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A Phase I Trial of Bevacizumab and Temsirolimus in Combination With Valproic Acid in Advanced Solid Tumors

Blessie Elizabeth Nelson[1], **Apostolia M. Tsimberidou[1], Xueyao Fu[1], Siqing Fu[1], Vivek Subbiah[1],**
Anil K. Sood[2], Jordi Rodon[1], Daniel D. Karp[1], George Blumenschein[3], Scott Kopetz[4],
Shubham Pant[4], Sarina A. Piha-Paul[*,1,†,]

[1]Department of Investigational Cancer Therapeutics, University of Texas MD Anderson Cancer Center, Houston, TX, USA
[2]Department of Gynecologic Oncology and Reproductive Medicine, University of Texas MD Anderson Cancer Center, Houston, TX, USA
[3]Department of Thoracic and Head and Neck Medical Oncology, University of Texas, MD Anderson Cancer Center, Houston, TX, USA
[4]Department of Gastrointestinal Medical Oncology, University of Texas MD Anderson Cancer Center, Houston, TX, USA
*Corresponding author: Sarina A. Piha-Paul, MD, Department of Investigational Cancer Therapeutics, [A phase I Clinical Trials Program], Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA. Email: spihapau@mdanderson.org
†Principal Investigator: Sarina A. Piha-Paul

Abstract

Background: Preclinical models suggest synergy between anti-angiogenesis therapy, mammalian target of rapamycin (mTOR), and histone deacetylase inhibitors to promote anticancer activity.

Methods: This phase I study enrolled 47 patients between April 2012 and 2018 and determined safety, maximum tolerated dose (MTD), and dose-limiting toxicities (DLTs) when combining bevacizumab, temsirolimus, and valproic acid in patients with advanced cancer.

Results: Median age of enrolled patients was 56 years. Patients were heavily pretreated with a median of 4 lines of prior therapy. Forty-five patients (95.7%) experienced one or more treatment-related adverse events (TRAEs). Grade 3 TRAEs were lymphopenia (14.9%), thrombocytopenia (8.5%), and mucositis (6.4%). Grade 4 TRAEs included lymphopenia (2.1%) and CNS cerebrovascular ischemia (2.1%). Six patients developed DLTs across 10 dose levels with grade 3 infection, rash, mucositis, bowel perforation, elevated lipase, and grade 4 cerebrovascular ischemia. The MTD was dose level 9 (bevacizumab 5 mg/kg days 1 and 15 intravenously (IV) plus temsirolimus 25 mg days 1, 8, 15, and 22 IV and valproic acid 5 mg/kg on days 1-7 and 15-21 per orally (PO)). Objective response rate (ORR) was 7.9% with confirmed partial response (PRs) in 3 patients (one each in parotid gland, ovarian, and vaginal cancers). Stable disease (SD) ≥+6 months was seen in 5 patients (13.1%). Clinical benefit state (CBR: PR + SD ≥+6 months) was 21%.

Conclusion: Combination therapy with bevacizumab, temsirolimus, and valproic acid was feasible, but there were numerous toxicities, which will require careful management for future clinical development (ClinicalTrials.gov Identifier: NCT01552434).

Key words: bevacizumab; temsirolimus; HIF-1 alpha; vascular endothelial growth factor; histone deacetylase inhibitors; valproic acid; clinical trial;

Lessons Learned
- This trial highlights the importance of understanding the biological rationale with resistance to anticancer therapies and designing a trial where 3 well-known pathways promoting oncogenesis and resistance via VEGF, mTOR, and histone deacetylase was successfully implemented with astute therapy selection, dose escalation scheme, and manageable toxicities without overt overlap.
- Objective responses were seen in high grade mucoepidermoid carcinoma of parotid, endometrioid ovarian carcinoma, and vaginal squamous cell carcinoma. Durable stable responses >6 months were seen in uveal melanoma, breast cancer, leiomyosarcoma, clear cell carcinoma of the kidney, and metastatic thymoma.
- Prospective studies to elucidate this subset of patients with translational or biomarker-based studies are vital to harness the potential of this combination with bevacizumab and temsirolimus and valproic acid.

Discussion

This is the first study to evaluate the combination of bevacizumab, temsirolimus, and valproic acid in patients with advanced malignancies. This combination demonstrated modest efficacy among various solid tumors, but at the expense of toxicity. Treatment-related adverse events were seen in 45/47 (95.7%) of patients with 26/47 (55%) patients having grade 3/4 TRAEs. The maximum tolerated dose (MTD) was determined to be dose level 9 which was bevacizumab (5 mg/kg IV once every 14 days), temsirolimus (25 mg IV weekly),

and valproic acid (5 mg/kg PO every other week). Except for temsirolimus, the other two drugs in this combination therapy were dosed well below their label indication including bevacizumab at 50% and valproic acid at 17% of the FDA approved doses (Table 1).

In our study, objective responses were observed in three patients. The first patient had high-grade mucoepidermoid carcinoma of parotid with no actionable mutations and had confirmed PR (−62%) after 2 cycles of treatment with a 2.5-month duration of response. The second patient had endometrioid ovarian carcinoma with no actionable mutations and achieved confirmed PR (−50%) with a duration of response of 17 months. The third patient who achieved confirmed PR (−35%), had P16 positive vaginal squamous cell carcinoma with 8-month duration of response. In terms of prolonged stable disease (≥6 months), one patient with uveal melanoma with no actionable mutations had SD for 9 months. Another patient with PIK3CA and KRAS aberrated, hormone receptor positive (HR+), human epidermal growth factor receptor-2 (HER-2) negative breast cancer had stable disease for 11 months. A patient with PTEN and TP53 aberrated metastatic leiomyosarcoma had SD for 7 months while another patient with PTEN aberrated metastatic clear cell carcinoma of the kidney had SD for 8 months. The last patient had APC and NF1 aberrated metastatic thymoma had SD for 8 months.

In conclusion, the combination of bevacizumab, temsirolimus, and valproic acid showed modest clinical efficacy across an array of advanced sold tumors; however, numerous toxicities were reported, which would require careful monitoring and management during future clinical development.

