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Phase I Study of Epigenetic Modulation with 5-Azacytidine and Valproic Acid in Patients with Advanced Cancers

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Abstract Purpose: 5-Azacytidine (5-AZA) is a DNA-hypomethylating agent. Valproic acid is a histone deacetylase inhibitor. Combining hypomethylating agents and histone deacetylase inhibitors produces synergistic anticancer activity *in vitro* and *in vivo*. On the basis of this evidence, we conducted a phase I study of the combination of 5-AZA and valproic acid in patients with advanced cancers.

Experimental Design: 5-AZA was administered s.c. daily for 10 days. Valproic acid was given orally daily with a goal to titrate to plasma levels of 75 to 100 $\mu\text{g}/\text{mL}$ (therapeutic for seizures). Cycles were 28 days long. 5-AZA was started at 20 mg/m^2 and escalated using an adaptive algorithm based on the toxicity profile in the prior cohort (6 + 6 design). Peripheral blood mononuclear cell global DNA methylation and histone H3 acetylation were estimated with the long interspersed nucleotide elements pyrosequencing assay and Western blots, respectively, on days 1 and 10 of each cycle when patients agreed to provide them.

Results: Fifty-five patients were enrolled. Median age was 60 years (range, 12-77 years). The maximum tolerated dose was 75 mg/m^2 of 5-AZA in combination with valproic acid. Dose-limiting toxicities were neutropenic fever and thrombocytopenia, which occurred at a dose of 94 mg/m^2 of 5-AZA. Stable disease lasting 4 to 12 months (median, 6 months) was observed in 14 patients (25%). A significant decrease in global DNA methylation and induction of histone acetylation were observed.

Conclusion: The combination of 5-AZA and valproic acid is safe at doses up to 75 mg/m^2 for 5-AZA in patients with advanced malignancies.

5-Azacytidine (5-AZA), a cytidine analogue, is a DNA methyltransferase inhibitor (1) that induces DNA hypomethylation (2) *in vitro* and *in vivo* (1). DNA methylation is a mechanism of epigenetic regulation of gene transcription. The term epigenetics describes stable alterations in gene expression

produced by several mechanisms, including DNA methylation and histone modifications (2). Epigenetic modifications are functional alternatives to genetic changes such as mutations and deletions that inactivate tumor suppressor genes, thereby silencing their expression (3). DNA methylation involves adding a methyl group to cytosine in a cytosine-guanine dinucleotide pair. These pairs, also called CpG islands, are abundantly located in and near specific gene promoters and in DNA-repetitive elements such as long interspersed nucleotide elements (4). There is an inverse relationship between the transcriptional activity of a promoter and its methylation status. Promoter DNA hypermethylation is a common hallmark of cancer (2, 3), and in contrast to deletions or mutations of tumor suppressor genes, this phenomenon can be modulated with DNA methyltransferase inhibitors such as 5-AZA.

The biochemical modification of chromatin-associated histone proteins, such as acetylation of histone H3 or H4, is another epigenetic regulatory mechanism of gene expression (2). This process is controlled by histone acetyl transferases and deacetylases (2, 5). Inhibiting histone deacetylase activity results in histone acetylation, which is associated with up-regulated gene transcription (5). Valproic acid, a short-chain fatty acid widely used in the treatment of epilepsy and other neurologic disorders, is also a histone deacetylase inhibitor. By promoting gene transcription, valproic acid induces differentiation, growth inhibition, and apoptosis in different cellular systems (6). The combination of decitabine, a DNA methyltransferase analogue

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Translational Relevance

This study confirms that epigenetic modulation is achievable with a combination of the histone deacetylase inhibitor valproic acid and the hypomethylating agent azacytidine, at doses that are safe and tolerable. The maximum tolerated dose is defined, and preliminary evidence of biological activity described. The study provides the foundation for phase II studies of this and similar combinations of epigenetic modulators.

inhibitor of 5-AZA, and valproic acid is synergistic in leukemic systems *in vitro* (7) and has significant clinical activity in patients with leukemia (8).

On the basis of this information, we designed a clinical trial to find the maximum tolerated dose and to assess the safety of the combination of 5-AZA and valproic acid in patients with advanced cancers.

