	12 (43)	23 (53)	
Hemoglobin g/dL, median (range)	8.6 (5.1-14)	8.6 (5.2-14)	8.5 (5.1-12.9)	0.4
Leukocyte count x10^9/L, median (range)	4.4 (0.5-117)	6 (0.7-117)	4.3 (0.5-107)	0.3
Platelet count x10^9/L, median (range)	60 (7-444)	62 (10-444)	52 (7-239)	0.3
Circulating blasts %, median (range)	14 (0-92)	18 (1-86)	14 (0-92)	0.4
Bone marrow blasts %, median (range)	48 (20-95)	49 (20-95)	43 (20-91)	0.2
HMA therapy, N (%)				
Azacitidine	26 (36)	7 (25)	19 (44)	0.1
Decitabine	45 (63)	21 (75)	24 (55)	0.1
Venetoclax dose in mg, median (range)	200 (50-400)	200 (100-400)	200 (50-400)	0.1
Azole antifungal, N (%)	59 (83)	26 (93)	33 (76)	0.1
Number of cycles of Ven+HMA, median (range)	3 (1-16)	6 (1-16)	2 (1-9)	<0.01

AML: acute myeloid leukemia; t-AML: therapy-related AML: ELN: European Leukemia Net; HMA: hypomethylating agents; mg: miligram; Ven: venetoclax.

a median follow-up of 6 months (range, 1-40 months) from initial treatment with Ven+HMA, 67 (94%) deaths were recorded. Median overall survival was 5.9 months (95% confidence interval [CI]: 2.7-14.1), and was superior in patients that were relapsed *versus* those refractory to Ven+HMA (median overall survival 11.2 *vs.* 3.1 months; *P*<0.01).

Online Supplementary Table S1 provides a description of 103 AML patients treated with frontline Ven+HMA including a comparison of clinical and laboratory characteristics of patients with or without relapsed/refractory disease following Ven+HMA. As expected, patients relapsed/refractory to Ven+HMA compared to responders were more likely to harbor adverse cytogenetics (49% *vs.* 34%; *P*=0.15), *TP53* (32% *vs.* 6%; *P*=0.002) and *FLT3*-internal tandem duplications (ITD) (14% *vs.* 0%; *P*=0.005) mutations.

Outcomes from the time of relapsed or refractory disease

Molecular testing at the time of relapsed/refractory disease was obtained in a subset of patients (n=18) which revealed persistence of *TP53* in all six (100%) *TP53*-mutated patients and one of two (50%) *NRAS*-mutated patients that were tested. New emergent clones in the remainder of ten patients tested included mutations in *NRAS, CEPBA* and

BCOR in one patient each. Salvage therapy was pursued in 11 of 71 (15%) patients with gilteritinib (n=6), ivosidenib (n=2), Ven+gilteritinib (n=1), CPX-351 (n=1), and clinical trial (n=1) of which three patients (27%) achieved CR with one patient proceeding to allogeneic stem cell transplant. Notably, none of the patients received standard induction salvage chemotherapy which was not surprising given the median age of study patients was 74 years. On the other hand, in a recent single institution series of younger patients (median age 62 years) and predominantly ELN adverse risk disease (79%), 19 of 208 (9%) went on to receive intensive chemotherapy following failure of Ven+HMA.[6] A total of 21 patients had *FLT3*-ITD (n=10) and *IDH1/2* mutations (n=11), of which two patients were co-mutated for *FLT3*-ITD and *IDH*. Seven of ten *FLT3*-ITD-mutated patients received FLT3 inhibitors, three patients did not receive salvage therapy because of mortality from sepsis (n=2) and fungal pneumonia (n=1). On the other hand, two of 11 *IDH1/2* mutated patients received IDH inhibitors, the remainder of patients passed away from sepsis (n=3), fungal pneumonia (n=1), intracranial hemorrhage (n=1) or transitioned to hospice due to poor performance status/medical comorbidities (n=4). However, it is to be noted that although survival was not significantly different in patients that received or did not receive targeted therapy (8.6 *vs.* 3.0 months; *P*=0.71), the observed difference was likely a reflection of the superior performance status/medical condition of patients that went on to receive salvage therapy.

