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Therapeutics, Targets, and Chemical Biology

Cancer Research

PIK3CA Mutation H1047R Is Associated with Response to PI3K/AKT/mTOR Signaling Pathway Inhibitors in Early-Phase Clinical Trials

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Abstract

PIK3CA mutations may predict response to PI3K/AKT/mTOR inhibitors in patients with advanced cancers, but the relevance of mutation subtype has not been investigated. Patients with diverse cancers referred to the Clinical Center for Targeted Therapy were analyzed for *PIK3CA* and, if possible, *KRAS* mutations. Patients with *PIK3CA* mutations were treated, whenever possible, with agents targeting the PI3K/AKT/mTOR pathway. Overall, 105 (10%) of 1,012 patients tested harbored *PIK3CA* mutations. Sixty-six (median 3 prior therapies) of the 105 *PIK3CA*-mutant patients, including 16 individuals (of 55 *PIK3CA*-mutant patients tested) with simultaneous *KRAS* mutations, were treated on a protocol that included a PI3K/AKT/mTOR pathway inhibitor; 17% (11/66) achieved a partial response (PR). Patients with a *PIK3CA* H1047R mutation compared with patients who had other *PIK3CA* mutations or patients with wild-type *PIK3CA* treated on the same protocols had a higher PR rate (6/16, 38% vs. 5/50; 10% vs. 23/174, 13%, respectively; all $P \leq 0.02$). None of the 16 patients with coexisting *PIK3CA* and *KRAS* mutations in codon 12 or 13 attained a PR (0/16, 0%). Patients treated with combination therapy versus single-agent therapies had a higher PR rate (11/38, 29% vs. 0/28, 0%; $P = 0.002$). Multivariate analysis showed that H1047R was the only independent factor predicting response [OR 6.6, 95% confidence interval (CI), 1.02–43.0, $P = 0.047$). Our data suggest that interaction between *PIK3CA* mutation H1047R versus other aberrations and response to PI3K/AKT/mTOR axis inhibitors warrants further exploration. *Cancer Res; 73(1); 276–84. ©2012 AACR.*

Introduction

The PI3K/AKT/mTOR pathway is frequently dysregulated in human cancers by virtue of a variety of molecular aberrations, including *PIK3CA* mutations, which are frequently found in diverse cancers (1–7). Preclinical models and early clinical data suggested that *PIK3CA* mutations may predict sensitivity to treatment with PI3K/AKT/mTOR inhibitors in multiple tumor types (8–14).

Patients with diverse tumors and *PIK3CA* mutations showed a response rate of 35% in early-phase clinical trials with PI3K/AKT/mTOR inhibitors compared with 6% in patients without *PIK3CA* mutations (11). It is, however, conceivable that only subsets of patients with *PIK3CA* mutations derive benefit from therapy targeting the PI3K/AKT/mTOR pathway. Resistance might be determined by the presence of simultaneous mutations in the mitogen-activated protein kinase (MAPK) pathway or by the type of *PIK3CA* mutation. An analogous situation exists for *EGFR* mutations in non–small cell lung cancer (NSCLC), *KIT* mutations in gastrointestinal stromal cancers, and others, where differential sensitivity to targeting compounds is of critical importance (15, 16). In the preclinical setting, *PIK3CA* mutation H1047R was a stronger driver of tumor development than E545K or E542K and showed sensitivity to the mTOR inhibitor everolimus (17). In addition, immortalized fibroblasts with the H1047R *PIK3CA* mutation resulted in greater activation of AKT than E545K and E542K mutations (18). Finally, preclinical characterization of PWT33597, a dual inhibitor of PI3K and mTOR, showed a lower IC_{50} for H1047R (21 nmol/L) than for E545K (86 nmol/L) or E542K (87 nmol/L; ref. 19). Therefore, we investigated treatment outcomes with respect to the type of *PIK3CA* mutation in patients with advanced cancer who were referred to the Clinical Center for Targeted Therapy (CCTT) at The University of Texas MD Anderson Cancer Center (MD Anderson, Houston, TX).

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Materials and Methods

Patients

PIK3CA mutations were investigated in patients with advanced tumors and available tissue referred to the CCTT at MD Anderson for clinical trials of targeted therapeutic agents starting in October 2008. The registration of patients in the database, pathology assessment, and mutation analysis were conducted at MD Anderson. The study and all treatments have been conducted according to the principles expressed in the Declaration of Helsinki and approved by the MD Anderson Institutional Review Board.

Tumor tissue 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 MD Anderson. Mutation testing was carried out 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 microdissected paraffin-embedded tumor sections and analyzed using a PCR-based DNA sequencing method for *PIK3CA* mutations in codons 532 to 554 of exon 9 (helical domain) and codons 1011 to 1062 of exon 20 (kinase domain). This included the mutation hotspot region of the *PIK3CA* proto-oncogene denoted by Sanger sequencing, following amplification of 276 bp and 198 bp amplicons, respectively, by using primers designed by the MD Anderson Molecular Diagnostic Laboratory.

