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ORIGINAL ARTICLE

Phase I study of 5-aza-2′-deoxycytidine in combination with valproic acid in non-small-cell lung cancer

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Abstract
Purpose Non-small-cell lung cancer (NSCLC) accounts for the majority of lung cancer and is the most common cause of cancer death in industrialized countries. Epigenetic modifications are observed universally during the tumorigenesis of lung cancer. The development of epigenetic-modulating agents utilizing the synergism between hypomethylating agents and histone deacetylase (HDAC) inhibitors provides a novel therapeutic approach in treating NSCLC.
Methods We performed a phase I trial combining 5-aza-2′-deoxycytidine (decitabine) and valproic acid (VPA), in patients with advanced stage NSCLC. Patients were treated with escalating doses of decitabine (5–15 mg/m^2) IV for 10 days in combination with VPA (10–20 mg/kg/day) PO on days 5–21 of a 28-day cycle. Pharmacokinetic and pharmacodynamic analysis included decitabine pharmacokinetics and fetal hemoglobin expression.
Results Eight patients were accrued to this phase I study. All patients had advanced NSCLC and had received prior chemotherapy. Eastern Cooperative Oncology Group performance status was 0–2. Major toxicities included myelosuppression and neurotoxicity. Dose-limiting toxicity was seen in two patients suffering grade 3 neurotoxicity during cycle one including disorientation, lethargy, memory loss, and ataxia at dose level 1. One patient had grade 3 neutropenia at the de-escalated dose. No objective response was observed, and stable disease was seen in one patient. Fetal hemoglobin levels increased after cycle one in all seven patients with evaluable results.
Conclusions We observed that decitabine and valproic acid are an effective combination in reactivating hypermethylated genes as demonstrated by re-expressing fetal hemoglobin. This combination in patients with advanced stage IV NSCLC, however, is limited by unacceptable neurological toxicity at a relatively low dosage. Combining hypomethylating agents with alternative HDAC inhibitors that lack the toxicity of VPA should be explored further.

Keywords Methylation · Histone acetylation · Epigenetics · Lung cancer

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Introduction

Lung cancer continues to be the leading cause of cancer-related deaths in the United States. Despite advancements in treatment options over the past decades, there has been little change in the 5-year disease-specific mortality. Non-small-cell lung cancer (NSCLC) represents the majority of

lung cancer (85 %) with surgery being the mainstay of treatment for early disease and the use of radiation and chemotherapy in more advanced settings. The standard treatments for stage IV NSCLC provide modest palliative benefits for the majority of patients [1, 2]. The recent development of targeted therapies has initiated a new era of antineoplastic therapy based upon a thorough understanding of the molecular events that lead to cancer [3]. Despite the impressive responses and durations of response, the benefits of targeted therapies are typically limited to subsets of populations [4]. In addition, the presence of the specific mutations does not always translate into response, and initial response inevitably succumbs to resistance [5].

Our understanding of epigenetic modifications in cancer cell biology has introduced a novel strategy in cancer treatments [6–8]. Epigenetics can be loosely defined as biologic processes that regulate gene expression without actual changes in gene sequences. It is accomplished by modification of the transcriptional microenvironment via DNA methylation in conjunction with dynamic posttranslational histone modifications. DNA methylation occurs in CpG islands near gene promoter regions. The cytosine residues of these CpG islands can be methylated and lead to subsequent transcriptional silencing. This process is initiated and maintained by several DNA methyltransferases (DNMTs). Methylation of CpG islands by DNMTs allows the formation of a silencing protein complex that physically inhibits proximal gene expression [9]. One group of proteins involved in the silencing complex are histone deacetylases (HDACs). By removing the acetyl group on the histone *N*-terminal lysine residues, HDACs promote a more condensed chromatin configuration and therefore suppress gene expression [10]. Conversely, if HDACs are inhibited, lysines at the histone tails tend to remain acetylated and confer a more open and accessible configuration.

