Published in final edited form as:

Cancer Chemother Pharmacol. 2012 August ; 70(2): 251–258. doi:10.1007/s00280-012-1911-1.

Phase I Study of Bevacizumab, Everolimus, and Panobinostat (LBH-589) in Advanced Solid Tumors

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Abstract

Purpose—To define the maximum tolerated dose, clinical toxicities, and pharmacodynamics of bevacizumab, everolimus and panobinostat (LBH-589) when administered in combination in patients with advanced solid tumor malignancies.

Experiment Design—Subjects received 10 mg of panobinostat three times weekly, 5 or 10 mg everolimus daily and bevacizumab at 10 mg/kg every two weeks. Dose limiting toxicities (DLTs) were assessed in cycle 1; toxicity evaluation was closely monitored throughout treatment. Treatment continued until disease progression or undesirable toxicity. Protein acetylation was assessed in peripheral blood mononuclear cells (PBMC) both at baseline and on-treatment.

Results—Twelve subjects were evaluable for toxicity and nine subjects for response. DLTs in cohort 1 included grade 2 esophagitis and grade 3 oral mucositis; DLTs in cohort -1 were grade 2 ventricular arrhythmia and grade 2 intolerable skin rash. Common adverse events were diarrhea (50%), headache (33%), mucositis/stomatitis (25%), hyperlipidemia (25%), and thrombocytopenia (25%). There was 1 partial response; an additional 2 subjects had stable disease as best response. No consistent changes in protein acetylation in PBMC were observed in samples available from eight patients on treatment compared to baseline.

Conclusions—Bevacizumab, everolimus, and panobinostat in combination at the lowest proposed doses did not have an acceptable safety and tolerability profile, and did not consistently inhibit HDAC activity; therefore we do not recommend further evaluation.

Keywords

Bevacizumab; Everolimus; Panobinostat; Phase I; Advanced Cancer

Introduction

The role of angiogenesis in the pathogenesis and growth of solid tumors is well established [20, 28]. Tumor hypoxia is an important driver of angiogenesis, and hypoxia inducible factor-1 (HIF-1) is a central mediator of responses to hypoxia. HIF-1 promotes transcription of genes related vascular endothelial growth factor (VEGF), leading to endothelial cell recruitment and proliferation [32, 34]. HIF-1 expression is regulated at multiple levels, including the phosphatidylinositol 3-kinase-related kinase (PI3K) signaling pathway [29]. The mammalian target of rapamycin (mTOR) is a member of the PI3K

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signaling cascade, and modulates HIF-1 expression via mTOR-S6K-dependent translation. The mTOR inhibitor everolimus is FDA and EMEA approved for the treatment of renal cell carcinomas, pancreatic neuroendocrine tumors, and subependymal giant-cell astrocytomas associated with tuberous sclerosis [21, 38, 55]. Histone deacetylase inhibitors (HDACi) represent another class of pharmacological agents that regulate HIF-1 function in tumor cells. Mechanisms of HDACi-mediated regulation of HIF-1 include repression of HIF-1 DNA binding ability, destabilization of the active form of the protein, and inhibition of nuclear translocation of HIF-1 [18, 33, 36]. Direct HIF-1 inhibitors have been developed, but many of these inhibitors are either poorly selective for HIF-1, have had pharmacologic limitations, or have had unacceptable toxicity [39, 48, 49]. Indirect inhibition of HIF-1 by targeting mTOR and HDAC represents a novel approach to anti-angiogenesis therapy.

Vascular endothelial growth factor (VEGF-A or VEGF) is the ligand for VEGF receptor-1 (VEGFR-1) and VEGFR-2, and is an important mediator of angiogenesis [3, 6-8]. Bevacizumab is a monoclonal antibody against VEGF, and is approved by the United States Food and Drug Administration (FDA) and European Medicines Agency (EMA) for the treatment of multiple solid tumors [17, 30, 47, 54]. The safety and activity of bevacizumab in combination with everolimus has been established in several clinical studies [4, 10, 26, 27]. The most common toxicities for this combination included fatigue, rash, nausea, diarrhea, mucositis, hyperlipidemia, anemia, and thrombocytopenia. Grade 3 or 4 toxicities were uncommon, but included fatigue, hypertension, bleeding, proteinuria, and perforation events.

