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PIK3CA Mutations in Patients with Advanced Cancers Treated with PI3K/AKT/mTOR Axis Inhibitors

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Abstract

Preclinical data suggest that *PIK3CA* mutations predict response to PI3K/AKT/mTOR inhibitors. Concomitant *KRAS* or *BRAF* mutations may mediate resistance. Therefore tumors from patients referred to the Phase I Program for targeted therapy starting in October 2008 were analyzed for *PIK3CA* mutations using PCR-based DNA sequencing of exons 9 and 20. Consecutive patients with diverse tumor types and *PIK3CA* mutations were treated whenever possible with agents targeting the PI3K/AKT/mTOR pathway. Overall, *PIK3CA* mutations were detected in 25 of 217 patients (11.5%) (exon 9, n=11; exon 20, n=14). In tumor types with >10 patients tested, *PIK3CA* mutations were most frequent in endometrial (3/14, 21%), ovarian (5/30, 17%), colorectal (9/54, 17%), breast (2/14, 14%), cervical (2/15, 13%), and squamous cell cancer of head and neck (1/11, 9%). Seventeen of the 25 patients (68%) with *PIK3CA* mutations were treated on a protocol that included a PI3K/AKT/mTOR pathway inhibitor, and 6 (35%) achieved a partial response. In contrast, only 15 of 241 patients (6%) without documented *PIK3CA* mutations treated on the same protocols responded (p=0.001). Six of the 17 (35%) patients with *PIK3CA* mutations had simultaneous *KRAS* or *BRAF* mutations (colorectal, n=4; ovarian, n=2). Colorectal cancer patients with *PIK3CA* and *KRAS* mutations did not respond to therapy, while both ovarian cancer patients with *PIK3CA* and *KRAS* or *BRAF* mutations did. In conclusion, *PIK3CA* mutations were detected in 11.5% of patients with diverse solid tumors. The response rate was significantly higher for patients with *PIK3CA* mutations treated with PI3K/AKT/mTOR pathway inhibitors than for those without documented mutations.

Keywords

PIK3CA mutation; *RAS* mutation; *RAF* mutation; Cancer; Clinical trial

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Introduction

Recently, major therapeutic advances have been made in tumors with druggable targets (1-4). These include the highly successful use of KIT kinase inhibitors in *KIT* mutation-positive gastrointestinal stromal tumors (GIST), ABL kinase inhibitors in *BCR-ABL*-positive chronic myelogenous leukemia (CML), and *BRAF* inhibitors in *BRAF* mutation-positive melanoma (1,2,4). Common solid tumors, such as breast, lung, and colorectal cancer remain difficult to treat, perhaps in part because they are heterogeneous, with each subset of patients having different molecular abnormalities (3). Identifying relevant molecular subtypes within heterogeneous diseases, and matching patients with appropriate targeted agents or combinations of them is crucial to future therapeutic progress (5).

The phosphatidylinositol 3-kinase (PI3K)/AKT/mTOR signaling pathway is activated in many different cancers (Supplementary Figure 1) (6). Activation is frequently mediated by mutations in the p110α subunit of *PI3K* called *PIK3CA*, with most mutations (>80%) occurring either in exon 9, which codes for the helical domain, or exon 20, which codes for the kinase domain (Supplementary Figure 2) (7). Preclinical studies suggested that *PIK3CA* mutations may predict for response to PI3K inhibitors (8).

We investigated the *PIK3CA* mutation status of patients referred to the Phase I Clinical Trials Program clinic (known as the Clinical Center for Targeted Therapy). Whenever possible, patients with *PIK3CA* mutations were offered treatment targeting the PI3K/AKT/mTOR pathway and their clinical outcomes were analyzed.

Patients and Methods

Patients

We investigated the *PIK3CA* mutation status of patients with advanced tumors and available tissue referred to the Department of Investigational Cancer Therapeutics (Phase I Clinical Trials Program) at The University of Texas M. D. Anderson Cancer Center (M. D. Anderson) starting in October 2008. The registration of patients in the database, pathology assessment, and mutation analysis were performed at M. D. Anderson. Eligible patients were those referred for clinical trials of targeted therapeutic agents. The study and all treatments were conducted in accordance with the guidelines of the M. D. Anderson Institutional Review Board.