5-Aza-2′-deoxycytidine (decitabine) is a nucleoside analog that inhibits DNMTs' ability to transfer methyl groups to hemimethylated DNA strands typically during replication, leading to DNA demethylation and ultimately upregulation of gene expression [11]. Its activity to inhibit DNA methylation has been observed initially in vitro, but subsequently in patients with hematological malignancies after low-dose exposure to decitabine. HDAC inhibitors, such as romidepsin, vorinostat, MGCD103, HMBA, and valproic acid, have also been found to be active epigenetic modulators in causing gene re-expression and inducing differentiation of transformed cell [12, 13]. A number of investigators have explored manipulation of the epigenetic mechanism through combinatorial approaches including the strategy of combining DNMT and HDAC inhibitors and have found that these agents can produce synergistic antineoplastic activity in vitro as seen in increased apoptotic cell death, increased gene re-expression, and alteration of histone acetylation [6, 14–16].

One of the potential agents to be used in the strategy of combining DNA methyltransferase inhibitors and HDAC inhibitors is valproic acid which is used in the treatment for simple and complex seizures. Valproic acid exhibits activity as an HDAC inhibitor at levels consistent with the therapeutic dose for seizure disorders and as a commonly used agent in the treatment for epilepsy has an extensive safety profile [17]. Investigations of HDAC inhibitors with myelodysplastic syndrome and cutaneous T cell lymphomas have shown promising results [9].

With the preclinical data showing promise in both solid and liquid tumors, and the wide applicability of epigenetic therapy in hematologic malignancies, we embarked upon a combination trial of DNMT and HDAC inhibitors in a common solid tumor [13, 18–20]. In this phase I trial, we explored dose, toxicity, biologic response, and preliminary clinical response of the combination of decitabine and valproic acid in patients with advanced stage NSCLC.

Patients and methods

Eligibility criteria and study design

This study enrolled patients (age ≥18 years) with relapsed histologically or cytologically confirmed non-small-cell lung cancer. Patients were required to have an ECOG performance status ≤2, adequate organ and marrow function: leukocytes >3,000, ANC >1,500, platelets >100,000, total bilirubin ≤1.5× institutional upper limit of normal (ULN), AST(SGOT)/ALT(SGPT) ≤2.5× institutional ULN, creatinine ≤1.5× institutional ULN (or calculated creatinine clearance >60 mL/min/1.73 m2 for patients with creatinine levels above 1.5× institutional normal). Informed written consent approved by The Ohio State University Human Studies Committee was obtained from all patients before study entry.

The primary objectives were to determine the safety and tolerability of decitabine and valproic acid and to recommend phase II dosing. Secondary objectives included determining the ability of decitabine and valproic acid to re-express methylated targets, analyze pharmacokinetic parameters, and provide preliminary evidence of antitumor activity.

Up to three prior chemotherapy treatments, including molecular targeted agents and cytotoxic agents, were allowed with at least 3-week lapse since last treatment and at least 6 weeks since prior nitrosoureas or mitomycin C. Prior radiation therapy and surgery were allowed, including

Table 1 Dose level

Dose level	Decitabine (mg/m^2) IV D1–10	Valproic acid (mg/kg/day) PO D5−21
Level −1	5	10
Level 0	5	15
Level 1	10	15
Level 2	10	20
Level 3	15	20

definitive or palliative therapy. Treatment was administered on an outpatient basis. A 3 + 3 phase I design was used, and patients were treated with escalating doses of 5-aza-CdR (5–15 mg/m^2) IV over 1 h for 10 days in combination with VPA (10–20 mg/kg/day) PO on days 5–21 of a 28-day cycle. Valproic acid was given by mouth starting day 5 through day 21. The dose was rounded to the closest 250 mg tablet size and administered three times per day (~8 h apart). The dose escalation scheme is shown in Table 1.

Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0. Dose-limiting toxicity was defined by occurrence of any of the following: grade 4 neutropenia lasting more than 7 days or accompanied by ≥grade 2 fever, grade 4 thrombocytopenia, grade 3 non-hematologic toxicity, with the exception of nausea and vomiting controllable with standard antiemetic therapy, or any grade 4 non-hematologic toxicity.