The HDACi panobinostat (LBH-589), a cinnamic hydroxamic acid analog, not only inhibits HIF-1, but also targets angiogenesis directly through the alteration of VEGF signaling [14, 35, 42, 43]. Several clinical trials have investigated panobinostat with conventional chemotherapy or with other targeted agents for the treatment of solid tumors [12, 16, 19, 22, 25, 31, 40, 45]. The combination of bevacizumab and panobinostat is safe and well-tolerated. In a phase I dose escalation study, grade 3 or 4 toxicities for the combination were rare, and included thrombocytopenia, venous thromboembolism, bleeding, and QTc prolongation [16]. In addition, the combination of everolimus and panobinostat is currently being investigated in patients with advanced renal cell cancer [2]. The tolerability and efficacy of bevacizumab, everolimus, and panobinostat in combination have not yet been evaluated.

The primary objective of this study was to define the maximal tolerated dose (MTD) and the recommended phase II dose (RPTD) for the combination of bevacizumab, everolimus, and panobinostat in subjects with advanced solid tumors. The secondary objectives were to investigate the pharmacodynamic characteristics of panobinostat and evaluate for signs of clinical activity. A prior study in prostate cancer assessed the pharmacodynamic properties of panobinostat by testing histone acetylation in peripheral blood mononuclear cells (PBMC) [45]. At doses under 20mg three days per week, panobinostat demonstrated incomplete inhibition of histone deacetylase. This study provides additional information regarding the pharmacodynamic properties of panobinostat in patients with advanced solid tumors.

Patients and Methods

Study Design

This was a dose-escalation phase I study to assess the triplet regimen of bevacizumab (Genentech, South San Francisco, CA, USA), everolimus and panobinostat (Novartis, East Hanover, NJ, USA) in patients with advanced solid tumors. A standard phase I “3 + 3” design was used to establish the MTD/ RPTD of the combination. The MTD was defined

around toxicities in the first 28-day cycle; the RPTD was selected based upon toxicities occurring in all cycles. The dose escalation schema is listed in Table 1.

A cycle was defined as 28 days. Treatment was continued until: disease progression, intercurrent illness that prevented further treatment, unacceptable toxicity, patient withdrawal from the study, or general or specific changes in the patient's condition that rendered further treatment inappropriate per judgment of the investigator or treating physician.

Patient Selection

Eligible patients were required to have a histologically confirmed solid malignancy refractory to standard therapy or for which standard therapies did not exist; subjects must have had at least one measurable site of disease according to Response Evaluation Criteria in Solid Tumors (RECIST) criteria (version 1.0) [52]. Additional eligibility requirements included: age ≥ 18 years; Karnofsky performance status (KPS) performance status $\geq 80\%$; previous radiation therapy, hormonal therapy, biologic therapy or chemotherapy for cancer permitted ≤ 4 weeks prior to study drug; surgery permitted ≤ 4 weeks prior to study drug. Adequate organ and marrow function was defined as: absolute neutrophil count (ANC) $\geq 1,500/\mu\text{L}$; platelets $\geq 100,000/\mu\text{L}$; hemoglobin ≥ 9 g/dL; total bilirubin ≤ 1.5 times the upper limit of normal (ULN); aspartate aminotransferase/alanine aminotransferase (AST/ALT) ≤ 2.5 times ULN or ≤ 5 times ULN if known hepatic metastases; fasting serum cholesterol ≤ 300 mg/dL; fasting blood sugar ≤ 160 mg/dL; creatinine clearance ≥ 50 mL/min/1.73 m²; left ventricular ejection fraction $\geq 50\%$; thyroid stimulating function and free T4 within institutional normal limits. Additional eligibility parameters included: absence of pregnancy; absence of central nervous system metastases, no clinically significant cardiovascular disease with intervention within six months; no history of HIV or hepatitis B or C seropositivity; no full dose anti-coagulation other than low molecular weight heparin; no invasion or encasement of a major artery; no history of abdominal fistula or gastrointestinal perforation within 6 months of study drug; no thrombosis, or bleeding diathesis; no significant vascular or peripheral vascular disease; no congestive heart failure or uncontrolled hypertension ($>150/100$ mmHg) despite supportive care. No intra-thoracic lung carcinoma of squamous cell histology or hypersensitivity/intolerance with bevacizumab, everolimus or panobinostat was permitted. CYP3A4 inhibitors and inducers and drugs with a risk of causing torsades de pointes were not permitted. Serious medical conditions that might have significantly affected patient safety or toxicity assessment were prohibited.

This was a single-center study (NCT 1055795) approved by the Duke Institutional Review Board (IRB) and followed the guidelines of the Helsinki Declaration. All patients provided informed written consent prior to any study-related procedure and were treated at Duke University Medical Center. Subject accrual took place from March 2010 to July 2011.