Table 1. Dose-escalation schedule for bevacizumab/temsirolimus and valproic acid.

Dose level	Dose and schedule (28-day cycle)		
	Temsirolimus*	Bevacizumab	Valproic acid
Level -1	5 mg days 1, 8, 15, and 22	2.5 mg/kg day 1 and 15	5 mg/kg rounded to nearest 250 mg daily on days 1-7 and 15-21
Level 0	5 mg days 1, 8, 15, and 22	5 mg/kg day 1 and 15	5 mg/kg rounded to nearest 250 mg daily on days 1-7 and 15-21
Level 1	5 mg days 1, 8, 15, and 22	10 mg/kg day 1 and 15	5 mg/kg rounded to nearest 250 mg daily on days 1-7 and 15-21
Level 2	12.5 mg days 1, 8, 15, and 22	2.5 mg/kg day 1 and 15	5 mg/kg rounded to nearest 250mg daily on days 1-7 and 15-21
Level 3	12.5 mg days 1, 8, 15, and 22	7.5 mg/kg day 1 and 15	10 mg/kg rounded to nearest 250 mg daily on days 1-7 and 15-21
Level 4	12.5 mg days 1, 8, 15, and 22	10 mg/kg day 1 and 15	10 mg/kg rounded to nearest 250 mg daily on days 1-7 and 15-21
Level 5	20 mg days 1, 8, 15, and 22	2.5 mg/kg day 1 and 15	5 mg/kg rounded to nearest 250 mg daily on days 1-7 and 15-21
Level 6	20 mg days 1, 8, 15, and 22	7.5 mg/kg day 1 and 15	10 mg/kg rounded to nearest 250 mg daily on days 1-7 and 15-21
Level 7	20 mg days 1, 8, 15, and 22	10 mg/kg day 1 and 15	10 mg/kg rounded to nearest 250 mg daily on days 1-7 and 15-21
Level 8	25 mg days 1, 8, 15, and 22	2.5 mg/kg day 1 and 15	5 mg/kg rounded to nearest 250 mg daily on days 1-7 and 15-21
Level 9	25 mg days 1, 8, 15, and 22	5 mg/kg day 1 and 15	5 mg/kg rounded to nearest 250 mg daily on days 1-7 and 15-21
Level 10	25 mg days 1, 8, 15, and 22	10 mg/kg day 1 and 15	10 mg/kg rounded to nearest 250 mg daily on days 1-7 and 15-21

TRIAL INFORMATION	
Disease	All solid tumors
Stage of disease/treatment	Metastatic/advanced
Prior therapy	Allowed/no limit
Type of study	Phase I
Primary endpoint	safety and tolerability and to determine the maximum tolerated dose (MTD) of the combinational treatment of bevacizumab, and temsirolimus with valproic acid
Secondary endpoints	Anti-Tumor Efficacy
Investigator's analysis	Level of activity did not meet planned endpoint

Additional Details of Endpoints or Study Design

This was a phase I open-label, dose-escalation study that enrolled adult patients with advanced malignancies and was conducted between April 2012 and April 2018. The primary endpoint of this study was to access safety and tolerability and to determine the MTD of the combinational treatment of bevacizumab, and temsirolimus with valproic acid.

Patients were recruited and treated at the University of Texas, MD Anderson Cancer Center (MDACC). The study was approved by the Institutional Review Board (IRB) in accordance with the Declaration of Helsinki, Good Clinical Practice, and all federal, state and local regulatory guidelines. Consent was obtained from all patients prior to study enrollment. Each cycle was 28 days. Bevacizumab was administered at 2.5, 5, 7.5, or 10 mg/kg by IV infusion on days 1 and 15. Temsirolimus was given at 5, 12.5, 20, or 25 mg by IV infusion on days 1, 8, 15, and 22. Valproic acid was administered PO daily at a dose of either 5 mg/kg or 10 mg/kg rounded to nearest 250 mg on days 1-7 and 15-21. Re-staging scans were performed every 8 weeks to evaluate patient responses. During the study period, no other investigational, commercial agents or therapies were allowed with the intent to treat the patient's malignancy.

This protocol utilized a standard 3 + 3 dose escalation design.[1] Ten dose levels were explored between bevacizumab (IV) 10 mg/kg Q2W + temsirolimus (IV) 5 mg QW + valproic acid (PO) 5 mg/kg (days 1-7 and 15-21 of a 28-day cycle) and bevacizumab (IV) 10 mg/kg Q2W + temsirolimus 25 mg IV QW + valproic acid (PO) 10 mg/kg IV QD (days 1-7 and 15-21 of a 28-day cycle). Initially, three patients were enrolled to one dose cohort and were evaluated for toxicity. If one of these three patients experienced a dose-limiting toxicity (DLT) during the first cycle, three additional patients were enrolled and treated at the same cohort. If, at any time, more than 33% of patients in that cohort experienced DLT, the cohort was closed, and dose escalation stopped. In this study, adverse events were evaluated and graded per Common Terminology Criteria for Adverse Events v3.0 (CTCAE v3.0). A DLT event was defined as a clinically significant adverse event that occurred during the first cycle and was possibly, probably, or definitely related to any of the study medications; including any grade 3 or 4 non-hematologic toxicity (except nausea and vomiting responsive to appropriate regimens, correctable electrolyte imbalances, or alopecia); any grade 4 hematologic toxicity lasting > 14 days despite supportive care; any

grade 4 nausea of vomiting > 4 days despite maximum anti-nausea regiment; any other grade 3 non-hematologic toxicity including symptoms/signs of vascular leak or cytokine release syndrome; any severe or life-threatening complication or abnormality not defined in CTCAE v3.0 that was attributable to the therapy.

