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A Phase I Protocol of Hydralazine and Valproic Acid in Advanced, Previously Treated Solid Cancers

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Abstract

Smokers experience aberrant gene promoter methylation in their bronchial cells, which may predispose to the development of neoplasia. Hydralazine is a DNA demethylating agent, and valproic acid is a histone deacetylase inhibitor, and both have modest but synergistic anticancer activity *in vitro*. We conducted a phase I trial combining valproic acid and hydralazine to determine the maximally tolerated dose (MTD) of hydralazine in combination with a therapeutic dose of valproic acid in patients with advanced, unresectable, and previously treated solid cancers. Twenty females and nine males were enrolled, with a median age of 57 years and a median ECOG performance status of 0. Grade 1 lymphopenia and fatigue were the most common adverse effects. Three subjects withdrew for treatment-related toxicities occurring after the DLT observation period, including testicular edema, rash, and an increase in serum lipase accompanied by hyponatremia in one subject each. A true MTD of hydralazine in combination with therapeutic doses of valproic acid was not reached in this trial, and the planned upper limit of hydralazine investigated in this combination was 400 mg/day without grade 3 or 4 toxicities. A median number of two treatment cycles were delivered. One partial response by Response Evaluation Criteria In Solid Tumors criteria was observed, and five subjects experienced stable disease for 3 to 6 months. The combination of hydralazine and valproic acid is simple, nontoxic, and might be appropriate for chemoprevention or combination with other cancer treatments. This trial supports further investigation of epigenetic modification as a new therapeutic strategy.

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Introduction

Epigenetics is the study of a stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence [1]. DNA methylation and histone modifications are essential epigenetic processes of normal cellular differentiation and function. Dysregulation of epigenetic modifications can lead to neoplasia [2]. In cancer, aberrant regulation of DNA methylation leads to global hypomethylation, though many gene promoters, including those of tumor suppressor genes are abnormally hypermethylated. Silencing of tumor suppressors by hypermethylation of their gene promoters, which inhibits transcription, is nearly universal in neoplasia. Genes encoding proteins that modify histones have emerged to be some of the most commonly mutated sequences associated with neoplasia [3]. These various epigenetic changes are targetable. Efforts have focused on DNA-demethylating drugs and inhibitors of histone deacetylases (HDACs). Cytidine analogs such as 5-azacytidine (azacitidine) and 5-aza-deoxycytidine (decitabine) are demethylating agents, which inhibit DNA methyltransferases (DNMTs) [4]. These drugs have

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been approved for the treatment of myelodysplastic syndrome and are currently under investigation in solid tumors [5]. Their potential mutagenic properties prevent use for cancer prevention. HDACs remove acetyl groups from the histone lysine residues (as well as other nonhistone proteins), leading to the formation of a condensed and transcriptionally silenced chromatin. HDAC inhibitors that are used for cancer therapy include romidepsin and vorinostat, both of which have been approved for cutaneous T cell lymphoma. Belinostat is currently under review by the United States Food and Drug Administration (US FDA) for various indications.

Of interest to the current trial, multiple older drugs have activity as DNMT or HDAC inhibitors. The antihypertensive drug hydralazine is a demethylating agent [6,7]. Reversal of promoter hypermethylation *in vitro* can be achieved at pharmacological concentrations of hydralazine [8]. Valproic acid is an HDAC inhibitor with modest anticancer activity. The combination of hydralazine and valproic acid demonstrates synergistic *in vitro* antineoplastic activity and increases the cytotoxicity of several chemotherapy agents, such as gemcitabine, cisplatin, and doxorubicin [9]. We conducted a phase I trial combining valproic acid and hydralazine. The primary end point was to determine the maximally tolerated dose (MTD) of hydralazine in combination with a therapeutic dose of valproic acid, on the basis of observed adverse events in patients with advanced, refractory, and previously treated solid cancers.

Methods

The trial was approved by the University of New Mexico Institutional Review Board, and patients were enrolled after signing an informed consent. This trial was registered with ClinicalTrials.gov (Identifier No. NCT0096060) (United States National Institutes of Health, Bethesda, MD).

Patient Population

Eligible patients included those with solid tumors who were previously treated, for whom no acceptable standard treatment regimen was available, and could not be cured with either surgery or radiotherapy. All patients had to be able to provide informed consent, be ≥18 years old, have an Eastern Cooperative Oncology Group (ECOG) performance status of ≤2 at the time of the initiation of therapy, have adequate end-organ function, have a life expectancy >8 weeks, and have no severe comorbidities.

