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A Phase I Study of Bevacizumab, Everolimus, and Panitumumab in Advanced Solid Tumors

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Abstract

Purpose—Preclinical data suggests concurrent inhibition of VEGF, mTOR and EGFR pathways may augment anti-tumor and anti-angiogenic effects compared to inhibition of each pathway alone. This study evaluated the maximum tolerated dose /recommended phase II dose and safety and tolerability of bevacizumab, everolimus and panitumumab drug combination.

Methods—Subjects with advanced solid tumors received escalating doses of everolimus and flat dosing of panitumumab at 4.8 mg/kg 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.

Results—Thirty-two subjects were evaluable for toxicity; 31 subjects were evaluable for tumor response. DLTs were observed in cohorts with everolimus at 10 and 5 mg daily and included grade 3 mucositis, skin rash and thrombocytopenia. Therefore, everolimus was dose-reduced to 5 mg three times weekly which improved tolerability of the treatment regimen. Common adverse events were skin rash/pruritus (91%), mucositis/stomatitis (75%), hypomagnesemia (72%), hypocalcemia (56%) and hypokalemia (50%). There were 3 partial responses; an additional 10 subjects had stable disease ≥6 months. Three subjects with ovarian cancer and one with endometrial cancer achieved prolonged disease control ranging from 11 to >40 months.

Conclusions—The recommended phase II dose is everolimus at 5 mg three times weekly plus panitumumab at 4.8 mg/kg and bevacizumab at 10 mg/kg every two weeks. This dosing regimen has an acceptable safety and tolerability profile and appears to have moderate clinical activity in refractory tumors.

Keywords

Bevacizumab; Everolimus; Panitumumab; Phase I; Advanced Cancer

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Introduction

Targeted inhibition of multiple cellular pathways involved in tumor growth is a major focus of anticancer treatments. Vascular endothelial growth factor (VEGF), mammalian Target of Rapamycin (mTOR), and epidermal growth factor receptor (EGFR) and their respective signaling pathways, have each been clinically validated as single agent therapies [1-3]. However, preclinical data suggests that strategic combinations of therapies against these targets may enhance clinical activity compared to each agent alone [4-9].

All three targets involve intracellular signaling pathways that interact together to promote tumor growth, proliferation, angiogenesis and metastasis. The VEGF pathway is considered one of the crucial mediators of tumor angiogenesis [10]. VEGF signals through phosphatidylinositol 3-kinase (PI3K) and Akt as well as the extracellular regulated kinase (ERK 1/2), a mitogen-activated protein kinase (MAPK) [2, 11]. mTOR inhibition exhibits robust antiproliferative effects by causing G1 cell cycle arrest in rapidly dividing cells as well as mediating anti-angiogenesis activity [12-17]. EGFR activation triggers the RAS-RAF-MEK- MAPK and PI3K-Akt pathways which regulate expression of many cell proliferation and survival signals [18-20] . Moreover, EGFR inhibition has been shown to have down-regulatory effects on VEGF expression and angiogenesis [3].

When this study was designed, safety and efficacy data for VEGF plus EGFR inhibitors in different tumor types suggested this combination was effective and well-tolerated [21-25]. Furthermore, preliminary results from our phase I study of bevacizumab, a humanized monoclonal antibody targeting VEGF (10 mg/kg every two weeks) plus everolimus, a highly specific mTOR inhibitor (10 mg daily) confirmed tolerability of combined mTOR and VEGF inhibition [26]. Therefore, we hypothesized that the potent antiproliferative and antiangiogenic activity and tolerability of simultaneous VEGF, mTOR and EGFR inhibition would be well-tolerated and provide potentially useful clinical activity. For these reasons, we performed a phase I dose escalation study to determine the maximum tolerated dose (MTD) and recommended phase II dose (RPTD) and to preliminarily evaluate the clinical activity of bevacizumab, everolimus and EGFR inhibitor panitumumab (BEP) in advanced solid tumors. Biomarker studies, which include serial plasma sampling for angiogenic markers and skin biopsies, will be reported separately.