Response was assessed after at least 2 cycles of treatment. If clinical benefit was seen after 2 cycles, therapy continued every 4 weeks until progression, unacceptable toxicity, or withdrawal of consent.

Pharmacokinetic analysis

Decitabine pharmacokinetic (PK) analysis was performed on day 1 and day 10 of cycle one. The results were compiled, and AUC, Css, $t_{1/2}$ (half-life), and clearance were calculated for decitabine. Pharmacokinetic analysis was performed using nonlinear regression software program PCNONLIN (PCNONLIN, SCI Consultants, Apex NC) [4, 8, 17].

Correlative studies

Hemoglobin F determination

We assessed HbF levels on day one of each cycle and 4+ weeks after completion of treatment [18]. The assay performed was a standard clinical laboratory test.

Table 2 Patient demographics

Characteristics	*N* (%)
Age	
30–39	1 (13)
50–59	4 (50)
60–69	3 (37)
Gender	
Male	3 (38)
Female	5 (62)
Stage	
IV	8 (100)
Previous chemotherapy	
Yes	8 (100)
Performance status	
0	5 (62)
1	2 (25)
2	1 (13)
Race	
Black or African American	1 (13)
White	7 (87)

Results

Patient characteristics and treatment groups

Eight patients were enrolled in this study. Patient characteristics are shown in Table 2. Median age was 55 years with a range of 35–66. All had stage IV disease. Performance status ranged from ECOG of 0–2. All of the patients received previous chemotherapy before starting on this trial. The number of prior treatments ranged from 1 to 4 with medium of 2.2.

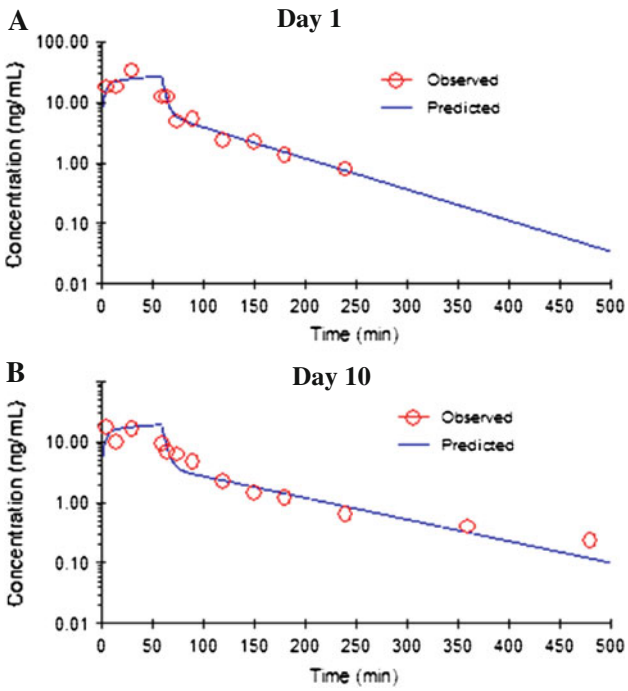
Toxicities

Of the eight patients, two were treated at dose level 0 (decitabine at 5 mg/m^2 daily × 10 and VPA 15 mg/kg/day in three divided doses), and both experienced grade 3 neurologic dose-limiting toxicity of disorientation, lethargy, somnolence, memory loss, and ataxia. Toxicity began on day 10 and day 14, respectively (day 5 and day 9 for VPA), and resolved within 24–48 h of discontinuation of valproic acid. Six patients were then treated at the de-escalated dose level −1 cohort (decitabine at 5 mg/m^2 combined with valproic acid at 10 mg/kg/day). Patients received one to seven cycles. One of six patients developed grade 4 neutropenia on day 2 of the second cycle at this dose level. Decitabine at 5 mg/m^2 and VPA 10 mg/kg/day was determined as the maximum tolerated dose (MTD). Toxicities are summarized in Table 3.

