

Safety and Efficacy: Clinical Experience of Venetoclax in Combination With Hypomethylating Agents in Both Newly Diagnosed and Relapsed/Refractory Advanced Myeloid Malignancies

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Abstract

Hypomethylating agents (HMAs) in combination with venetoclax have been widely adopted as the standard of care for patients who cannot tolerate induction chemotherapy and for patients who have relapsed/refractory (R/R) acute myeloid leukemia (AML). This study retrospectively analyzed the outcomes of all patients with AML (n = 65) or myelodysplastic syndrome (n = 7) who received the combination of HMA and venetoclax at our institution. Outcomes measured included complete remission (CR) and CR with incomplete hematologic recovery (CRI) rates, duration of response (DOR), and overall survival (OS). Patient mutational profiles and transfusion requirements were also assessed. Of 26 newly diagnosed AML patients, the CR/CRI rate was 53.8%. The median DOR and OS were 6.9 months and not reached, respectively. Of 39 R/R AML patients, the CR/CRI rate was 38.5%. The median DOR and OS were both 8.1 months. Responders to HMA and venetoclax were enriched for *TET2*, *IDH1*, and *IDH2* mutations, while nonresponders were associated with *FLT3* and *RAS* mutations. Adaptive resistance was observed through various mechanisms including acquired *RAS* pathway mutations. Of transfusion-dependent patients, 12.2% and 15.2% achieved red blood cell (RBC) and platelet transfusion independence, respectively, while 44.8% and 35.1% of RBC and platelet transfusion independent patients, respectively, became transfusion dependent. In total 59.1% of patients developed a \geq grade 3 infection and 46.5% neutropenic fever. HMA + venetoclax can lead to impressive response rates with moderately durable remissions and survival. However, the benefits of this combination are diminished by the significant toxicities from infection, persistent cytopenias, and transfusion requirements.

Introduction

Acute myeloid leukemia (AML) continues to be a devastating illness. While 10 new drugs have been approved by the Food and Drug Administration (FDA) over the past 5 years, the overall prognosis of most patients with AML remains dire, particularly in patients \geq 65 years of age.¹ However, one of the newly approved therapies, venetoclax (VEN) in combination with the hypomethylating agents (HMA) azacitidine or decitabine, has led to noteworthy impacts on disease management.² VEN is a B-cell leukemia/lymphoma 2 inhibitor first approved for

the treatment of chronic lymphocytic leukemia.³ Initial clinical studies with VEN monotherapy in AML showed unimpressive results.⁴ However, subsequent preclinical studies demonstrated synergistic activity and the potential for the combination to eradicate leukemia stem cells.⁵ Within a few years, HMA + VEN has become the treatment of choice for patients with newly diagnosed AML who are unable to tolerate intensive chemotherapy, reflective of widespread adoption of this regimen.⁶ While only approved in the induction setting, it is frequently utilized to lesser effect in patients who are relapsed/refractory (R/R) to induction chemotherapy as well.⁷ HMA + VEN has been the practice changing innovation in AML, and there are reasonable debates as to whether HMA + VEN should be first-line treatment for subsets of younger and more fit patients with newly diagnosed AML.⁸

HMA + VEN was FDA approved in late 2018 based on early phase trials that exhibited outstanding response rates and preliminary survival data.^{9,10} Since initial approval, 2 randomized phase III trials comparing either HMA (azacitidine)¹¹ or low-dose cytarabine (LDAC)¹² with VEN have confirmed the superiority of the addition of VEN. However, it is important to validate the real-world safety and efficacy of HMA + VEN outside of clinical trials and in an uncontrolled, less-fit population, which more closely approximates the actual benefit and risk of this novel therapeutic combination in the larger oncology community. Several retrospective studies have been published confirming the efficacy of HMA + VEN in a variety of settings, including

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newly diagnosed,¹³ (R/R),¹⁴⁻¹⁶ therapy related,¹⁷ myeloproliferative neoplasms (MPNs) in blast phase,¹⁸ HMA monotherapy failure,¹⁹ post-allogeneic stem cell transplant (allo-SCT) patients with AML,²⁰ and myelodysplastic syndromes (MDS).²¹

Here, we report the clinical experience at a large tertiary academic medical center with HMA + VEN therapy in treatment-naïve and R/R AML patient subgroups as well as patients with high-risk MDS.

Methods

This study was approved by the Program for Protection of Human Subjects (PPHS) of the Icahn School of Medicine at Mount Sinai. We reviewed the electronic medical records of all patients at our multihospital New York City health system who have received VEN between April 2018 and January 2020. We narrowed the patients further into those with pathology confirmed myeloid malignancies who had received VEN in combination with azacitidine or decitabine.

Outcomes assessed included overall survival (OS), progression-free survival, and overall response rates (ORRs). For patients with AML, ORR was calculated via 2017 European LeukemiaNet (ELN) criteria.²² Patients without a bone marrow biopsy after initiation of treatment were deemed non-evaluable but peripheral blast responses were annotated. These patients were included in the intent to treat population. For patients with MDS, ORR was calculated via 2006 International Working Group (IWG) criteria.²³ Transfusion dependence was determined by Gale²⁴ criteria: greater than or equal to 2 units per month over the prior 3 months. Risk stratification was assigned by the Revised International Prognostic Scoring System (IPSS-R) for patients with MDS²⁵ and via ELN 2017 criteria for AML.²² Duration of hospitalization was determined as the number of days a patient was admitted for an adverse event and within 30 days of the last dose of HMA + VEN. Length of admission excluded 10 days in the setting of HMA + VEN treatment and days after change in therapy. Cytogenetic analysis was performed by the Tumor CytoGenomics Laboratory at our institution with determinations of risk status according to published criteria. Next-generation sequencing (NGS) to evaluate for myeloid neoplasms-associated gene mutations was performed by Genoptix (Carlsbad, CA) and Foundation Medicine (Cambridge, MA) panels. Patients without NGS data (n = 3) were excluded from mutational analysis. Only mutations of likely or known pathogenicity were included in this analysis. Descriptive statistics were used and the Kaplan-Meier method utilized to estimate OS and duration of response (DOR) with censoring at the last date known alive and at time of allo-SCT for DOR. Survival comparisons were done using the Log-rank (Mantel-Cox) method for multiple curves (not pairwise comparisons). Clonal responses and relapses were analyzed and depicted using the fishplot R package.²⁶