Tissue samples and mutation analyses

PIK3CA mutations were investigated in archival formalin-fixed, paraffin-embedded tissue blocks or material from fine needle aspiration biopsy obtained from diagnostic and/or therapeutic procedures. All histologies were centrally reviewed at M. D. Anderson. *PIK3CA* mutation testing was performed in the Clinical Laboratory Improvement Amendment–certified Molecular Diagnostic Laboratory (MDL) within the Division of Pathology and Laboratory Medicine at M. D. Anderson. DNA was extracted from microdissected paraffin-embedded tumor sections and analyzed using a polymerase chain reaction (PCR)-based DNA sequencing method for *PIK3CA* mutations in codons [c]532-554 of exon 9 (helical domain) and c1011-1062 of exon 20 (kinase domain), which included the mutation hot spot region of the *PIK3CA* proto-oncogene by Sanger sequencing following amplification of 276 bp and 198 bp amplicons, respectively, utilizing primers designed by the M.D. Anderson MDL. Whenever possible, in addition to *PIK3CA*, mutation analysis was done for *KRAS* and *NRAS* c12, c13, and c61 mutations of exon 2; and *BRAF* codon 595-600 mutations of exon 15 by pyrosequencing as previously described (9).

Treatment and evaluation

Consecutive patients with underlying *PIK3CA* mutations were enrolled whenever possible in clinical trials containing inhibitors of the PI3K/AKT/mTOR pathway, particularly protocols with anti-mTORC1 (rapalog)-based regimens or regimens containing PI3K inhibitors. Treatment continued until disease progression or unacceptable toxicity occurred.

Treatment was carried out according to the specific requisites in the treatment protocols selected.

Assessments, including history, physical examination, and laboratory evaluations, were performed as specified in each protocol, typically before the initiation of therapy, weekly during the first cycle, and then, at a minimum, at the beginning of each new treatment cycle. Efficacy was assessed from computed tomography (CT) scans and/or magnetic resonance imaging (MRI) at baseline before treatment initiation and then every 2 cycles (6-8 weeks). All radiographs were read in the Department of Radiology at M. D. Anderson and reviewed in the Department of Investigational Cancer Therapeutics tumor measurement clinic. Responses were categorized per RECIST 1.0 criteria and were reported as best response (10). In brief, complete response (CR) was defined as the disappearance of all measurable and non-measurable disease; partial response (PR) was defined as at least a 30% decrease in the sum of the longest diameter of measurable target lesions; progressive disease (PD) was defined as at least a 20% increase in the sum of the longest diameter of measurable target lesions, or unequivocal progression of a non-target lesion, or the appearance of a new lesion; and stable disease (SD) was defined as neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progressive disease.

Statistical analysis

Statistical analysis was verified by our statistician (XW). Fisher's exact test was used to assess the association among categorical variables and *PIK3CA* mutation status. The Wilcoxon rank-sum test assessed the association between age and *PIK3CA* mutation status. Time to progression (TTP) was defined as the time interval from the start of therapy to the first observation of disease progression or death, whichever occurred first. All tests were two-sided, and a P value less than 0.05 was considered statistically significant. All statistical analyses were carried out using SAS 9.1 software (SAS Institute, Cary, NC).

Results

Patients

A total of 217 patients with advanced tumors were analyzed for the presence of *PIK3CA* mutations. One-hundred-and-thirty-one (60%) patients were women and 86 (40%) were men (Table 1). The median age was 56 years (range 13 to 91 years). One-hundred-and-seventy-six (81%) were Caucasians, 19 (9%) African Americans, 12 (6%) Hispanic, and 10 (4%) Asians. Fifty-four (25%) had colorectal cancer, 30 (14%) ovarian cancer, 18 (8%) melanoma, 14 (6%) breast cancer, 14 (6%) endometrial cancer, 8 (4%) squamous cell cervical cancer, 7 (3%) cervical adenocarcinoma, 8 (4%) soft tissue sarcoma (excluding GIST), and 11 (5%) squamous cell cancer of head and neck. A variety of other tumors made up the rest of the patients (Table 1).

PIK3CA mutations

PIK3CA proto-oncogene mutations were detected in 25 of the 217 patients (11.5%). In 11 patients, a mutation in exon 9 was detected: 8 in c545, 1 in c542, 1 in c546, and 1 in c545/c549. Exon 20 mutations were found in the 14 remaining individuals: 10 in c1047, 3 in c1049, and 1 in c1043 (Table 2). In tumor types with more than three patients tested,

PIK3CA mutations were most frequent in endometrial cancer (3 of 14 patients [21%]). Mutations were also present in 5 of 30 patients (17%) with ovarian cancer, in 9 of 54 patients (17%) with colorectal cancer, in 2 of 14 patients (14%) with breast cancer, in 2 of 15 patients (13%) with cervical cancer (both of whom had squamous histology), in 1 of 7 patients (14%) with non-small cell lung cancer, and in 1 of 11 patients (9%) with squamous cell cancer of head and neck (Table 1). *PIK3CA* mutation status was not significantly associated with age, gender, or race (Fisher's exact test).

Simultaneous RAS and PIK3CA mutations

Preclinical data suggest that activation of RAS mediates resistance to PI3K inhibitors (8). Therefore, *RAS* mutation status was investigated whenever possible.

