A phase I study of pevonedistat, azacitidine, and venetoclax in patients with relapsed/refractory acute myeloid leukemia

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Abstract

Azacitidine/venetoclax is an active regimen in patients with newly diagnosed acute myeloid leukemia (AML). However, primary or secondary resistance to azacitidine/venetoclax is an area of unmet need and overexpression of MCL1 is suggested to be a potential resistance mechanism. Pevonedistat inhibits MCL1 through activation of NOXA, and pevonedistat/azacitidine has previously shown activity in AML. To assess the tolerability and efficacy of adding pevonedistat to azacitidine/venetoclax in relapsed/refractory AML, we conducted a phase I, multicenter, open-label study in 16 adults with relapsed/refractory AML. Patients were treated with azacitidine, venetoclax along with pevonedistat intravenously on days 1, 3 and 5 of each 28-day cycle at doses of 10, 15 or 20 mg/m^2 in successive cohorts in the dose escalation phase. The impact of treatment on protein neddylation as well as expression of pro-apoptotic BCL2 family members was assessed. The recommended phase II dose of pevonedistat was 20 mg/m^2. Grade 3 or higher adverse events included neutropenia (31%), thrombocytopenia (13%), febrile neutropenia (19%), anemia (19%), hypertension (19%) and sepsis (19%). The overall response rate was 46.7% for the whole cohort including complete remission in five of seven (71.4%) patients who had not previously been treated with the hypomethylating agent/venetoclax. No measurable residual disease was detected in 80.0% of the patients who achieved complete remission. The median time to best response was 50 (range, 23-77) days. Four patients were bridged to allogeneic stem cell transplantation. The combination of azacitidine, venetoclax and pevonedistat is safe and shows encouraging preliminary activity in patients with relapsed/refractory AML. (NCT04172844).

Introduction

Outcomes of patients with relapsed/refractory acute myeloid leukemia (AML) have remained poor.[1] While salvage cytotoxic chemotherapy is commonly used, the availability of targeted agents is changing the treatment landscape of relapsed/refractory AML. Currently, gilteritinib (an FLT3 inhibitor), ivosidenib and olutasidenib (IDH1 inhibitors) and enasidenib (an IDH2 inhibitor) are approved for the management of subsets of patients with relapsed/refractory AML.[2-4] In addition, the B-cell lymphoma 2 (BCL2) pathway has been explored as a therapeutic target in AML, with encouraging efficacy of venetoclax (a BCL2 inhibitor) in combination with hypomethylating agents or cytarabine in the frontline management of AML.[5-8] However, in contrast to the high efficacy of venetoclax-based therapy in newly diagnosed AML, the rates of response to venetoclax remain low in the relapsed/refractory setting.[9,10] Preclinical studies investigating the mechanisms of BCL2 inhibitor resistance suggest overexpression of the anti-apoptotic protein MCL1 as a potential mechanism blocking the downstream effects of BCL2 inhibition.[11,12]

Pevonedistat (also known as TAK-924 and MLN4924) is a first-in-class, small-molecule inhibitor of the neural precursor cell expressed, developmentally downregulated 8 (NEDD8)-activating enzyme (NAE) which is important in the regulated turnover of proteins by Cullin RING ligases.[12-14] As a consequence of its impact on protein turn-

over, pevonedistat upregulates the pro-apoptotic protein NOXA, leading to neutralization of MCL1 and facilitating apoptosis.[15,16] In preclinical models, the combination of pevonedistat and venetoclax has shown a synergistic effect in leukemia cell lines and clinical AML isolates.[16] Prior clinical trials have investigated the safety and efficacy of pevonedistat in the setting of newly diagnosed as well as relapsed/refractory AML and myelodysplastic syndrome.[17-20] Because primary or secondary resistance to the hypomethylating agent/venetoclax combination can result from MCL1 overexpression and pevonedistat was shown to contribute to MCL1 neutralization through NOXA upregulation, we designed a clinical trial to assess the safety and preliminary efficacy of adding pevonedistat to the azacitidine/venetoclax backbone in patients with relapsed/refractory AML.

Methods

Patients

We conducted a phase I, multicenter, open-label study to determine the safety and recommended phase II dose (RP2D) of the combination of pevonedistat, venetoclax and azacitidine in patients with relapsed/refractory AML. The study enrolled patients from two US centers (Medical College of Wisconsin, Milwaukee and Mayo Clinic, Rochester). Key inclusion and exclusion criteria are included in *Online Supplementary Table S1*. Notably, patients with prior exposure to a hypomethylating agent and/or venetoclax therapy were eligible for the study. The study was approved by the institutional review board of each participating institution and was conducted according to International Council for Harmonization Good Clinical Practice guidelines and the ethical principles of the Declaration of Helsinki. All patients provided written informed consent. The study included a dose escalation phase (to determine the RP2D) and a dose expansion phase (with the RP2D) (*Online Supplementary Figure S1*). The primary endpoint was to determine the RP2D and toxicity profile of pevonedistat, azacitidine and venetoclax. Secondary endpoints included response rate,[21] survival and correlative studies (*Online Supplementary Methods*).