At a median follow up duration of 3 months from the time of relapsed or refractory disease, median overall survival was similar for relapsed *versus* refractory patients (median survival 3.1 *vs.* 2.8 months; *P*=0.82). In univariate survival analysis, age <60 years (2.5 *vs.* 3.2 months; *P*=0.02), presence of *TP53* mutation (2.2 *vs.* 3.6 months; *P*=0.01), presence of *K/NRAS* mutation (0.7 *vs.* 3.2 months; *P*<0.01), presence of *ASXL1* mutation (2.2 *vs.* 3.2 months; *P*=0.1), and ELN adverse cytogenetics (2.7 *vs.* 3.4 months; *P*=0.04) predicted inferior survival following Ven+HMA failure (Table 2). In subsequent multivariable analysis, presence of *TP53* mutation (hazard ratio [HR]=3.1; 95% CI: 1.7-5.7; *P*<0.01), *K/NRAS* mutation (HR=3.2; 95% CI: 1.5-6.8; *P*<0.01) and *ASXL1* mutation (HR=2.2; 95% CI: 1.1-4.3; *P*=0.03) retained significance. Accordingly, *TP53/RAS/ASXL1* mutational status predicted survival with median survival of 4.6 months (1-year survival 42%) in the absence of all three mutations (n=33), *versus* 2.2 months (1-year survival <1%) in the presence of one or more mutations (n=38) (Figure 1A). Similar results were obtained when survival was assessed in 103 AML patients from time of initiation of frontline Ven+HMA with median survival of 16 months *versus* 5.4 months in the absence *versus* in the presence of *TP53/RAS/ASXL1* mutations (*P*<0.01) (Figure 1B).

The current study confirms the grim prognosis of AML patients that are relapsed/refractory to upfront Ven+HMA therapy. Moreover, salvage therapy was pursued in a minority (15%) of patients and demonstrated limited ability to induce CR with one patient proceeding to allogeneic stem cell transplant. The Mayo clinic cohort comprises AML patients treated outside the context of clinical trials

Table 2. Predictors of inferior survival* following failure of venetoclax plus hypomethylating agents in 71 patients with acute myeloid leukemia relapsed or refractory to frontline therapy with venetoclax plus hypomethylating agents.

Variables obtained at diagnosis	Overall survival Univariate *P*	Overall survival	
		Multivariate *P*	HR (95% CI)
Age <60 years	0.02	0.13	
Sex	0.16		
Secondary/therapy-related AML	0.60		
Bone marrow blasts %	0.41		
Complex or monosomal karyotype	0.11		
ELN adverse karyotype	0.04	0.37	
Presence of *NPM1* mutation	0.59		
Absence of *IDH1/2* mutation	0.10		
Presence of *RAS* mutation	0.03	<0.01	3.4 (1.5-7.5)
Presence of *TP53* mutation	<0.01	0.01	2.5 (1.2-5.3)
Presence of *ASXL1* mutation	0.10	0.02	2.2 (1.1-4.3)
Absence of *FLT3*-ITD mutation	0.07		

*Survival was determined from time of relapsed/refractory disease. HR: hazard ratio; CI: confidence interval; AML: acute myeloid leukemia; ELN: European Leukemia Net; ITD: internal tandem duplication.
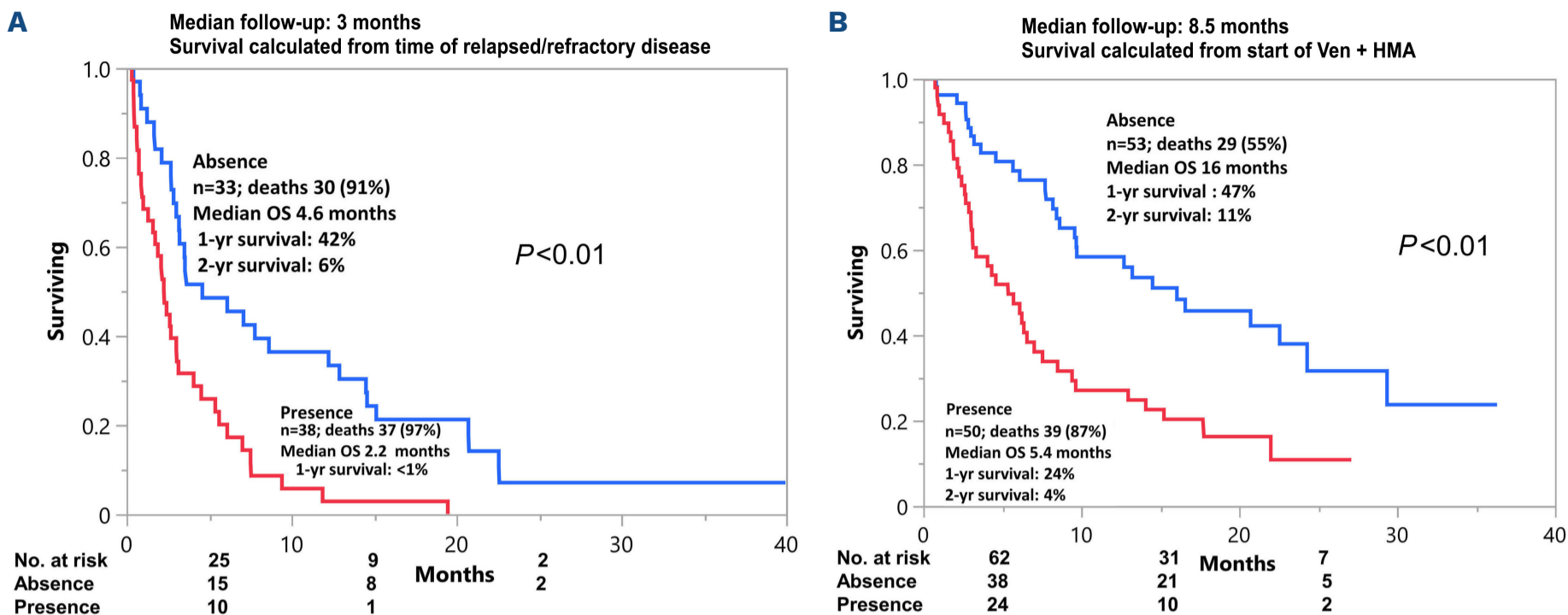
Figure 1. Overall survival of patients with acute myleoid leukemia (AML) following frontline venetoclax plus hypomethylating agent stratified by presence/absence of *TP53, K/NRAS, ASXL1* mutations. (A) Overall survival of 71 patients with acute myeloid leukemia (AML), relapsed/refractory following frontline venetoclax (Ven) plus hypomethylating agents (HMA) stratified by presence/absence of *TP53, K/NRAS, ASXL1* mutations. (B) Overall survival of 103 patients with AML treated with frontline Ven plus HMA stratified by presence/absence of *TP53, K/NRAS, ASXL1* mutations.