Study Design

The study was an open-label, nonrandomized, dose-escalation phase I trial that enrolled patients in sequential cohorts. The drugs were given in 28-day cycles. Valproic acid was initiated at day −14 of the first cycle to achieve a steady state level, and subsequently, both drugs were given continuously for the subsequent cycles. The initial dose of valproic acid was 250 mg orally three times a day for days −14 through −8, then 500 mg orally three times each day daily for days −7 through 28, with the dose titrated to keep the serum level between 0.4 and 0.7 μg/ml. Hydralazine (immediate-release formulation) was initiated at 25 mg per day in the first dosing cohort and then dose-escalated in divided doses through the day in subsequent cohorts of patients as long as the blood pressure values were tolerated by patients. Table 1 shows the cohorts representing hydralazine dose escalation. To avoid neurotoxicity and excessive sedation, there was no plan to escalate the dose of valproic acid to achieve a steady state level higher than 0.7 μg/ml. A 3 + 3 design was followed for transition from one cohort to the next. If none of the first three patients in one cohort experienced dose-limiting toxicity (DLT) by day 28 of cycle 1, then the dose was escalated in the next cohort to the next higher hydralazine dose level. A DLT consisted of one or more grade 3 or greater nonhematologic toxicities or any grade 4 or greater hematologic toxicities lasting longer than 10 days during the first cycle and must have been at least possibly attributed to the treatment regimen. If one of the three patients experienced DLT by day 28 of cycle 1, then the cohort was expanded to six patients. If none of these three additional patients experienced DLT, then the dose was escalated to the next higher dose level in the subsequent cohort. The MTD was the dose level at which none of six or one of six patients experienced a DLT during the first 4-week cycle with the next higher dose having at least two of six patients experiencing a DLT. At the MTD, a total of six additional patients were enrolled to better assess potential toxicities. A standard 3 + 3 design was used in this setting with toxicity end points rather than pharmacodynamic end points due to the potential differences in the panel of epigenetically silenced tumor suppressors between the various tumor types, as well as within tumor types. A pharmacodynamic end point was deemed to be more appropriate for evaluation in a controlled phase II trial.

Results

Patients Characteristics

A total of 29 patients were enrolled, and 27 were treated. One withdrew consent before initiating any therapy, and one never received therapy due to a rapid decline in performance status. Of those treated, there were 19 females and 8 males, with a median age of 57 years (range = 29-75 years), and a median ECOG performance status of 0. These subjects had received a median of four prior regimens (range = 1-12). The data are summarized in Table 2.

Toxicity

This combination was largely well tolerated. Twenty-seven patients received the combination through six consecutive cohorts with increasing doses of hydralazine. The potential toxicities associated with hydralazine are known to be associated with formulation and acetylator phenotype; whereas the formulation was controlled (immediate *vs* sustained release preparations), the limited number of subjects involved in this study precluded adequate stratification or assessment by acetylator phenotype (slow *vs* fast). Each subject was able to take the valproic acid at therapeutic levels. Lymphopenia and fatigue were the most common adverse effects (Table 3*A, B, C, D*), and adverse effects required reducing the dose of valproic acid in three patients; subsequent serum levels were not recorded. Hydralazine caused edema in five subjects but resulted in treatment discontinuation in only one of the subjects who experienced testicular edema at the dose level of 50 mg per day (the other four experienced lower

Table 1. Dose Cohort Strategy.

Dosing Cohort	Hydralazine Dose	No. of Patients Treated
0	25 mg	3
1	50 mg	6
2	100 mg (25 mg QID)	3
3	200 mg (50 mg QID)	3
4	300 mg (75 mg QID)	9
5	400 mg (100 mg QID)	3

Table 2. Subject Demographics (N = 27).

Age (Years)	
Median	57
Range	29-75
Gender	
Males	8
Females	19
ECOG Performance Status	
0	21
1	5
2	1
No. of Prior Chemotherapy Regimens	
Median	4
Range	1-12
Tumor Histology	
Colorectal	4
Cutaneous melanoma	4
Ovary	4
Breast	4
Soft-tissue sarcoma	3
Non–small cell lung	2
Head and neck	2
Cervix	2
Ocular melanoma	1
Gastric	1
TOTAL	27

extremity edema). Two other subjects withdrew for treatment-related toxicities occurring after the DLT observation period, including rash in the one subject (dose level of 25 mg per day) and hyponatremia and an increase in serum lipase in the other subject (dose level of 300 mg per day). Although hypotension was anticipated to be a DLT at higher doses, the patients in the highest planned dosing cohort tolerated hydralazine at a dose of 400 mg per day with no clinically relevant hypotension. No MTD of hydralazine was observed in this trial, but as the maximum recommended dose of hydralazine for the treatment of hypertension or congestive heart failure is 300 mg per day, the phase II dose of hydralazine in combination with valproic acid at therapeutic doses was defined as 300 mg per day; six additional patients were enrolled at this dose level (total of nine) to better define any potential toxicities, without any DLTs observed.