Results

Baseline characteristics

A total of 72 patients received a combination of HMA + VEN, 65 with a diagnosis of AML and 7 with MDS. Forty-four patients were treated in the R/R setting; of these, 39 had a diagnosis of AML and 5 had MDS. The median age was 61.5 years, 65.9% were male, 23.3% had a documented Eastern Cooperative Oncology Group (ECOG) 0, and 58.1% ECOG 1. In total 59.1% of patients had prior HMA exposure with a median of 5 cycles (22.7% received HMA for an antecedent hematologic disorder prior to transformation to AML, and 25.0% received HMA for AML) and 29.5% were treated after allo-SCT. Of the AML patients, 51.3% had de novo disease and

28.2%/17.9% were secondary to MDS/MPN; 41.0% met ELN 2017 intermediate risk and 48.7% were adverse risk (Table 1).

Among the 28 newly diagnosed patients, the median age was 72 years, 64.3% were male, 21.4% had a documented ECOG 0, and 53.6% were ECOG 1. Four (14.3%) patients had prior HMA exposure for an antecedent hematologic neoplasm with a median of 6.5 cycles. Of the 26 AML patients, 50.0% had de novo disease, 34.6% were secondary to MDS, MPN, or chronic myelomonocytic leukemia, and 15.4% were therapy related. Per ELN 2017 criteria, 46.2% were intermediate risk and 42.3% were adverse risk.

Concurrent azole fungal prophylaxis was administered in 84.1% of R/R patients and 78.6% of newly diagnosed patients (Table 1). VEN dosing was adjusted to 100 mg for CYP3A inhibition pharmacokinetic effects. Twenty patients who were not fully evaluable in terms of response had multiple reasons for a lack of a second bone marrow biopsy, including persistent disease in the peripheral blood, poor clinical status, death, and provider preference.

Response and survival

In newly diagnosed AML (n = 26), the complete remission (CR) + CR with incomplete hematologic recovery (CRi) rate was 53.8%. Of the 20 evaluable patients (all patients with follow-up bone marrow biopsies available), the CR + CRi rate was 70.0%. Among 6 nonevaluable patients, 1 (16.7%) had clearance of peripheral blood blasts. In de novo AML patients (n = 13), the CR + CRi rate was 77% (See STable 1, <http://links.lww.com/HS/A142>). Median time to response was 2.4 months and median DOR was 6.9 months, with 33.3% of responders relapsed at time of data cutoff. In total 7.7% of patients went on to receive an allo-SCT (Table 2). Median OS was not reached at a median duration of follow-up of 7.0 months (Figure 1A). Death occurred in 42.3% of patients.

In R/R AML (n = 39), the CR + CRi rate was 38.5%. Among the 25 evaluable patients, the CR + CRi rate was 60.0%. Of the nonevaluable patients, 71.4% cleared peripheral blood blasts. In de novo AML patients (n = 20), the CR + CRi rate was 60% (See STable 1, <http://links.lww.com/HS/A142>). Median time to response was 1.8 months and median DOR was 8.1 months, with 26.7% of responders relapsed at time of data cutoff. Of these patients 20.5% went on to receive an allo-SCT (Table 2). Median OS was 8.1 months at a median duration of follow-up of 6.5 months (Figure 1A). Death occurred in 56.4% of patients. There was no statistical difference in OS based on choice of HMA in combination with VEN (Figure 1B). Notably, in the 18 HMA-naïve patients, the CR + CRi rate was 66.7%, while for the 21 patients who had prior HMA exposure, the CR + CRi rate was 14.3%.

Overall, for AML patients who achieved a response (CR/CRi), the median OS was not reached; for nonresponders, the median OS was 8.1 months; and for those who were not evaluable, the median OS was 2.1 months. The hazard ratio (HR) between responders and all others (HR, 0.24; 95% CI, 0.12-0.49) was the only one that reached statistical significance ($P < 0.001$) (Figure 1C). There were only 7 MDS patients in this cohort and excellent responses were seen in the R/R setting, with 5 treated patients achieving a CR/marrow complete remission (mCR) (Table 2).

Mutational characterization

Molecular profiles have been shown to be associated with response to VEN, and thus, we explored the molecular characteristics of those patients (n = 35) who had responded (CR/CRi/mCR) (Table 3).²⁷ Of these patients, 40% (n = 14) had a decrease in the variant allele frequency (VAF) of the mutations

Table 1**Patient Characteristics Newly Treated and Relapsed/Refractory AML/MDS.**