Key inclusion criteria were, advanced or metastatic cancer that was refractory to standard treatment, relapsed after standard treatment or had no standard treatment available; Eastern Cooperative Oncology Group (ECOG) performance $\leq$ 2; absolute neutrophil count $\geq 1 \times 10^9$/L; platelet count $\geq 50 \times 10^9$/L; creatinine $\leq 3 \times$ the upper limit of normal (ULN); total bilirubin $\leq$ 3.0 mg/dL; aspartate transaminase (AST) and alanine transaminase (ALT) $\leq 5 \times$ ULN; fasting level of total cholesterol $\leq$ 350 mg/dL; triglyceride level $\leq$ 400 mg/dL. Key exclusion criteria were clinically significant unexplained bleeding with 28 days prior to study entry; uncontrolled systemic vascular hypertension (systolic blood pressure > 140 mmHg, diastolic pressure > 90 mmHg on medication); clinically significant cardiovascular disease; history of hypersensitivity to bevacizumab, temsirolimus, or valproic acid; major surgery within 6 weeks of the study enrollment; pregnancy. Patients who had prior treatments with bevacizumab, temsirolimus, and/or valproic acid were allowed to participate.

Computed tomography or magnetic resonance imaging scans were performed at baseline and every two cycles (8 weeks) thereafter. Tumor measurements were performed according to Response Evaluation Criteria In Solid Tumors (RECIST) v1.0 to evaluate measurable target lesions for response.[2] Prolonged stable disease (SD) was defined as lasting $\geq$+ 6 months.

Genetic analysis was performed to analyze phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA), phosphatase and tensin homolog (PTEN), rapidly accelerated fibrosarcoma (RAF), rat sarcoma virus (RAS), and tumor protein p53 (TP53) aberrations for patients who had archival tissue samples available. Deoxyribonucleic acid (DNA) was extracted and purified from formalin-fixed, paraffin-embedded archival tumor tissue or blood samples. Polymerase chain reaction (PCR)-based primer extension assay, or next generation sequencing (NGS)-based analysis were used to screen for genetic mutations and copy number variations in target genes. All tests were performed in Clinical Laboratory Improvement Amendment (CLIA)-certified molecular diagnostics laboratories at MD Anderson Center and Foundation Medicine.

DRUG INFORMATION—DRUG 1

Generic/working name	Bevacizumab
Company name	Genentech
Drug type	Antibody
Drug class	Anti-angiogenic; Anti-VEGF-A
Dose	5
Unit	mg/kg
Route	IV
Schedule of administration	day 1 and 15 every 28 days

DRUG INFORMATION—DRUG 2

Generic/working name	Temsirolimus
Company name	Wyeth Pharmaceuticals, Inc.
Drug type	Small molecular inhibitor
Drug class	specific inhibitor of mTOR
Dose	5
Unit	mg
Route	IV
Schedule of administration	days 1, 8, 15, and 22 every 28 days

DRUG INFORMATION—DRUG 3

Generic/working name	Valproic acid
Company name	Various
Drug type	organic weak acid
Drug class	Anti-epileptic
Dose	5
Unit	mg/kg
Route	PO
Schedule of administration	Daily on days 1-7 and 15-21 every 28 days

PATIENT CHARACTERISTICS

Number of patients, male	22
Number of patients, female	25
Stage	IV
Age: median (range)	56 (21-78) years
Number of prior systemic therapies: median (range)	4 (0-9)
Performance status: ECOG	0: 2 1: 43 2: 2 3: 0 4: 0

CANCER TYPES OR HISTOLOGIC SUBTYPES

Cancer Types or Histologic Subtypes	Number
Adrenocortical	1
Bartholin's gland adenoid cystic carcinoma	1
Breast	4
Cervix	2
Colorectal	6
Endometrial	2
Esophageal	2
Glioblastoma	4
Kidney	1
Lung	1
Melanoma	2
Salivary gland	1

CANCER TYPES OR HISTOLOGIC SUBTYPES	NUMBER
Pituitary carcinoma	1
Parotid	1
Ovarian	4
Sarcoma	5
Squamous cell carcinoma head and neck	6
Squamous cell carcinoma of skin	1
Thymoma	1
Vaginal	1

PRIMARY ASSESSMENT METHOD	
Title	Objective response rate
Number of patients screened	69
Number of patients enrolled	47
Number of patients evaluable for toxicity	47
Number of patients evaluated for efficacy	38
Evaluation method	RECIST 1.0
Response assessment, CR	0 (0%)
Response assessment, PR	3 (7.9%)
Response assessment, SD	23 (60%)
Response assessment, PD	12 (31.5%)

Outcome Notes

Patient Demographic and Clinical Characteristics

Between April 2012 and April 2018, 47 patients with advanced, metastatic tumors were enrolled onto this study. Median age for enrolled patients was 56 years (range 21-78) and the majority were female (53%). The most frequent tumor types enrolled were Head and Neck Squamous Cell Carcinoma (HNSCC; 7 patients, 14.9%) and colorectal (6 patients, 12.8%), followed by breast (4 patients, 8.5%), glioblastoma (4 patients, 8.5%), and ovarian (4 patients, 8.5%). Patients were heavily pretreated with 28 patients (59.6%) having received at least four prior lines of therapy. Five patients (10.6%) had received prior mTOR inhibitors (1 patient received temsirolimus, 2 patients received everolimus, and 2 patients received MLN0128. Twenty patients (42.6%) had received prior bevacizumab therapy, 4 patients had prior pazopanib exposure, 3 patients had aflibercept previously, and one patient each had prior regorafenib, sorafenib, and axitinib therapy. No patients had received prior valproic acid-based therapies, but 2 patients had treatment with vorinostat and KA-2507 (histone deacetylase, HDAC6 inhibitor), respectively.

The median number of treatment cycles (cycle = 28 days) was 2 (range, 1-22). Twenty-one patients (44.7%) received at least 3 cycles of treatment. All patients have discontinued the treatment. The primary reasons for discontinuation were disease progression (22 patients, 46.8%), clinical progression (11 patients, 23.4%), toxicities (7 patients, 14.8%) death unrelated to therapy (3 patients, 6.3%), withdrew consent (2 patients; %), lost to follow up (1 patient; 2.3%%), and holding therapy for quality of life (1 patient; 2.3%).

