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PI3K/AKT/mTOR Inhibitors in Patients With Breast and Gynecologic Malignancies Harboring *PIK3CA* Mutations

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A B S T R A C T

Purpose
Mutations of the *PIK3CA* gene may predict response to phosphatidylinositol 3-kinase (PI3K)/AKT/ mammalian target of rapamycin (mTOR) inhibitors. Concomitant mutations in the mitogen-activated protein kinase (MAPK) pathway may mediate resistance.

Patients and Methods
Tumors from patients with breast, cervical, endometrial, and ovarian cancer referred to the Clinical Center for Targeted Therapy (Phase I Program) were analyzed for *PIK3CA*, *KRAS*, *NRAS*, and *BRAF* mutations. Patients with *PIK3CA* mutations were treated, whenever feasible, with agents targeting the PI3K/AKT/mTOR pathway.

Results
Of 140 patients analyzed, 25 (18%) had *PIK3CA* mutations, including five of 14 patients with squamous cell cervical, seven of 29 patients with endometrial, six of 29 patients with breast, and seven of 60 patients with ovarian cancers. Of the 25 patients with *PIK3CA* mutations, 23 (median of two prior therapies) were treated on a protocol that included a PI3K/AKT/mTOR pathway inhibitor. Two (9%) of 23 patients had stable disease for more than 6 months, and seven patients (30%) had a partial response. In comparison, only seven (10%) of 70 patients with the same disease types but with wild-type *PIK3CA* treated on the same protocols responded ($P = .04$). Seven patients (30%) with *PIK3CA* mutations had coexisting MAPK pathway (*KRAS*, *NRAS*, *BRAF*) mutations (ovarian cancer, n = 5; endometrial cancer, n = 2), and two of these patients (ovarian cancer) achieved a response.

Conclusion
PIK3CA mutations were detected in 18% of tested patients. Patients with *PIK3CA* mutations treated with PI3K/AKT/mTOR inhibitors demonstrated a higher response rate than patients without mutations. A subset of patients with ovarian cancer with simultaneous *PIK3CA* and MAPK mutations responded to PI3K/AKT/mTOR inhibitors, suggesting that not all patients demonstrate resistance when the MAPK pathway is concomitantly activated.

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INTRODUCTION

Activating oncogenic mutations are attractive drug targets in many malignancies.[1-5] Mutations in the p110α subunit of *PI3K*, called *PIK3CA*, are often responsible for activation of the phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway and have been reported in various human cancers.[6] *PIK3CA* mutations can cause neoplastic transformation and promote cancer progression.[7,8] The PI3K/AKT/mTOR pathway is often dysregulated in gynecologic and breast cancers, and *PIK3CA* mutations have

been reported in approximately 18% of breast,[9] 17% to 33% of cervical,[10,11] 39% of endometrial,[12] and 12% of ovarian cancers.[9] Preclinical studies suggested that *PIK3CA* mutations could predict response to PI3K and mTOR inhibitors, although mutations in the mitogen-activated protein kinase (MAPK) pathway (*KRAS*, *NRAS*, *BRAF*) might mediate resistance.[13-15]

We investigated *PIK3CA* mutation status, and when enough tissue permitted, we also assessed the MAPK pathway (*KRAS*, *NRAS*, *BRAF*) mutation status of patients with advanced breast, cervical, endometrial, and ovarian cancers referred to the Phase I

I Clinical Trials Program clinic (known as the Clinical Center for Targeted Therapy). When feasible, the results of molecular matching were used for treatment selection, and in those cases, patients with *PIK3CA* mutations were offered treatment targeting the PI3K/AKT/mTOR pathway.

PATIENTS AND METHODS

Patients

Patients with advanced breast, cervical, endometrial, and ovarian cancers who experienced treatment failure with standard therapy and who had tissue available for mutation analysis were eligible. The study was carried out in the Department of Investigational Cancer Therapeutics (Phase I Clinical Trials Program) at The University of Texas MD Anderson Cancer Center (MD Anderson). The registration of patients in the database, pathology assessment, and mutation analysis were performed at MD Anderson. Eligible patients were those referred for phase I clinical trials for targeted therapeutic agents. The study and all treatments were conducted in accordance with the guidelines of the MD Anderson Institutional Review Board.