Patients and Methods

Study Design

This was a dose-escalation phase I and biomarker study to assess the triplet regimen of bevacizumab (Genentech, South San Francisco, CA, USA), everolimus (Novartis, East Hanover, NJ, USA) and panitumumab (Amgen, Thousand Oaks, CA, USA) in patients with advanced solid tumors. A standard phase I “3 + 3” design was used to establish the MTD/RPTD of the combinations [27]. The MTD was defined around toxicities in the first 28-day cycle; the RPTD was selected based upon toxicities occurring in all cycles. Once the MTD was determined, twenty additional patients were enrolled at MTD in an expanded cohort to ensure the tolerability of the study drug regimen. 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. Additional eligibility requirements included: age >18 years; Karnofsky performance status (KPS) performance status ≥70%; life expectancy >12 weeks; 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) >1,500/μl; platelets >100,000/μl; hemoglobin >9 g/dL; magnesium ≥1.2 mg/dL; calcium (corrected for albumin) ≥8.7 mg/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; urine protein to creatinine ratio (UPCR) < 1.0; creatinine clearance >50 mL/min/ 1.73 m^2. Additional eligibility parameters included: absence of pregnancy; absence of central nervous system metastases, no clinically significant cardiovascular disease with intervention within six months; no thrombosis or bleeding diathesis; no significant vascular or peripheral vascular disease; no uncontrolled hypertension (>150/100 mmHg) or any history of hypertensive crisis or hypertensive encephalopathy. No history of interstitial lung disease or hypersensitivity/intolerance with bevacizumab, panitumumab or everolimus was permitted. Serious medical conditions that might have significantly affected patient safety or toxicity assessment were prohibited.

This was a single-center study (NCT 00586443) 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 December 2007 to February 2010.

Patient Evaluation

All patients completed an extensive medical history, baseline physical examination and clinical assessment prior to receiving study drug. Toxicity and safety assessments were performed every two weeks prior to treatment and as clinically indicated. These assessments included vital signs, KPS, medical history, physical examination including neurosensory assessment, complete blood count (CBC), biochemistries including creatinine, AST, ALT, bilirubin, magnesium, UPCR and fasting lipid profile. An electrocardiogram, thyroid stimulating hormone and prothrombin time/ partial thromboplastin time/international normalized ratio (PT/PTT/INR) were performed at baseline and every two cycles. Cardiac ejection fraction was assessed every six months. Tumor response was assessed via computed tomography scan or magnetic resonance imaging every two cycles (8 weeks) using Response Evaluation Criteria in Solid Tumors (RECIST) criteria (version 1.0) [28]. General

symptom management and supportive care such as anti-diarrheal and anti-emetics agents were provided as clinically indicated to ensure optimal patient care.

Safety

The National Cancer Institute Common Toxicity Criteria (NCI CTCAE) version 3.0 was used to grade adverse events [29]. The following adverse events were considered DLT in cycle 1: hematologic toxicity ≥ grade 4 neutropenia or thrombocytopenia; nausea/vomiting or diarrhea ≥ grade 3 and lasting ≥ 4 days despite adequate supportive measures; hypertension ≥ grade 4; febrile neutropenia where ANC <500/μl and temperature > 101°F; other non-hematologic toxicity ≥ grade 3, excluding alopecia, anorexia, hyperbilirubinemia due to biliary obstruction or progressive disease, acne form rash, nail changes, erythema, pruritus, paronychia and ulcerations; 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.

Clinical and Radiographic Assessment

Baseline evaluations, including a complete history, physical examination, routine laboratories, and EKG were conducted within 14 days prior to start of protocol therapy. Labs included CBC, serum chemistries with blood urea nitrogen, creatinine, albumin, alkaline phosphatase, total bilirubin, bicarbonate, calcium, magnesium, chloride, glucose, phosphorus, potassium, sodium, total protein, lactate dehydrogenase, AST, ALT, PT/PTT/INR, fasting lipid panel, UPCR, and serum beta-human chorionic gonadotropin (for women of child bearing potential).

Radiologic scans were completed within four weeks prior to the start of therapy and every two cycles. Clinical activity was defined as complete response (CR), partial response (PR), minor response (<30% tumor decrease from baseline) (MR) or stable disease (SD) ≥6 months).

Results

Patient demographics are summarized in Table 2. A total of 32 patients were evaluable for toxicity; 31 were evaluable for radiographic tumor response. The median age was 51.5 years (range 23-76) and the median number of prior treatments was three (range 0-7).