Toxicity Assessment

Standard 3 + 3 dose-escalation design was followed to enroll patients in this study up to dose level 10. Table 2 summarizes numbers of patients treated and DLT events observed in each dose level. A total of six patients experienced DLTs. At dose level 2, one patient experienced a G3 elevated lipase. At dose level 5, one patient experienced G3 mucositis. At dose level 6, one patient with metastatic SCC of the tongue with no bowel issues experienced a G3 bowel perforation secondary to therapy. At dose level 10, two out of six patients experienced DLT events: one patient experienced a G3 infection, and the other patient experienced multiple adverse events (AEs) resulting in death (eg, 3 dizziness, G3 neuropathy, G3 weakness, G3 gait abnormality and G4 cerebrovascular ischemia). Per protocol, dose escalation was stopped at dose level 10 and 4 additional patients were enrolled to dose level 9. One of these 4 additional patients enrolled at dose level 9 experienced one G3 rash DLT event.

Forty-five patients (95.7%) experienced one or more adverse events that were at least possibly related to the treatment. Most AEs were grades 1 and 2 and were reversible. Table 3 summarizes treatment-related AEs (TRAEs) at all dose levels. The most common grades 1 and 2 TRAEs reported in more than 20% of patients were hypercholesterolemia (57.4%), hypertriglyceridemia (53.2%), thrombocytopenia (53.2%), hyperglycemia (38.3%), mucositis (38.3%), anorexia (36.2%), elevated AST (34%), leukopenia (34%), anemia (31.9), fatigue (29.8%), nausea (29.8%), rash (29.8%), headache (27.7%), diarrhea (23.4%), and proteinuria (23.4%). Grade 3 TRAEs included lymphopenia (14.9%, DL6, 7, 9, 10), thrombocytopenia (8.5%; DL1, 5, 6, 7), mucositis (6.4%, DL2, 5, 6), hypophosphatemia (4.3%; DL1 and 10), elevated lipase (4.3%, DL 2 and 6), hypertriglyceridemia (.3%, DL4 and 9), bowel perforation (4.3%, DL6 and 10), neutropenia (4.3%, DL6 and 9), anemia (2.1%, DL6), hypokalemia (2.1%, DL8), rash (2.1%, DL9), perirectal abscess (2.1%s, DL9), pericoronitis (2.1%, DL9), intestinal obstruction (2.1%, DL10), hyperglycemia (2.1%, DL10), infection (2.1%, DL10), dizziness (2.1%, DL10), neuropathy (2.1%, DL10), weakness (2.1%, DL10), and gait abnormality 2.1%, DL10). Grade 4 treatment-related AEs include lymphopenia

(2.1%, DL8), and cerebrovascular ischemia (2.1%, DL10). At dose level 10, one patient diagnosed with GBM experienced 5 DLT events during the first cycle of therapy (G3 dizziness, G3 neuropathy, G3 weakness, G3 gait-walking and G4 CNS cerebrovascular ischemia). She did not finish the first cycle and was taken off study due to toxicities.

Antitumor Activity

All 47 patients had disease that was measurable per RECIST v1.0 on baseline scans. However, 9 patients were taken off the study before completing cycle 2 and did not reach restaging assessment. Three patients developed clinical progression before reaching the first restaging scans, one patient withdrew consent, and another was lost to follow up. Four patients came off secondary to toxicity related to therapy (one patient each with G2 pneumonitis, G3 bowel micro-perforation, G3 perineal skin and soft tissue infection, and G4 cerebrovascular ischemia and gait abnormality). For the 38 patients who had at least one post-baseline restaging scan, the best RECIST response for each patient is depicted in Figure 1. In this waterfall plot, 7 patients were assigned a value of +21% for clinical progression or new lesions upon restaging. objective response rate (ORR) was 7.9% as confirmed partial response (PR) was observed in 3 patients. Stable disease (SD) ≥+ 6 months were observed in 5 patients (13.1%). Clinical Benefit Rate (CBR: CR+ PR + SD ≥+ 6 months) was 21% (8/38). Table 4 provides detailed information of patients with PR or SD ≥+ 6 months.

One patient with high grade mucoepidermoid carcinoma of parotid, treated at dose level 5 achieved PR (−62%). This patient had a 2.5-month duration of response. A second patient with endometrioid ovarian carcinoma, treated at dose level achieved PR (−50%) with a duration of response of 17 months. The third patient who achieved PR (−35%) had P16 positive vaginal squamous cell carcinoma and was treated at dose level 9. She had a duration of response of 8 months. This patient had PIK3CA E545K and I391M mutations.

The six patients who had SD ≥+ 6 months included one with ocular melanoma treated at dose level 2, one with breast cancer treated at dose level 3 who had PIK3CA E452K and KRAS G12S mutations, one with leiomyosarcoma treated at dose level 6 who had PTEN deletion, and TP53 V147fs*23 mutations, one with clear cell kidney cancer treated at dose level 9 who had PTEN I150fs*4 mutation, and one patient with thymoma treated at dose level 10. Please see Table 4 for additional details.

Molecular Analysis and Association with Response

For patients who had archival tissue samples available, genetic testing was performed to analyze PIK3CA, PTEN, RAF, RAS, and TP53 mutations. Table 5 is a summary of genetic analysis of patients tested with detailed molecular aberration, cancer type, and best response information.

Forty-three patients were tested for PIK3CA aberrations, and 10 patients were positive (10/43, 23.3%). The most commonly detected PIK3CA mutations were E545K (3/43, 7%) and I391M (3/43, 7%). Of the 3 patients detected with PIK3CA E545K mutation, one with vaginal cancer (#120, also had PIK3CA I391M mutation) had PR with 35% decrease and was treated for a total of 11 cycles. One patient with esophageal cancer (#111, also had KRAS G12D mutation) had SD for 6 cycles. One patient with breast cancer (#66, also had TP53 R196* mutation) was taken off study due to toxicities after 1 cycle of treatment.

Forty patients were tested for PTEN gene aberrations and 12 patients had molecular alterations (12/40, 30%) of which PTEN deletion (3/12, 25%) was the predominant alteration. For 3 patients with PTEN deletion, one patient with HNSCC (#99) was treated for 3 cycles with stable disease before withdrawing consent. One leiomyosarcoma patient (#107, also had TP53 V147fs*23 mutation) had SD for 7 cycles of treatment. Another leiomyosarcoma patient (#124) had SD for 4 cycles of treatment.