Tissue Samples and Mutation Analyses

PIK3CA, *KRAS*, *NRAS*, and *BRAF* 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 MD Anderson. *PIK3CA*, *KRAS*, *NRAS*, and *BRAF* mutation testing was performed in the Clinical Laboratory Improvement Amendment–certified Molecular Diagnostic Laboratory within the Division of Pathology and Laboratory Medicine at MD Anderson. DNA was extracted from micro-dissected, paraffin-embedded tumor sections and analyzed using a polymerase chain reaction–based DNA sequencing method for *PIK3CA* mutations in codons [c]532 to [c]554 of exon 9 (helical domain) and c1011 to c1062 of exon 20 (kinase domain), which included the mutation hotspot region of the *PIK3CA* proto-oncogene by Sanger sequencing after amplification of 276– and 198–base pair amplicons, respectively, using primers designed by the MD Anderson Molecular Diagnostic Laboratory. Whenever possible, in addition to *PIK3CA*, mutation analysis was done for *KRAS* and *NRAS* c12, c13, and c61 mutations of exons 1 and 2 and *BRAF* c595 to c600 mutations of exon 15 using pyrosequencing as previously described.[16]

Treatment and Evaluation

Starting in October 2008, consecutive patients (N = 140) with advanced breast, cervical, endometrial, and ovarian cancers were studied. Patients with *PIK3CA* mutations were enrolled, whenever possible, onto clinical trials containing inhibitors of the PI3K/AKT/mTOR pathway. These clinical trials included temsirolimus, bevacizumab, and liposomal doxorubicin[17] (ClinicalTrials.gov identifier: NCT00761644); single-agent temsirolimus (ClinicalTrials.gov identifier: NCT00877773); temsirolimus and bevacizumab (ClinicalTrials.gov identifier: NCT00610493); sirolimus and docetaxel (ClinicalTrials.gov identifier: NCT01054313); and PX866[18] (ClinicalTrials.gov identifier: NCT00726583). 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 using computed tomography scans and/or magnetic resonance imaging at baseline before treatment initiation and then every two cycles (6 to 8 weeks). All radiographs were read in the Department of Radiology at MD Anderson and reviewed in the Department of Investigational Cancer Therapeutics tumor measurement clinic. Responses were categorized per Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0 and were reported as best response.[19] In brief, complete response (CR) was defined as the disappearance of all measurable and nonmeasurable disease. Partial response (PR) was defined as at least a 30% decrease in the sum of the longest diameter of measur-

able target lesions. Progressive disease was defined as at least a 20% increase in the sum of the longest diameter of measurable target lesions, unequivocal progression of a nontarget lesion, or the appearance of a new lesion. Stable disease (SD) was defined as neither sufficient shrinkage of tumor burden to qualify as a PR nor sufficient increase in tumor volume to qualify as progressive disease. A confirmation of CR/PR required repeat imaging at least 28 days after the initial response assessment.

Statistical Analysis

Statistical analysis was verified by our statistician (J.J.L.). The 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 $P < .05$ was considered statistically significant. All statistical analyses were carried out using SPSS 17 software (SPSS, Chicago, IL).

RESULTS

Patients

A total of 140 patients with advanced breast, cervical, endometrial, and ovarian cancers were analyzed for the presence of *PIK3CA* mutations. Their median age was 56.5 years (range, 25 to 91 years), and 113 patients (81%) were white, 11 (8%) were African American, seven (5%) were Hispanic, and nine (6%) were Asian. Sixty patients (43%) had ovarian cancer, 29 (21%) had endometrial cancer, 29

Table 1. Demographics and Clinical Characteristics of All Enrolled Patients With Breast, Cervical, Endometrial, and Ovarian Cancers (N = 140)

Demographic or Clinical Characteristic	Wild-Type *PIK3CA* (n = 115)*		Mutant *PIK3CA* (n = 25)*	
	No. of Patients	%	No. of Patients	%
Age, years				
Median	58		52	
Range	25-91		35-72	
< 50	36	31.3	11	44.0
50-70	67	58.3	13	52.0
> 70	12	10.4	1	4.0
Ethnicity				
White	95	82.6	18	72.0
African American	7	6.1	4	16.0
Hispanic	6	5.2	1	4.0
Asian	7	6.1	2	8.0
Tumor type, histology				
Breast	23	20.0	6	24.0
Cervical	17†	14.8	5‡	20.0
Endometrial	22	19.1	7	28.0
Ovarian	53	46.1	7	28.0
Site of mutation analysis				
Primary tumor	54	47.0	15	60.0
Metastatic tumor	61	53.0	10	40.0
No. of prior therapies				
Median	4		3	
Range	1-14		1-12	
≤ 2	31	27.0	11	44.0
> 2	84	73.0	14	56.0

*There was no significant difference in any of the listed characteristics between patients with wild-type and mutant *PIK3CA*.
†Adenocarcinoma, n = 8; squamous cell carcinoma, n = 9.
‡All squamous cell carcinomas.