KRAS mutations in exon 2 were assessed in 130 patients and identified in 33 individuals (25%) (Table 3). The presence of *KRAS* mutations was significantly associated with *PIK3CA* mutations (p=0.03; Fisher's exact test). Indeed, forty-five percent (9 of 20) of patients with a *PIK3CA* mutation (who were also tested for a *KRAS* mutation) also had a *KRAS* mutation, whereas only 22% of patients (24 of 110) without a *PIK3CA* mutation (who were also tested for *KRAS*) harbored a *KRAS* mutation. Of the 33 patients with *KRAS* mutations, 9 (27%) had simultaneous *PIK3CA* mutations. In contrast, of the 97 patients without a *KRAS* mutation, only 11 (11%) had a *PIK3CA* mutation (p = 0.03). Of the nine patients with simultaneous *PIK3CA* and *KRAS* mutations, disease distribution was as follows: colorectal cancer, n=7; pancreatic cancer, n=1; ovarian cancer, n=1 (Table 2).

NRAS c61 mutations were detected in 2 patients (3%) of 62 tested. Both patients had melanoma with wild-type *PIK3CA*.

Simultaneous BRAF and PIK3CA mutations

BRAF exon 15 mutations were assessed in 122 patients, of whom 11 (9%) had a c600 mutation (Table 3). *BRAF* mutations were found in patients with melanomas, n=7; colorectal cancer, n=3; and ovarian cancer, n=1. Only one patient had a simultaneous *PIK3CA* and *BRAF* mutation detected (ovarian cancer) (Table 2).

Response in patients with PIK3CA mutations treated with PI3K/AKT/mTOR inhibitors

Seventeen of 25 patients with an underlying *PIK3CA* mutation were enrolled in clinical trials that included a PI3K/AKT/mTOR inhibitor. These patients were refractory to a median of four prior therapies (range,1 to 12). Of these patients, 6 had colorectal cancer, 4 had ovarian cancer, 3 had endometrial cancer, 2 had squamous cell cervical cancer, 1 had small intestine cancer, and 1 had breast cancer (Table 2). Sixteen patients received anti-mTORC1-based regimens and 1 patient was treated with an anti-PI3K-based regimen (Table 4) (11). A PR was observed in 6 (35%) patients. Duration of response was 8.9, 17.9+, 25+, 30.6+, 35.3, and 59+ weeks (with the plus sign indicating ongoing response at the time period noted). (Figures 1 and 2). Seven (41%) patients had stable disease (SD), including 4 (23.5%) patients with prolonged stable disease lasting for more than 16 weeks. In total, 10 patients (59%) achieved either stable disease for over 16 weeks or a PR. Four (23.5%) patients had progressive disease (PD) (two with radiological and two with clinical progression). In comparison, patients without documented *PIK3CA* mutations (meaning they had no mutation or tumor tissue was unavailable for testing) treated on the same protocols demonstrated a significantly lower response rate of 6% (15 of 241) (p=0.001). Fisher's exact test was used to assess the associations among response (PR, SD or PD) and other patient characteristics, such as age, gender, race, number of prior therapies (> 3 prior therapies vs. ≤ 3 therapies), type of *PIK3CA* mutation (exon 9 vs. exon 20), and *KRAS* mutation. None of these variables was significantly associated with response.

Discussion

We determined that mutations in exon 9 or exon 20 of the *PIK3CA* proto-oncogene were present in 25 of 217 patients (11.5%) with diverse tumor types, with the incidence being highest (9 – 21%) in patients with endometrial, ovarian, colorectal, breast, cervical cancer, non-small cell lung cancer, and squamous cell cancer of head and neck. Although the number of patients in each tumor type is limited in our study, previous reports have also documented *PIK3CA* mutations in these tumor types with an incidence as follows: 23%-36% of endometrial cancers (12,13), 14%-32% of colon cancers (12,14,15), 4%-12% of ovarian cancers (16-18), 18-40% of breast cancers usually associated with expression of hormone receptors or HER2/neu (12,16,17,19), 8%-14% of cervical squamous cell cancers (12,20), and in 11%-33% of squamous cell cancers of the head and neck (12,21).