Responses

A median number of two treatment cycles were administered on this protocol (range = 1 -29). There were no complete responses. One partial response by Response Evaluation Criteria In Solid Tumors (RECIST) criteria was observed in a patient who had metastatic mutant B-RAF V600E-positive melanoma (before the availability of vemurafenib). They received this regimen as a second-line systemic therapy after a combination of temozolomide, paclitaxel, and carboplatin and remained on therapy for 29 months. They initially had stable disease for 4 months, which slowly evolved into a partial response. They developed vitiligo on this experimental combination. On disease progression, they received ipilimumab without response. Five additional subjects experienced stable disease for 3 to 6 months: two with soft-tissue sarcoma (3 and 4 months), ovarian cancer (3 months), squamous cell cancer of the head and neck (4 months), and breast cancer (6 months). At the time of this report, 24 of the 27 subjects have died, with a median overall survival of 3 months (range = 1-18 months); the three survivors are alive at 16, 18, and 18 months.

Table 3A. All Related Hematologic Adverse Events.

Adverse Event	Grade 1	Grade 2	Grade 3	Grade 4	Total Events
Lymphopenia	9	4	2	0	15
Anemia	6	0	1	0	7
Thrombocytopenia	6	0	0	0	6
Decreased leukocytes	3	0	0	0	3
Hemolysis	1	0	0	0	1

Table 3B. All Related Laboratory Value Adverse Events.

Adverse Event	Grade 1	Grade 2	Grade 3	Grade 4	Total Events
Hyperglycemia	5	0	0	0	5
Hyponatremia	4	0	0	0	4
Hypokalemia	3	0	0	0	3
Hypocalcemia	2	0	0	0	2
Hypomagnesemia	2	0	0	0	2
Increased aspartate aminotransferase	2	0	0	0	2
Increased alkaline phosphatase	1	0	0	0	1
Increased lipase	1	0	0	0	1
Increased alanine transaminase	1	0	0	0	1
Hypoalbuminemia	1	0	0	0	1

Discussion

Although the primary objective of this phase I study was to identify the MTD of the combination of escalating doses of hydralazine with a fixed, steady-state concentration of valproic acid, the significance of the study was to design and test a tolerable combination of agents that may subsequently be evaluated as a regimen for the chemoprevention of lung cancer. Chromatin-modifying agents have demonstrated activity *in vitro* and *in vivo* against non–small cell lung cancer. However, the adverse event profiles of current FDA-approved chromatin-modifying agents are not justifiable for chronic delivery in healthy patients at risk for lung cancer. In our trial, the recommended dose for further study is hydralazine at 300 mg per day and valproic acid with a target serum concentration of 0.4 to 0.7 μg/ml. Although the dose of 400 mg per day of hydralazine did not exceed DLT as defined, the rates of mild, symptomatic hypotension and edema were considered unacceptable for the purpose of prolonged administration. This study demonstrates that pharmacological doses of hydralazine and valproic acid may be delivered to patients with heavily pretreated malignancies, with evidence of potential clinical activity in melanoma, soft-tissue sarcoma, and carcinomas of the breast, ovary, and head and neck. The study was

Table 3C. All Related Systemic Adverse Events.

Adverse Event	Grade 1	Grade 2	Grade 3	Grade 4	Total Events
Fatigue	7	5	0	0	12
Edema (leg)	4	0	0	0	4
Nausea	2	2	0	0	4
Vomiting	3	0	0	0	3
Headache	1	2	0	0	3
Heartburn	2	1	0	0	3
Rash	2	1	0	0	3
Decreased level of consciousness	1	1	0	0	2
Tremor	0	2	0	0	2
Somnolence	0	2	0	0	2
Pruritis	0	1	0	0	1
Anorexia	1	0	0	0	1
Hypopigmentation	1	0	0	0	1
Neuropathy	0	1	0	0	1
Constipation	1	0	0	0	1
Edema (testicles)	0	0	1	0	1
Bruising after fall	1	0	0	0	1
Hypotension	1	0	0	0	1
Rhinitis	1	0	0	0	1
Confusion	1	0	0	0	1

Table 3D. Related Adverse Events, All Grades, by Cohort.