Patients and Methods

Study group. Patients entered in the study were required to have a pathologically confirmed cancer that was metastatic or unresectable and refractory to standard therapy or for whom there was no standard therapy for their cancer that resulted in a 3-month survival advantage. Other eligibility criteria were adequate performance status (Eastern Cooperative Oncology Group ≤ 2 ; ref. 9), adequate cardiac function (New York Heart Association classes III and IV were excluded; ref. 10) and adequate bone marrow, liver, and kidney function (absolute neutrophil count $>1,500/\mu\text{L}$, platelets $>100,000/\mu\text{L}$, total bilirubin <2.0 mg/dL, and creatinine <2.0 mg/dL). The study was conducted at the University of Texas M. D. Anderson Cancer Center and all patients were enrolled after giving written informed consent in accordance with our Institutional Review Board requirements.

Interventions. 5-AZA was provided by Pharmion Corporation and was administered on an outpatient basis, *s.c.*, daily for 10 days. The starting dose was 20 mg/m²/d and was escalated according to a toxicity-based adaptive algorithm (11) and continued until dose-limiting toxicities were produced. Initially, six patients were treated at the starting dose and evaluated for toxicity. If none of the six patients experienced toxicity, the next cohort of six patients was treated with a 100% dose escalation. If grade 1 toxicity was seen, the next cohort was treated at a 50% dose escalation. If grade 2 toxicity was observed or if one of the six patients treated at a determined dose experienced a dose-limiting toxicity or toxicities, the next cohort of six patients was treated with a 25% dose-escalation. If two or more of the six patients at a determined dose experienced dose-limiting toxicities, the maximum tolerated dose was exceeded. The prior lower dose level was established as the maximum tolerated dose. The maximum tolerated dose was defined as the highest dose studied in which the incidence of dose-limiting toxicities was $<33\%$. In order to obtain adequate correlative data, more patients were added to specific dose levels following Institutional Review Board approval.

Valproic acid was administered orally at a starting dose of 10 mg/kg/d once daily and titrated by 5 mg/kg/d every week with a maximum dose of 60 mg/kg/d, with the goal to achieve a therapeutic plasma level of 75 to 100 $\mu\text{g}/\text{mL}$. Courses of therapy were repeated not earlier than every 28 days as indicated by follow-up studies. Patients continued on treatment until disease progression or unacceptable toxicities occurred. Toxicity for dose escalation was assessed at the end of the first cycle (28 days) and adverse events were recorded and coded based on the National Cancer Institute Common Terminology Criteria for Adverse

Events v3.0. Six patients were enrolled per dose level. Intracohort dose escalation was not permitted. Patients who experienced a dose-limiting toxicity could resume treatment if the toxicity resolved to grade 1 or less with the dose of 5-AZA reduced by 50%. 5-AZA was stopped when a patient experienced grade 4 toxicity or a second episode of grade 3 toxicity. For toxicities most likely related to valproic acid (central nervous system effects), the dose was held until resolution of toxicity to grade 1, and then the reduced dose was resumed to maintain a trough level of 50 to 75 $\mu\text{g}/\text{mL}$ or less. Patients received two courses of treatment (56 days) before their initial re-evaluation for response.

Monitoring and treatment assessment. Patients were assessed at baseline and during treatment as per standard of care. Regular physical examination, complete blood counts with differential, serum chemistry (liver function tests, electrolytes, urea, and creatinine), and valproic acid levels were done at least bimonthly. We did measure free valproic acid plasma levels, using plastic transport tubes, and the ultrafiltrate was assayed by immunoassay. We ordered imaging studies for tumor measurement, *i.e.*, computed tomography scan, magnetic resonance imaging, positron-emission tomography scan, and plain radiographs. Patients were seen on average every week during the first month and every 2 to 4 weeks during the subsequent cycles. Response and progression were evaluated according to the Response Evaluation Criteria in Solid Tumors (12).