	Newly Diagnosed	R/R
Total (n)	28	44
Age, median (y)	72	61.5
Male/female, N (%)	18/10 (64.3)	29/15 (65.9)
ECOG performance status, N (%)		
0	6 (21.4)	10 (23.3)
1	15 (53.6)	25 (58.1)
2	5 (17.9)	5 (11.6)
3	2 (7.1)	3 (7.0)
AML, (N)	26	39
Subtype, N (%)		
De novo	13 (50.0)	20 (51.3)
Secondary to MDS	3 (11.5)	11 (28.2)
Secondary to MPN	3 (11.5)	7 (17.9)
Secondary to CMML	3 (11.5)	1 (2.6)
Therapy related	4 (15.4)	0
ELN criteria, N (%)		
Favorable	3 (11.5)	4 (10.3)
Intermediate	12 (46.2)	16 (41.0)
Adverse	11 (42.3)	19 (48.7)
MDS, (N)	2	5
IPSS-R very high, N (%)	2 (100)	3 (60)
IPSS-R high, N (%)	0	2 (40)
Cytogenetics, N (%)		
Favorable	1 (3.6)	1 (2.3)
Intermediate	15 (53.6)	26 (59.1)
Adverse	12 (42.9)	17 (38.6)
Baseline counts, median		
WBC $\times 10^9/L$	5.9	3.7
ANC $\times 10^9/L$	1.0	1.6
Hemoglobin, g/dL	8.3	8.9
Platelet $\times 10^9/L$	60	57
Peripheral blood blast (%)	15	2.0
Bone marrow blast (%)	44	13
Lines of prior therapy, median	NA	2
Concurrent azole, N (%)	22 (78.6)	37 (84.1)
Post allo-SCT, N (%)	NA	13 (29.5)
Previous HMA use		
Overall HMA exposure, N (%)	4 (14.3)	26 (59.1)
MDS/CMML pts, N (%)	0	5 (11.4)
Antecedent HM prior to AML, N (%)	4 (14.3)	10 (22.7)
AML, N (%)	NA	11 (25.0)
Median cycles (N)	6.5	5

allo-SCT = allogeneic stem cell transplant; AML = acute myeloid leukemia; ANC = absolute neutrophil count; CMML = chronic myelomonocytic leukemia; ECOG = Eastern Cooperative Oncology Group; ELN = European LeukemiaNet; HM = hematologic malignancy; HMA = hypomethylating agent; IPSS-R = Revised International Prognostic Scoring System; MDS = myelodysplastic syndromes; MPN = myeloproliferative neoplasm; NA = not available; R/R = relapsed/refractory; WBC = white blood cell.

in the initial mutant clone to less than 5% at the time of best response, which is the lower limit of clinically reportable single nucleotide variants. In these 14 patients, this took a median of 9.5 weeks to achieve. With additional analysis of NGS data using a lower threshold of detection of 1% to 5% VAF, 11% of patients (n = 4) still had detectable mutations, while in 26% of patients (n = 9), the VAF was undetectable or at less than 1% (1 patient could not be further analyzed). For the 4 patients with mutations between 1% and 5% VAF, all had AML, with 3 having newly diagnosed disease. Two patients had adverse risk disease, and 1 each with intermediate and favorable risk, per ELN criteria. For the 9 patients who cleared their mutations to less than 1%, 7 had AML (5 newly diagnosed, 2 R/R) and 2 had MDS (1 newly diagnosed and 1 R/R). For the AML patients, 4 had adverse and 3 had intermediate-risk disease. For the

Table 2**Efficacy of Hypomethylating Agents + Venetoclax in AML/MDS.**

	Newly Diagnosed	R/R
AML (N = 65)		
ORR (CR + CRi + PR) total	57.7% (15/26)	38.5% (15/39)
ORR evaluable	75.0% (15/20)	60.0% (15/25)
CR (n)	19.2% (5)	12.8% (5)
CRi (n)	34.6% (9)	25.6% (10)
PR (n)	3.8% (1)	0
SD (n)	15.4% (4)	10.3% (4)
PD (n)	3.8% (1)	15.4% (6)
NE (n)	23.1% (6)	35.9% (14)
PB blast clearance in NE	16.7% (1/6)	71.4% (10/14)
Median time to CR/CRi/PR, mo	2.4	1.8
Median DOR, mo	6.9	8.1
Relapse in responders	33.3% (5/15)	26.7% (4/15)
Allo-SCT post-treatment	7.7% (2/26)	20.5% (8/39)
Death	42.3% (11/26)	56.4% (22/39)
Median OS, mo	NR	8.1
HR OS newly diagnosed vs R/R: 0.7108 ($P = 0.3362$)		
Median duration of follow-up, mo	7.0	6.5
ORR HMA naive	68.1% (15/22)	66.7% (12/18)
ORR prior HMA exposure	0% (0/4)	14.3% (3/21)
ORR azacitidine (total)	2/6	2/11
ORR azacitidine (evaluable)	2/4	2/8
mOS azacitidine	5.9 mo	8.1 mo
ORR decitabine (total)	12/20	13/27
ORR decitabine (evaluable)	12/16	13/17
mOS decitabine, mo	NR	8.1
HR mOS azacitidine vs decitabine, newly diagnosed: 1.510 ($P = 0.5822$)		
HR mOS azacitidine vs decitabine, R/R: 1.137 ($P = 0.7863$)		
MDS (N = 7), azacitidine (N = 6), decitabine (N = 1)		
ORR (CR + mCR) newly diagnosed (N = 2)	50.0% (1/2)	
ORR (CR + mCR) R/R (N = 5)	100.0% (5/5)	
CR	2	
mCR	4	
SD	1	
Median time to response, mo	1.6	
Relapse in responders	50.0% (3/6)	
Allo-SCT post-treatment	14.3% (1/7)	
Death	57.1% (4/7)	

allo-SCT = allogeneic stem cell transplant; AML = acute myeloid leukemia; CR = complete remission; CRi = complete remission with incomplete hematologic recovery; DOR = duration of response; HR = hazard ratio; mCR = marrow complete remission; MDS = myelodysplastic syndromes; mOS = median overall survival; NE = nonevaluable; NR = not reached; ORR = overall response rate; OS = overall survival; PB = peripheral blood; PD = progressive disease; PR = partial remission; R/R = relapsed/refractory; SD = stable disease.