Treatment

Pevonedistat was administered by intravenous infusion on days 1, 3 and 5 of each 28-day cycle along with standard doses of intravenous azacitidine (75 mg/m^2 daily for 7 days) and oral venetoclax (400 mg daily for 28 days). During the dose escalation phase, pevonedistat was given in escalating doses (10 mg/m^2, 15 mg/m^2 and 20 mg/m^2) in successive treatment cohorts without intrapatient dose escalation. Dose escalation was overseen by the data safety monitoring committee of the Medical College of Wisconsin. Dose-limiting toxicities (DLT) were assessed during cycle 1 of therapy. Definitions of DLT are included in *Online Supplementary Table S2*. Dose expansion was conducted with the RP2D of pevonedistat. We did not attempt to go beyond 20 mg/m^2 as this was the RP2D in the azacitidine/pevonedistat study.[20]

Correlative studies

Correlative studies included: (i) examination of NAE inhibition; (ii) correlation of pretreatment levels of BCL2, BCLXL, MCL1, BAX and BAK with response; (iii) determination of levels of NOXA (PMAIP1) mRNA and protein expression before and after pevonedistat treatment; (iv) evaluation of BH3 mimetic profiling on bone marrow samples by flow cytometry; and (v) assessment of the sensitivity of leukemia and leukemic stem/progenitor cells to pevonedistat. Details regarding the methods for these assays can be found in the *Online Supplementary Methods*.[16,22]

Statistical analyses

The study followed a 3 + 3 design for the dose-escalation phase. The dose expansion phase utilized the RP2D of pevonedistat determined during the dose escalation phase. The baseline characteristics, toxicity profile, and response rate of the subjects at each dose level were analyzed using descriptive statistics. The Kaplan-Meier method was used to estimate survival. Event-free survival was defined from the time of achievement of complete remission (CR), CR with incomplete blood count recovery (CRi) or complete remission with partial hematologic recovery (CRh) to the time of relapse/progression/death. Overall survival was defined from the time of initiation of treatment until death from any cause. The maximum tolerated dose was the highest dose level at which none of the first three patients treated or none or one of the first six patients treated had a DLT during cycle 1 of the dose escalation phase. Statistical analysis was conducted with a level of significance set at $P<0.05$.

Results

Baseline characteristics

Sixteen patients with relapsed/refractory AML participated in the study (15 were evaluable for response), 13 in the dose escalation phase and three in the dose expansion phase (reported together with those given the RP2D in the dose escalation phase). The baseline characteristics of the study cohort are presented in Table 1. The patients' median age was 73 (range, 61-91) years, seven patients (43.8%) had secondary or therapy-related AML, 11 (68.7%) had adverse risk, nine (56.3%) had received prior therapy with venetoclax/hypomethylating agent and three (18.8%) had relapsed after prior allogeneic hematopoietic stem cell transplantation.

Table 1. Baseline demographic and clinical characteristics of the patients.

Characteristic	N=16
Age in years, median (range)	73 (61-91)
Gender, N (%)	
Male	7 (43.8)
Female	9 (56.3)
AML subtype, N (%)	
De novo	9 (56.3)
Secondary	5 (31.3)
Therapy-related	2 (12.5)
ELN 2017 risk status, N (%)	
Favorable	0 (0)
Intermediate	5 (31.3)
Adverse	11(68.7)
N of prior lines of therapy, median (range)	2 (1-6)
Prior hypomethylating agent/venetoclax, N (%)	
No	7 (43.8)
Yes	9 (56.3)
Prior allogeneic stem cell transplant, N (%)	
No	13 (81.3)
Yes	3 (18.8)
Gene mutations, N (%)	
DNMT3A	6 (37.5)
TP53	3 (18.7)
ASXL1	3 (18.7)
RUNX1	3 (18.7)
NRAS	2 (12.5)
JAK2	2 (12.5)
IDH2	1 (6.2)
FLT3	1 (6.2)
BCOR	1 (6.2)
BCORL1	1 (6.2)
KMT2A	1 (6.2)
SRSF2	1 (6.2)
ETV6	1 (6.2)
NF1	1 (6.2)
PTPN11	1 (6.2)
CHEK2	1 (6.2)
TET2	1 (6.2)
CSF3R	1 (6.2)
PHF6	1 (6.2)
DDX41	1 (6.2)
SMC1A	1 (6.2)
MUTYH1	1 (6.2)
EZH2	1 (6.2)
SETBP1	1 (6.2)
SMC1A	1 (6.2)
PASK	1 (6.2)
ZNF703	1 (6.2)

N: number; AML: acute myeloid leukemia; ELN: European LeukemiaNet.