Previous preclinical observations have demonstrated that activation of the RAS/RAF/MEK pathway mediates resistance to PI3K inhibitors in *PIK3CA*-mutant tumors (8). Therefore, we examined our patients for co-existence of *PIK3CA* mutations with *RAS (K- or N-)* or *BRAF* mutations. Forty-five percent of patients (9 of 20) with a *PIK3CA* mutation (who were also tested for a *KRAS* mutation) also had a *KRAS* mutation, whereas only 22% of patients (24 of 110) without a *PIK3CA* mutation (who were also tested for *KRAS*) harbored a *KRAS* mutation. Of the 33 patients with *KRAS* mutations, 9 (27%) had simultaneous *PIK3CA* mutations. In contrast, of the 97 patients without *KRAS* mutation, only 11 (11%) had a *PIK3CA* mutation ($p = 0.03$). These results suggest that these mutations (*PIK3CA* and *RAS*) commonly co-exist. We also observed that 7 of 9 patients (78%) with colorectal cancer who harbored a *PIK3CA* mutation also had a *KRAS* mutation. This rate of dual mutations is similar to that in a previously reported study, which showed mutant *KRAS* in 56% of patients with colorectal cancer and *PIK3CA* mutations (14). In one of five patients with *PIK3CA*-mutant ovarian cancer, we detected a simultaneous *KRAS* mutation and, in another, a simultaneous *BRAF* mutation. In contrast, a study from the Middle East showed no coexistence of mutated *KRAS* or *BRAF* mutations with *PIK3CA* mutations in ovarian cancer, though the incidence of *PIK3CA* mutations in the population studied was quite low (4%) (18).

Whenever possible, our patients with *PIK3CA* mutations were entered on trials utilizing targeted inhibitors of the PI3K/AKT/mTOR pathway. Their overall response rate on these trials was 35%. Responses were seen in patients with cervical, endometrial, ovarian cancer, and breast cancer (Figure 1). In contrast, of the 241 patients without documented *PIK3CA* mutations treated on the same protocols, only 15 (6%) responded ($p=0.001$). The latter response rate is similar to the 4% to 11% response rate reported by our group and others when patients are treated on Phase I trials without molecular selection (22-24). It should be noted that it is conceivable that some of the small group of patients without *PIK3CA* mutations who responded to PI3K/AKT/mTOR axis inhibitors had other aberrations in *PIK3CA* not detected by our assay or had other abnormalities such as PTEN loss, that are known to activate *PIK3CA* (6). Indeed, we have previously shown that PTEN loss can be detected in about 20% of patients in the phase I setting (25).

Consistent with our data, clinical trials with therapies directed against well-defined targets have shown improved results when patients are selected for the presence of those targets, even in the phase I setting (where patients tend to be heavily pretreated and refractory/ resistant to multiple conventional drugs), though mostly these trials have reported results in a disease-specific setting. Examples include imatinib mesylate (a KIT and BCR-ABL kinase inhibitor), which demonstrated response rates of over 50% in patients with gastrointestinal stromal tumors (a disorder characterized by *KIT* kinase mutations) or *BCR-ABL*-positive chronic myelogenous leukemia (1,26). More recently, patients with NSCLC and an

underlying *EML4-ALK* fusion also demonstrated a response rate over 50% after treatment with the ALK inhibitor crizotinib as did patients with metastatic malignant melanoma who had an underlying *BRAF* mutation responded to the BRAF inhibitor PLX4032 (4,27). In contrast, epidermal growth factor (EGFR) receptor tyrosine kinase inhibitors were initially tested in an unselected patient population and had only modest activity (28). Subsequent "bench to the bedside" forays demonstrated that anti-EGFR tyrosine kinase inhibitors are far more effective in patients with lung cancer and an underlying *EGFR* mutation (29).

One question that arises is whether or not the detection of additional mutations that might confer resistance would provide more predictive information. In this regard, our patients with colorectal cancer and a simultaneous *KRAS* mutation did not respond to PI3K/AKT/mTOR axis therapy, which is in agreement with preclinical data suggesting that *KRAS* activation mediates resistance to PI3K inhibitors (8). In contrast, two patients with ovarian cancer and simultaneously occurring *KRAS* or *BRAF* mutations achieved a PR with PI3K/AKT/mTOR axis inhibitors, thus suggesting that such resistance is not absolute or that *RAS*- or *RAF*-mutant colorectal cancers behave differently than *RAS*- or *RAF*-mutant ovarian cancers.