Analysis of DNA methylation. To study the dynamics of DNA hypomethylation sequentially during treatment, we collected peripheral blood mononuclear cells (PBMC) from patient blood samples on days 1 and 10 of each cycle (whenever patients agreed to provide samples) and we assessed the methylation status of long interspersed nuclear elements, a surrogate marker of global DNA methylation. To do so, we extracted DNA from the PBMC and submitted the cells to a bisulfite pyrosequencing assay (13). Bisulfite modification of DNA converts unmethylated CpG sites to UpG without modifying methylated sites, thus allowing methylated and unmethylated sites to be distinguished from each other. In this procedure, DNA was extracted using a standard phenol-chloroform method in which 2 μg of DNA were denatured in 0.2 N NaOH at 37°C for 10 min and incubated with 3 mol/L of Na-bisulfite at 50°C for 16 h. DNA was then purified using the Wizard PCR Clean-Up System (Promega), washed with 80% isopropanol and desulfonated with 0.3 N NaOH at 25°C for 5 min. DNA was then precipitated with ammonium acetate, glycogen, and 100% ethanol, washed with 70% ethanol, dried and resuspended in H₂O. The details of these techniques have been described elsewhere (8).

After bisulfite treatment of the DNA, we did a PCR amplification of long interspersed nuclear elements. The PCRs were carried out in a 50 μL mix. In each reaction, 1 μL of bisulfite-treated DNA was mixed with 5 μL of PCR buffer, 0.4 μL of 25 mmol/L deoxynucleotide triphosphate mix, 0.5 μL of 10 $\mu\text{mol}/\text{L}$ TTTTGAGTTAGTGTGGGA-TATA forward primer, 0.5 μL of 10 $\mu\text{mol}/\text{L}$ AAAATCAAAAATTCCCTTTC reverse universal biotinylated primer and 1 unit of Taq polymerase. Pyrosequencing, a method of direct sequencing by DNA synthesis, was done after DNA amplification using a PCR. The long interspersed nuclear element amplicon was purified and the methylation status was quantified with the PSQ HS 96 Pyrosequencing System (Pyrosequencing, Inc.). The sequencing primer used was AGT-TAGGTGTGGGATATAGT. This method is highly reproducible, with a SD of 2% (13). Long interspersed nuclear element methylation was reported in percentage units.

Analysis of histone acetylation. To confirm the induction of histone acetylation by valproic acid, we did a standard Western blot analysis on PBMC. Proteins from PBMCs were isolated by sonication using a lysis buffer [10 mmol/L HEPES (pH 7.9), 1.5 mmol/L MgCl₂, 10 mmol/L KCl, 0.5 mmol/L DTT, 1 mmol/L phenylmethylsulfonyl fluoride]. Protein concentrations were measured using the Bio-Rad protein assay kit. A total of 30 μg of protein was then loaded in a 12% SDS-polyacrylamide gel, transferred to Immobilon-P nitrocellulose membranes (Millipore), and blocked with 3% nonfat milk. The nitrocellulose membrane was incubated overnight with a 1:2,000 dilution of

Table 1. Patients' characteristics

	No. of patients (%)
Total	55 (100)
Sex	
Male	30 (55)
Female	25 (45)
Age (y)	
Median	60
Range	12-77*
Cancer diagnosis	
Breast	4 (7)
Colon	11 (20)
Glioblastoma	2 (4)
Head and neck	3 (5)
Lung	2 (4)
Melanoma of the skin	7 (13)
Uveal melanoma	3 (5)
Rectal	2 (4)
Renal	2 (4)
Urothelial	3 (5)
Thymic	3 (5)
Thyroid	3 (5)
Uterine leiomyosarcoma	2 (4)
Cervical small cell cancer	1 (2)
Neuroendocrine	2 (4)
Appendiceal	1 (2)
Liposarcoma	1 (2)
Unknown primary	1 (2)
Prostate	2 (3)

*There were three children enrolled in the trial (12- and 15-year-old girls, and a 17-year-old boy).

polyclonal rabbit antiacetylated histone 3 (Upstate Biotechnology, Inc.). Afterwards, washing out membranes with a solution of PBS and Tween 0.1%, incubated for 1 h in anti-rabbit horseradish peroxidase secondary antibody diluted to 1:2,000 (Amersham Biosciences) and washed again with PBS and Tween 0.1%. Chemiluminescence was induced using a SuperSignal West Dura kit from Pierce Biotechnology, Inc., according to the manufacturer's instructions. We used β -actin (1:5,000; Sigma) as an internal control.