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 (n=6) included grade 3 mucositis (n=2) and grade 3 thrombocytopenia (n=1). In cohort −1, everolimus was dose reduced to 5 mg daily, however, all three subjects in this cohort developed treatment-related DLTs: grade 3 mucositis (n=1), grade 3 mucositis and skin rash (n=1) and grade 3 skin rash and thrombocytopenia (n=1). One subject in this cohort withdrew during cycle 1 due to dose limiting mucositis and rash. In cohort −2, everolimus was further dose reduced to 5 mg three times weekly and had no DLTs. As a result, everolimus three times weekly, bevacizumab at

10 mg/kg and panitumumab at 4.8 mg/kg every two weeks was deemed the MTD and used in the expanded cohort, which was well-tolerated with an acceptable toxicity profile.

Treatment-related toxicities for all subjects are summarized in Table 3. Overall, the most common nonhematological adverse events of any grade were skin rash/pruritis (91%), mucositis/stomatitis (75%), hypomagnesemia (72%), hypocalcemia (55%) and hypokalemia (50%). 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, hypophosphatemia (19%), skin rash/pruritis (16%), hypokalemia (16%), hypertension (16%) and mucositis/stomatitis (13%) were most common. Grade 3 skin rash was seen across all dosing cohorts throughout during protocol therapy, whereas all grade ≥3 mucositis/ stomatitis only occurred in cohorts 1 and −1, primarily during cycle 1 and were associated with DLTs. Two subjects had recurrent grade 3 mucositis which resulted in additional dose holdings and modifications. No subjects developed >2 mucositis at the MTD. One subject discontinued protocol therapy for persistent grade 3 proteinuria after 13 cycles of treatment (Table 4). Bleeding events (34%) were limited to grade 1 or 2 epistaxis (n=9), grade 2 hematuria (n=1) and grade 1 rectal bleeding (n=1). There was only one grade 4 treatment-related event, asymptomatic hypophosphatemia. Of the 5 grade 3 hypokalemia events, 4 occurred in cohort 1. Other less common grade 3 toxicities included: hyponatremia (9%), fatigue (6%), diarrhea (3%), anorexia (3%), voice changes (3%), pancreatitis (3%), proteinuria (3%) paronychia (3%), hyperglycemia (3%), and hyperlipidemia (3%). There were no treatment-related deaths.

Most hematological toxicities were grade 1 or 2 and included neutropenia, thrombocytopenia and anemia, each occurring with similar frequency. There were no grade 4 hematologic events; all grade hematologic 3 events were seen in cohorts 1 and −1. One subject in the −1 cohort experienced recurrent grade 3 thrombocytopenia during prolonged therapy.

Efficacy

Table 4 summarizes clinical activity for this study population. Clinical activity was seen in 13 subjects (41%), 6 of whom had progressed on prior anti-VEGF or anti-EGFR therapy. Three subjects, all enrolled at MTD, demonstrated PRs with response durations ranging from 2 months to >18 months. These included a 61 year old woman with endometrial cancer with 2 prior therapies, a 64 year old woman with non-small cell lung cancer with 3 prior therapies including bevacizumab and erlotinib and a 53 year old man with rectal cancer with 1 prior therapy including bevacizumab. Two subjects, both with ovarian cancer, experienced a prolonged MR (each with approximately 26% reduction in tumor burden) for 16 and 11 months, respectively. Ten subjects experienced SD ≥6 months as best response, ranging from 6 months to >40 months; 9 subjects had SD < 6 months and 9 subjects had progressive disease as best response. There were no CRs.

Discussion

For the past decade, targeting specific molecular pathways has been the focus of drug discovery in oncology. However, tumor heterogeneity and potential crosstalk between