Table 2. Distribution of *PIK3CA*, *KRAS*, *NRAS*, and *BRAF* Mutations

Oncogene	Mutated No.	Mutated %	Total Tested (No.)	*P*
PIK3CA	25	18	140	NA
KRAS	10	10	98	NA
NRAS	2	4	53	NA
BRAF	2	2	84	NA
KRAS/NRAS or *BRAF*	14	17	81	NA
KRAS in mutant *PIK3CA*	5	23	22	.04
KRAS in wild-type *PIK3CA*	5	7	76	
KRAS/NRAS or *BRAF* in mutant *PIK3CA*	7*	35	20	.04
KRAS/NRAS or *BRAF* in wild-type *PIK3CA*	7	11	61	

Abbreviation: NA, not applicable.
*Simultaneous mutations in *PIK3CA* and *KRAS* (n = 5), *PIK3CA* and *BRAF* (n = 1), or *PIK3CA* and *NRAS* (n = 1).

H1047R (a mutation in c1047 of *PIK3CA* that changes the encoded amino acid from histidine to arginine) detected in 11 patients (Table 3). *PIK3CA* mutations were detected in five (36%) of 14 patients with squamous cell cervical cancer, seven (24%) of 29 patients with endometrial cancer, six (21%) of 29 patients with breast cancer, and seven (12%) of 60 patients with ovarian cancer. *PIK3CA* mutation status was not significantly associated with age, disease type, or ethnicity.

Simultaneous RAS and PIK3CA Mutations

KRAS mutations in exons 1 or 2 were assessed in 98 patients who had enough tissue available for mutation analysis and were identified in 10 patients (10%; Table 2). The mutations were most frequent in codon 12 (*G12D* [changes the encoded amino acid from glycine to aspartic acid], n = 2; *G12A* [changes the encoded amino acid from glycine to alanine], n = 1; *G12V* [changes the encoded amino acid from glycine to valine], n = 1; Table 3). The presence of *KRAS* mutations was significantly associated with *PIK3CA* mutations. Indeed, 23% of patients (five of 22 patients) with a *PIK3CA* mutation (who had enough tissue for *KRAS* mutation analysis) also had a *KRAS* mutation, whereas only 7% of patients (five of 76 patients) without a *PIK3CA* mutation (who were also tested for *KRAS*) harbored a *KRAS* mutation (P = .04; Table 2). Of the 10 patients with *KRAS* mutations, five (50%) had simultaneous *PIK3CA* mutations. In contrast, of the 88 patients without a *KRAS* mutation, only 17 (19%) had a *PIK3CA*

(21%) had breast cancer, 14 (10%) had squamous cell cervical cancer, and eight (5%) had cervical adenocarcinoma. Detailed patient characteristics are listed in Table 1.

PIK3CA Mutations

PIK3CA proto-oncogene mutations were detected in 25 (18%) of the 140 study patients (Table 2). The most frequent mutation was