Safety and recommended phase II dose

The triplet combination of pevonedistat, azacitidine and venetoclax was well tolerated by participants. A summary of the common adverse events is provided in Table 2. The most common grade 3 or higher adverse events included neutropenia (31%), thrombocytopenia (13%), febrile neutropenia (19%), anemia (19%), hypertension (19%) and sepsis (19%). Atrial fibrillation was the only DLT that occurred in a patient who received pevonedistat at the 10 mg/m^2 dose. This cohort was subsequently expanded to a total of six patients, and no further DLT was observed. Pevonedistat 20 mg/m^2 was established as the RP2D. The regimen was also well tolerated beyond the DLT period, and no unexpected toxicities were observed when the regimen was continued beyond the first cycle. Patients received a median of two cycles of therapy (range, 1-6 cycles).

Efficacy

Fifteen patients were evaluable for response (one patient dropped out of the study prior to completion of cycle 1 of therapy because of active central nervous system disease). Among patients who were evaluable, the overall response rate was 46.7% (Table 3). The CR rate was 33.3% for the overall cohort and 71.4% in patients with venetoclax/hypomethylating agent-naïve relapsed/refractory AML (*Online Supplementary Table S3*). Among nine patients with prior exposure to venetoclax/hypomethylating agent, one patient achieved a morphological leukemia-free status (MLFS). The median time to achieve best response was 50 (23-77) days. In patients achieving CR, 80.0% were negative for minimal residual disease by flow cytometry. Among patients who responded to treatment, 1-year overall survival was 80.0% (*vs.* 0% in those who did not achieve CR) (*Online Supplementary Figure S3*). For the overall population, the median progression-free survival was 2.4 months, and median overall survival was 6.3 months (*Online Supplementary Figures S4* and *S5*). Four patients subsequently underwent allogeneic hematopoietic stem cell transplantation.

Correlative studies

Several studies were conducted to examine pharmacodynamic biomarkers of drug action (Figures 1-3, *Online Supplementary Figures S6-S8*). Serial samples of bone marrow or peripheral blood were available from eight patients to assess the impact of treatment on protein neddylation as well as expression of pro-apoptotic BCL2 family members. Immunoblotting showed that CUL1 neddylation was diminished, indicative of pevonedistat action, at 6 h after pevonedistat administration in five of the eight patients. Importantly, cells from the two patients who achieved a CR displayed decreased CUL1 neddylation that persisted at 24 hours, whereas cells from those who did not achieve CR had recurrent CUL1 neddylation by 24 hours (Figure 1A). Despite preclinical data suggesting that mRNA encoding PMAIP1 (NOXA) is upregulated by pevonedistat in AML cells *ex vivo*, quantitative reverse transcriptase polymerase chain reaction on RNA isolated from serial samples failed to demonstrate a consistent pattern of changes in mRNA encoding pro-apoptotic BCL2 family members after therapy initiation (Figure 1B). Nonetheless, immunoblotting revealed treatment-associated upregulation of two proteins that bind MCL1 and promote apoptosis, i.e., p53 upregulated

Table 2. Adverse events.