Since January 2011, the assay has been changed to mass spectrometric detection (Sequenom MassARRAY) to screen for the mutational hotspots in exon 1 (Q60K, R88Q, E110K, and K111N), exon 4 (N345K), exon 6 (S405S), exon 7 (E418K, C420R, and E453K), exon 9 [P539R, E542 (bases 1 and 2), E545 (all 3 bases), and Q546 (bases 1 and 2)], exon 18 (F909L), and exon 20 [Y1021 (bases 1 and 2), T1025 (base 1), M1043I, M1043V, A1046V, H1047Y, H1047, and G1049R]. The mutations identified during the initial screening were confirmed by Sanger sequencing assay. The lower limit of detection is approximately 10%. Whenever possible, in addition to *PIK3CA*, mutation analysis using PCR-based DNA sequencing was done for *KRAS* and *NRAS* codons 12, 13, and 61 mutations of exons 1 to 2 (20). The lower limit of detection was approximately 20%. In addition, whenever possible, PTEN expression was evaluated with immunohistochemistry (monoclonal mouse anti-human PTEN antibody clone 6H2.1; Dako), and complete loss of expression was considered as PTEN loss.

Treatment and evaluation

Consecutive patients with underlying *PIK3CA* mutations were enrolled, whenever possible, in clinical trials containing inhibitors of the PI3K/AKT/mTOR pathway (Supplementary Table S1). 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 carried out 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 scans and/or magnetic resonance imaging at baseline before treatment initiation and then every 2 cycles (6–8 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 according to RECIST 1.0 criteria (21). 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 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 nontarget lesion, or the appearance of a new lesion; and stable disease (SD) was defined as neither sufficient shrinkage to qualify for a PR nor sufficient increase to qualify for PD.

Statistical analysis

Two-way contingency tables were used to summarize the relationship between 2 categorical variables. Fisher exact test was used to assess the association among categorical variables and mutation status. A Wilcoxon rank-sum test was applied to assess the association among continuous variables and mutation status. Multicovariable logistic regression was applied to identify the multiple predictors associated with the response outcome and number of prior therapies, histology, type of therapy, *PIK3CA* mutation types, and *KRAS* mutations, etc. Progression-free survival (PFS) was defined as the time interval from the start of therapy to the first observation of disease progression or death, whichever occurred first. Patients alive and without disease progression were censored at the last follow-up date. Overall survival (OS) was defined as the time interval from the start of therapy to the date of death or the date of last follow-up, whichever occurred first. OS and PFS were estimated using the method of Kaplan and Meier and were compared among the subgroups of patients using a log-rank test. Cox proportional hazards regression models were fit to assess the association between patient characteristics and PFS or OS. All tests were 2-sided, and P values less than 0.05 were considered statistically significant. All statistical analyses were carried out using SPSS 17 computer software (SPSS).

Results

Patients

A total of 1,012 patients with diverse advanced cancers were analyzed for the presence of *PIK3CA* mutations. Their median age was 58 years (range, 13–91 years) and 796 (79%) were white, 96 (9%) African American, 74 (7%) Hispanic, and 46 (5%) Asian. Of the 1,012 patients, 195 (19%) had colorectal cancer, 112 (11%) ovarian cancer, 88 (9%) melanoma, 58 (6%)

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Table 1. Patient characteristics ($N = 1,012$)

Variable	Number (%)	*PIK3CA* mutation (%)	Wild-type *PIK3CA* (%)	*P*
All	1,012 (100)	105 (100)	907 (100)	NA
Gender				
Men	462 (46)	37 (35)	425 (47)	0.029[a]
Women	550 (54)	68 (65)	482 (53)	
Median age	58; range, 13–91	56; range, 16–81	59; range,13–91	0.26[b]
Ethnicity				
White	796 (79)	81 (77)	715 (79)	0.24[c]
African-American	96 (9)	13 (12)	83 (9)	
Hispanic	74 (7)	4 (4)	70 (8)	
Asian	46 (5)	7 (7)	39 (4)	
Site of biopsy				
Primary tumor	514 (51)	59 (56)	455 (50)	0.26[d]
Metastatic tumor	498 (49)	46 (44)	452 (50)	
Tumor type				
Colorectal	195 (19)	32 (30)	163 (18)	NA
Ovarian	112 (11)	11 (10)	101 (11)	
Melanoma	88 (9)	2 (2)	86 (9)	
Non–small cell lung	58 (6)	5 (5)	53 (6)	
Breast	54 (5)	15 (14)	39 (4)	
Head and neck: squamous	53 (5)	8 (8)	45 (5)	
Endometrial	46 (5)	12 (11)	34 (4)	
Thyroid	40 (4)	2 (2)	38 (4)	
Soft tissue sarcomas	37 (4)	0 (0)	37 (4)	
Renal cell	29 (3)	2 (2)	27 (3)	
Pancreatic	26 (3)	1 (1)	25 (3)	
Gastric	25 (2)	1 (1)	24 (3)	
Prostate	24 (2)	0 (0)	24 (3)	
Neuroendocrine	24 (2)	1 (1)	23 (3)	
Cervical: squamous	20 (2)	6 (6)	14 (2)	
Biliary tract	20 (2)	0 (0)	20 (2)	
Salivary gland	17 (2)	0 (0)	17 (2)	
Head and neck: nonsquamous	15 (1)	2 (2)	13 (1)	
Hepatocellular	12 (1)	0 (0)	12 (1)	
Cervical: adenocarcinoma	10 (1)	0 (0)	10 (1)	
Ewing sarcoma	10 (1)	0 (0)	10 (1)	
Other	97 (10)	5 (5)	92 (10)	
KRAS mutations — total tested	717 (100)	86 (100)	631 (100)	
KRAS mutated[e,f]	137 (19)	31 (36)	106 (17)	<0.001[e]
KRAS wild-type[e,f]	580 (81)	55 (64)	525 (83)	

Abbreviation: NA, not applicable.