In conclusion, mutations in *PIK3CA* occur in a subset of patients with several common cancers. In the current study, the response rate for patients with heavily-pretreated, diverse, advanced cancers and *PIK3CA* mutations who were given PI3K/AKT/mTOR axis inhibitors was significantly higher than that for patients without documented *PIK3CA* mutations treated on the same trials. The latter observation is consistent with data that demonstrate low response rates on traditional phase I trials, where molecular testing is not used. One hypothesis that could be generated from this data is that selecting *PIK3CA*-mutant patients for treatment with PI3K/AKT/mTOR axis inhibitors may predict response independent of underlying histology. Patients with colorectal cancer and the concomitant presence of *KRAS* and *PIK3CA* mutations did not respond, consistent with previous experiments indicating that the RAS/RAF/MEK pathway serves as a driver of resistance to PI3K inhibitors. Since the number of patients in our series was small and no randomization occurred, these data must be interpreted cautiously. However, it appears that screening for *PIK3CA* (and *RAS* or *RAF)* mutations warrants further investigation in the application of targeted PI3K/AKT/mTOR inhibitors to the clinic.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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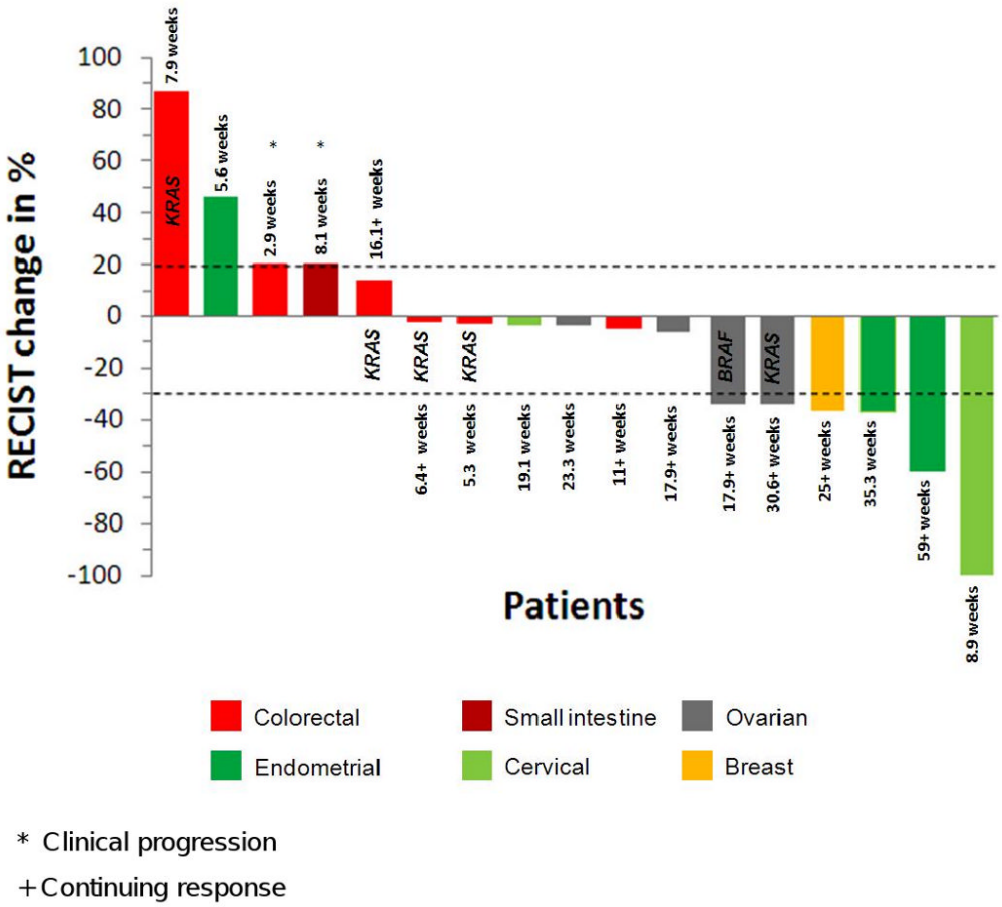


* Clinical progression

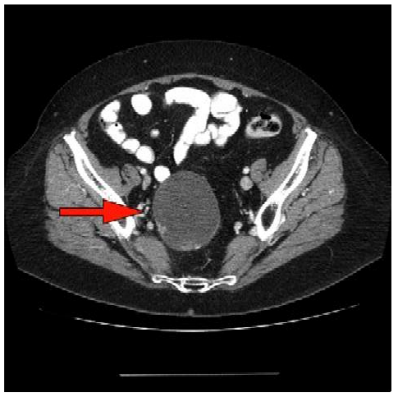
+ Continuing response

Figure 1. Waterfall plot of patients with *PIK3CA* mutations treated with PI3K/AKT/mTOR inhibitors

Six PRs (5 confirmed) and 6 minor responses less than PR were observed. The overall response rate was 35%. The best response was complete resolution of all measurable disease with persistence of non-measurable disease in a patient with squamous cell cervical carcinoma treated with an mTOR-based regimen. Four patients with colorectal cancer and 1 patient with ovarian cancer had simultaneous *KRAS* mutations. One patient with ovarian cancer had a simultaneous *BRAF* mutation.