Cohort	Overall		Pevonedistat 10 mg/m²		Pevonedistat 15 mg/m²		Pevonedistat 20 mg/m²	
Toxicity, N (%)	**All N=16**	**Grade 3+ N=16**	**All N=7**	**Grade 3+ N=7**	**All N=3**	**Grade 3+ N=3**	**All N=6**	**Grade 3+ N=6**
Diarrhea	6 (38)	0	3 (43)	0	1 (33)	0	2 (33)	0
Hypotension	5 (31)	1 (6)	2 (29)	1 (14)	1 (33)	0	2 (33)	0
Neutropenia	5 (31)	5 (31)	2 (29)	2 (29)	1 (33)	1 (33)	2 (33)	2 (33)
Anemia	4 (25)	3 (19)	1 (14)	1 (14)	0	0	3 (50)	2 (33)
Constipation	4 (25)	0	1 (14)	0	1 (33)	0	2 (33)	0
Fatigue	4 (25)	0	1 (14)	0	0	0	3 (50)	0
Hypertension	4 (25)	3 (19)	2 (29)	2 (29)	1 (33)	0	1 (17)	1 (17)
Dizziness	3 (19)	0	2 (29)	0	0	0	1 (17)	0
Febrile neutropenia	3 (19)	3 (19)	2 (29)	2 (29)	0	0	1 (17)	1 (17)
Fever	3 (19)	0	1 (14)	0	0	0	2 (33)	0
Hyperglycemia	3 (19)	0	0	0	0	0	3 (50)	0
Oral mucositis	3 (19)	0	0	0	0	0	3 (50)	0
Nausea	3 (19)	0	1 (14)	0	0	0	2 (33)	0
Sepsis	3 (19)	3 (19)	2 (29)	2 (29)	0	0	1 (17)	1 (17)
Abdominal pain	2 (13)	0	0	0	0	0	2 (33)	0
Anorexia	2 (13)	0	0	0	0	0	2 (33)	0
Back pain	2 (13)	0	1 (14)	0	0	0	1 (17)	0
Dyspepsia	2 (13)	0	2 (29)	0	0	0	0	0
Dyspnea	2 (13)	1 (6)	1 (14)	1(14)	0	0	1 (17)	0
Flatulence	2 (13)	0	0	0	0	0	2 (33)	0
Headache	2 (13)	0	1 (14)	0	0	0	1 (17)	0
Hyperuricemia	2 (13)	0	0	0	1 (33)	0	1 (17)	0
Hypoalbuminemia	2 (13)	0	0	0	0	0	2 (33)	0
Hypocalcemia	2 (13)	1 (6)	0	0	0	0	2 (33)	1 (17)
Hypokalemia	2 (13)	0	0	0	1 (33)	0	1 (17)	0
Hyponatremia	2 (13)	1 (6)	1 (14)	1 (14)	0	0	1 (17)	0
Oral pain	2 (13)	0	1 (14)	0	0	0	1 (17)	0
Pain	2 (13)	0	0	0	1 (33)	0	1 (17)	0
Platelet count decreased	2 (13)	2 (13)	1 (14)	1 (14)	0	0	1 (17)	1 (17)
Sinus tachycardia	2 (13)	0	2 (29)	0	0	0	0	0
Skin infection	2 (13)	0	1 (14)	0	0	0	1 (17)	0

modulator of apoptosis (PUMA) at 24 hours for two patients who achieved a CR (patients 021 and 022) and NOXA at 6 hours for patient M002 who achieved MLFS (Figure 1A). One patient (PAVE 20) whose leukemia was studied at the time of regrowth had significant upregulation of BCL2 and less prominent upregulation of MCL1 and $BCLX_L$ (Figure 1A, *Online Supplementary Figure S6*). Proximity ligation assays indicated the presence of preformed complexes of BAK with various anti-apoptotic family members, indicative of a "primed for apoptosis" state that was reflected in BH3 mimetic profiling assays (*Online Supplementary Figure S7*). In the one patient whose samples could be studied at progression, there was a decrease in the preformed BAK/MCL1 and BAK/BCL2 complexes compared to baseline (*Online Supplementary Figure S8*), suggesting a less primed state. Additional studies were undertaken to search for potential predictive biomarkers in the pretreatment samples. Samples from four patients were treated with pevonedistat and

Table 3. Response assessment.

Variables	Total N=16*	Dose group		
		Pevonedistat 10 mg/m² N=7	Pevonedistat 15 mg/m² N=3	Pevonedistat 20 mg/m² N=6
N of responders	7	1	2	4
Best response, N (%)				
Complete remission	5 (33.3)	1 (16.7)	2 (66.7)	2 (33.3)
Morphological leukemia-free state	1 (6.7)	0 (0.0)	0 (0.0)	1 (16.7)
Partial remission marrow	1 (6.7)	0 (0.0)	0 (0.0)	1 (16.7)
Treatment failure	8 (53.3)	5 (83.3)	1 (33.3)	2 (33.3)
Time from start of treatment to best response in days, median (range)	50.0 (23.0-77.0)	52.0 (52.0-52.0)	45.0 (23.0-67.0)	38.5 (27.0 - 77.0)
Overall response, N (%)				
No response	8 (53.3)	5 (83.3)	1 (33.3)	2 (33.3)
Response**	7 (46.7)	1 (16.7)	2 (66.7)	4 (66.7)
CR/CRi, N (%)				
No	11 (68.8)	6 (85.7)	1 (33.3)	4 (66.7)
Yes	5 (31.3)	1 (14.3)	2 (66.7)	2 (33.3)
MRD among patients achieving CR/CRi, N (%)				
CR/CRi with MRD-	4 (80.0)	1 (100.0)	2 (100.0)	1 (50.0)
CR/CRi with MRD+	1 (20.0)	0 (0.0)	0 (0.0)	1 (50.0)

*15 patients were evaluable for response. **Achieving any type of complete remission, partial remission of morphological leukemia-free state. N: number; CR: complete remission; CRi: complete remission with incomplete blood count recovery; MRD: minimal residual disease.