[a]*PIK3CA* mutations were more prevalent in women than in men.

[b]No difference in prevalence of *PIK3CA* mutations and age.

[c]No difference in prevalence of *PIK3CA* mutations among different ethnic groups.

[d]No difference in prevalence of *PIK3CA* mutations and site of biopsy.

[e]*KRAS* mutations were more prevalent in patients with *PIK3CA* mutations.

[f]Tested for *KRAS*, $n = 717$ (*PIK3CA* mutation, $n = 86$; wild-type *PIK3CA*, $n = 631$).

NSCLC, 54 (5%) breast cancer, 53 (5%) squamous cell head and neck cancer, 46 (5%) endometrial cancer, and 406 (40%) other tumor types. Detailed patient characteristics are listed in Table 1.

Mutations: types and associations

PIK3CA mutations were detected in 105 (10%) of the 1,012 patients. *PIK3CA* mutations were more frequent in women than men (68/550, 12% vs. 37/462, 8%; $P = 0.029$), which is not

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unexpected taking into account the high prevalence of *PIK3CA* mutations in malignancies such as breast, endometrial, squamous cervical, and ovarian cancer (Table 1). Mutations in exon 9 of *PIK3CA* were found in 67 (64%) patients, in exon 20 in 36 (34%) patients, and in other or more than one exons in 2 (2%) patients. The most frequent mutation was E545K (1633G>A) in 35 (33%) patients, followed by H1047R (3140A>G) in 20 (19%) patients, and E542K (1624G>A) in 19 (18%) patients (Table 2).

Of the 1,012 patients, 717 were tested for *KRAS* and 137 (19%) were found to have mutations. The most prevalent was the G12D mutation (35G>A) present in 41 (30%) patients, G12V mutation (35G>T) in 26 (19%), G13D mutation (38G>A) in 18 (13%), G12A mutation (35G>C) in 12 (9%) patients, and G12C (34G>T) in 12 (9%) patients (Table 2).

Table 2. Types of *PIK3CA* and *KRAS* mutations

Mutation type	*N* (%)
PIK3CA mutations[a]	105
N345K	1 (<1)
E542K	19 (18)
E542V	1 (<1)
E545K	35 (33)
E545A	1 (<1)
E545G	2 (2)
Q546K	2 (2)
Q546P	1 (<1)
Q546R	1 (<1)
S553N	1 (<1)
P539R, E545A	1 (<1)
E545K, D549H	1 (<1)
Exon 9 deletion	1 (<1)
E545A, H1047Y	1 (<1)
Y1021C	1 (<1)
R1023Q	1 (<1)
T1025A	2 (2)
M1043I	2 (2)
M1043V	2 (2)
N1444K	1 (<1)
D1045N	1 (<1)
H1047L	4 (4)
H1047R	20 (19)
G1049R	3 (3)
KRAS[b] mutations	137
G12A	12 (9)
G12C	12 (9)
G12D	41 (30)
G12F	1 (<1)
G12R	5 (4)
G12S	5 (4)
G12V	26 (19)
G13C	1 (<1)
G13D	18 (13)
Q61H	5 (4)
Q61L	2 (1)
Not specified	9 (7)

[a]Tested for *PIK3CA*, $N = 1{,}012$
[b]Tested for *KRAS*, $n = 717$

Patients with *PIK3CA* mutations had a higher prevalence of *KRAS* mutations than patients with wild-type (wt) *PIK3CA* (31/86, 36% vs. 106/631, 17%; $P < 0.001$; Supplementary Table S2). Interestingly, *PIK3CA* mutations in exon 9, compared with others (wt *PIK3CA*, other *PIK3CA* mutation), showed a strong association with *KRAS* mutations (21/53, 40% vs. 116/664, 17%; $P < 0.001$). This trend was not significant for exon 20 *PIK3CA* mutations (9/31, 29% vs. 128/686, 19%; $P = 0.16$).

Of the 1,012 patients, 367 were tested for *NRAS* and 24 (7%) had mutations. The most prevalent was the Q61R mutation (182A>G) present in 13 (54%) patients with a *NRAS* mutation, Q61K mutation (181C>A) in 6 patients (25%), Q61L mutation (182A>T) in 3 patients (13%), G12S mutation (34G>A) in 1 (4%) patient, and G13D (38G>A) in 1 (4%) patient. *NRAS* mutations were not associated with *PIK3CA* mutations.

Furthermore, of the 1,012 patients, 586 were tested for PTEN expression and 88 (15%) showed complete loss of staining. PTEN status was not associated with *PIK3CA* or *KRAS* mutation status.