MDS patients, both had IPSS-R very high-risk disease. In 31% of patients, despite a morphologic remission as determined by pathology, the VAF associated with the mutant clone persisted at time of response. In 17% of the patients, there was a lack of molecular markers, and in 11% of patients, there was insufficient follow-up. In 11% of these patients, a new mutation was detectable at the time of response.

Molecular characterization was also associated with response and resistance to HMA + VEN therapy. Among patients with NGS data available, the mutations associated with response included *TET2* (9/13; 69%) and *IDH1* or *IDH2* (9/11; 81%) (Figure 2A). Most other mutations occurred too infrequently to evaluate prognostic association. Among the mutations that were found more frequently in responding patients than nonresponders were *PHF6* (3 versus 0), *BCOR* (4 versus 1), and *DNMT3A* (6 versus 1); however, in all but 1 case, these mutations were associated with concurrent *TET2*, *IDH1*, or *IDH2* mutations. It has been reported that *FLT3* and *RAS* mutations are associated with primary and adaptive resistance.²⁷ In concordance,

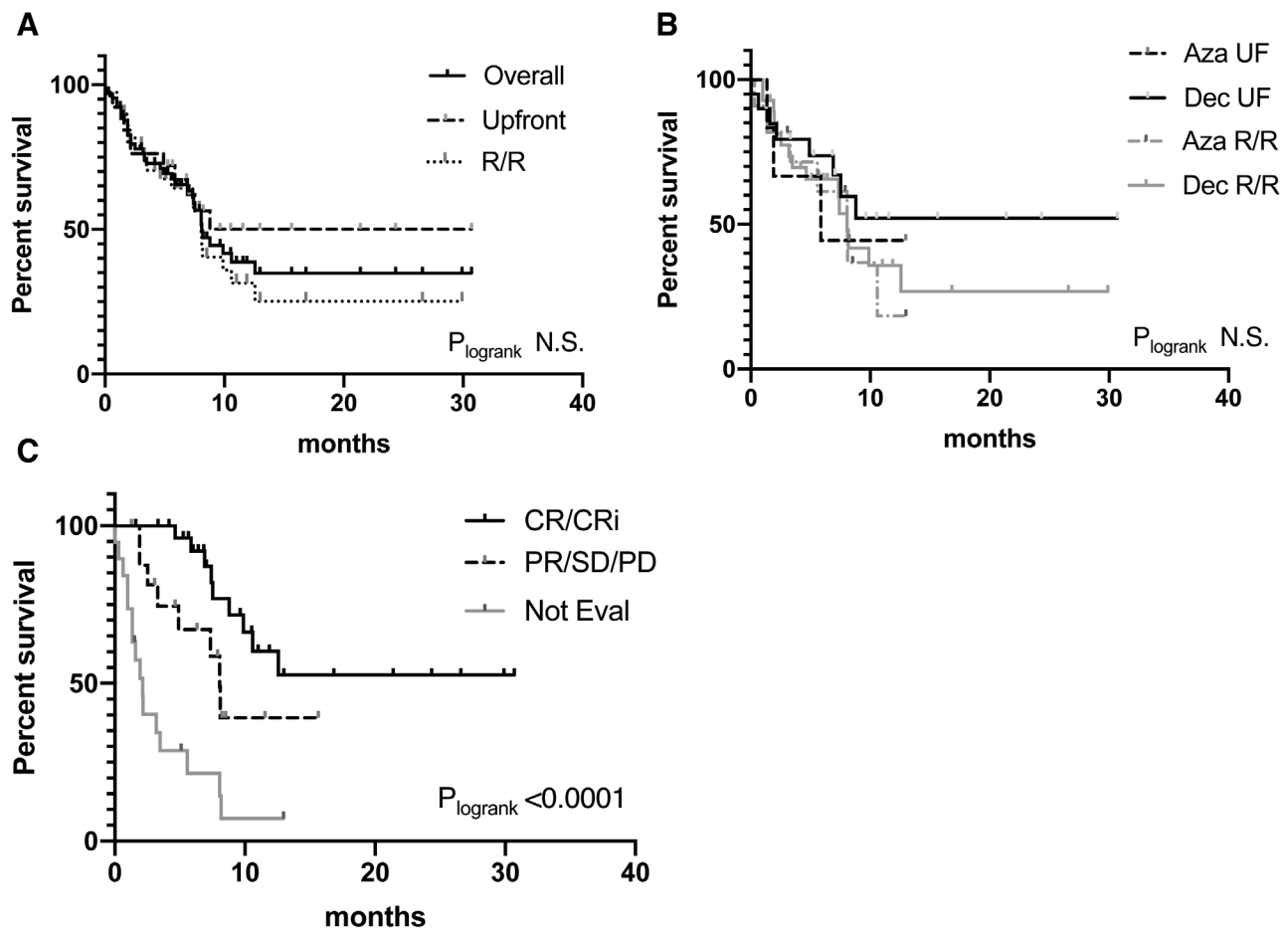


Figure 1. Survival curves of patients receiving HMA + VEN. (A), Overall, AML UF treatment and AML R/R cohorts. (B), Treatment cohorts divided by UF or R/R; Aza or Dec cohorts. (C), Patients divided by response, responder (CR/CRi), nonresponders (PR, SD, PD), and Not Eval. AML = acute myeloid leukemia; Aza = azacitidine; CR = complete remission; CRi = complete remission with incomplete hematologic recovery; Dec = decitabine; HMA = hypomethylating agent; N.S. = not significant; Not Eval = nonevaluable; PD = progressive disease; PR = partial remission; R/R = relapsed/refractory; SD = stable disease; UF = upfront; VEN = venetoclax.