pathways which share common downstream effectors suggest single targeted therapies are less likely to sustain disease control. Therefore, simultaneous inhibition of multiple, interrelated signaling pathways which regulate tumor growth, progression and cell death may be more effective than inhibition of a single target. To test this rationale, we examined the safety, tolerability and MTD/RPTD of the antiangiogenic and antiproliferative triplet combination of bevacizumab, everolimus and panitumumab, targeting the VEGF, mTOR and EGFR pathways, respectively. Full doses of everolimus and bevacizumab and 4.8 mg/kg of panitumumab were not well-tolerated, resulting in two dose de-escalations of everolimus due to dose-limiting grade 3 mucositis, rash and thrombocytopenia. Early onset of grade 3 mucositis at 5 and 10 mg daily has been reported with other mTOR and anti-EGFR combinations and may suggest a potential pharmacodynamic or a possible, though less likely, pharmacokinetic interaction between everolimus and anti-EGFR agents [26]. Once everolimus was dose-reduced to 5 mg three times weekly, the frequency and severity of mucositis and thrombocytopenia was greatly decreased. Overall, this regimen was well-tolerated at the MTD with grades 1 to 2 gastro-intestinal and hematological toxicities. Expected class-related toxicities for bevacizumab, everolimus and panitumumab were consistent with each drug's prescribing information and were readily managed by early and aggressive dose hold/reductions and supportive clinical care.

In this heavily pretreated population, there was moderate clinical activity observed in a variety of tumor types and in subjects who had previously progressed on prior bevacizumab therapy including one subject with rectal cancer who was KRAS wild type (Table 4). Interestingly, one subject with NSCLC (adenocarcinoma) who progressed on bevacizumab and erlotinib therapies and who was positive for an EGFR deletion mutation in exon 19 (E746_A750del5) achieved a PR of 45% by month 4, which was sustained for an additional 10 months. This subject's response is remarkable as over 50% of NSCLC patients with mutations in exons 18-21 have good initial clinical response to EGFR inhibitors but eventually develop resistance within 12 months [30]. Therefore, despite developing tumor resistance to both VEGF and EGFR tyrosine kinase inhibition, disease control was maintained for over one year thus emphasizing the importance of how strategic combinations of targeted therapy with cross-talking pathways might restore tumor sensitivity.

Interestingly, four subjects with refractory gynecologic cancers, 1 with endometrial and 3 with ovarian cancer achieved prolonged disease control (PR and SD) ranging from 11 to >40 months with two subjects remaining on active therapy (Table 4). Preclinically, both tumor types have been shown to favorably respond to mTOR monotherapy or mTOR in combination with anti-VEGF agents [31, 32]. Given the significance of VEGF-regulated tumor angiogenesis and increased PI3K/AKT activity observed in almost half of studied ovarian tumor samples [33, 34], inhibition of these interrelated pathways appears biologically promising. Similarly, the loss of functional phosphatase and tensin homologue (PTEN) which occurs in 35 to 80% of endometrial tumors is associated with an increase in mTOR activation and increased angiogenesis [35-37]. Phase II trials are currently evaluating mTOR inhibition in combination with bevacizumab in a refractory setting for both of these tumor types [38, 39].

In conclusion, the MTD/RPTD for the BEP study drug combination is bevacizumab at 10 mg/kg, panitumumab at 4.8 mg/kg every two weeks and everolimus at 5 mg three times weekly. Daily doses of 5 and 10 mg of everolimus were intolerable due to unacceptable mucositis and thrombocytopenia. Prolonged clinical activity was seen in several tumor types which suggest that concurrent inhibition of the VEGF, EGFR and mTOR axes may provide clinically meaningful benefit for subjects with refractory tumors.

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Table 1

Dose Escalation Schema and Dose Limiting Toxicity Events

Cohort Level	Bevacizumab IV, mg/kg	Everolimus oral, mg	Panitumumab IV, mg/kg	No. of Subjects	No. of Dose limiting toxicity events	Dose limiting toxicity
−2*	10	5, three times weekly	4.8	3	0	
−1	10	5, daily	4.8	3	3	Grade 3 rash and thrombocytopenia Grade 3 mucositis Grade 3 mucositis and grade 3 rash
1	10	10, daily	4.8	6	3	Grade 3 mucositis (n=2) Grade 3 thrombocytopenia
Expanded*	10	5, three times weekly	4.8	20	−	

*MTD

Table 2

Patient Characteristics

Characteristics	No. of patients
Total Patients	32
Gender	
Male	12
Female	20
Age (Years)	
Median	51.5
Range	23-76
Karnofsky performance status, (%)	
Median	100
Range	80-100
Number of Prior Treatments	
0	1
1- 2	10
3	9
≥ 4	12
Primary Tumor Type	
Colorectal	10
Ovarian	4
Chondrosarcoma	3
Non-small cell lung	2
Other[a]	13

[a]Includes one patient each with the following: pancreatic, small bowel, leiomyosarcoma, small cell lung cancer, adrenal cortical carcinoma, Merkle cell, neuroendocrine, endometrial, transitional cell, squamous cell of head and neck, gastroesophageal, adenocarcinoma of unknown primary, liposarcoma.