Table 3. *PIK3CA*, *KRAS*, *NRAS*, and *BRAF* Mutations

Patient No.	Histology	*PIK3CA* Mutation	*KRAS* Mutation	*NRAS* Mutation	*BRAF* Mutation
1	Breast: lobular ER positive/PR positive/HER2 negative	*H1047R*	None	None	None
2	Breast: metaplastic, triple negative	*H1047R*	None	Not done	None
3	Breast: ductal, ER positive/PR positive/HER2 negative	*E542K*	None	None	None
4	Breast: ductal, ER negative/PR negative/HER2 positive	*H1047R*	None	Not done	Not done
5	Breast: ductal, ER positive/PR positive/HER2 positive	*M1043I*	Not done	Not done	Not done
6	Breast: ductal, ER positive/PR positive/HER2 negative	*H1047R*	None	None	Not done
7	Breast: ductal, ER positive/PR positive/HER2 negative	None	*Q61L*	None	None
8	Cervix: squamous	*E545K*	None	None	None
9	Cervix: squamous	*E545K/D549H*	None	None	None
10	Cervix: squamous	*E545K*	None	None	None
11	Cervix: squamous	*E542K*	None	Not done	None
12	Cervix: squamous	*E545K*	None	Not done	None
13	Cervix: squamous	None	*G12D*	None	None
14	Endometrial: clear cell	*H1047R*	Not done	Not done	None
15	Endometrial: endometrioid	*H1047R*	None	None	None
16	Endometrial: endometrioid	*G1049R*	Not done	Not done	Not done
17	Endometrial: papillary	*H1047R*	None	*G13D*	None
18	Endometrial: endometrioid	*E545G*	*G12A*	None	None
19	Endometrial: endometrioid	*H1047L*	None	Not done	None
20	Endometrial: endometrioid	*H1047R*	None	None	None
21	Endometrial: endometrioid	None	*G13D*	Not done	None
22	Endometrial: clear cell	None	*Q61L*	Not done	Not done
23	Endometrial: endometrioid	None	None	*Q61L*	Not done
24	Ovarian: endometrioid	*Q546K*	*Q61H*	None	None
25	Ovarian: high-grade serous	*E542K*	None	None	None
26	Ovarian: clear cell	*G1049R*	None	None	None
27	Ovarian: clear cell	*H1047R*	None	None	*V600E*
28	Ovarian: endometrioid	*H1047R*	*G12D*	None	None
29	Ovarian: high-grade serous	*H1047R*	*G13D*	None	None
30	Ovarian: endometrioid	*M1043V*	*G12V*	None	None
31	Ovarian: clear cell	None	*Q61H*	Not done	Not done
32	Ovarian: low-grade serous	None	None	None	*V600E*

Abbreviations: ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; PR, progesterone receptor.

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mutation ($P = .04$). Of the five patients with simultaneous *PIK3CA* and *KRAS* mutations, four had ovarian cancer, and one had endometrial cancer (Table 3).

NRAS mutations (*G13D* [changes the encoded amino acid from glycine to aspartic acid] in codon 13 and *Q61L* [changes the encoded amino acid from glutamine to leucine] in codon 61) were detected in two patients (4%) of 53 tested who had enough tissue available for *NRAS* mutation analysis (Table 2), and one of those two patients had a simultaneous *PIK3CA* mutation (endometrial cancer; Table 3).

Simultaneous BRAF and PIK3CA Mutations

BRAF exon 15 mutations were assessed in 84 patients (Table 2) who had enough tissue available for *BRAF* mutation analysis. Two patients (2%) had a *V600E* mutation (a mutation in c600 of *BRAF* that changes the encoded amino acid from valine to glutamic acid), and one of those two patients (ovarian cancer) had a simultaneous *PIK3CA* mutation (Table 3).

Simultaneous MAPK Pathway (KRAS, NRAS, BRAF) Mutations and PIK3CA Mutations

We analyzed associations between *PIK3CA* mutations and all tested MAPK pathway mutations (*KRAS, NRAS, BRAF*). This analysis included patients tested for *RAS* (*KRAS* or *NRAS*) and *BRAF* mutations. Not all patients could be tested for all mutations because of the limited amount of available tumor tissue. Mutations in *KRAS, NRAS,* and *BRAF* are considered mutually exclusive[20]; therefore, patients with mutations in *RAS* (*KRAS* or *NRAS*) or *BRAF* were included, even if they were not tested for both *RAS* and *BRAF*. In total, 81 patients were included in this analysis. *RAS* (*KRAS* or *NRAS*) or *BRAF* mutations were identified in 14 (17%) of these 81 patients. The presence of *RAS* (*KRAS* or *NRAS*) or *BRAF* mutations was significantly associated with *PIK3CA* mutations. Thirty-five percent of patients (seven of 20 patients) with a *PIK3CA* mutation also had a *RAS* (*KRAS* or *NRAS*) or *BRAF* mutation, whereas only 11% of patients (seven of 61 patients) without a *PIK3CA* mutation harbored a *RAS* (*KRAS* or *NRAS*) or *BRAF* mutation ($P = .04$).