Cohort 0	Adverse Event	Grade 1	Grade 2	Grade 3	Grade 4	Total Events
	Hyperglycemia	5	0	0	0	5
	Lymphopenia	4	1	0	0	5
	Fatigue	2	1	0	0	3
	Hypokalemia	2	0	0	0	2
	Hypomagnesemia	2	0	0	0	2
	Increased aspartate aminotransferase	2	0	0	0	2
	Increased alanine transaminase	1	0	0	0	1
	Edema (leg)	2	0	0	0	2
	Nausea	1	1	0	0	2
	Anemia	1	0	0	0	1
	Hyponatremia	1	0	0	0	1
	Hypoalbuminemia	1	0	0	0	1
	Increased lipase	1	0	0	0	1
	Muscle twitch	0	1	0	0	1
	Neuropathy	0	1	0	0	1
	Vomiting	1	0	0	0	1
	Constipation	1	0	0	0	1
	Headache	1	0	0	0	1

Cohort 1	Adverse Event	Grade 1	Grade 2	Grade 3	Grade 4	Total Events
	Lymphopenia	2	2	0	0	4
	Fatigue	0	3	0	0	3
	Edema (leg)	2	0	0	0	2
	Hypocalcemia	2	0	0	0	2
	Thrombocytopenia	2	0	0	0	2
	Hypokalemia	1	0	0	0	1
	Increased alkaline phosphatase	1	0	0	0	1
	Edema (testicles)	0	0	1	0	1
	Vomiting	1	0	0	0	1
	Bruise after fall	1	0	0	0	1

Cohort 2	Adverse Event	Grade 1	Grade 2	Grade 3	Grade 4	Total Events
	Anemia	2	0	0	0	2

Cohort 3	Adverse Event	Grade 1	Grade 2	Grade 3	Grade 4	Total Events
	Decreased level of consciousness	0	1	0	0	1
	Confusion	1	0	0	0	1
	Hyponatremia	1	0	0	0	1
	Rhinitis	1	0	0	0	1

Cohort 4	Adverse Event	Grade 1	Grade 2	Grade 3	Grade 4	Total Events
	Lymphopenia	2	1	2	0	5
	Fatigue	4	0	0	0	4
	Rash	2	1	0	0	3
	Hyponatremia	2	0	0	0	2
	Headache	0	2	0	0	2
	Leukopenia	2	0	0	0	2
	Thrombocytopenia	2	0	0	0	2
	Hypokalemia	0	0	1	0	1
	Decreased level of consciousness	0	0	1	0	1
	Hypotension	1	0	0	0	1
	Heartburn	1	0	0	0	1
	Pruritis	1	0	0	0	1
	Anemia	1	0	0	0	1
	Hemolysis	1	0	0	0	1

Table 3D (*continued*)

Cohort 5	Adverse Event	Grade 1	Grade 2	Grade 3	Grade 4	Total Events
	Fatigue	1	2	0	0	3
	Nausea	2	1	0	0	3
	Somnolence	0	2	0	0	2
	Anemia	2	0	0	0	2
	Thrombocytopenia	2	0	0	0	2
	Heartburn	1	1	0	0	2
	Leukopenia	1	0	0	0	1
	Vomiting	0	1	0	0	1
	Anorexia	1	0	0	0	1
	Tremor	0	1	0	0	1
	Hypopigmentation	1	0	0	0	1

limited by absence of a correlative pharmacodynamic surrogate of epigenetic reprogramming. Zambrano et al. conducted a phase I trial hydralazine in women with cervical cancer and were able to demonstrate partial reversal of a panel of aberrantly silenced genes at all dose levels tested, with gene re-expression in three-quarters of the informative cases [8].

Epigenetics are the processes that modulate DNA expression without changing the DNA code. At the DNA level, epigenetic changes modulate the genome through the covalent addition of a methyl group to the 5-position of the cytosine ring within the context of cytosine and guanine (CpG) dinucleotides. Although the majority of the genome is CpG poor, about three-quarters of the CpG residues in the mammalian genome are methylated. These areas of the genome are called CpG islands and are often found at the 5′ ends of genes. DNA methylation can promote oncogenesis through an increased mutation rate or by silencing transcription of tumor suppressor genes [10-12]. For example, some colorectal carcinomas with microsatellite instability have a high frequency of promoter region hypermethylation of the mismatch repair gene *hMLH1*. Colon cell lines containing a hypermethylated *hMLH1* gene re-express *hMLH1* when treated with 5-aza-2′-deoxycytidine and show restoration of mismatch repair ability, indicating that hypermethylation of the *hMLH1* CpG island could be the primary inactivating event [13]. In patients with heterozygous mutations in tumor suppressor genes, the second hit can occur by hypermethylation of the wild-type allele, leading to tumorigenesis. Five-methylcytosine itself may be mutagenic by undergoing spontaneous deamination to form thymine, leading to a C→T transition [10-12]. Hydralazine reverses aberrant gene promoter methylation *in vitro* at concentrations that are achieved clinically [6].