we observed that patients with *FLT3* mutations were less likely to achieve response (1/9; 11%) and similarly with *KRAS* or *NRAS* mutations (3/10; 30%). Furthermore, in 5 of 6 patients who did not achieve a response but had *TET2*, *IDH1*, or *IDH2* mutations, their disease had concurrent *FLT3*, *KRAS*, or *NF1* mutations. Nearly half of patients with *TP53* mutations had a response (7/15; 47%). Responses occurred despite the out-sized presence of complex cytogenetics among patients with a *TP53* mutation (13/15; 86.7%). Of the 15 patients, there were 6 patients with biallelic *TP53* mutations, but only 2 of 6 treated patients achieved a response. Other more commonly mutated genes in myeloid malignancies such as *ASXL1*, *NPM1*,

and splicing factor genes did not clearly predict for response in our cohort. In terms of cytogenetics, there was no clear association between cytogenetic risk group and response. Half of the treated patients with adverse risk cytogenetics (14/28) achieved a response to HMA + VEN (Figure 2A).

Molecular patterns could also inform upon clonal relapse or adaptive resistance mechanisms. Nine patients who had achieved response (CR/CRi) subsequently relapsed and had molecular profiling performed from the bone marrow aspirate. Of these patients, we observed 2 cases in which the VEN combination was able to substantially reduce the VAFs from the initial clone at time of response; however, this clone appears to return at relapse with additional mutations (*MPL*) or potential amplification of an existing mutation (*NRAS*) (Figure 2B). In 2 cases, the same clone persists from initial treatment to relapse (Figure 2C). We also observed a distinct new clone with new mutations (*NF1*) arising as a relapse mechanism (Figure 2D). Finally, we observed a case where the mutant VAF did not change with response, and resistance occurred with acquisition of additional mutations (*CEBPA*) (Figure 2E). In 3 cases, the molecular profiles were unrevealing due to lack of mutations or subclonal (<10% VAF) mutations.

Table 3

Molecular Mutation Patterns of Responders.

Pattern	N = 35 total	
Cleared original mutation <5% VAF	40%	14
Persistent mutation 1%-5% VAF	11%	4
<1% or not detectable	26%	9
Unknown	3%	1
Persistent mutation >5% VAF	31%	11
Uninformative	17%	6
Not done	11%	4
New mutation	11%	4

VAF = variant allele frequency.

Safety

Myelosuppression was frequently encountered and led to increased transfusion requirements, infection risk, and