Response in Patients With PIK3CA Mutations Treated With PI3K/AKT/mTOR Inhibitors

Twenty-three (92%) of 25 patients with an underlying *PIK3CA* mutation were enrolled onto clinical trials that included a PI3K/AKT/mTOR inhibitor.[17,18,21] Two patients were not enrolled because of eligibility or logistical reasons. These 23 patients were refractory to a median of two prior therapies (range, one to 12 prior therapies). Of the 23 patients, seven had ovarian cancer, six had endometrial cancer, five had breast cancer, and five had squamous cell cervical cancer. A response (six confirmed PRs, one unconfirmed PR) was observed in seven patients (30%; 95% CI, 16% to 51%; Figs 1 and 2). Duration of response in the seven responders was 2.0, 4.6, 8.2, 8.4, 8.5, 10.3, and 21.6 months (Fig 3). Two patients (9%; 95% CI, 2% to 27%) who did not have a PR experienced prolonged SD, which lasted for more than 6 months (Fig 3). In total, nine patients (39%) achieved either SD for more than 6 months or a PR. Of the seven patients who responded, six received a combination of a PI3K/AKT/mTOR inhibitor and a cytotoxic drug (liposomal doxorubicin). The responders had a median of two prior therapies (range, one to 12 prior therapies); five of the responders received prior platinum-based, five received prior taxane-based, and three received prior doxorubicin-based therapy and expe-
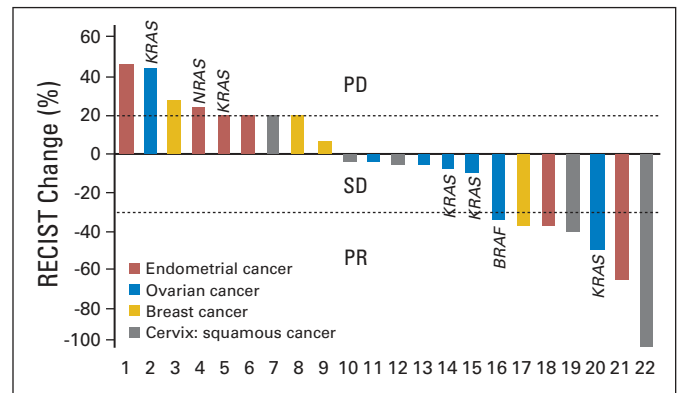

Fig 1. Waterfall plot of patients with *PIK3CA* mutations treated with phosphatidylinositol 3-kinase/AKT/mammalian target of rapamycin inhibitors. The overall response rate was 30%. Five patients with ovarian cancer and two patients with endometrial cancer had simultaneous *PIK3CA* and MAPK pathway (*KRAS, NRAS,* or *BRAF*) mutations. One patient with breast cancer who was never evaluated for response is not depicted. PD, progressive disease; PR, partial response; RECIST, Response Evaluation Criteria in Solid Tumors; SD, stable disease.

rienced progression. In comparison, only seven (10%; 95% CI, 5% to 19%) of 70 patients with the same disease types with wild-type *PIK3CA* treated on the same protocols responded (PR or CR; $P = .04$). Of those 70 patients, 68 received combination therapies, most commonly with cytotoxic drugs such as liposomal doxorubicin or docetaxel. In patients with *PIK3CA* mutations, there were no associations among response and other patient characteristics, such as age, race, number of prior therapies ($> v \leq$ two therapies), time from tumor sample collection to mutation analysis, and site of mutation analysis (primary tumor v metastasis). Patients with an *H1047R* *PIK3CA* mutation experienced a response rate of 44% (four of nine patients; 95% CI, 19% to 73%) compared with a response rate of 21% (three of 14 patients; 95% CI, 8% to 48%) in patients with other *PIK3CA* mutations ($P = .36$). Patients treated with combinations of agents demonstrated a trend to a higher response rate of 44% (seven of 16 patients; 95% CI, 23% to 67%) compared with a rate of 0% (zero of seven patients; 95% CI, 0% to 35%) in patients treated with single-agent therapies ($P = .06$). Response rates per tumor type were as follows: breast, 20% (one of five patients); squamous cell cervical, 40% (two of five patients); endometrial, 33% (two of six patients); and ovarian, 29% (two of seven patients; Fig 1).