At the histone level, posttranslational modification of amino acids can alter the histone conformation. Modification of histones ensures that a differentiated cell remains differentiated and does not convert back into a stem cell. Histone recognition by protein complexes called readers, writers, and erasers of the histone code helps shape the structural determinants of histone functions. Although histone modifications occur throughout the entire sequence, the "histone tails" (unstructured N termini) are the targets of most modifications. These include acetylation, methylation, ubiquitylation, phosphorylation, and sumoylation. Of particular interest to the current study design, acetylation leads to transcriptional competence. HDAC inhibitors represent a novel class of therapeutic agents that increase histone acetylation to maintain the chromatin structure in a more open conformation. This conformational change may lead to restoration of transcriptionally silenced pathways or suppression of aberrantly expressed genes through recruitment of repressor proteins

[14]. Thus, the balance of acetylation and methylation plays an important regulatory role in the transcription of a number of genes. Nonhistone proteins, including p53, p63, and GATA-1, are also influential substrates of HDACs [15-20]. HDAC inhibitors block proliferation of transformed cells in culture by inducing cell cycle arrest, differentiation, and/or apoptosis and inhibit tumor growth in animal models. Various mechanisms of actions are continuously being discovered. Approximately 2% of genes are functionally altered after exposure to HDAC inhibitors; some genes, like the cell cycle inhibitors *p21WAF1/CIP1*, *gelsolin, p27Kip, p16INK4a*, and *p15INK4b* are induced after exposure to HDAC inhibitors, whereas other genes, such as *cyclin D1* and *NFκB*, are repressed [21-32].

Valproic acid, a short-chain fatty acid that has been in clinical use for more than three decades for the therapy of seizures and bipolar disorder, also inhibits HDAC. At therapeutic levels, valproic acid directly inhibits class I and II HDACs (except HDAC6 and HDAC10), with resultant hyperacetylation of histones H3 and H4. After treatment with valproic acid, there is altered expression of multiple genes, including the cyclin-dependent kinase inhibitor *p21Cip1*, *glycogen synthase kinase-3ß,* and *peroxisome proliferator-activated receptors*, and down-regulation of the expression of the antiapoptotic *protein kinase C α and ε* isoforms [33-39]. Valproic acid has displayed potent *in vitro* and *in vivo* antitumor activities against neuroblastoma, glioma, leukemia, breast cancer, multiple myeloma, and prostate cancer lines [9,40-47]. Even though valproic acid is a potent teratogen in noncommitted cell lineages, it is otherwise usually well tolerated; in fact, it may even protect against neurotoxicity observed with some drugs. However, although it has been incidentally used in some patients with malignancies, to date, there are no reported trials of valproic acid alone or with other agents in a controlled clinical trial setting. *In vitro*, the cytotoxicity of valproic acid is potentiated by hydralazine, a noncytotoxic drug.

Clinical efforts to evaluate epigenetic modulation in solid tumors are in very early stages. Juergens et al. reported the outcome of a phase I-II trial in heavily pretreated patients (more than three lines of chemotherapy) with non–small cell lung cancer treated with a combination of the DNMT and HDAC inhibitors 5-azacytidine and entinostat, respectively, and noted a 35% clinical benefit rate, with two objective responses and ten subjects with disease stabilization [48]. As in most phase I trials, the current investigation was conducted in heavily pretreated patients with limited standard therapeutic options, and nonetheless, intriguing activity was seen. In smokers, the risk for development of non–small cell lung cancer is associated with a promoter methylation signature that is detectable in sputum [49]. Given the potential for synergistic epigenetic modulation between hydralazine and valproic acid, as well as the safety track record for long-term administration in nononcology patients, we conducted this trial to identify a dose appropriate for chronic administration for lung cancer chemoprevention.

The results of our trial support further investigation of epigenetic modification as a new therapeutic strategy. The combination of hydralazine and valproic acid is simple, nontoxic, and lends itself to chemoprevention or combination with other treatments. Future studies will need to be conducted with pharmacodynamic end points, such as the re-expression of defined panels of tumor suppressor genes as a function of therapy. Furthermore, if hydralazine is used, then study patients will need to be stratified by acetylator phenotype, as it is possible that toxicity, and even efficacy, may be determined by such phenotypic expression. Prospective trials will need to assess the role of epigenetic modification through newly discovered epigenetic mechanisms of action that could be used as biomarkers of efficacy.

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