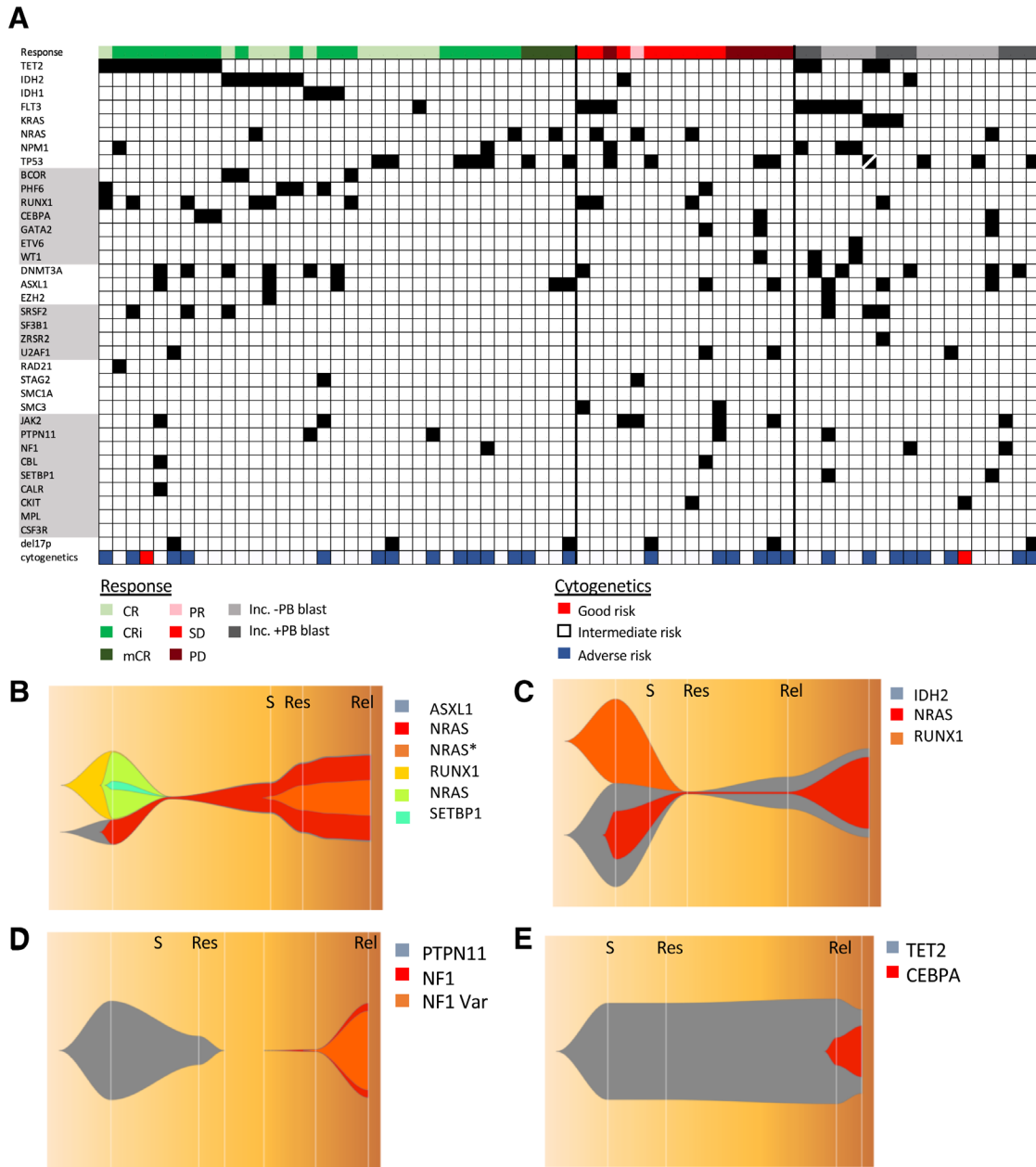


Figure 2. Mutational profile of patients receiving HMA + VEN. (A), Individual patients are profiled based on their disease-associated responses, mutations, and cytogenetics per column. Black boxes indicate presence of mutation. Patients are clustered based on responses: CR, CRi, mCR, PR, SD, PD, Inc, and +/- PB blast. Cytogenetics are classified as good, intermediate, and adverse risk and del17p involving *TP53* deletion. Hashed box represents dual *TP53* mutations. Genes are clustered based on those reported to affect HMA + VEN response, transcription factors, epigenetic modifiers, splicing factors, cohesion components, and signaling components. (B–E), Initial, response, and relapse profiles of patients were determined through fishplot analysis of variant allele frequencies. (B), A patient whose clone was selected after induction therapy, responded to HMA + VEN, but then relapsed with rising *NRAS* allele frequency (* >50% suggesting amplification or loss of heterozygosity). (C), A patient whose clone persisted after 7 + 3, but went into remission with HMA + VEN, responded with decrease in VAF of mutations, but relapsed with same clone. (D), A patient who responded to HMA + VEN and relapsed with a new mutant clone. (E), A patient who responded to HMA + VEN but had no decrease in VAF of the mutant clone and relapsed with additional mutation. +/- PB blast = with and without peripheral blood blast; CR = complete remission; CRi = complete remission with incomplete hematologic recovery; HMA = hypomethylating agent; Inc = incomplete evaluation; mCR = marrow complete remission; PD = progressive disease; PR = partial remission; Rel = relapse; Res = response; S = start of venetoclax; SD = stable disease; VAF = variant allele frequency; VEN = venetoclax.

dosing modifications for patients receiving HMA + VEN. Only 5 patients who were red blood cell (RBC) (n = 41) or platelet (n = 33) transfusion dependent (per Gale criteria) prior to treatment became independent afterward (12.2%, 15.2%, respectively), with 3 patients overlapping between the 2 groups, leading to only 7 unique patients (10.0%) who became transfusion independent. Furthermore, 44.8% and 35.1% of RBC and platelet transfusion independent patients, respectively, became transfusion dependent after starting therapy. Overall, 70.0% of

patients remained RBC transfusion dependent and 58.6% of patients remained platelet transfusion dependent during/after HMA + VEN treatment. While only 5.6% of patients experienced ≥grade 3 bleeding, 59.1% developed a ≥grade 3 infection, with 46.5% suffering neutropenic fever. This likely stems from the fact that 71.8% of patients remained neutropenic for ≥30 days during treatment (Table 4). Severe and persistent hypoplastic bone marrow biopsies were frequently observed with HMA + VEN therapy, as witnessed by the high rate of CRi. Patients

Table 4

Adverse Events.

RBC transfusion dependence status (N = 70), N (%)	
Dep before Dep after	36 (51.4)
Dep before Ind after	5 (7.1)
Ind before Dep after	13 (18.6)
Ind before Ind after	16 (22.9)
Platelet transfusion dependence status (N = 70), N (%)	
Dep before Dep after	28 (40.0)
Dep before Ind after	5 (7.1)
Ind before Dep after	13 (18.6)
Ind before Ind after	24 (34.3)
Treatment-related AEs (N = 71), N (%)	
Bleeding ≥ grade 3	4 (5.6)
Infection ≥ grade 3	42 (59.1)
Febrile neutropenia	33 (46.5)
Invasive fungal infection	2 (2.8)
Neutropenia ≥30 d	51 (71.8)
Tumor lysis syndrome	4 (5.6)
Hospitalization for AE	31 (43.7)
Treatment stopped due to AE	25 (35.2)
30-d mortality (N = 72), %	
Newly diagnosed	7.1
R/R	6.8
60-d mortality (N = 72), %	
Newly diagnosed	17.9
R/R	13.6
Venetoclax dosing, N (%)	
Ramp-up strategy (N = 64)	
400 mg daily (N = 70)	6 (9.4)
100 mg daily (N = 70)	17 (24.3)
Noncontinuous dosing (N = 71)	53 (75.7)
	39 (54.9)

AE = adverse event; Dep = dependent; Ind = independent; R/R = relapsed/refractory; RBC = red blood cell.

can remain transfusion dependent well after stopping VEN or the combination therapy. As an example, a patient achieved clearance of AML blasts in the refractory setting (Figure 3A–C), but serial bone marrows displayed significant aplasia despite remaining off VEN for over 10 months (Figure 3, D–F). The majority of patients (54.9%) required intermittent therapy or a delay in the treatment cycle and did not tolerate continuous daily dosing. In total, 43.7% of treated patients required a hospitalization for a treatment-related adverse event separate from treatment initiation and 35.2% of patients had to stop VEN due to adverse events (Table 4). Of those hospitalizations, newly diagnosed patients were hospitalized for a median of 16 days, while R/R treated patients were hospitalized for a median of 20 days (excluding days for initiating or continuing treatment). Invasive fungal infections (2.8%) and tumor lysis syndrome (5.6%) remained rare events. Patients with de novo AML faced a similar rate of adverse events as compared with the overall cohort (See STable 2, <http://links.lww.com/HS/A142>). VEN was mostly dosed at 100 mg (75.7%) due to concurrent azole administration, and a ramp-up strategy was used in 6 patients (9.4%). Thirty-day and 60-day mortality rates were 7.1%/17.9% in upfront patients and 6.8%/13.6% in R/R patients (Table 4).