Clinical and Radiographic Assessment

All patients completed an extensive medical history, baseline physical examination and clinical assessment prior to receiving study drug. Toxicity and safety assessments were performed weekly during cycle 1, then every two weeks prior to treatment and as clinically indicated. These assessments included vital signs, KPS, medical history, physical examination including complete blood count (CBC), biochemistries including creatinine, AST, ALT, bilirubin, fasting lipid profile and serum beta-human chorionic gonadotropin for women of child bearing potential. Urine protein-creatinine ratio, thyroid stimulating hormone/free T4 and prothrombin time/ partial thromboplastin time/international normalized ratio (PT/PTT/INR) were assessed at baseline and every two cycles. During cycle 1, electrocardiograms (ECGs) were performed in triplicate at the following time

points: 30 minutes pre-dosing, then 1, 2, 4, 8 and 24 hours after dosing on days 1, 8 and 15; ECGs were performed weekly during cycles 2 and 3, then on day 1 of each subsequent cycle. Cardiac ejection fraction was assessed every six months. General symptom management and supportive care such as anti-diarrheal and anti-emetics agents were provided as clinically indicated to ensure optimal patient care.

Computed tomography (CT) scan or magnetic resonance imaging (MRI) were completed within four weeks prior to the start of therapy and every two cycles (8 weeks) using RECIST criteria (version 1.0).

Safety

The National Cancer Institute Common Toxicity Criteria (NCI-CTC; version 4.0) was used to grade adverse events. The following adverse events were considered DLT in cycle 1: any grade 4 neutropenia or grade 3 thrombocytopenia lasting over 4 days; nausea/vomiting or diarrhea grade 3 and lasting 4 days despite adequate supportive measures; febrile neutropenia where ANC <500/ μ l and temperature > 101°F; other non-hematologic toxicity grade 3 excluding anorexia and hypertension; anorexia and hypertension were considered DLT if they reached grade 4 or were considered unmanageable; any treatment-related death or hospitalization; receiving less than 85% of planned study medication due to toxicity. Patients were considered evaluable for toxicity if they received any treatment; patients were evaluable for DLT and MTD determinations if they completed cycle 1 or experienced a DLT in cycle 1; patients not evaluable for DLT and MTD were replaced.

HDAC activity

PBMC were collected by Ficoll-Paque PLUS (GE healthcare, Piscataway, NJ) 1 day prior to cycle 1 (day -1), cycle 1 day 15 prior to treatment, and cycle 3 day 1 prior to treatment. HDAC activity of PBMC whole cell protein extracts (50ug) was determined using the Fluor de Lys (Fluorogenic Histone Deacetylase Lysyl Substrate/Developer) fluorescent assay kit (Enzo Life Sciences International, Inc, Plymouth Meeting, PA). The assay was performed as recommended by the manufacturer. Fluorescence was measured using a fluorometric plate reader (Biotek Synergy HT), with an excitation wavelength of 360nm and emission wavelength of 460nm.

Results

Patient demographics are summarized in Table 2. A total of 12 patients were treated and evaluable for toxicity; 9 were evaluable for DLT and radiographic tumor response. The median age was 57 years (range 41-71). All patients had at least one prior chemotherapy treatment and/or radiation; the median number of prior treatments was three (range 1-11).

The dose escalation schema and corresponding DLTs are listed in Table 1. Dose findings were based on overall safety and tolerability of the investigational drug combination. Treatment-related DLTs in cohort 1 included grade 2 esophagitis (n=1) and grade 3 oral mucositis (n=1). One subject in cohort 1 voluntarily withdrew after 4 days of protocol treatment due to disease-related pain and anorexia. In cohort -1 (n=8), everolimus was dose reduced to 5 mg daily, however, two out of six evaluable subjects in this cohort developed treatment-related DLTs which resulted in protocol discontinuation: grade 2 ventricular arrhythmia (n=1) and grade 2 intolerable skin rash (n=1). Two subjects in cohort -1 were not evaluable due to intercurrent illness and clinical progression. Cohort -1 was the lowest cohort proposed for this drug combination; therefore study accrual was halted due to excess toxicity.

Treatment-related toxicities for all subjects are summarized in Table 3. Overall, the most common non-hematological adverse events of any grade were diarrhea (50%), headache (33%), oral mucositis (25%), anorexia (25%), skin rash (acneiform, maculo-papular) (25%) and hyperlipidemia (25%). Most adverse events were mild to moderate and resolved with supportive clinical care and protocol-specified dose holdings and reductions. For grade 3 adverse events, hypertension (17%), hyperglycemia (17%) and thrombocytopenia (17%) were most common. There were no treatment-related deaths.