Table 3

Treatment-related Adverse Events (highest event per subject)

	N=32	
Toxicity	**Grade 1-2 (%)**	**Grade 3-4 (%)***
Non-Hematological		
Nausea/Vomiting	10 (31)	0
Diarrhea	10 (31)	1 (3)
Constipation	2 (6)	0
Anorexia	12 (38)	0
Fatigue	8 (25)	2 (6)
Skin rash/pruritus	24 (75)	5 (16)
Mucositis/stomatitis (including enteritis, vaginitis)	20 (63)	4 (13)
Hypertension	3 (9)	5 (16)
Proteinuria	9 (28)	1 (3)
Headache	4 (13)	0
Bleeding/Epistaxis	11 (34)	0
Voice changes/hoarseness	8 (25)	1 (3)
Dry eyes	9 (28)	0
Dysgeusia	2 (6)	0
Paronychia	2 (6)	1 (3)
Pancreatitis	0	1 (3)
Heartburn/Indigestion	3 (9)	0
Metabolic		
Adrenal Insufficiency	1 (3)	0
Hyperglycemia	10 (31)	1 (3)
Hypokalemia	11 (34)	5 (16)
Hypomagnesemia	22 (69)	1 (3)
Hypophosphatemia	4 (13)	6 (19)
Hyponatremia	6 (19)	3 (9)
Hypocalcemia	18 (56)	0
Hypoalbuminemia	7 (22)	0
Elevated ALT/AST	8 (25)	0
Hyperlipidemia	10 (31)	1(3)
Hematological		
Anemia	9 (28)	1 (3)
Thrombocytopenia	10 (31)	2 (6)
Neutropenia	9 (28)	1 (3)

* There was only one grade 4 adverse event: asymptomatic hypophosphatemia.

Table 4

Characteristics of patients with clinical responses and prolonged disease control ≥6 months

Primary Tumor Type	Cohort	Prior therapies	Tumor biomarker	Response	Duration (months)
Endometrial	Expanded	Doxorubicin/Cisplatin/Taxol Taxol/Carboplatin		PR	>21 (stable disease for 6 months prior to PR)*
NSCLC	−2	Carboplatin/Taxol/Avastin Tarceva Tarceva/Alimta/Avastin	EGFR mutant	PR	10 (stable disease for 4 months prior to PR)
Rectal	Expanded	FOLFIRI/Avastin	KRAS wild type	PR	2 (stable disease for 10 months prior to PR)
Ovarian	Expanded	Carboplatin/Taxol Doxorubicin/Cisplatin Gemcitabine Taxol Doxil CEP11981		MR	16
Ovarian	Expanded	Taxol/Cisplatin Doxil XL880 Methylgene/Gemcitabine		MR	11
Ovarian	1	Carboplatin Gemcitabine/Doxil Taxol		SD	>40*
Unknown primary	Expanded	Carboplatin/Taxol Carboplatin/Etoposide		SD	13**
Small bowel	−1	XELOX/Avastin	KRAS mutant	SD	12
Leiomyosarcoma	1	Adriamycin/Ifosfamide Gemcitabine/Taxotere Dacarbazine Trabectedin AGO12736/Xeloda		SD	10
Pancreatic	1	Gemcitabine Gemcitabine/Tarceva Gemcitabine/Cetuximab/Dasatinib		SD	8
Chrondrosarcoma	−1	none		SD	8
Squamous cell of the head and neck	1	Cisplatin/Tarceva/Avastin Taxotere/Cisplatin/Cetuximab Xeloda Tarceva Tarceva/Avastin		SD	6

Primary Tumor Type	Cohort	Prior therapies	Tumor biomarker	Response	Duration (months)
Neuroendocrine	Expanded	Pazopanib		SD	6

FOLFIRI – 5FU, Leucovorin, Irinotecan

* Subject still active on protocol therapy

** Subject came off study due to toxicity

MR-minor response (<30% tumor decrease from baseline)

XELOX – Xeloda, Oxaliplatin