and was enriched with secondary/therapy-related AML (40%) and adverse cytogenetics (49%). The corresponding figures for secondary AML and adverse cytogenetics in the VIALE-A study were lower at 25% and 36%, respectively.[1] These differences explain the shorter CR duration and inferior median overall survival observed in our study in comparison to VIALE-A study.[1,7]

Our findings differ from an MD Anderson study which included 41 clinical trial patients, in which 24 of 41 (59%) of patients with AML that were relapsed/refractory disease following upfront Ven+HMA therapy received salvage therapy.[2] However, akin to our study, only 21% of patients responded to salvage therapy and one patient underwent allogeneic stem cell transplant.[2] Previous studies on the impact of mutations on response and survival in treatment-naïve and relapsed/refractory AML patients treated with Ven+HMA did not provide detailed information on survival following treatment failure. In our prior report on Ven+HMA-treated newly diagnosed AML patients, the presence of *ASXL1* mutations and absence of *FLT*-ITD and *TP53* mutations were associated with superior response; on the other hand, the presence of *ASXL1* mutations, adverse karyotype and absence of CR/CRi predicted inferior survival.[7] In contrast, in clinical trial patients with AML receiving frontline Ven+HMA or low-dose cytarabine, durable remissions and prolonged survival were reported with *NPM1* and *IDH2* mutations while *TP53* and *FLT3*-ITD mutations were associated with adaptive resistance.[8] Similarly, in relapsed/refractory AML patients treated with Ven combination therapy, responses were superior with *NPM1* mutation while survival was shortened with *TP53, K/NRAS* and *SF3B1*

mutations.[9] In addition, in a Mayo Clinic study of relapsed/refractory AML patients treated with Ven+HMA, the presence of *ASXL1* mutation and absence of adverse karyotype predicted superior response, while survival was negatively impacted by the presence of *TP53* mutations and absence of *IDH1/2* mutations.[10] In an exploratory analysis of AML patients treated on Ven+azacitidine clinical trials, stratified according to ELN 2017 risk, adverse risk patients with *TP53* mutation had distinctly poor outcome with median overall survival of 5.42 months.[11]

The current study unveils the prognostic impact of *TP53, RAS* and *ASXL1* mutations on survival in the setting of Ven+HMA failure in treatment-naïve patients with AML. Our observations provide a practically relevant survival prediction model based on the presence *versus* absence of *TP53, RAS* and *ASXL1* mutations (1-year survival <1% *vs.* 42% in their presence *vs.* absence) which should be incorporated in patient counseling. However, whether these findings are specific to Ven+HMA therapy remains to be determined. Taken together, the current study underscores the prognostic relevance of *TP53, RAS* and *ASXL1* mutations in treatment-naïve AML patients following failure of frontline Ven+HMA therapy.

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