DISCUSSION

We detected helical and kinase domain *PIK3CA* mutations in 18% of 140 patients with advanced breast, cervical, endometrial, and ovarian cancers. The prevalence was highest in squamous cell cervical cancer (36%), followed by endometrial cancer (24%), breast cancer (21%), and ovarian cancer (12%). Although the small number of patients precludes definitive conclusions regarding absolute difference in mutation rates, our results are consistent with those in the literature.[9,11,12] Eligible patients received treatments, if feasible, containing a PI3K/AKT/mTOR inhibitor. In 23 *PIK3CA*-mutant patients with breast and gynecologic cancers who experienced treatment failure with standard therapies, we observed a response rate of 30%. This response rate is favorable compared with a 10% response rate in patients with advanced breast, cervical, endometrial, and ovarian cancers and wild-type *PIK3CA* treated on the same protocols ($P = .04$). The latter is
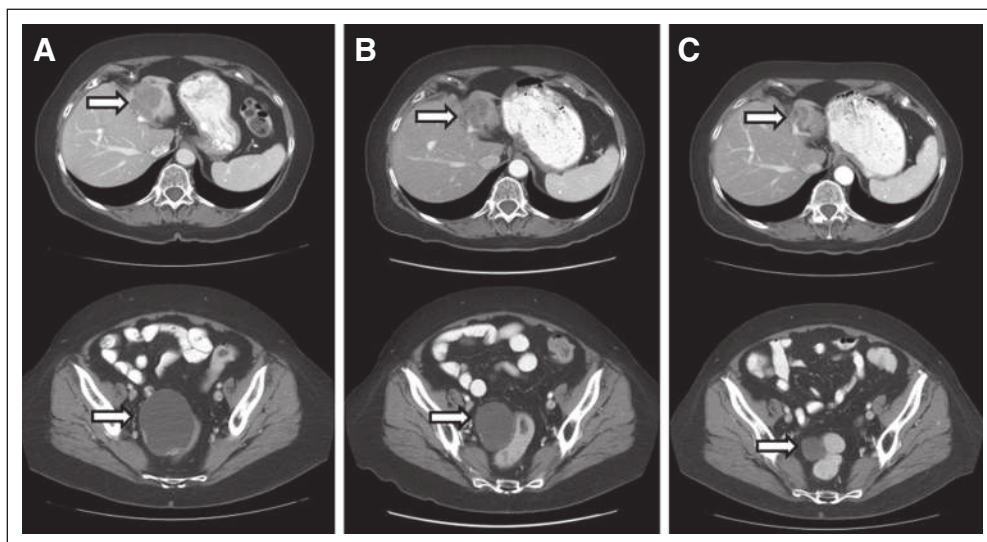
Fig 2. Computed tomography scans of a responding patient (previously treated with cisplatin/gemcitabine, paclitaxel, carboplatin, liposomal doxorubicin, and cetuximab) with endometrial cancer demonstrating response on therapy with temsirolimus, bevacizumab, and liposomal doxorubicin. (A) Pretreatment scans of liver metastasis and pelvic mass. (B) Restaging scans after two cycles (6 weeks) demonstrating response in liver and pelvic mass. (C) Restaging scans after six cycles (18 weeks) demonstrating continuing response in liver and pelvic mass.

comparable to the response rate of 4% to 11% reported by our group and others when patients were treated on phase I trials without molecular selection.[22-25] It is also conceivable that the response rate of 10% in unselected patients was on the high end compared with response rates reported previously from other phase I clinics,[22,23] because even when mutation status was negative, physicians tended to select patients for PI3K/AKT/mTOR inhibitor studies based on the known frequency of other mutations in the PI3K/AKT/mTOR pathway not detected by our assay (eg, *PIK3R1*, *PTEN*, *AKT*).[26] Previously published oncogene-driven clinical trials with a BRAF inhibitor in *BRAF*-mutant melanoma, epidermal growth factor receptor (EGFR) inhibitors in *EGFR*-mutant non–small-cell lung cancer, and an ALK inhibitor in non–small-cell lung cancer with an underlying *EML4-ALK* fusion also show a relatively high response rate, even in the phase I setting.[4,5,27] In our study, most observed responses were durable, with five (70%) of seven responses lasting longer than 8 months.