KRAS testing was performed on tumor samples from 43 patients, and 9 were positive for a KRAS aberration. The most commonly detected KRAS mutation was G12V (3/9, 33%) and G12S (2/9, 22.2%). All three patients with KRAS G12V mutated tumor had colorectal cancer. One patient (#34) had SD for 4 cycles of treatment. One patient (#131, also had concurrent PIK3CA I391M, PTEN S338fs*6, and TP53 R248Q mutations) had SD for 4 cycles of treatment. One patient (#133, also had PTEN splice site 254-2A>G and TP53 I232_N235del mutations) had clinical progression before finishing cycle 1 treatment. Of the two patients with KRAS G12S mutation, one breast cancer patient (#50, with concurrent PIK3CA E542K mutation) had SD > + 6 months. One patient with colorectal cancer (#122, also had TP53 R248W mutation) had clinical progression before finishing cycle 1 treatment. HRAS mutation was tested in 39 patients and 1 patient was positive (1/39, 2.6%). NRAS mutation was tested in 41 patients and 2 patients were positive (2/41, 4.9%).

ASSESSMENT, ANALYSIS, AND DISCUSSION	
Completion	Study completed
Investigator's Assessment	Level of activity did not meet planned endpoint

In this study, the most common non-hematologic adverse events (observed in ≥ 20% of patients) were anorexia, diarrhea, elevated AST, fatigue, headache, hypercholesterolemia, hyperglycemia, hypertriglyceridemia, mucositis, nausea, proteinuria, and cutaneous rash. Hyperglycemia and hyperlipidemia have been reported as common adverse events after temsirolimus treatment, affecting 17-26% and 6-27% treated patients, respectively.[3] In our study, hyperglycemia, hypercholesterolemia and hypertriglyceridemia were noted in 67%, 57% and 53% of patients, respectively. Dermatitis occurs in 47-75% of patients treated with temsirolimus in prior studies.[4] We observed dermatitis in 37% of our patients, which is lower than the reported incidence for single agent temsirolimus. Although, a phase II temsirolimus trial reported fatigue in 71% of their patients, in our study, fatigue was observed in only 30% of patients. Mucositis was also observed at a lower incidence (38%) compared to the 46% incidence rate reported in a phase 3 trial of single agent temsirolimus in renal cell carcinoma.[5] Proteinuria has been reported to occur in 32% of patients with ovarian cancer treated with single agent bevacizumab.[6] In our study, proteinuria was observed at a lower rate in our patients at 23% likely secondary to the lower dose of bevacizumab at 50% of FDA approved dosing.

The most common hematologic toxicities were leukopenia and neutropenia which occurred in 26 (55%) patients however most of these AEs were of lower grade (G1/G2). Only two patients had G3/G4 neutropenia which was reversible with dose hold. We also observed thrombocytopenia, lymphopenia, and anemia in 25 (53%), 17 (36%) and 16 (34%) patients, respectively. In another trial examining combination therapy with temsirolimus, bevacizumab and cetuximab, the incidence of thrombocytopenia was 24% which was lower than observed in our study. This is likely attributable to the addition of valproic acid to bevacizumab and temsirolimus as valproic acid has an incidence of thrombocytopenia between 1%-27%.[7] No patients developed thromboembolic complications.

Although there are no clinical data on the combination of bevacizumab, temsirolimus and valproic acid, preclinical studies have supported the rationale for combining these drugs. Brugarulos et al. found that Tuberous Sclerosis Complex-2 (TSC2) tumor suppressor protein regulates VEGF through mTOR-dependent and independent pathways.[8] TSC2 loss leads to increase in HIF-1α and upregulates HIF-responsive genes like VEGF. The authors were able to demonstrate that rapamycin homogenized HIF levels in TSC2–/– cells, indicating that TSC2 regulates HIF by inhibiting mTOR. VEGF overproduction by TSC2–/– cells was suppressed by the HDAC inhibitor agent, Trichostatin A indicating its anti-VEGF properties. This study demonstrated that a synergistic interplay and crosstalk occurred in inactivated TSC2 between rapamycin and trichostatin regulating the mTOR, HIF, VEGF and HDAC pathways.[8] However, it should be noted that there are emerging studies that demonstrated AKT/mTOR activation in tumor endothelial cells can contribute to antiangiogenic resistance by increasing the activity of mitogen-activated protein kinase (MAPK) and expression of the serine/threonine-protein kinase PIM-1which counteracts the anti-angiogenic efficacy of mTOR inhibitors.[9-11] Further characterization of the complex interplay between the AKT/mTOR and VEGF pathway is needed.

Clinically, the safety of bevacizumab and everolimus in combination has been demonstrated in prior clinical trials with anti-tumor activity in refractory colorectal cancer, melanoma and renal cell carcinoma.[12-14] Strickler et al. performed a phase I study of bevacizumab, everolimus, and panobinostat (LBH-589) in advanced solid tumors where patients received 10 mg of panobinostat three times weekly, 5 or 10 mg of everolimus daily, and bevacizumab at 10 mg/kg every 2 weeks.[15] However, the combination regimen did not have an acceptable safety and tolerability profile with 2 DLTs in DL1 (Grade 3 oral mucositis; G2 esophagitis) and 2 DLTs in DL-1 (G2 ventricular arrythmias; G2 refractory skin rash). Regarding responses seen in this study, one patient (1/9, 11%) with advanced breast cancer had PR for 2 months. In contrast, in our study, we were able to establish an MTD and objective responses were observed in three patients.

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Ethics Approval

The authors obtained MD Anderson Cancer Center Institutional Review Board approval and have followed the principles outlined in the Declaration of Helsinki for all human experimental investigations. For investigations involving human subjects, informed consent has been obtained from the participants involved.

Conflict of Interest

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Data Availability

The data underlying this article cannot be shared publicly for the privacy of individuals that participated in the study. The data will be shared on reasonable request to the corresponding author.