Statistical methods/considerations. The primary objective of this study was to assess the safety, tolerability, and maximum tolerated dose of 5-AZA in combination with valproic acid in patients with advanced cancer. Secondary objectives included the assessment of global DNA methylation, histone acetylation, and evaluation of responses. Dose-limiting toxicity was defined as any grade 4 thrombocytopenia, grade 4 febrile neutropenia, or any clinically significant grade 3 or higher nonhematologic toxicity, as defined in the National Cancer Institute Common Terminology Criteria for Adverse Events v3.0 that was

attributable to the therapy. Dose escalation proceeded as explained above (see Interventions). The effects of 5-AZA and valproic acid on DNA methylation and histone acetylation, respectively, were evaluated at days 1 and 10 of each 28-day cycle (whenever patients agreed to provide samples). Descriptive statistics (mean, SD, minimum, median, and maximum) were computed for each evaluation time of each cycle for each dose cohort. The methylation data was expressed as a percentage, which showed a normal distribution. The *P* values provided were two-sided and obtained by Wilcoxon signed-rank test and Fisher's exact test as appropriate. *P* = 0.05 was considered significant.

Results

Patient characteristics. Fifty-five patients were enrolled. The 5-AZA dose levels used were 20, 25, 37.5, 47, 59, 75, and 94 mg/m². Median age was 60 years (range, 12-77 years). Patient characteristics are shown in Table 1. The most common malignancies were colorectal cancer (11 patients), melanoma (10 patients; 3 of them with ocular melanoma), and breast cancer (4 patients).

Dose escalation and toxicity. Side effects in each cohort are shown in Table 2. One patient had a dose-limiting toxicity at an AZA-5 dose of 37.5 mg/m² (neutropenic fever) and at 94 mg/m², three of six patients experienced dose-limiting toxicities (two patients with neutropenic fever; one with grade 3 thrombocytopenia). The maximum tolerated dose was 75 mg/m² s.c. daily for 10 days. At that dose, which was expanded, 3 of 16 patients (18%) showed dose-limiting toxicity (neutropenic fever). Other toxicities were grades 1 and 2 somnolence (six patients), tremor (six patients), hypomagnesemia (one patient), anemia (two patients), and vomiting (one patient), as detailed in Table 3. We could not achieve plasma levels of 75 to 100 μ g/mL in the majority of patients. Patients had side effects such as tremulousness and somnolence that limited dose escalation to what would otherwise be deemed therapeutic doses in the setting of seizures. The mean plasma valproic acid level achieved by the end of the first cycle was 65.3 \pm 22.2 μ g/mL (SD); by the end of the second cycle, the mean valproic acid achieved was 63.4 \pm 24.5 μ g/mL (SD). Even so, epigenetic modulation effects were apparently achieved (see below).

Objective response. To date, there have been no complete or partial remissions. Nonetheless, stable disease with a median duration of 6 months (4-12 months) was achieved in 14 patients (25%; Table 4). A patient with papillary carcinoma of the thyroid has remained stable for 12 months and continues on the study. One patient with leiomyosarcoma of the uterus had stable disease for 8 months. One patient with melanoma

Table 2. Number of dose-limiting toxicities observed in each cohort (drug-related, first course)

5-AZA dose (mg/m ²)	No. of patients	Grade 3 or 4 toxicities	No. of patients
20	6		
25	6		
37.5	8	Neutropenic fever	1
47	7		
59	6		
75	16	Neutropenic fever	3
94	6	Neutropenic fever	2
		Thrombocytopenia	1
Total	55		7

Table 3. Grade 1 or 2 toxicities observed during treatment

Toxicity (grade 1 or 2)	No. of patients
Somnolence	6
Tremor	6
Anemia	2
Nausea/vomiting	1
Electrolyte imbalance	1

NOTE: These toxicities were evenly distributed across dose levels, probably because they were related to valproic acid.

has been stable for 10 months and remains on the study. Two patients with ocular melanoma had stable disease (one for 6 months and the other for 4 months). A patient with rapidly progressive renal cell carcinoma achieved stable disease for 6 months until progression. Stable disease was also observed in patients with thymoma ($n = 2$), prostate cancer ($n = 1$), colorectal cancer ($n = 1$), ethmoidal carcinoma ($n = 1$), salivary gland pleomorphic adenoma ($n = 1$), thymic carcinoid ($n = 1$), and breast cancer ($n = 1$; Table 4). Mixed responses were observed in three patients with an overall classification of stable disease.