Patients with H1047R PIK3CA mutation respond to PI3K/AKT/mTOR inhibitors

Response rate. Of the 105 patients with *PIK3CA* mutations, 66 (63%) were prospectively enrolled in clinical trials that included a PI3K/AKT/mTOR inhibitor. These patients were refractory to a median of 3 prior therapies (range, 1–12). Of these 66 patients, 17 (26%) had colorectal cancer, 12 (18%) breast cancer, 10 (15%) ovarian cancer, 9 (14%) endometrial cancer, 6 (9%) squamous cell cervical cancer, 4 (6%) squamous cell head and neck cancer, 2 (3%) renal cancer, and 6 (9%) other cancers (adenoid cystic head and neck cancer, anal squamous cell cancer, appendiceal carcinoma, carcinoma of unknown primary, papillary thyroid cancer, and small intestine cancer; Fig. 1). Most patients (52/66, 79%) received mTORC1 inhibitor (rapalog)-based therapy, 9 (14%) PI3K inhibitor-based therapy, 3 (4%) dual PI3K and mTOR kinase inhibitor-based therapy, and 2 (3%) AKT inhibitor-based therapy (Supplementary Table S1). Single-agent therapies were given to 28 (42%) patients and 38 (58%) received combination therapy. Overall, 11 [17%; 95% confidence interval (CI), 0.10–0.27] patients achieved a PR and an additional 4 (6%; 95% CI, 0.02–0.15) had SD $\geq$ 6 months (rate of SD $\geq$ 6 months/PR 23%; 15/66; 95% CI, 0.14–0.34).

Patients with a *PIK3CA* H1047R mutation compared with patients having other *PIK3CA* mutations had a higher PR rate (6/16, 38% vs. 5/50, 10%; $P = 0.018$) and a higher rate of SD of 6 or more months/PR (7/16, 44% vs. 8/50, 16%; $P = 0.037$). Patients with a *PIK3CA* E545K mutation had an identical PR rate (3/18, 17% vs. 8/48, 17%; $P = 1.00$), and not a significantly different rate of SD of 6 or more months/PR (5/18, 28% vs. 10/48, 21%; $P = 0.53$) compared with patients having other *PIK3CA* mutations. In addition, patients with a *PIK3CA* E542K mutation did not have a significantly different PR rate (1/11, 9% vs. 10/55, 18%; $P = 0.67$), and not a significantly different rate of SD of 6

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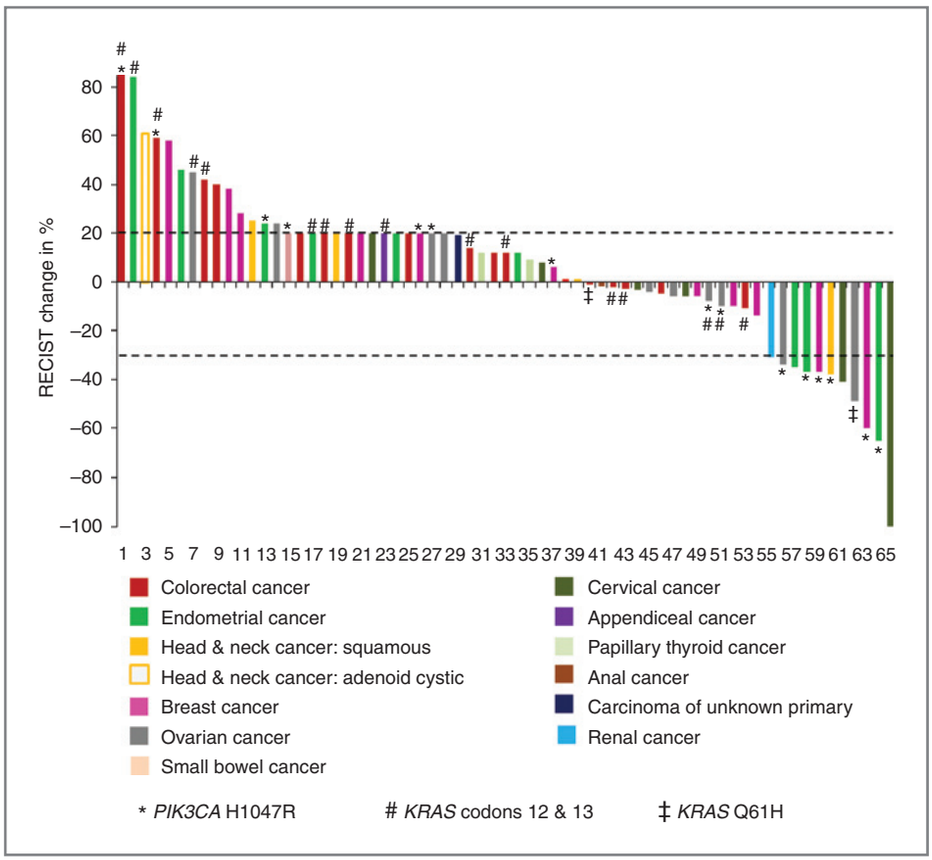

Figure 1. Waterfall plot shows best response for patients with *PIK3CA* mutations treated with PI3K/AKT/mTOR inhibitors. Of the 66 treated patients, 65 are depicted in the waterfall plot (1 patient died of unrelated causes before her first restaging). A total of 11 PRs and 11 minor regressions less than PR were observed. The overall PR rate was 17%. Patients with a H1047R mutation (*) had a PR rate of 38% (6/16 — denominator includes the patient who is not depicted), whereas none of the patients with *KRAS* mutations in codons 12 and 13 (#) had a PR. Two patients with *KRAS* mutation Q61H had a SD of 6 or more months and a PR, respectively (‡).

or more months/PR (1/11, 9% vs. 14/55, 25%; $P = 0.43$) compared with patients having other *PIK3CA* mutations. Finally, we analyzed response in 174 patients with wt *PIK3CA* who received the same therapies as patients with a H1047R *PIK3CA* mutation and found that patients with a *PIK3CA* H1047R mutation compared with patients having wt *PIK3CA* had a higher PR rate (6/16, 38% vs. 23/174, 13%; $P = 0.02$), but not an SD of 6 or more months/PR rate (7/16, 44% vs. 49/174, 28%; $P = 0.25$). Characteristics of patients with H1047R versus other *PIK3CA* mutations are listed in Table 3.