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X-axis: individual patient/disease site. Y-axis: the best RECIST response (%)
All 47 patients were measurable by RECIST
* 7 patients were assigned to a value of +21% for clinical progression or new lesions
9 patients not included due to non-evaluability for response
Dotted line shows -30% response by RECIST
Abbreviations: SCC: Squamous Cell Carcinoma; NSCLC: Non-Small Cell Lung Cancer

Figure 1. Waterfall plot depicting best RECIST response by patient. *X*-axis: individual patient/disease site. *Y*-axis: the best RECIST response (%). All 47 patients were measurable by RECIST * 7 patients were assigned to a value of +21% for clinical progression or new lesions. 9 patients not included due to non-evaluability for response dotted line shows −30% response by RECIST. Abbreviations: SCC: squamous cell carcinoma; NSCLC: non-small cell lung cancer.

Table 2. Dose level (28-day cycle), toxicities (G3/4),* and response.

Dose level	N	Temsirolimus IV on days 1, 8, 15, 22	Bevacizumab IV on days 1, 15	Valproic acid on days 1-7, and 15-21	SD≥6 months or PR/total treated	Grade (G) 3/4 Toxicity (N)*
1	3	5 mg	10 mg/kg	5 mg/kg rounded to nearest 250 mg	0/3	G3 Hypophosphatemia (1) G3 Thrombocytopenia (1)
2	6	12.5 mg	2.5 mg/kg	5 mg/kg rounded to nearest 250 mg	1/6	G3 **Elevated Lipase (1)^** G3 Mucositis (1)
3	4	12.5 mg	7.5 mg/kg	10 mg/kg rounded to nearest 250 mg	1/4	G3 Hypertriglyceridemia (1)
4	3	12.5 mg	10 mg/kg	10 mg/kg rounded to nearest 250 mg	0/3	G3 **Mucositis (1)^** G3 Thrombocytopenia (1)
5	6	20 mg	2.5 mg/kg	5 mg/kg rounded to nearest 250 mg	1/6	
6	6	20 mg	7.5 mg/kg	10 mg/kg rounded to nearest 250 mg	2/6	G3 Anemia (1) G3 **Bowel Perforation (1)^** G3 Mucositis (1) G3 Lymphopenia (2) G3 Elevated Lipase (1) G3 Thrombocytopenia (1) G3 Neutropenia (1)
7	3	20 mg	10 mg/kg	10 mg/kg rounded to nearest 250 mg	0/3	G3 Thrombocytopenia (1) G3 Lymphopenia (1)
8	3	2.5 mg	2.5 mg/kg	5 mg/kg rounded to nearest 250 mg	0/3	G3 Lymphopenia (1) G3 Hypokalemia (1) G4 Lymphopenia (1)
9	7	2.5 mg	5 mg/kg	5 mg/kg rounded to nearest 250 mg	2/7	G3 Lymphopenia (2) G3 Neutropenia (1) G3 Hypertriglyceridemia (1) G3 **Rash (1)^** G3 Perirectal Abscess (1) G3 Pericoronitis (1)
10	6	25 mg	10 mg/kg	10 mg/kg rounded to nearest 250 mg	1/6	G3 Hypophosphatemia (1) G3 Lymphopenia (1) G3 Intestinal Obstruction (1) G3 Bowel Perforation (1) G3 Hyperglycemia (1) G3 **Infection (1)^** G3 **Dizziness (1)^** G3 **Neuropathy (1)^** G3 **Weakness (1)^** G3 **Gait-walking (1)^** G4 **CNS Cerebrovascular Ischemia (1)^**

*Adverse events deemed at least possibly related to treatment, graded based on Common Terminology Criteria for Adverse Events, version 3.0 (CTCAEv3.0).
^A dose-limiting toxicity.
Abbreviation: N, number of patients experiencing toxicity.

Table 3. Adverse events at all dose levels.

Dose level, temsirolimus (mg) and bevacizumab (mg/kg) and valproic acid (mg/kg)

Adverse event of all grades / Dose level / Grade	1, (5/10/5) (n=3)		2, (12.5/2.5/5) (n=6)		3, (12.5/7.5/10) (n=4)		4, (12.5/10/10) (n=3)		5, (20/2.5/5) (n=6)		6, (20/7.5/10) (n=6)		7, (20/10/10) (n=3)		8, (25/2.5/5) (n=3)		9, (25/5/5) (n=7)		10, (25/10/10) (n=6)		Total (n=47)	
	1/2	3/4	1/2	3/4	1/2	3/4	1/2	3/4	1/2	3/4	1/2	3/4	1/2	3/4	1/2	3/4	1/2	3/4	1/2	3/4	1/2	3/4
Abdominal pain	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	2	0
Acne	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0
Anemia	1	0	3	0	3	0	1	0	3	0	1	1	1	0	2	0	0	0	0	0	15	1
Anorexia	0	0	4	0	2	0	0	0	3	0	3	0	0	0	0	0	3	0	2	0	17	0
Basophilia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
Bilateral lower extremity edema	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Bleeding (vaginal)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
Bowel perforation	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	2
Chest pain	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0
Cough	0	0	0	0	1	0	0	0	1	0	2	0	0	0	0	0	0	0	0	0	4	0
CNS cerebrovascular ischemia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
Confusion	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	2	0
Constipation	1	0	1	0	0	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	5	0
Cognitive disturbance	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0
Decreased PT/INR	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0
Dermatitis	0	0	0	0	0	0	0	0	1	0	2	0	0	0	0	0	0	0	0	0	3	0
Diarrhea	0	0	3	0	3	0	0	0	0	0	1	0	1	0	1	0	2	0	0	0	11	0
Diffuse gallbladder wall edema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0
Dizziness	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	2	1
Dry eye	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0
Dysgeusia	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	2	0
Dyspnea	1	0	0	0	1	0	1	0	0	0	1	0	0	0	1	0	0	0	1	0	6	0
Dysuria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
Edema	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	3	0
Elevated ALT	1	0	4	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	9	0
Elevated ALP	1	0	2	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	3	0	8	0
Elevated amylase	1	0	0	0	0	0	0	0	0	0	2	0		0	0	0	0	0	1	0	4	0
Elevated AST	3	0	3	0	3	0	1	0	2	0	1	0	1	0	1	0	0	0	1	0	16	0
Elevated bilirubin	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0
Elevated BUN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	3	0
Elevated Creatinine	2	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	8	0
Elevated D-dimer	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0
Elevated LDH	1	0	1	0	0	0	0	0	4	0	1	0	0	0	1	0	0	0	1	0	9	0
Elevated Lipase	0	1	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	2	0	4	2
Epistaxis	0	0	0	0	1	0	1	0	0	0	2	0	1	0	1	0	1	0	1	0	8	0