Analysis of DNA methylation. To study DNA methylation dynamics during treatment, global DNA methylation was analyzed on days 1 and 10 of each 28-day treatment cycle (whenever patients agreed to provide samples). Adequate data were available mostly from the first cycle, because patients refused to provide further blood samples or because they came off study during subsequent cycles. We used a pyrosequencing assay to estimate the methylation of long interspersed nuclear elements as a surrogate marker of global methylation. Median methylation pretreatment was 63.4% (59-70%); it decreased to 57.4% (53-63%) by day 10, the last day of treatment with 5-AZA, and returned close to baseline, 60.6% (46%-68%) by day 0 of the next cycle ($P < 0.001$) as shown in Fig. 1. Global DNA methylation was not significantly different between

patients achieving stable disease and those who did not (data not shown). Global DNA methylation observed in each cohort at different doses of 5-AZA are shown in Fig. 1 and differences seen in the figure do not achieve statistical significance.

Induction of histone acetylation. To assess the effect of valproic acid on histone acetylation, we did Western blots of acetylated histone H3 on days 1 and 10 of every cycle (whenever patients agreed to provide samples). Acetylation of histone H3 (at least doubling over baseline by densitometry) was observed in 20 of 33 evaluable patients (61%; Fig. 2A and B). Evaluable patients achieving stable disease had a higher frequency of acetylation than those not achieving it (7 of 10 versus 13 of 23, $P = 0.0003$).

Discussion

We showed in this study that the combination of 75 mg/m² of 5-AZA daily for 10 days, and valproic acid titrated to achieve a plasma level of 75 to 100 µg/mL daily, is safe. Administration of this combination was associated with stable disease, demonstrating a median duration of 6 months (4-12 months) in 14 patients (25%) in this cohort of individuals with advanced cancers who had progressive disease after standard treatment. This therapy also resulted in the induction of global DNA hypomethylation and histone acetylation. We defined the maximum tolerated dose of 5-AZA in combination with valproic acid to be 75 mg/m² daily for 10 days. This is consistent with the experience of the use of single-agent 5-AZA in myelodysplastic syndromes (14).

Here, we evaluated the induction of histone acetylation by valproic acid and global DNA hypomethylation by 5-AZA. Consistent with the *in vitro* histone deacetylase inhibitory effect of valproic acid, we observed evidence of histone acetylation in 61% of the study patients. Interestingly, the frequency of histone acetylation was higher in patients achieving stable disease. Fourteen patients had stable disease. Of these patients, histone acetylation was measured in 10, and 7 of the 10 patients had acetylation of their PBMC histones (Table 4). Of note, the three patients without a documented absence of histone acetylation showed decreased levels of global DNA

Table 4. Characteristics of patients achieving stable disease

Age	Sex	Cancer diagnosis	No. of prior therapies	5-AZA cohort/dose (mg/m ²)	SD duration (mo)	Decrease in methylation (%)	Histone acetylation
72	M	Renal cell	6	20	6	n/a	Yes
44	F	Ethmoidal	1	20	4	7	No
72	F	LMS of uterus	3	25	8	9	Yes
61	F	Thymic carcinoid	3	25	4	6	No
41	F	Breast	6	25	4	6	No
50	F	Ocular melanoma	1	47	6	4	Yes
64	M	Colorectal	1	47	4	3	n/a
58	M	Papillary thyroid	2	59	12	5	Yes
49	M	Thymoma	1	59	4	17	Yes
62	F	Melanoma	9	75	10	16	Yes
43	F	Thymoma	3	94	6	13	n/a
36	F	Ocular melanoma	0	94	4	9	Yes
60	M	Prostate	4	75	6	n/a	n/a
61	F	Salivary	2	75	6	n/a	n/a

Abbreviations: SD, stable disease; n/a, not available; LMS, leiomyosarcoma.