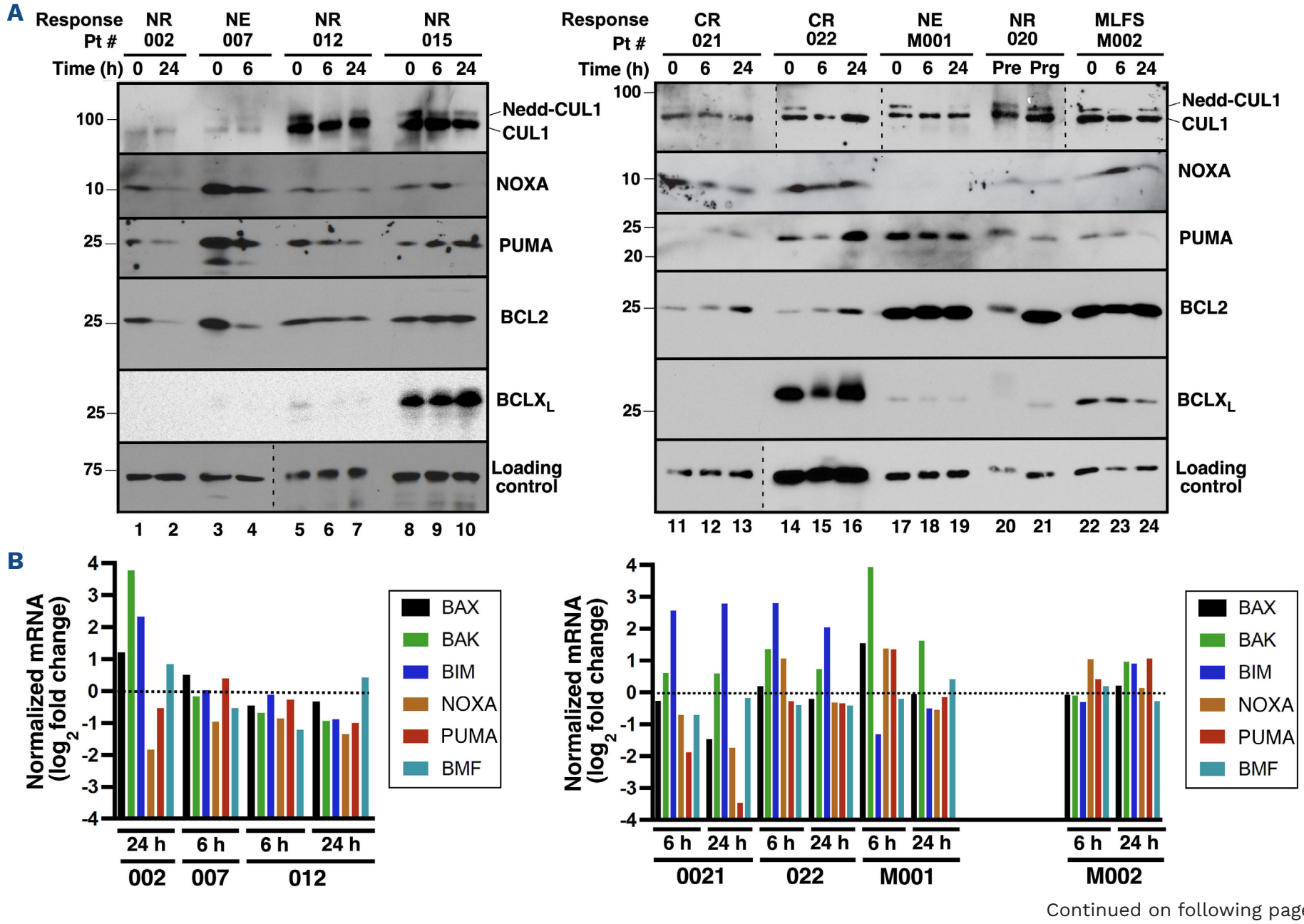

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Figure 1. Impact of treatment on protein and mRNA levels. (A) Whole cell lysates harvested before (0 h) or at the indicated times after chemotherapy administration on day 1 or on progression (Prg) were subjected to immunoblotting for the indicated antigens. LMNB1 or GAPDH served as a loading control. Response is indicated above each patient's number. Dashed lines indicate juxtaposition of different exposures of the same blot (upper panels) or a different loading control (bottom panels). (B) mRNA levels determined by quantitative reverse transcriptase polymerase chain reaction at the indicated time points were compared to mRNA levels in the pretreatment samples. Pt #: patient's number; NR: no response; NE: not evaluable; CR: complete response; MLFS: morphological leukemia-free state.

venetoclax *ex vivo* for 24 hours and examined for survival of leukemic stem and progenitor cells by ten-color flow cytometry.[22] This assay demonstrated that leukemia stem-like cells from a patient achieving CR (PAVE 021) were the most sensitive to pevonedistat at 50-100 nM and the pevonedistat/venetoclax combination (Figure 2). In contrast, BH3 mimetic profiling of the bulk leukemia showed variable baseline sensitivity to BH3 mimetics (*Online Supplementary Figure S7*). Pretreatment samples from 13 of the 16 treated patients were also examined by immunoblotting. Results of this assay demonstrated that levels of DNMT1 and DNMT3A were generally higher in samples from patients with CR or MLFS than in samples from patients who did not respond, whereas pretreatment levels of pro- or anti-apoptotic proteins did not correlate with response (Figure 3, *Online Supplementary Figure S6B*).