Figure 2A

I. Before treatment



II. After 18 weeks of treatment

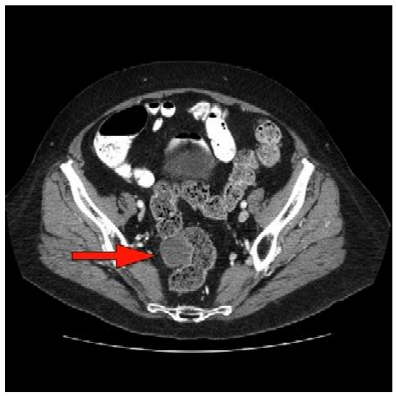
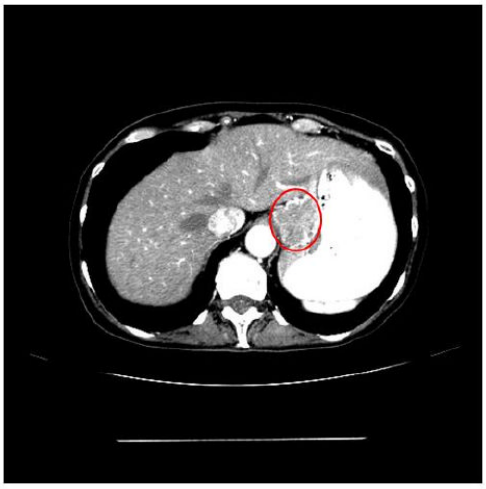


Figure 2B

I. Before treatment



II. After 4 weeks of treatment

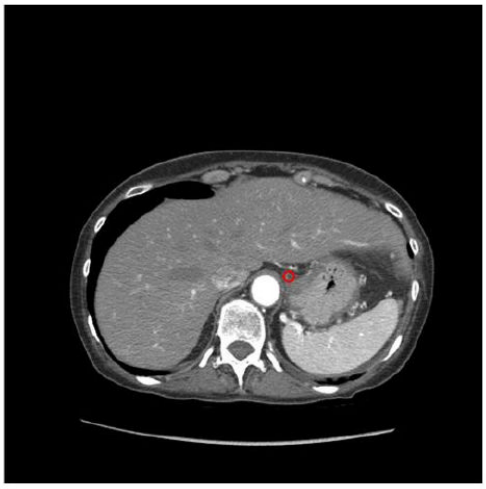
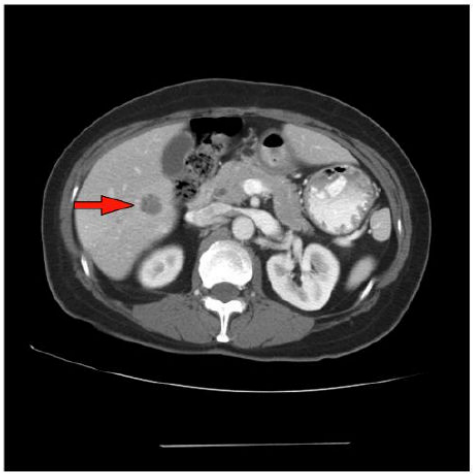


Figure 2C

I. Before treatment



II. After 18 weeks of treatment

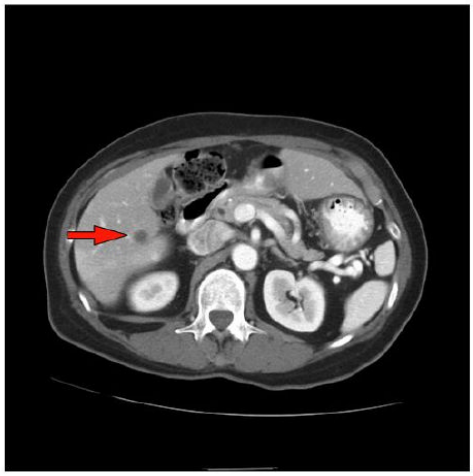
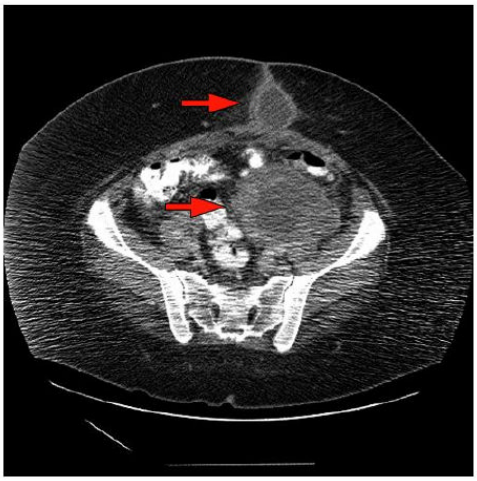
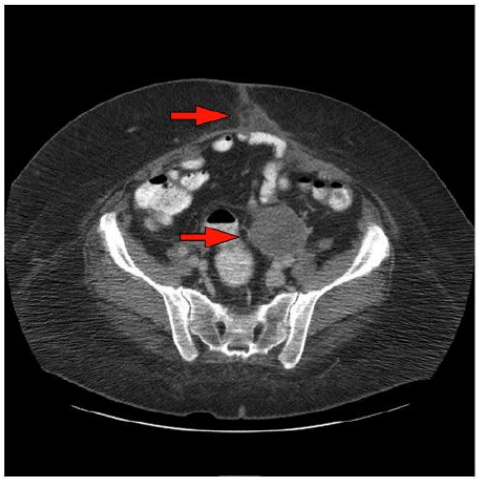