Table 3 Adverse effect

	Grade of adverse effect	
	3 = Severe	4 = Life threatening
Neurological		
Neuropathy	1	
Anxiety/depression	2	
Dizziness	2	
Ataxia	1	
Confusion	2	
Visual changes	1	
Constitutional		
Fatigue	1	
Weakness	3	
Pulmonary		
Effusion	2	1[a]
Dyspnea	1	

[a] Acute respiratory distress due to volume overload, atrial fibrillation, and a left-side malignant effusions that required intubation and chest tube placement



Assays	2 ng/mL
Css for 5 mg/m^2 (Day1)	0.14 +/- 0.05 μM
Css for 5 mg/m^2 (Day 10)	0.13 +/- 0.08 μM
AUC for 5 mg/m^2 (Day 1)	9.17 +/- 3.0 μM*min

Fig. 1 Pharmacokinetics of decitabine. **a** Plasma decitabine concentration versus time in a representative patient treated with decitabine 5 mg/m^2 intravenously over 1 h as a single agent. **b** Summary of PK parameters for decitabine

Correlative studies

The pharmacokinetics of decitabine is shown in Fig. 1. When comparing PK parameters between day 1 and day 10 for all patients, there was no meaningful difference in parameters evaluated. There was no significant difference in drug steady state and area under the curve between day 1 and day 10 for these patients, suggesting that the plasma level of decitabine does not accumulate. In addition, similar pharmacokinetic parameters were noted in AML patients reported by our colleagues, suggesting that there is no significant difference in terminal half-life (Tβ) and clearance (CL) of decitabine between NSCLC and AML patients [10].

Fetal hemoglobin expression

We examined the ability of decitabine and valproic acid to re-express fetal hemoglobin. Hemoglobin F or fetal hemoglobin is normally present in uterus and disappears shortly after birth. Decitabine treatment has been shown to be of some benefit in improving HbF levels in patients with hydroxyurea refractory sickle cell anemia [19]. After one cycle of treatment, all seven evaluable patients had increasing trends of HbF level (Fig. 2).

Discussion

DNA mutations found in tumor cells are drivers of tumor initiation and progression. With the understanding of epigenetics and the dynamic modulation of the nucleosome through DNA methylation, posttranslational histone modifications, and other changes, we have come to an understanding that cancer is a disease of epigenetic abnormalities as much as one of genetic alterations.

Several genes have been associated with altered methylation in lung cancer including putative tumor suppressor genes O^6-MGMT, $p16^{INK4A}$, and RASSF1A [8, 20]. Methylation of these genes, mediated by DNMTs, prevents recruitment of chromatin remodeling proteins including HDAC and therefore renders the region inaccessible to the transcriptional machinery leading to subsequent gene silencing. DNMT and HDAC have been studied as potential targets to reverse altered epigenetic programming with the development of several DNMT and HDAC inhibitors. By targeting both DNMT and HDAC, we and other groups have observed in vitro potentiating effects of gene reactivation, nucleosomal remodeling, and tumor suppression [18, 21]. The strategy utilizing the synergism of DNMT and HDAC inhibitors provides a novel approach in treating advanced stage NSCLC.

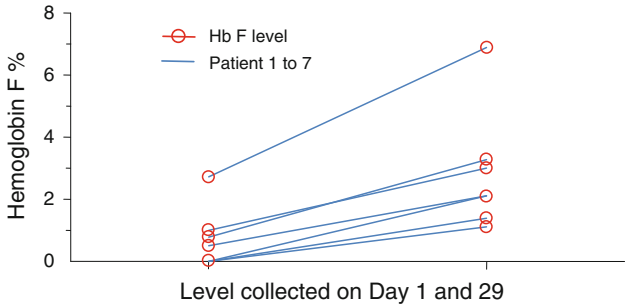


Fig. 2 Re-expression of fetal hemoglobin after treatment of decitabine and valproic acid using standard laboratory evaluation for Hb F. Seven evaluable patients showed increasing trends in Hb F level after cycle 1

The evaluation of combination therapy with decitabine and valproic acid was limited by the dose-limiting toxicity of neurological side effects likely related to valproic acid. These symptoms include memory loss, ataxia, somnolence, and confusion that resolved within 24–48 h of discontinuation. After dose reduction of the valproic acid, the maximum tolerated dose was determined at dose level −1 (decitabine 5 mg/m^2 IV combined with valproic acid 10 mg/kg/day). Only one patient remained on trial after cycle 4 with stable disease.