Genomic mutations

Baseline mutation status at the time of study entry was known in 13 patients. Most commonly mutated genes included *DNMT3A, TP53, ASXL1, RUNX1, JAK2*, and *NRAS* (Table 1). One patient had an *IDH2* mutation, and none of the patients had an *NPM1* mutation. *TP53* mutation was seen in 18.7% of patients. Despite the limited sample size, no significant association was noted between baseline mutation profile and treatment response.

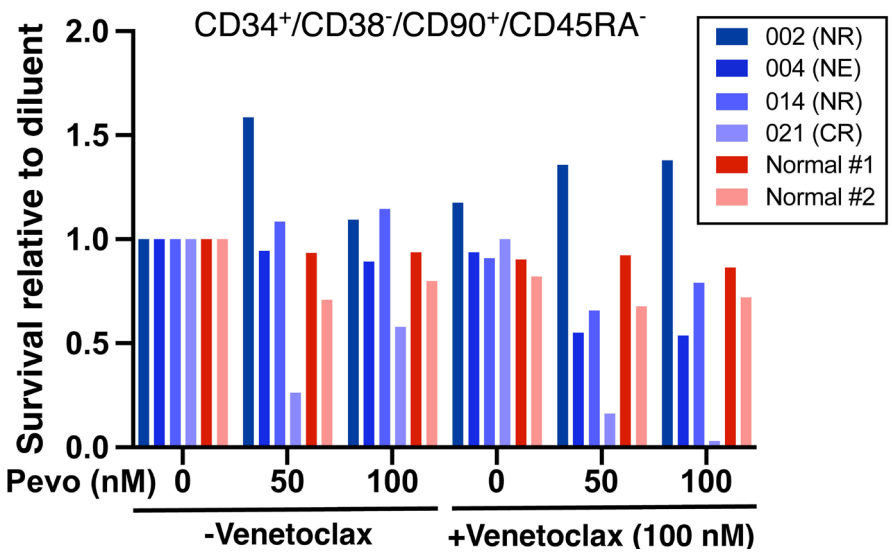

Figure 2. Relative survival of cells with a hematopoietic stem cell-like immunophenotype after 24 hours of exposure to the indicated treatments *ex vivo*. Bone marrow mononuclear cells isolated prior to treatment from the indicated study patient (various shades of blue) or from normal individuals (red shades) were incubated for 24 hours with the indicated concentrations of pevonedistat (pevo) and/or venetoclax and subjected to multiparameter flow cytometry as described in the *Online Supplementary Methods*. Results for each patient were normalized to those of the diluent control. NR: no response; NE: not evaluable; CR: complete remission; pevo: pevonedistat.

Discussion

The optimal salvage strategy for patients with relapsed/refractory AML remains unclear. While cytotoxic chemotherapy and targeted agents remain as tools in the armamentarium, there are limitations to the application of these strategies. For example, intensive cytotoxic salvage chemotherapy regimens such as CLAG-M (cladribine, cytarabine, granulocyte colony-stimulating factor and mitoxantrone) or MEC (mitoxantrone, etoposide and cytarabine) are most useful in fit patients with adequate performance status.[23] Targeted agents such as IDH1 inhibitors, IDH2 inhibitors and FLT3 inhibitors are most effective in patients harboring mutations in the targeted proteins.[3,24,25] This leaves a large group of AML patients who are older, have comorbidities and lack targetable mutations in need of better salvage therapy. While hypomethylating agent/venetoclax-based salvage therapy has been investigated in patients with AML, the limited efficacy of this regimen in the relapsed/refractory setting underscores the need for further research to improve long-term outcomes. Toward this end, we investigated the potential role of adding pevonedistat to the azacitidine/venetoclax backbone in patients with relapsed/refractory AML, and our results demonstrate a potentially important role for this combination in the management of these patients.

The present study found that the addition of pevonedistat to azacitidine and venetoclax was safe and well tolerated in patients with relapsed/refractory AML. When administered with a standard 7-day azacitidine and 28-day venetoclax regimen, pevonedistat 20 mg/m^2 was established as the recommended dose. Most patients included in our study were older adults (median age 73 years) with AML harboring poor-risk features, and over half of the participants had been previously treated with hypomethylating agent/venetoclax combinations before study enrollment. The side-effect profile of pevonedistat was consistent with that reported in prior clinical trials and no new safety concerns were noted when pevonedistat was added to azacitidine + venetoclax and given as a continued therapy.[26] Because of the small sample size, the clinical efficacy data of this combination need to be confirmed in larger studies. We