Table 3. Continued

Adverse event of all grades — Dose level, temsirolimus (mg) and bevacizumab (mg/kg) and valproic acid (mg/kg)

Adverse event / Dose level	1 (5/10/5) n=3 1/2	1 3/4	2 (12.5/2.5/5) n=6 1/2	2 3/4	3 (12.5/7.5/10) n=4 1/2	3 3/4	4 (12.5/10/10) n=3 1/2	4 3/4	5 (20/2.5/5) n=6 1/2	5 3/4	6 (20/7.5/10) n=6 1/2	6 3/4	7 (20/10/10) n=3 1/2	7 3/4	8 (25/2.5/5) n=3 1/2	8 3/4	9 (25/5/5) n=7 1/2	9 3/4	10 (25/10/10) n=6 1/2	10 3/4	Total n=47 1/2	Total 3/4
Facial numbness	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0
Fall	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
Fatigue	0	0	4	0	1	0	1	0	1	0	1	0	1	0	0	0	1	0	4	0	14	0
Fever	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	2	0
Fistula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
Gait-walking	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
Gallstone	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0
Glycosuria	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	2	0
Hand and foot syndrome	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0
Headache	0	0	2	0	1	0	1	0	3	0	1	0	0	0	0	0	5	0	0	0	13	0
Hematuria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	3	0
Hemoglobinuria	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0
Hemorrhage	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0
Hypercalcemia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
Hypercholesterolemia	3	0	4	0	3	0	2	0	3	0	4	0	2	0	1	0	1	0	4	0	27	0
Hyperglycemia	2	0	4	0	2	0	0	0	1	0	2	0	2	0	1	0	1	0	3	1	18	1
Hyperkalemia	1	0	0	0	1	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	5	0
Hypertension	0	0	0	0	2	0	2	0	0	0	1	0		0	0	0	0	0	1	0	6	0
Hypernatremia	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0	1	0	1	0	5	0
Hypertriglyceridemia	3	0	5	0	2	0	2	0	2	1	3	0	2	0	1	0	2	1	3	0	25	2
Hyperphosphatemia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0
Hypoalbuminemia	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	3	0
Hypocalcemia	1	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	4	0
Hypokalemia	1	0	2	0	3	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	8	1
Hypomagnesemia	1	0	3	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0
Hyponatremia	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	2	0	0	0	5	0
Hypophosphatemia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	2	2
Hypothermia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	2	0
Indigestion	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Infection	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1	2	1
Insomnia	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	0	2	0	5	0
Intestinal obstruction	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
Jaw pain	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0
Ketonuria	0	0	0	0	0	0	0	0	0	0	2	0	1	0	1	0	1	0	2	0	7	0

Table 3. Continued

Adverse event of all grades	Dose level, temsirolimus (mg) and bevacizumab (mg/kg) and valproic acid (mg/kg)																					
Dose level	1, (5/10/5) (n = 3)		2, (12.5/2.5/5) (n = 6)		3, (12.5/7.5/10) (n = 4)		4, (12.5/10/10) (n = 3)		5, (20/2.5/5) (n = 6)		6, (20/7.5/10) (n = 6)		7, (20/10/10) (n = 3)		8, (25/2.5/5) (n = 3)		9, (25/5/5) (n = 7)		10, (25/10/10) (n = 6)		Total (n = 47)	
Grade	1/2	3/4	1/2	3/4	1/2	3/4	1/2	3/4	1/2	3/4	1/2	3/4	1/2	3/4	1/2	3/4	1/2	3/4	1/2	3/4	1/2	3/4
Leukopenia	3	0	2	0	1	0	0	0	2	0	2	0	1	0	3	0	1	0	1	0	16	0
Lip infection (fever blister)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
Lymphopenia	0	0	1	0	0	0	0	0	0	0	2	2	0	1	2	2	0	2	4	1	9	8
Malaise	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0
Mucositis	0	0	1	0	1	0	3	0	3	1	1	1	1	0	2	0	3	1	3	0	18	3
Myositis	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0
Myalgia	0	0	1	0	0	0	1	0	1	0	0	0	2	0	0	0	1	0	0	0	6	0
Nausea	1	0	3	0	4	0	0	0	2	0	2	0	0	0	1	0	0	0	1	0	14	0
Neuropathy	1	0	1	0	2	0	2	0	0	0	1	0	0	0	0	0	0	0	1	1	8	1
Neutropenia	1	0	0	0	0	0	0	0	0	0	2	1	1	0	2	0	2	1	0	0	8	2
Neutrophilia	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2	0
Pain	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	3	0
Pericoronitis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	1
Perirectal abscess	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
Pneumonitis	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
Positive urine bacteria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
Positive urine bilirubin	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	2	0
Positive urine casts	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	2	0
Positive urine glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	2	0
Positive urine leuk. est.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	3	0
Positive urine mucous	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	2	0
Positive urine RBCs	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
Positive urine uric acid crystals	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
Positive urine WBCs	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	2	0
Positive urine urobilinogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0
Presyncope	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
Proteinuria	0	0	1	0	2	0	0	0	0	0	3	0	2	0	1	0	1	0	1	0	11	0
Pruritic scalp rash	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0
Pruritis	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	2	0
Radiation recall wound	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0
Rash	1	0	1	0	1	0	1	0	4	0	0	0	0	0	1	0	4	1	1	0	14	1
Sinusitis	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Tachycardia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0
Thinning of nails	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0

