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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