Limited clinical activity was seen in this trial. 4 subjects demonstrated modest clinical benefit. One subject with breast cancer with 11 prior therapies developed a partial response (2 months in duration) and 3 additional subjects with metastatic colorectal cancer had stable disease as best response.

Eight patients were evaluable for peripheral blood assessment of HDAC activity. Seven of eight patients had PBMC samples drawn on day 15 of cycle 1. Among these seven patients, three exhibited decreases (< 70%) in HDAC activity, three exhibited increases (20%-82%) in HDAC activity, and one patient had HDAC activity that remained essentially unchanged. HDAC activity on day 15 of cycle 1 was not significantly different from baseline ($P=0.5781$) as revealed by Wilcoxon signed rank test (data not shown). Four of eight evaluable patients had PBMC samples drawn before treatment on day 1 of cycle 3. At this later time point, we observed a reduction in HDAC activity in three of the four patients examined. Two patients showed further decreases in HDAC activity compared with day 15 of cycle 1, while one patient's HDAC activity reverted back to baseline levels.

Discussion

The mechanisms of resistance to anti-VEGF therapy are not well understood. One proposed mechanism is compensatory upregulation of other pro-angiogenic signals [5]. HIF-1 is a transcription factor that has been implicated in preclinical models as a mediator of resistance to anti-angiogenic therapy [11, 13, 44, 53]. Attempts at developing direct HIF-1 inhibitors are currently ongoing [9, 48]. In this study, we attempted to combine the anti-VEGF agent bevacizumab with two indirect HIF-1 inhibitors, everolimus and panobinostat. In addition, panobinostat has been shown to directly inhibit expression of pro-angiogenic proteins VEGFR1, VEGFR2, and neuropilin-1 [14].

Although the safety of bevacizumab and everolimus in combination have been demonstrated in prior clinical trials [4, 10, 26, 27], the addition of panobinostat in this trial added toxicity which compromised the tolerability of the full combination. Several of the most common side effects in this trial, including diarrhea, headache, mucositis, anorexia, rash and hyperlipidemia, are observed with everolimus monotherapy [1]. Diarrhea and anorexia are also observed with panobinostat [41]. It is possible that overlapping toxicity between panobinostat and everolimus contributed to the number and severity of side effects. Alternatively, panobinostat may have altered cellular processes involved in response to mTOR inhibition, thereby increasing the toxicity of otherwise tolerable therapies. Most DLTs encountered in this study were due to the additive toxicity of panobinostat, everolimus and bevacizumab in combination. For example, a patient with recurrent non-cardiac chest and abdominal pain prior to study enrollment developed worsening pain on day 15 of cycle 1. Upper gastrointestinal endoscopy discovered esophagitis involving more than 75% of esophageal circumference (grade D), which necessitated discontinuation of all study drugs. Hematologic toxicity was also observed in this study, and is likely related to the combination of everolimus and panobinostat. Two patients (17%) experienced grade 3 or 4 thrombocytopenia. In both cases, platelets recovered promptly upon dose hold and modification of everolimus and dose hold and discontinuation of panobinostat.

Thrombocytopenia is a known side effect of everolimus and panobinostat, and in the case of panobinostat is thought to be mediated by transient maturational arrest of megakaryocyte development [24].

HDAC inhibitors as a class are associated with QTc prolongation and cardiac arrhythmias [41]. Panobinostat is also associated with QTc prolongation in prior clinical trials [15, 23, 37], although the severity of QTc prolongation may be mitigated with modifications to treatment dose and schedule [56]. Preclinical models suggest that HDAC inhibitors cause QTc prolongation by inhibiting the human ether-a-go-go related gene (hERG) potassium ion channel [46, 50, 51]. In this trial, a patient with a documented history of rare asymptomatic premature ventricular contractions (PVCs) was found to have asymptomatic ventricular bigeminy while receiving panobinostat, which resolved upon cessation of the drug. As a precaution the panobinostat was discontinued. No further cardiac conduction disturbances were noted. This patient's case illustrates the need to monitor cardiac conduction abnormalities closely when patients receive HDAC inhibitors.

In this study PBMC were collected to assess HDAC activity at multiple timepoints. At day 15 of cycle 1, four patients had HDAC activity that was higher than pretreatment levels, and 3 patients had HDAC activity decreases of 70%, 74%, and 82% respectively. There was no apparent relationship between HDAC inhibition and disease response, intensity of treatment, or treatment tolerability. Notably, a subject with a paradoxical 45% increase in HDAC activity on day 15 of cycle 1 experienced clinical benefit and remains on study with stable disease after 6 cycles.