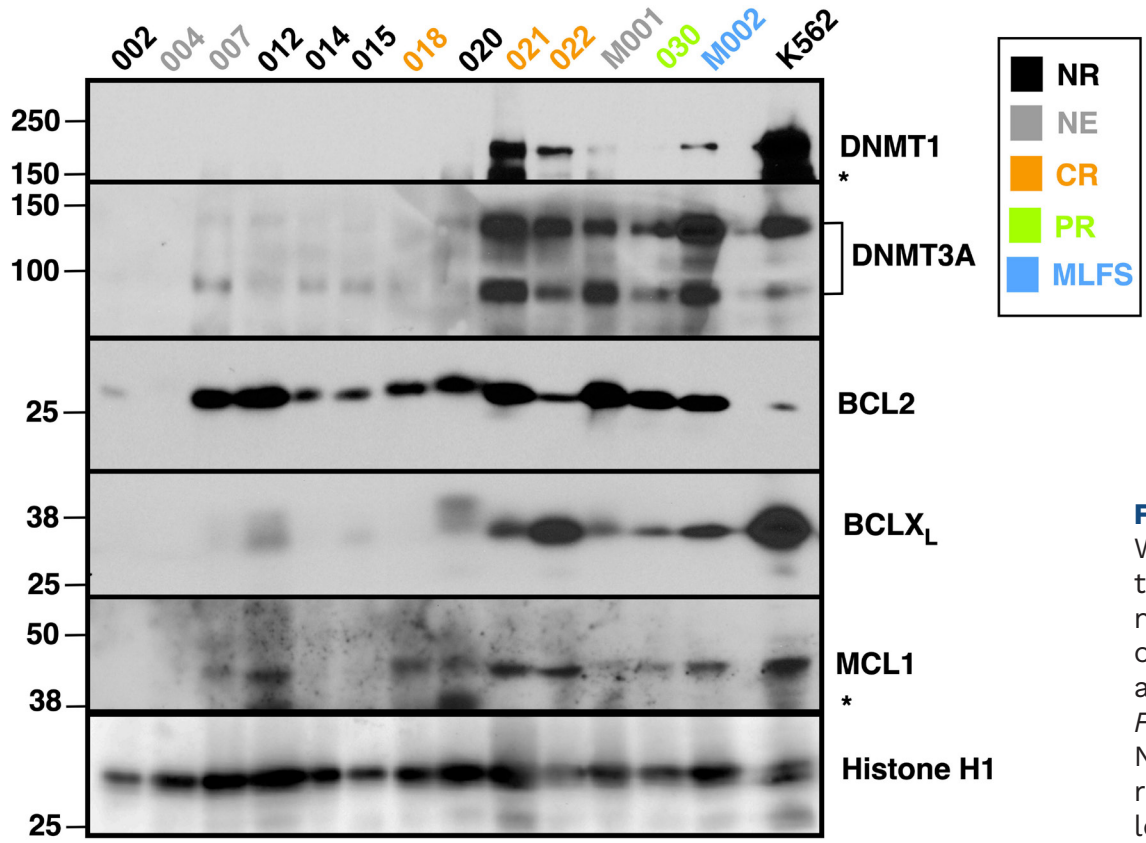

Figure 3. Potential predictive markers of response. Whole cell lysates harvested prior to therapy from the indicated patients were subjected to immunoblotting for the indicated antigens. The response of each patient is color coded. Additional correlative studies are shown in *Online Supplementary Figures S2* and *S6-S8*. *indicates nonspecific band. NR: no response; NE: not evaluable; CR: complete response; PR: partial response; MLFS: morphological leukemia-free state.

noted a variability in response based on prior exposure to venetoclax and hypomethylating agents. Among the patients with relapsed/refractory AML who had not previously been exposed to venetoclax therapy, CR was observed in five of the seven (71.4%) patients treated with the triplet combination, which is numerically higher than previously reported for the azacitidine/venetoclax doublet, albeit with a small sample size. For example, a large retrospective series reported a CR/CRi of 24% with venetoclax-based salvage therapy (37% with azacitidine/venetoclax) in relapsed/refractory AML patients who had not previously received venetoclax.[9] In contrast, among patients who had previously been exposed to venetoclax, one patient out of nine in the present study achieved MLFS with the addition of pevonedistat to azacitidine/venetoclax. While the results are limited by the small size of this study, these observations suggest that this strategy might work best with the upfront addition of pevonedistat to azacitidine/venetoclax rather than sequential addition of pevonedistat after the onset of resistance to azacitidine/venetoclax.

Based on preclinical data,[15,16] several correlative studies were performed in serial samples to assess the impact of this regimen on apoptotic pathways. Immunoblotting demonstrated that CUL1 neddylation was inhibited at 6 and 24 hours in a subset of cases (Figure 1A), as would be expected if NAE were inhibited by pevonedistat. However, upregulation of PMAIP1/NOXA at the mRNA and protein levels was not routinely observed in these cases (Figure 1A, B). While this might reflect loss of cells with the greatest PMAIP1/NOXA upregulation due to rapid killing, it is also possible that preclinical studies have failed to uncover important aspects of the antileukemic mechanism of pevonedistat.