Figure 2D

I. Before treatment



II. After 18 weeks of treatment



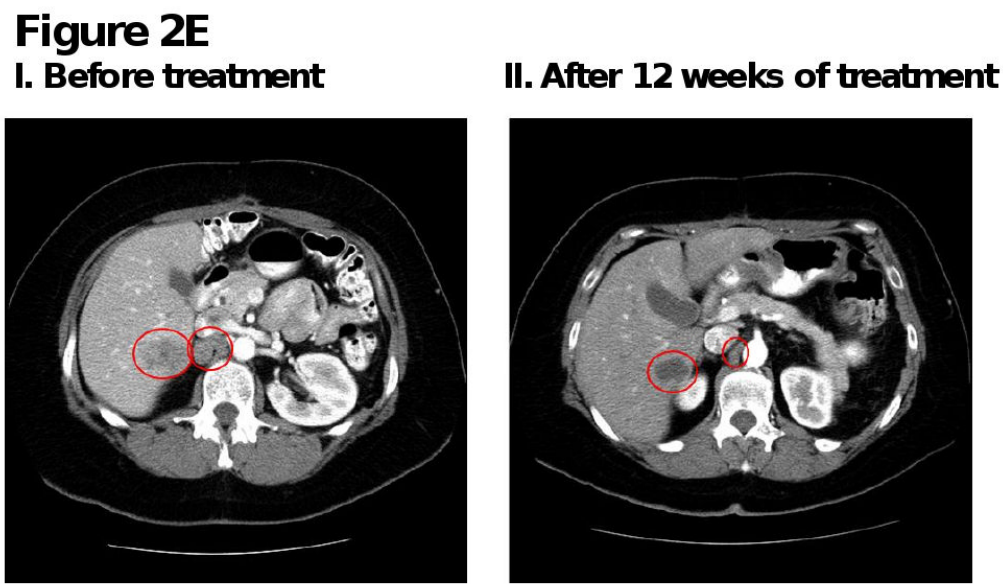


Figure 2. Computed tomography (CT) scans of responding patients. Red arrows or red circles indicate locations of metastases

A. Patient with endometrial cancer demonstrating partial response in pelvic mass.

B. Patient with squamous cell cervical cancer demonstrating partial response in gastrohepatic metastasis.

C. Patient with endometrial cancer demonstrating partial response in liver metastases.

D. Patient with ovarian cancer demonstrating partial response in pelvic and subcutaneous metastases.

E. Patient with ovarian cancer demonstrating partial response in liver and aortocaval metastases.

Table 1
Patients characteristics and distribution of *PIK3CA* mutations

Variable	Patients (n=217)		Patients with *PIK3CA* mutations in the category	
	N	%	N	%
Sex				
Male	86	40	11	13
Female	131	60	14	11
Age				
< 50 years	66	30	8	12
50-70 years	127	59	17	13
> 70 years	24	11	0	0
Ethnicity				
Caucasian	176	81	17	10
African American	19	9	4	21
Hispanic	12	6	1	8
Asian	10	4	3	30
Tumor type				
Colorectal	54	25	9	17
Ovarian	30	14	5	17
Melanoma	18	8	0	0
Breast	14	6	2	14
Endometrial	14	6	3	21
Cervix	15	7	2	13
Soft tissue sarcomas	8	4	0	0
Non-small cell lung cancer	7	3	1	14
Small cell lung cancer	2	<1	0	0
Head & Neck: squamous	11	5	1	9
Head & Neck: adenoid cystic	3	1	0	0
Renal	4	2	0	0
Parotid	4	2	0	0
Thymoma	3	1	0	0
Pancreatic	3	1	1	33
Neuroendocrine	3	1	0	0
Vulvar: squamous	2	<1	0	0
Adrenocortical	2	<1	0	0
Small intestine	1	<1	1	100
Other cancers	19	9	0	0