The results of this study suggest that HDAC activity was not consistently inhibited in patients at the 10mg (3 days per week) dose level. In a separate study of oral panobinostat with docetaxel in patients with castration-resistant prostate cancer, increased histone acetylation was noted in 7 of 7 patients treated at the 20mg dose level, but only 2 of 4 patients treated at the 15mg dose level [45]. Findings from these studies support 20mg three times per week as the minimum dose level to ensure consistent HDAC inhibition.

Combination anti-angiogenesis strategies are of interest for patients with solid tumors, but limited evidence is available on the tolerability and efficacy of this approach. This clinical trial did not demonstrate acceptable safety and tolerability of bevacizumab, everolimus, and panobinostat in combination at the lowest proposed dose level. In this heavily pretreated patient population, treatment related side effects limited the ability to escalate doses of study drugs to efficacious levels. Additional investigation of lower doses is not recommended, as there is no evidence that panobinostat provides sustained and consistent HDAC inhibition at lower dose levels. Moreover, it is unknown whether everolimus provides clinical benefit at doses and schedules below 5mg daily. Inhibition of tumor angiogenesis through the targeting of VEGF and HIF-1 remains an attractive, although largely untested strategy.

Acknowledgments

We gratefully acknowledge the invaluable contributions of the patients and their families. We would also like to acknowledge the Duke University GI Oncology clinical trials team.

This research was supported by National Institute of Health Grant 5K24-CA113755-05 (H Hurwitz).

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Figure 1.
HDAC activity in patient PBMC whole cell protein extracts (50 μ g) at baseline, on day 15 of cycle 1, and day 1 of cycle 3. Each line represents a unique patient.

Table 1
Dose Escalation Schema and Dose Limiting Toxicity Events

Cohort Level	Bevacizumab IV, mg/kg	Everolimus oral, mg	panobinostat oral, mg	No. of Subjects	No. of Dose limiting toxicity events	Dose limiting toxicity
-1	10	5, daily	10, 3 times weekly	8*	2	Received <85% of study drug due to: <ul style="list-style-type: none">• Grade 2 ventricular arrhythmia• Grade 2 intolerable skin rash
1	10	10, daily	10, 3 times weekly	4*	2	Received <85% of study drug due to: <ul style="list-style-type: none">• Grade 2 esophagitis• Grade 3 oral mucositis
2	10	10, daily	20, 3 times weekly	0	0	
3	10	10, daily	30, 3 times weekly	0	0	

* For cohort 1, one subject was not evaluable due to subject withdrawal; for cohort -1, two subjects were not evaluable due to intercurrent illness and clinical progression

Table 2**Patient Characteristics**

Characteristics	No. of patients
Total Patients	12
Gender	
Male	6
Female	6
Age (Years)	
Median	57
Range	41-71
Karnofsky performance status, (%)	
Median	100
Range	80-100
Number of Prior Treatments	
0	0
1- 2	6
3	4
4	2
Primary Tumor Type	
Colorectal	9
Breast	2
GE junctional	1

Table 3**Treatment-related Adverse Events (Highest event per subject)**

Toxicity	N=12	
	Grade 1-2 (%)	Grade 3-4 (%)
Non-Hematological		
Nausea/Vomiting	1 (8)	0
Diarrhea	6 (50)	0
Anorexia	3 (25)	0
Fatigue	1 (8)	0
Weight loss	1 (8)	0
Skin rash (acneiform, maculo-papular)	3 (25)	0
Mucositis/stomatitis	3 (25)	1 (8)
Hypertension	2 (17)	2 (17)
Abdominal pain	1 (8)	0
Proteinuria	1 (8)	0
Headache	4 (33)	0
Esophageal ulcer	1 (8)	0
Peripheral edema	1 (8)	0
Peripheral sensory neuropathy	1 (8)	0
Ventricular arrhythmia	1 (8)	0
Hyperglycemia	2 (17)	2 (17)
Hyperhidrosis	1 (8)	0
Hypokalemia	1 (8)	0
Hypophosphatemia	0	1 (8)
Hypomagnesemia	1 (8)	0
Elevated creatinine	1 (8)	0
Hyperlipidemia	3 (25)	0
Hematological		
Thrombocytopenia	3 (25)	2 (17)
Neutropenia	2 (17)	1 (8)