Similar neurologic toxicity has been seen in other trials utilizing valproic acid. While the study age group is comparable, we experienced a much lower dose tolerance in this trial [22, 23].

Epigenetic changes require prolonged exposure during S phase of the cell cycle [24]. Clinical experience has also shown that decitabine treatment in myelodysplastic disorder produced optimal effects with more prolonged, multicycle, low-level dosing below MTD in order to generate the theoretical exposure in S phase of the cell cycle and to elicit the expected clinical response [25]. Because of early disease progression and toxicity, the average on-study period of this trial was only 1.6 months. We suspect that the lack of response can be partially attributed to suboptimal duration of treatment as the majority of our patients received less than two cycles of treatments. Pharmacodynamic analysis did demonstrate an increase in HbF expression which is encouraging. Whether the combination of DNMT and HDAC inhibition was necessary for this effect is, however, unclear.

Another potential explanation for the lack of efficacy can be derived from an interesting observation in breast cancer cell lines when treated with an alternative schedule with HDAC and DMNT inhibitor combination. An unexpected antagonistic effect was observed in a breast carcinoma cell line subjected to concurrent exposure to HDAC and DMNT inhibitors; however, sequential administration (of DNMT inhibitor first, followed by HDAC inhibition) was able to reintroduce the expected synergistic effects [26]. This phenomenon was much less pronounced in cell lines whose cell cycle was not affected by HDAC inhibitor [27]. This may be due to the fact that the HDAC inhibitor blocks cell progression into S phase while DMNT inhibitors require DNA incorporation during S phase. Sequential scheduling with DNMT inhibitor pretreatment followed by administration of an HDAC inhibitor has been utilized in the treatment of patients with hematological malignancies [28]. Although sequential administration may be an attractive approach to avoid the toxicity associated with combination therapy and potentially to improve efficacy, this concept has not been proven in solid tumors and further examination is warranted. With the development of next-generation HDAC inhibitors such as AR42 or depsipeptide, future trials should explore alternative schedule using newer and more potent agents.

Trials investigating epigenetic agents in solid tumors have been generally disappointing [29]. HDAC inhibitors such as romidepsin and vorinostat have been studied in lung cancer with minimal clinical efficacy [15, 30]. Trials examining the synergistic effects of demethylating agents and HDAC inhibitors similarly have shown mixed results [21, 27–29]. Candelaria et al. [18] observed 25 % clinical response with the combination of hydralazine and valproic acid in solid tumors (among seventeen patients, two with NSCLC). Stathis et al. [31] showed that decitabine and vorinostat combination had no antitumor activity in patients with advanced solid tumors and non-Hodgkin's lymphomas.

The experience in myelodysplastic syndromes in which response is often more delayed, along with the theoretical requirement of cycling cells and DNA incorporation for DNMT inhibition to be effective, suggests that the conventional strategy of drug development for these agents may be the wrong place to apply these agents—that is, advanced, refractory disease following exhaustion of standard therapies. Given the abundant preclinical data, consideration of alternative clinical situations such as adjuvant or maintenance therapy (after achieving some level of response) may allow sufficient time for epigenetic therapy to have a more robust effect. To address this, investigators have initiated a study of adjuvant 5-azacytidine and entinostat in patients with resected stage 1 NSCLC (NCT01207726).

We conclude that the combination of decitabine and valproic acid is limited by toxicity, specifically by neurologic symptoms arising early, presumably related to valproic acid. The synergistic activity of combining a DNMT and a HDAC inhibitor seen in vitro remains an attractive strategy. Alternative combinations or schedules with improved toxicity should be explored in clinical settings including alternative settings such as adjuvant or maintenance epigenetic therapy.

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