Of the 66 treated patients with *PIK3CA* mutations, 55 (83%) had available tissue for *KRAS* mutation testing. Moreover, of the 16 patients with *PIK3CA* and simultaneous *KRAS* mutations in codon 12 or 13, none had a PR compared with 9 PRs in 39 patients with *PIK3CA* mutations and wt *KRAS* or codon 61 *KRAS* mutations (0/16, 0% vs. 9/39, 23%; $P = 0.046$). Similarly, no patient with *PIK3CA* and simultaneous *KRAS* mutations in codon 12 or 13 had SD of 6 or more months/PR compared with 13 SD of 6 or more months/PR in patients with *PIK3CA* mutations and wt *KRAS* or a codon 61 *KRAS* mutation (0/16, 0% vs. 13/39, 33%; $P = 0.011$). Interestingly, patients ($n = 2$) with *PIK3CA* mutations and simultaneous *KRAS* mutations in codon 61 (Q61H) had either a PR (ovarian cancer, $n = 1$) or SD of 6 or more months (colorectal cancer, $n = 1$).

Furthermore, of the 66 treated patients with *PIK3CA* mutations, 23 (35%) had available tissue for *NRAS* testing and only 1 (4%) had the mutation. This patient had PD after 1.6 months.

Of the 66 treated patients with *PIK3CA* mutations, 32 (48%) had available tissue for PTEN expression testing and 5 (16%) had a complete loss of staining. These 5 patients had SD ranging from 3.7 months to 4.4 months ($n = 2$) or PD ($n = 3$).

Among the other factors considered, patients with *PIK3CA* mutations treated with combination therapies including a PI3K/AKT/mTOR inhibitor had a higher PR rate (11/38, 29% vs. 0/28, 0%; $P = 0.002$) and a higher rate of SD of 6 or more months/PR (14/38, 37%, vs. 1/28, 4%; $P = 0.002$) than patients treated with PI3K/AKT/mTOR inhibitor monotherapies. In addition, patients with colorectal cancer and *PIK3CA* mutations had a trend toward having a lower PR rate (0/17, 0% vs. 11/49, 22%; $P = 0.05$) and a lower rate of SD of 6 or more months/PR (1/17, 6% vs. 14/49, 29%; $P = 0.09$) than patients with *PIK3CA* mutations and other histologies. There was a trend to a higher PR rate (9/38, 24% vs. 2/28, 7%; $P = 0.10$) and rate of SD of 6 or more months/PR (12/38, 32% vs. 3/28, 11%; $P = 0.07$) in patients with *PIK3CA* mutations with up to 3 prior therapies compared with patients who had more than 3 prior therapies. Patients with *PIK3CA* mutations with mutation analysis in primary tumor tissue had similar PR rates (7/39, 18% vs. 4/27, 15%; $P = 1.00$) and rates of SD of 6 or more months/PR (10/39, 26% vs. 5/27, 19%; $P = 0.56$) compared with patients who had mutation analysis done in tissue from metastatic sites.

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Table 3. Characteristics of patients treated with PI3K/AKT/mTOR inhibitors according to the presence of H1047R mutation

Variable	H1047R mutation (%)	Other *PIK3CA* mutations (%)	*P*
Number	16 (100)	50 (100)	
Median of prior therapies, range	3, 1–12	3, 1–10	0.67
Colorectal cancer	2 (13)	15 (30)	0.20
Ovarian cancer	4 (25)	6 (12)	0.24
Breast cancer	5 (31)	7 (14)	0.14
Endometrial cancer	3 (19)	6 (12)	0.68
Head and neck squamous cell cancer	1 (6)	3 (6)	1.00
Small intestine cancer	1 (6)	0 (0)	0.24
Cervical squamous cell cancer	0 (0)	6 (12)	0.32
Renal cell cancer	0 (0)	2 (4)	1.00
Other cancers	0 (0)	5 (10)	0.32
Treatment with PI3K/AKT/mTOR inhibitor-based combinations	12 (75)	26 (52)	0.15
Treatment with rapalogs	14 (88)	38 (76)	0.49

Because most patients (52/66, 79%) received treatment with rapalogs, we conducted a separate analysis for this group and found that patients with a H1047R mutation compared with other *PIK3CA* mutations had a strong trend to a higher rate of PR (6/14, 43% vs. 5/38, 13%; $P = 0.05$) and a higher rate of SD of 6 or more months/PR (7/14, 50% vs. 7/38, 18%; $P = 0.026$). Patients treated with rapalogs in combination with other therapies had a higher PR rate (11/38, 29% vs. 0/28, 0%; $P = 0.002$) and a higher rate of SD of 6 or more months/PR (14/38, 37%, vs. 1/28, 4%; $P = 0.002$) than patients treated with rapalog monotherapies.