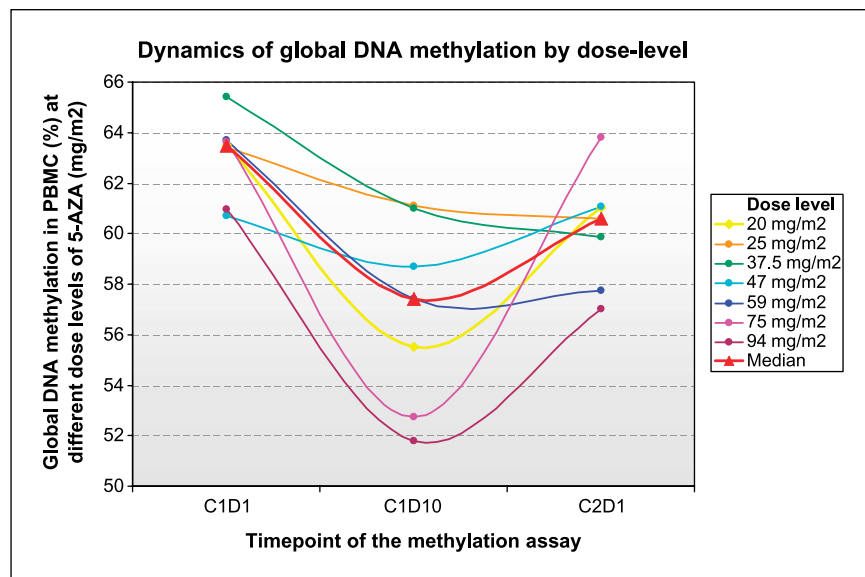


Fig. 1. Global DNA methylation was observed at different 5-AZA doses (mg/m²). Red, median percentage of methylated CpG sites on day 0 [63.4% (range, 59-70%)] and day 10 [57.4% (range, 53-63%)] before returning close to baseline by day 1 of the next cycle [60.6% (range, 46-68%)]. $P < 0.001$ by Wilcoxon signed-rank test. C1D1, cycle 1/day 1; C1D10, cycle 1/day 10; C2D1 cycle 2/day 1 (which is day 29 from the start of therapy).

methylation (Table 4). Prior studies in hematologic malignancies have not shown an association between histone acetylation and response (8, 15). An explanation for the latter observation includes the possibility that the unrelated molecular effects of valproic acid occur downstream of histone deacetylase inhibition. However, the presence of histone acetylation in most of the patients achieving stable disease suggests that the relevance of this biological effect in patients with solid tumors requires further study. We confirmed the *in vivo* hypomethylating activity of 5-AZA, which we assessed using a long interspersed nuclear element bisulfite pyrosequencing assay. As described for decitabine in leukemia (8), we documented global methylation as a transient phenomenon, which peaks shortly after 5-AZA exposure and returns gradually to baseline. Although all patients achieving stable disease had decreased levels of global DNA methylation, we did not observe a statistically significant difference in the induction of hypomethylation by 5-AZA between patients with stable disease and those without it, which is not surprising because the study was not powered to evaluate this. Interestingly, evidence of global DNA hypomethylation was found at doses as low as 20 mg/m², a dose at which stable disease was observed in two of six patients. This analysis showing the dynamics of global methylation could be considered as a marker of the biological activity of the drug but it is not known if this marker is necessarily related to the clinical activity of 5-AZA. It is possible that specific gene methylation studies may identify markers of response. It is also possible that the clinical activity of 5-AZA is not related to the induction of DNA methylation.

Despite the safety and clinical benefit of the combination, we have not been able to show that the combination is superior to single-agent 5-AZA, as this study was not designed to test that hypothesis, and we observed only stable disease and not partial or complete responses. That said, previous studies of 5-AZA in solid tumors had largely negative results (16). In contrast, our study showed clinical benefit (stable disease) in 25% of cases, a response that lasted for several months. It is possible that this relatively high rate of disease stability could be due to the contribution of valproic acid and induction of acetylation, although the small number of patients and the pilot nature of

the data preclude any certainty as to the relative contribution of azacytidine alone. Because of the heterogeneous characteristics and different cancer diagnoses of our study group, it is difficult