Discussion

The results here confirm prior reports regarding the real-world use of HMA + VEN at a tertiary academic medical center,²⁸ but also raise new concerns regarding safety, as our clinical experience with HMA + VEN has shown it to be less well tolerated than previously reported. As expected, we did not achieve in the overall cohort the outcomes noted in the VIALE-A phase III randomized

study for newly diagnosed AML in which the CR rate was noted to be 36.7% and CR/CRi rate of 66.4%, although incidence of febrile neutropenia (42%) was quite similar when compared with our overall cohort.¹¹ As would be expected, R/R AML patients tended to do worse while de novo AML patients had response rates similar to what was reported. The therapy could be used as a bridge to transplant, as it did for 20% of R/R patients. It is difficult to compare OS and DOR data as the median duration of follow-up was substantially longer in the clinical trial than observed here.

This study has importantly corroborated the available literature in terms of responses and survival data in both the newly diagnosed and R/R patient populations, although there are additional findings described here. Importantly, previous papers have not stressed the significant bone marrow failure state that can be observed in a meaningful number of patients receiving HMA + VEN. While these patients are technically in CRi, without any sign of AML, they also are transfusion dependent and remain at risk for infection. This is reflected in both the high rate of treatment-emergent transfusion dependence (70%) seen during and after treatment as well as how many patients encountered neutropenia for >30 days. Of note, under alternative criteria such as the stricter IWG 2018 criteria, an even greater proportion of the patients would be expected to be transfusion dependent. This significant treatment-related pancytopenia resulted in 12.5% of the overall cohort dying from infection-related (not associated with progressive disease) causes. It would be unlikely that patients receiving either HMA monotherapy or intensive chemotherapy would face such significant transfusion requirements as studies have shown reduced transfusion dependence with HMAs.^{29,30} As a tertiary referral center, our patient population is different from other settings and contributed to outcomes; however, toxicities were observed across all patient groups.

A notable finding from our review was the disparate outcomes in the R/R AML patients between those who did and did not have previous exposure to HMA. Analyses of HMA + VEN have mostly noted only minimally worse responses in those with previous HMA treatment, ranging from a 40%-50% CR/CRi rate.^{19,28,31} One exception is the report by Dinardo et al.¹⁴ Among their cohort of 31 HMA + VEN patients, 21 of whom had received prior HMA, the ORR rate was only 23.8%. A systematic review has yielded an ORR (including partial remissions and morphologic leukemia-free state) of 31.1% for patients treated with HMA/LDAC + VEN with prior HMA exposure.³² While the sample size here is small (n = 21), we observed a response (CR/CRi) rate of 14.3% in this population. Further studies will be necessary to see what the true response rate is for these patients and whether prior HMA exposure affects outcome.

In terms of choice of HMA, with our limited patient sample size and varying individual provider preferences, decitabine was noninferior to azacitidine in terms of response rates and OS (Table 2). While azacitidine was chosen as the HMA backbone for the VIALE-A trial, decitabine should be included in future HMA + VEN trials and be studied for any response differences.

We also analyzed the impact of mutational status on therapeutic response as well as the change in mutational burden with HMA + VEN therapy. We show how in some instances, HMA + VEN is able to seemingly eradicate the mutant clone at time of CR (26% of responders), while in other instances, a small clonal population remains that can expand at time of morphologic relapse. There are also those, who despite morphologic response, have persistent mutant VAFs that would suggest response through differentiation of the mutant clone or selection of ancestral clones that retain select mutations.

With the caveat that numbers are fairly limited, responses were enriched in patients harboring mutated *TET2*, *IDH1*, and *IDH2* and less likely in those with *FLT3* mutations. *RAS* pathway mutations were also associated with primary and adaptive resistance. Single *TP53* mutations themselves did not predict for poor response, although biallelic patients did have lower response rates.