A multicovariable logistic regression model, which included number of prior therapies ($\leq$3 vs. >3), histology (colorectal vs. others), *PIK3CA* mutation type (H1047R vs. others), and type of therapy (combination vs. monotherapy), showed that the *PIK3CA* H1047R mutation was the only independent factor predicting a PR (OR 6.6; 95% CI, 1.02–43.0; $P = 0.047$). A separate multivariate model with 55 patients tested for *KRAS* mutations, which included number of prior therapies ($\leq$3 vs. >3), histology (colorectal vs. others), *KRAS* mutation (codons 12 and 13 vs. others), *PIK3CA* mutation type (H1047R vs. others), and type of therapy (combination vs. monotherapy), showed that the *PIK3CA* H1047R mutation was the only factor trending toward statistical significance to predict a PR (OR 9.3; 95% CI, 0.86–100.29; $P = 0.067$); however, this analysis was underpowered to give a definitive answer.

Progression-free survival. The median PFS for all 66 patients with *PIK3CA* mutations treated with PI3K/AKT/mTOR inhibitors was 2 months (95% CI, 1.4–2.6). Patients with *PIK3CA* H1047R mutations compared with patients having other *PIK3CA* mutations trended toward having a longer median PFS (5.7 vs. 2 months; $P = 0.06$; Fig. 2A). Patients with a *PIK3CA* E545K mutation did not have a significantly different median PFS compared with patients who had other *PIK3CA* mutations (3.1 vs. 1.9 months; $P = 0.54$; Fig. 2B). Patients with a *PIK3CA* E542K mutation compared with patients who had other *PIK3CA* mutations had a trend toward having a shorter median PFS (1.8 vs. 2.6 months; $P = 0.06$; Fig. 2C). Finally, we analyzed PFS in 174 patients with wt *PIK3CA* who received the same therapies as patients with H1047R *PIK3CA* mutations and found that patients with a *PIK3CA* H1047R mutation compared with patients having wt *PIK3CA* had a similar median PFS (5.7 vs. 3.5 months; $P = 0.34$).

Of the 55 patients with *PIK3CA* mutations tested for *KRAS* mutations, patients with codon 12 and 13 mutations had a shorter median PFS compared with patients who had wt *KRAS* or codon 61 mutations (1.8 vs. 2.6 months; $P = 0.046$; Fig. 2D).

Patients with *PIK3CA* mutations treated with combination therapies had a longer median PFS than patients treated with monotherapies (3.1 vs. 1.8 months; $P = 0.004$) and patients with *PIK3CA* mutations and colorectal cancer had a trend toward having a shorter median PFS than patients with *PIK3CA* mutations and other histologies (1.9 vs. 2.5 months; $P = 0.11$). There was no difference in median PFS between patients with up to 3 prior therapies compared with patients who had more than 3 prior therapies (1.9 months vs. 2.6 months; $P = 0.13$).

In a subgroup of patients treated with rapalogs, patients with a H1047R mutation compared with other *PIK3CA* mutations had a longer median PFS (8.2 vs. 2 months; $P = 0.023$). Patients treated with rapalogs in combination with other therapies had a longer median PFS (2.2 vs. 1.7 months; $P = 0.005$) than patients treated with rapalog monotherapies.

A multicovariable Cox regression model, which included, histology (colorectal vs. others), *PIK3CA* mutation type (H1047R vs. others and E542K vs. others), and type of therapy (combination vs. monotherapy), showed prolonged PFS in patients treated with combination therapies (HR 0.51; 95% CI, 0.27–0.94; $P = 0.03$). A separate multicovariable Cox regression model with 55 patients tested for *KRAS* mutations, which included histology (colorectal vs. others), *KRAS* mutation (codons 12 and 13 vs. others), *PIK3CA* mutation type

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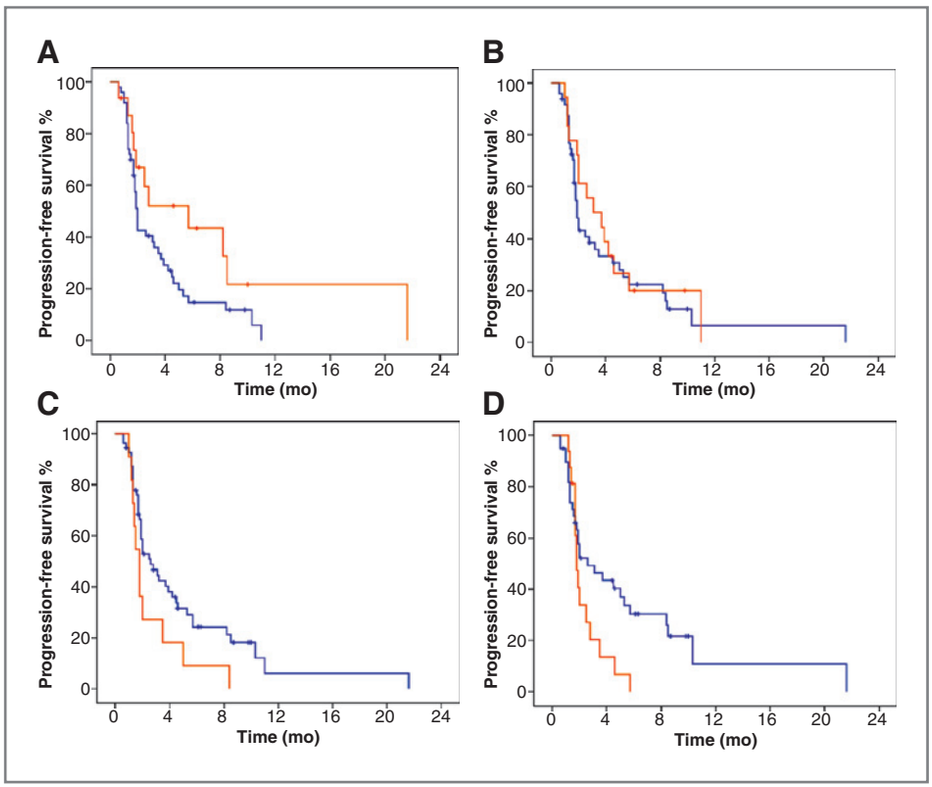