Table 2

Characteristics of 25 patients with *PIK3CA* mutations

Case number	Tumor type	Histology	*PIK3CA* mutations	Other mutations	Response (RECIST %)	TTP (weeks)
1	**Ovarian**	Clear cell carcinoma	c1047	*BRAF* c600	PR (-34)	17.9^{+}
2	**Ovarian**	High-grade endometrioid carcinoma	c1047	None	-	-
3	**Ovarian**	Clear cell carcinoma	c1049	None	SD (-4)	23.3
4	**Ovarian**	High-grade carcinoma	c542	None	SD (-6)	17.9^{+}
5	**Ovarian**	High-grade carcinoma	c546	*KRAS* c61	PR (-34)	30.6^{+}
6	**Colorectal**	Moderately differentiated adenocarcinoma	c1047	*KRAS* c12	PD ($^{+}$87)	7.9
7	**Colorectal**	Moderately differentiated adenocarcinoma	c545	*KRAS* c12	-	-
8	**Colorectal**	Moderately differentiated adenocarcinoma	c1049	*KRAS* c12, c13	SD (-2)	6.4^{+}
9	**Colorectal**	Moderately differentiated adenocarcinoma	c1047	*KRAS* c12	-	-
10	**Colorectal**	Moderately differentiated adenocarcinoma	c545	*KRAS* c12	SD (-3)	5.3
11	**Colorectal**	Moderately differentiated adenocarcinoma	c545	*KRAS* c12	SD ($^{+}$14)	16.1^{+}
12	**Colorectal**	Moderately differentiated adenocarcinoma	c545	None	SD (-5)	11^{+}
13	**Colorectal**	Moderately differentiated adenocarcinoma	c1047	None	PD[a]	2.9
14	**Colorectal**	Moderately differentiated adenocarcinoma	c545	*KRAS* c61	-	-
15	**Endometrial**	High-grade endometrial	c1047	None	PR (-37)	35.3
16	**Endometrial**	Endometrial adenocarcinoma, grade 2	c1047	None	PR (-60)	59^{+}
17	**Endometrial**	Endometrial adenocarcinoma, grade 2	c1049	None	PD ($^{+}$46)	5.6
18	**Breast**	Lobular carcinoma, ER^{+}, PR^{+}, HER2/neu-	c1047	None	PR (-37)	25^{+}
19	**Breast**	Ductal carcinoma, grade 2, ER^{+}, PR^{+}, HER2/neu-	c1047	None	-	-
20	**Cervix**	Moderately differentiated squamous cell carcinoma	c545	None	SD (-4)	19.1
21	**Cervix**	Moderately/poorly differentiated squamous cell carcinoma	c545,c549	None	PR (-100)	8.9
22	**Head & Neck**	Poorly differentiated squamous cell carcinoma	c1043	None	-	-
23	**Lungs**	Adenocarcinoma	c545	None	-	-
24	**Pancreas**	Poorly differentiated adenocarcinoma	c545	*KRAS* c12	-	-
25	**Small Intestine**	Poorly differentiated adenocarcinoma	c1047	None	PD[a]	8.1

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TTP, time to progression; c, codon; PR, partial response; SD, stable disease; PD, progressive disease; ER+, estrogen receptor positive; PR+, progesteron receptor positive; HER2/neu-, HER2/neu receptor negative.

[a] clinical progression

[+] not progressing at the time of analysis

Table 3

***PIK3CA, RAS* (*K*- or *N*-), and *BRAF* mutations**

Oncogene	Mutated (%)	Total tested
***PIK3CA* mutations**	25 (11.5)	217
***KRAS* mutations**	33 (25)	130
***NRAS* mutations**	2 (3)	62
***BRAF* mutations**	11 (9)	122
***RAS* or *BRAF* mutations**	46 (31)	145
KRAS* mutations in mutated *PIK3CA	9 (45)	20
RAS* or *BRAF* mutations in mutated *PIK3CA	10 (50)	20

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Table 4

Therapeutic regimens used to treat patients with *PIK3CA* mutations

Regimen	Mechanism of action	Patients (Case number*)	%	Reference
Temsirolimus	mTORC1 inhibitor	5 (4, 10, 11, 15, 17)	29	NCT00877773
Temsirolimus, bevacizumab	mTORC1 inhibitor, anti-VEGF monoclonal antibody	2 (8, 13)	12	NCT00610493
Temsirolimus, liposomal doxorubicin, bevacizumab	mTORC1 inhibitor, anti-VEGF monoclonal antibody, topo II alpha inhibitor	8 (1, 3, 5, 12, 16, 18, 20, 21)	47	NCT00761644
Temsirolimus, topotecan, bortezomib	mTORC1 inhibitor, topoisomerase I inhibitor, proteasome inhibitor	1 (25)	6	NCT00770731
XL147, carboplatin, paclitaxel	PI3K inhibtor, alkylating agent, microtubule stabilizing agent	1 (6)	6	Wheler et al.[11]

*Case numbers are depicted in Table 2

NCT: clinicaltrials.gov identifier