Additional studies were performed to identify potential biomarkers that are predictive of response. No association was noted between genomic mutations and response and the cohort was not enriched for venetoclax-sensitive mutations.[27] In an analysis of drug sensitivity *ex vivo*, higher sensitivity to pevonedistat and pevonedistat + venetoclax was observed in cells from a patient who achieved CR (Figure 2). Examination of immunoblotting data indicated that sustained inhibition of CUL1 neddylation at 24 hours and induction of BBC3/PUMA or PMAIP1/NOXA were associated with CR and prolonged MLFS, respectively (Figure 1A). Moreover, pretreatment levels of DNMT1 and DNMT3A tended to be higher in patients who achieved a response (Figure 3), consistent with previous suggestions that pretreatment DNMT1 levels might be a potential harbinger of response to hypomethylating agents in AML.[28,29] All of these correlative studies were limited to AML cases with sufficient bone marrow cellularity or enough circulating blasts to permit completion of the various assays. Moreover, immunoblotting to complete proof-of-mechanism studies and allow evaluation of potential predictive biomarkers at the protein level was prioritized over more exploratory biomarkers such as ten-color flow cytometry after drug exposure *ex vivo* and proximity ligation assays to assess the presence of constitutively activated BAK. Given the small sample sizes, the resulting observations require further assessment in future studies.

In addition to the encouraging results with this combination in venetoclax treatment-naïve patients with relapsed/refractory AML, prior studies have also demonstrated the efficacy of pevonedistat in myeloid malignancies. A phase I study by Swords *et al.* in patients with myelodysplastic syndrome and oligoblastic treatment-naïve AML showed that the combination of pevonedistat and azacitidine was

able to produce an overall response rate of 50% in newly diagnosed patients, including those with *TP53* mutations.[20] More recently, two randomized clinical trials in patients with treatment-naïve, high-risk myelodysplastic syndrome and oligoblastic AML showed significantly higher response rates with azacitidine/pevonedistat as compared to azacitidine monotherapy, although this finding did not translate into a significant improvement in overall survival.[18,30] In addition, a phase I/II study of pevonedistat/azacitidine/venetoclax in patients with newly diagnosed, secondary AML showed a 64% CR/CRi rate.[31] Together with the findings of our study, these results indicate a potential for improvement in response rates and good tolerability with the addition of pevonedistat to the azacitidine/venetoclax backbone. Achievement of CR is an important goal in patients with relapsed/refractory AML given the potential for then moving forward with potentially curative treatments such as allogeneic hematopoietic stem cell transplantation. Although limited by a small sample size and lack of planned cohort stratification based on prior venetoclax exposure, our study demonstrates the feasibility, safety and encouraging early efficacy of a potential triplet therapy for the management of these patients.

In conclusion, the addition of pevonedistat to a backbone of venetoclax and azacitidine is safe and well tolerated in patients with relapsed/refractory AML. Dose escalation yielded encouraging efficacy specifically in patients with relapsed/refractory AML not previously treated with the hypomethylating agent/venetoclax combination. Given this encouraging clinical activity, our results suggest that further study of NAE inhibitors in this setting is warranted. Future efforts to investigate novel agent combinations, including triplet therapy options, could potentially improve the response rates and outcomes of patients with relapsed/refractory AML, particularly those without a targetable mutation.

Disclosures

GSGM has received honoraria from Cardinal Health, DAVA Oncology, Aptitude Health, and Curio science; has sat on advisory boards for BMS, BeiGene, Pfizer, and Gilead/Kite; has participated in speakers' bureau for Amgen and Rigel; and has provided consultancy services for Cancerexpert Now, Qessential, and Techspert, all outside the submitted work. ML has received research support from AbbVie, Astellas, Actinium, Amgen, Pluristem, and Sanofi; has participated in speakers' bureau for Amgen and BeiGene; and has performed data safety monitoring for BioSight, all outside the submitted work. EA has received research support from AbbVie, Novartis and Takeda; has acted as a consultant for AbbVie, Novartis, and BMS; and has participated in speakers' bureau for AbbVie and BMS, all outside the submitted work. All the other authors report that they have no relevant conflicts of interest to disclose.

Contributions

GSGM, EA, ANS, and SHK conceived and designed the study. GSGM, EA, ANS, ML, ANS, and SHK collected, assembled and analyzed the data, and wrote the manuscript. All authors interpreted the data and approved the final version of the manuscript. GSGM and EA had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Data-sharing statement

De-identified datasets are available upon request to authors and after approval by the sponsors.

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