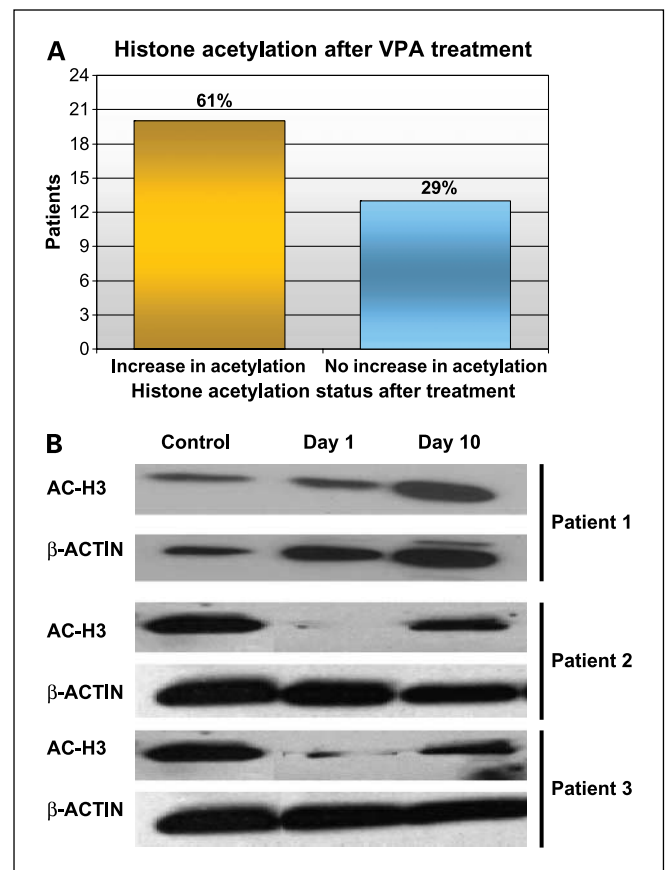


Fig. 2. *A*, increased acetylation of histone H3 was observed in 20 of 33 evaluable patients (61%). *B*, three representative Western blot of histone H3 acetylation analyzed using conventional Western blot techniques on days 1 and 10 of treatment. Histone acetylation was measured in positive controls (HL-60 cells treated with valproic acid), and in patients on day 1 (pretherapy) and day 10. Histone acetylation was considered if at least a 2-fold increase in histone acetylation was detected comparing day 10 with day 1. AC-H3, acetylated histone H3.

to draw conclusions about which patients benefited the most. The longest responses were observed in patients with papillary thyroid carcinoma, cutaneous and uveal melanoma, thymoma, leiomyosarcoma, and renal cell cancer. These observations will need to be studied further to more carefully assess clinical efficacy specific to diverse phenotypes.

In addition to the heterogeneous cancers of our study patients, there are several limitations in our study. First, valproic acid, the histone deacetylase inhibitor used in this study, is one of the least potent histone deacetylase inhibitors and it is not known if the dose used in this trial is the most clinically effective, however, the continuous administration used here constitutes an innovative intervention. In addition, most patients could not tolerate doses that would result in therapeutic blood levels for patients with a seizure disorder. Even so, evidence of histone acetylation was found in 61% of the cases. Prior studies in leukemia and myelodysplastic syndromes needed higher levels of valproic acid to achieve a similar frequency of histone acetylation, but were associated with dose-limiting neurologic toxicities (8, 15). In those studies, histone acetylation was also measured in PBMC. It may be that PBMC in patients with leukemia and myelodysplastic syndromes differ from those cells in patients with solid

tumors. The combination of 5-AZA with more effective and less toxic histone deacetylase inhibitors may improve the results observed in this trial. A study combining 5-AZA with MGCD0103, an oral isotype selective histone deacetylase inhibitor, is currently ongoing with promising interim results (17). Second, measurement of DNA methylation and histone acetylation was done on PBMCs, and not in malignant tissue. The correlation between measurements in cancer cells and those in PBMCs in solid tumors is not known. Our findings showing global DNA hypomethylation, histone acetylation, and clinical benefit encourage further molecular analysis of gene-specific methylation, gene expression, and effects downstream of DNA methylation and histone acetylation.

In summary, this study showed that the combination of 5-AZA and valproic acid is safe. Prolonged stable disease was observed in some patients. This therapy was associated with DNA hypomethylation and induction of histone acetylation. Together, these results suggest that this approach warrants further study.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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