Figure 2. Kaplan–Meier plot for PFS. Tick marks represent patients who were progression-free at last follow-up and are censored at that point. A, patients with a *PIK3CA* H1047R mutation (yellow) showed a trend toward having a longer median PFS compared with patients with other *PIK3CA* mutations (blue; 5.7 vs. 2 months; $P = 0.06$). B, patients with a *PIK3CA* E545K mutation (yellow) did not have a significantly different median PFS compared with patients with other *PIK3CA* mutations (blue; 3.1 vs. 1.9 months; $P = 0.54$). C, patients with a *PIK3CA* E542K mutation (yellow) compared with patients with other *PIK3CA* mutations (blue) had a trend toward having a shorter median PFS (1.8 vs. 2.6 months; $P = 0.06$). D, patients with *PIK3CA* and simultaneous *KRAS* mutations either in codon 12 or 13 (blue) had a shorter median PFS compared with patients with *PIK3CA* mutations and wt *KRAS* or codon 61 mutations (yellow; 1.8 vs. 2.6 months; $P = 0.046$).

(H1047R vs. others and E542K vs. others), and type of therapy (combination vs. monotherapy), showed that none of factors tested independently predicted prolonged PFS.

Overall survival. The median OS for all patients with *PIK3CA* mutations treated with PI3K/AKT/mTOR inhibitors was 9.6 months (95% CI, 7.1–12.1) and there were no significant differences among *PIK3CA* mutation subtypes (H1047R, E545K, E542K) and *KRAS* mutation subtypes (codon 12 and 13). In addition, there was no difference in a median OS between combinations versus monotherapies and in patients with up to 3 prior treatment regimens compared with patients who had more than 3 prior treatment regimens. However, patients with colorectal cancer compared with other histologies had a shorter median OS (5.4 vs. 11.1 months; $P = 0.025$).

In a subgroup of patients treated with rapalogs, patients with a H1047R mutation had a similar median OS as patients with other *PIK3CA* mutations (7.5 vs. 8.9 months, $P = 0.96$). Patients treated with rapalogs in combination with other therapies had a longer median OS (10.0 vs. 3.6 months; $P = 0.029$) than patients treated with rapalog monotherapies.

A multicovariable Cox regression model, which included number of prior therapies (≤ 3 vs. >3) and histology (colorectal vs. others), showed a trend to shorter OS in patients with colorectal cancer (HR 2.01; 95% CI, 0.91–4.74; $P = 0.08$).

Discussion

In the current study, in heavily pretreated patients with *PIK3CA* mutations, the overall SD of 6 or more months/PR rate following PI3K/AKT/mTOR inhibitor treatment was 23% (with 17% of all patients attaining a PR). It is known that EGFR inhibitors preferentially induce response in NSCLC with certain *EGFR* mutations and, similarly, KIT inhibitors are preferentially active in gastrointestinal stromal tumors only with specific *KIT* mutations (15, 16). It is, therefore, plausible that certain *PIK3CA* mutation types predict enhanced sensitivity to PI3K/AKT/mTOR inhibitors. Alternatively, resistance might be driven by molecular aberrations in other relevant pathways. Our group and others showed that, in colorectal and other cancers, *PIK3CA* mutations often coexist with *KRAS* mutations, and preclinical data suggested that having a concurrent *KRAS* mutation might account for resistance to PI3K/AKT/mTOR pathway inhibitors (8–10, 22–24). In agreement with these previous reports, we found in this study that cancers with *PIK3CA* mutations compared with wt *PIK3CA* have a higher prevalence of simultaneous *KRAS* mutations (36% vs. 17%; $P < 0.001$). This was mainly due to the prevalence of simultaneous *KRAS* mutations in patients with *PIK3CA* exon 9 mutations compared with prevalence of *KRAS* mutations in patients without *PIK3CA* exon 9 mutations (40% vs. 17%; $P < 0.001$. In contrast, *PIK3CA* exon 20 mutations were not statistically associated with simultaneous *KRAS* mutations (29% vs. 19%; $P = 0.16$). Similar observations have been reported in patients with metastatic colorectal cancer (23).