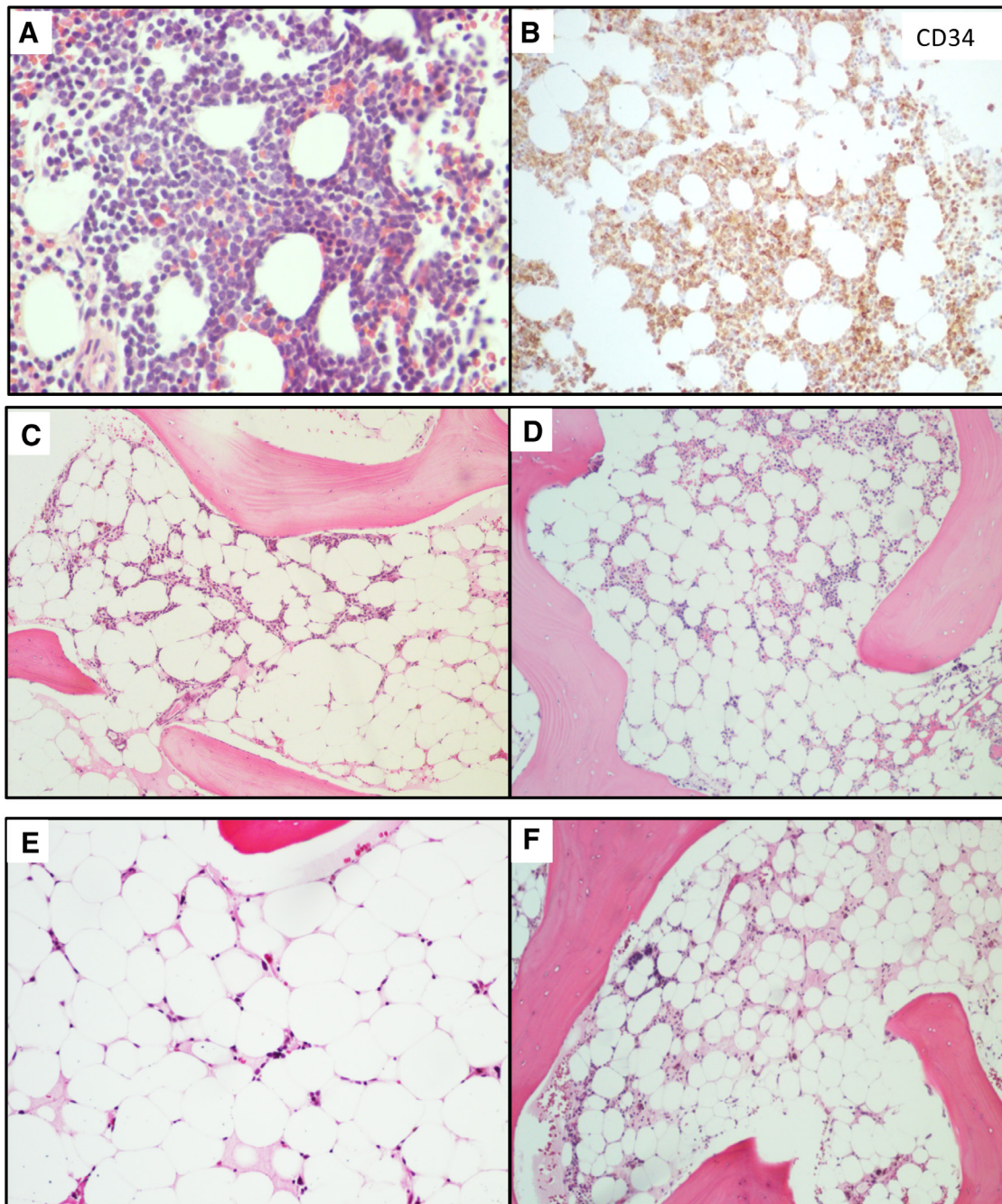


Figure 3. Patient bone marrow biopsy with persistent aplasia after HMA + VEN. (A), Bone marrow biopsy initial diagnosis showing hypercellular marrow with sheets of blasts and (B) the blasts are positive for CD34⁺. (C), Remission hypocellular bone marrow with no evidence of leukemia. (D and E), Continued remission marrow showing hypocellular marrow and VEN dose reduction. (F), Persistent hypocellular marrow aplasia after discontinuing VEN for 9 mo. HMA = hypomethylating agent; VEN = venetoclax.

Adverse or intermediate-risk cytogenetics also did not predict for response, as we observed responses in both risk groups. This included adverse risk patients who were able to achieve the deepest response with VAFs <1% at time of best response. Incompletely evaluated patients had poor outcomes, suggesting that the majority likely had nonresponsive disease or severe treatment-emergent side effects. These patients also had a higher proportion of poor response-associated mutations such as *FLT3* and *KRAS*.

In our cohort of patients, adaptive resistance to HMA + VEN did not have a consistent genetic pattern and instead involves various means of clonal evolution. Already at time of response, 11% of patients had new mutations. We observed evolution

including additional acquired mutations and expansion of new or previously undetected clones. These events were associated with mutations in *MPL*, *NRAS*, *CEBPA*, and *NF1*. We also observed persistent original clones throughout response and relapse. However, because these molecular profiles were obtained with targeted myeloid malignancy panels, it is possible that other mutations may be acquired at resistance that are not being measured. This suggests that adaptive resistance is multifaceted and can develop through a variety of genetic changes.

There are many current trials utilizing HMA + VEN as a backbone while adding on new agents. However, as we have shown here, the substantial cytopenias incurred with this regimen,

especially neutropenia, may make it difficult to successfully add on any novel therapies that have a negative impact on bone marrow function. One potential strategy that has not been formally studied may be to hold VEN dosing in patients who achieve a CRI in order to mitigate cytopenias and to continue HMA as monotherapy. Similarly, dose reductions or intermittent dosing of VEN (14/28 d) could be employed. While these strategies may lead to increased disease-related mortality, they may very well be offset by improvement in treatment-related mortality as well as improvements in quality of life. Studies that determine more limited VEN exposure with retained efficacy are needed. Patients starting these treatments need to fully understand the risks involved and supportive measures needed for prolonged cytopenias. Alternatively, these toxicities should be carefully considered to assess whether optimal supportive care may be a more appropriate option, especially in those with a very poor performance status or disease prognosis.

Our data are able to confirm prior reports of the efficacy of HMA + VEN in AML and MDS, while also highlighting the unique bone marrow suppressive effects of the combination. While HMA + VEN remains a potent new option in both the upfront and R/R settings, an alternative treatment strategy, including dosing regimens that mitigate cytopenias, may benefit the many AML patients who never achieve a hematological recovery and suffer morbidity and quality of life issues from frequent transfusions, infections, and hospitalizations. These findings require further investigation, including corroboration with larger cohorts of HMA + VEN treated patients, and need to be more systematically studied.

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