Table 3. Continued

Adverse event of all grades	Dose level, temsirolimus (mg) and bevacizumab (mg/kg) and valproic acid (mg/kg)																				
Dose level	1, (5/10/5) (n = 3)		2, (12.5/2.5/5) (n = 6)		3, (12.5/7.5/10) (n = 4)		4, (12.5/10/10) (n = 3)		5, (20/2.5/5) (n = 6)		6, (20/7.5/10) (n = 6)		7, (20/10/10) (n = 3)		8, (25/2.5/5) (n = 3)		9, (25/5/5) (n = 7)		10, (25/10/10) (n = 6)		Total (n = 47)	
Grade	1/2	3/4	1/2	3/4	1/2	3/4	1/2	3/4	1/2	3/4	1/2	3/4	1/2	3/4	1/2	3/4	1/2	3/4	1/2	3/4	1/2	3/4
Thrombocytopenia	0	1	3	0	3	0	0	0	4	0	5	1	2	1	3	0	2	0	3	0	25	4
Upper back pain	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
UTI	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Vision flashing lights, floater	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0
Vomiting	1	0	2	0	2	0	0	0	0	0	1	0	0	0	1	0	2	0	0	0	9	0
Wound (right foot)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
Weakness	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	2	1
Weight loss	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0	0	0	0	1	0	4	0
Xerostomia	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0

Table 4. Stable disease ≥ + 6 months or partial response (PR) by RECIST and characterization by patient.

Patient ID	Cancer type	Dose level	Best response by RECIST v1.0	Number of prior systemic regimens	Duration of treatment (weeks)	PTEN mut	PIK3CA mut	RAS mut	RAF mut	P53 mut
41	Ocular melanoma	2	SD (−3%)	4	36	ND	N	ND	ND	ND
50	HR+, HER2- Breast	3	SD (−9%)	9	43	N	E542K	KRAS G12S	N	N
73	Mucoepidermoid Parotid Cancer	5	PR (−62%)	1	19	N	N	N	N	N
101	Endometrioid ovarian carcinoma	6	PR (−50%)	6	97	N	N	N	N	N
107	Leiomyosarcoma	6	SD (−4%)	7	29	deletion	N	N	N	V147fs*23
120	Vaginal Squamous Cell carcinoma	9	PR (−35%)	8	50	N	E545K, I391M	N	N	N
138	Clear cell carcinoma of kidney	9	SD (−16%)	5	31	I1150fs*4	N	N	N	N
127	Thymoma	10	SD (−16%)	6	31	N	N	N	N	N

Abbreviations: mut, mutation; ND, not done; N, no; HR, hormone receptor; HER-2, human epidermal growth factor receptor 2.

Table 5. Tumor molecular analysis.

Gene	N/total tested (%)	Molecular aberration	Patient ID	Cancer type	Best response comments
PIK3CA	43/47	E542K	50	Breast	9% decrease
		E542K	114	Endocervical	3% increase with new lesion
		E545K	66	Breast	NE, off study for toxicities after completing cycle 1
		E545K	111	Esophageal	10% increase
		E545K, I391M	120	Vaginal SCC	35% decrease
		I391M	95	Leiomyosarcoma	NE, patient withdrew consent before restaging
		I391M	131	Colon	9% increase
		M1043T	96	Endometrial	27% increase
		H1047R	104	SCC of tongue	NE, patient did not finish cycle 1, patient was taken off study for G3 bowel perforation,
PTEN	40/47	K111_I112delinsN	122	Colon	Clinical progression
		Q110*	95	Leiomyosarcoma	NE, patient withdrew consent before restaging
		Deletion	99	SCC of tonsil	18% increase
		Deletion	107	Leiomyosarcoma	4% decrease
		L325P	108	GBM	27% increase
		R335*	112	GBM	66% increase
		Deletion	124	Leiomyosarcoma	4% decrease
		I50fs*4	138	Kidney	16% decrease
		K267N	139	GBM	Clinical progression
		S338fs*6	131	Colon	9% increase
		Rearrangement intron 4	132	SCC of oropharynx	26% decrease
		splice site 254-2A>G	133	Colon	NE, patient was taken off study due to clinical progression after 1 cycle of treatment
		R130*	134	GBM	NE, patient was taken off study due to toxicities, patient did not finish cycle 1 treatment
HRAS	39/47	G13R	113	SCC of Oropharynx	13% increase with new lesion
KRAS	43/47	G13D	7	Colon	13% increase
		KRAS AMP	14	Breast	1% decrease
		G12V	34	Colon	18% increase
		G12V	131	Colon	9% increase
		G12V	133	Colon	NE, patient was taken off study due to clinical progression after 1 cycle of treatment
		G12S	50	Breast	9% decrease
		Q61L	52	Colon	NE, patient was taken off study due to clinical progression, patient did not finish cycle 1 treatment
		G12D	111	Esophageal	10% increase
		G12S	122	Colon	Clinical progression

Table 5. Continued

Gene	N/total tested (%)	Molecular aberration	Patient ID	Cancer type	Best response comments
NRAS	41/47	Q61R	64	Melanoma	7% increase with new lesion
		G61K	113	SCC of Oropharynx	13% increase with new lesion
TP53	40/47	R175H	14	Breast	1% decrease
		R175H	96	Endometrial	27% increase
		R248W	33	SCC of Larynx	23% decrease
		F270C	34	Colon	18% increase
		R196*	66	Breast	NE, off study for toxicities after completing cycle 1
		G245D	67	Ovarian	7% decrease
		E11Q, R342*	72	SCC of Esophagus	14% increase
		E258K	104	SCC of tongue	NE, patient did not finish cycle 1, patient was taken off study for G3 bowel perforation,
		V147fs*23	107	Leiomyosarcoma	4% decrease
		L111P	109	Ovarian	3% increase
		R240W	112	GBM	66% increase
		H179Y	116	SCC of skin	11% decrease
		R248W	122	Colon	Clinical progression
		S121fs*11	147	Ovarian	NE, patient was taken off study due to newly discovered brain metastases, patient did not finish cycle 1 treatment
		I232_N235del	133	Colon	NE, patient was taken off study due to clinical progression after 1 cycle of treatment
		V274fs	151	Endometrium	20% increase
		R248Q	131	Colon	9% increase

Abbreviations: NE, no response evaluation; GBM, Glioblastoma Multiforme; SCC, squamous cell carcinoma; AMP, amplification.