Furthermore, in the current study, we analyzed treatment outcomes (PR, PFS, and OS) for patients with the most frequent *PIK3CA* mutations such as E545K and E542K in exon 9 coding for the helical domain, and H1047R in exon 20 coding for the

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kinase domain, and we found that patients with a *PIK3CA* H1047R mutation compared with patients with other *PIK3CA* mutations treated on the same protocols had a higher PR rate (38% vs. 10%; $P = 0.018$) and a trend toward having a longer median PFS (5.7 months vs. 2 months; $P = 0.06$). Similarly, patients with a *PIK3CA* H1047R mutation compared with patients with wt *PIK3CA* treated on the same protocols had a higher PR rate (38% vs. 13%; $P = 0.02$); they did not, however, have a statistically significant longer median PFS (5.7 months vs. 3.5 months; $P = 0.34$). In addition, patients with *PIK3CA* mutations and simultaneous *KRAS* mutations in codon 12 or 13 compared with patients who had *PIK3CA* mutations and wt *KRAS* or codon 61 *KRAS* mutations had a significantly lower PR rate (0% vs. 23%; $P = 0.046$). However, *KRAS* mutations in codon 12 or 13 were not confirmed as an independent factor for a PR in multivariate analysis. These observations regarding the relevance of *KRAS* aberrations have to be interpreted with caution because of the low numbers of patients and because differences in outcomes can be influenced by other factors such as type of therapy and histology. Of potential interest, both patients with *PIK3CA* and simultaneous *KRAS* mutations in codon 61 (Q61H) attained either a PR (ovarian cancer, $n = 1$) or SD of 6 or more months (colorectal cancer, $n = 1$). Patients with *PIK3CA* mutations and codon 12 and 13 *KRAS* mutations had a shorter median PFS compared with patients who had *PIK3CA* mutations and wt *KRAS* or codon 61 mutations (1.8 months vs. 2.6 months; $P = 0.046$). Patients with colorectal cancer and *PIK3CA* mutations treated with a PI3K/AKT/mTOR inhibitor had a strong trend toward having a lower PR rate compared with other cancers with *PIK3CA* mutations (0% vs. 22%; $P = 0.05$). This trend might be explained by the frequent presence of simultaneous *KRAS* mutations and low prevalence of H1047R mutations in the patients with colorectal cancer (22, 23). In addition, it is possible that *PIK3CA* mutations may have different roles in different histologies. A similar phenomenon has been reported for *BRAF* mutations. A *BRAF* V600E mutation is highly predictive for response to BRAF inhibitors in melanoma, but not in colorectal cancer (25, 26).

In our study, all PRs in *PIK3CA* mutant patients were observed when combination therapies including a PI3K/AKT/mTOR inhibitor were used (29% vs. 0%, $P = 0.002$; combination therapy including a PI3K/AKT/mTOR inhibitor vs. single-agent PI3K/AKT/mTOR inhibitor). However, combinations were used as frequently in the wt *PIK3CA* group, and the response rate was significantly lower, suggesting that it is not the use of combinations in and of itself that mediates response. The need for combination therapy, rather than treatment with single-agent PI3K/AKT/mTOR inhibitors, was not unexpected as patients with advanced cancer, including those with *PIK3CA* mutations, are likely to have other driver aberrations as well (9, 10, 27–30). Furthermore, preclinical models showed that breast cancer cell lines with *PIK3CA* mutations develop an apoptotic response to the single-agent BEZ235 PI3K inhibitor only in the presence of BIM expression, whereas paclitaxel was similarly effective irrespective of BIM expression (31). In addition, *PIK3CA* mutations can increase the expression of other factors such as heregulin, which lead to oncogenic pathway activation, independent of PI3K (29). Last but not least, single-agent mTOR inhibition with rapalogs or even with mTOR kinase inhibitors can lead to feedback activation of AKT signaling (27, 28).

There are several limitations to our data (32). First, this study was retrospective and not randomized, and our findings need to be confirmed in a more controlled fashion. Second, the number of patients treated with PI3K/AKT/mTOR inhibitors was relatively small. Third, the treatments used and the patient population were heterogeneous, although this might also imply that the relevance of H1047R is not limited to any one drug or histology. It remains plausible, however, that among distinct histologies, *PIK3CA* mutations may or may not function in precisely the same role (principal driver mutation vs. passenger mutation). Further studies are needed to explore the relationship between different agents and sensitivity based on specific mutations. Finally, improved response rates did not translate to improved survival, although the relatively small number of patients might preclude definitive conclusions.

In summary, we have shown that heavily pretreated patients with advanced cancers who harbor a *PIK3CA* H1047R mutation may be more sensitive to therapeutic targeting with PI3K/AKT/mTOR pathway inhibitors. In multicovariable analysis, having a *PIK3CA* H1047R mutation was the only independent factor predicting a response. Therefore, the role of *PIK3CA* H1047R mutations warrants further investigation in the setting of prospective controlled trials with the application of targeted PI3K/AKT/mTOR inhibitors in the clinic.

Disclosure of Potential Conflicts of Interest

Filip Janku has a commercial research grant from Novartis. Razelle Kurzrock has commercial research grants from GlaxoSmithKline, Novartis, Merck, and Bayer. A.M. Tsimberidou is a consultant/advisory board member of CTI. No potential conflicts were disclosed by the other authors.

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PIK3CA Mutation H1047R Is Associated with Response to PI3K/AKT/mTOR Signaling Pathway Inhibitors in Early-Phase Clinical Trials

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