Preclinical experiments suggested that *PIK3CA* mutations render tumors sensitive to PI3K and/or mTOR inhibitors, whereas simultaneous mutations in the MAPK pathway (*KRAS*, *NRAS*, *BRAF*) can
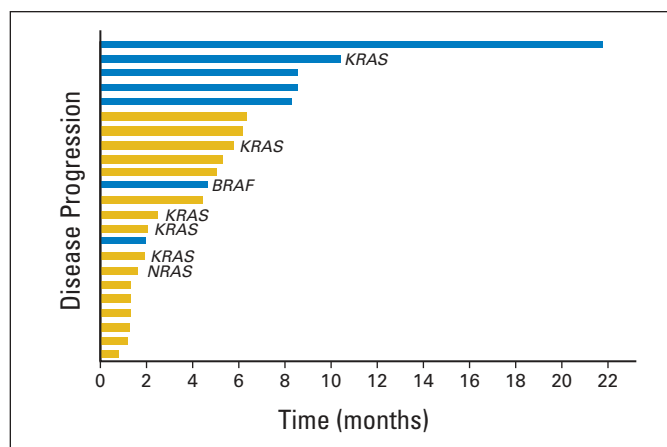

Fig 3. Time to progression (TTP). Seven patients (30%), of whom five had a partial response (blue bars), experienced TTP longer than 6 months. All patients were off therapy at the time of analysis (19 experienced progression, and four withdrew consent).

mediate resistance to therapy.[13-15] We demonstrated that mutations in the MAPK pathway (*KRAS*, *NRAS*, *BRAF*) are more frequent in patients with *PIK3CA* mutations compared with patients with wild-type *PIK3CA* (35% v 11%, respectively; $P = .04$). Of interest, two of our patients with ovarian cancer and coexisting *PIK3CA* and MAPK pathway mutations responded to PI3K/AKT/mTOR inhibitors.

Conceptually, the use of molecular profiling in early-phase clinical trials has the potential to accelerate the development of new therapies. A decade elapsed before implementation of molecular profiling to identify patients with advanced lung cancer who demonstrated benefit from EGFR tyrosine kinase inhibitors.[28] However, development of other targeted therapies, such as ALK inhibitors in lung cancer with an *EML4-ALK* fusion and BRAF inhibitors in *BRAF*-mutated melanoma, was more streamlined, with molecular profiling successfully incorporated in the clinical study designs, including phase I trials, which demonstrated striking responses.[4,5]

Other examples of successful implementation of molecular profiling include imatinib mesylate (a KIT and BCR-ABL kinase inhibitor), which demonstrated response rates greater than 50% in patients with GI stromal tumors (a disorder characterized by *KIT* kinase mutations) and *BCR-ABL*–positive chronic myelogenous leukemia.[1,29] The response rate in our study is lower than the rates described earlier. Future larger analyses should examine other variables such as coexisting *RAS* or *RAF* mutations and/or specific types of *PIK3CA* mutations to assess factors attenuating responsiveness. In addition, our patients were treated on different dose levels of several early-phase clinical trials. It is plausible that some patients may have received doses or drugs that did not adequately inhibit the pathway.

Finally, all responses in our study were observed with combination, but not single-agent, therapies (44% v 0%, respectively; $P = .06$). This is in agreement with preclinical data, which suggested that single-agent PI3K/AKT/mTOR pathway inhibition may not always be sufficient to induce a response because *PIK3CA* mutations often coexist with other concurrent molecular aberrations.[13,14] This observation is potentially important for further development of PI3K/AKT/mTOR inhibitors.

In conclusion, we have shown that *PIK3CA* mutations occur in a significant proportion of patients with advanced breast, cervical, endometrial, and ovarian cancers and that, even in a patient population

that has experienced failure with standard therapies, they are associated with response to treatments that include PI3K/AKT/mTOR inhibitors. Such responses have also been anecdotally reported in patients with other *PIK3CA*-mutant cancers, and we are now analyzing a larger group of such patients.[30] Because the number of patients in our series was small and no random assignment occurred, these data must be interpreted cautiously. However, it seems that screening for *PIK3CA* mutations warrants further investigation in the application of targeted PI3K/AKT/mTOR inhibitors in the clinic, especially in gynecologic and breast cancers, where these mutations are common.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

AUTHOR CONTRIBUTIONS

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