PIK3CA mutation H1047R is associated with response to PI3K/AKT/mTOR signaling pathway inhibitors in early phase clinical trials

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Short title: PI3K/mTOR inhibitors in cancers with PIK3CA H1047R mutation

Financial support: Supported by Grant Number RR024148 from the National Center for Research Resources, a component of the NIH Roadmap for Medical Research (http://nihroadmap.nih.gov/clinicalresearch/overview-translational.asp).

Conflict of interest: Filip Janku has research support from Novartis. Razelle Kurzrock has research support from Glaxo Smith Kline, Novartis, Merck, and Bayer.
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 to patients with 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 ≤ 0.02). None of the 16 patients with co-existing PIK3CA and KRAS mutations in codon 12 or 13 attained a PR (0/16, 0%). Patients treated with combination therapy vs. 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 (odds ratio (OR) 6.6, 95% CI 1.02-43.0, p = 0.047). Our data suggest that interaction between PIK3CA mutation H1047R vs other aberrations and response to PI3K/AKT/mTOR axis inhibitors warrants further exploration.
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.\textsuperscript{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.\textsuperscript{8-14} Patients with diverse tumors and PIK3CA mutations demonstrated a response rate of 35\% in early phase clinical trials with PI3K/AKT/mTOR inhibitors compared to 6\% in patients without PIK3CA mutations.\textsuperscript{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.\textsuperscript{15, 16} In the preclinical setting, PIK3CA mutation H1047R was a stronger driver of tumor development than E545K or E542K and demonstrated sensitivity to the mTOR inhibitor everolimus.\textsuperscript{17} In addition, immortalized fibroblasts with the H1047R PIK3CA mutation resulted in greater activation of AKT than E545K and E542K mutations.\textsuperscript{18} Finally, preclinical characterization of PWT33597, a dual inhibitor of PI3K and mTOR demonstrated a lower IC50 for H1047R than E545K (86nM) or E542K (87nM) (21nM vs. 86 nM and 87 nM, respectively).\textsuperscript{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).
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 performed 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 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 microdissected paraffin-embedded tumor sections and analyzed using a polymerase chain reaction-based DNA sequencing method for PIK3CA mutations in codons 532-554 of exon 9 (helical domain) and codons 1011-1062 of exon 20 (kinase domain). This included the mutation hot spot region of the PIK3CA proto-oncogene denoted by Sanger sequencing, following amplification of 276 bp and 198 bp amplicons, respectively; utilizing 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 hot spots in exon 1 (Q60K, R88Q, E110K and K111N), exon 4 (N345K), exon 6 (S405S), exon 7 (E418K, C420R, E453K), exon 9 (P539R, E542 [base 1 and 2], E545 [all 3 bases] and Q546 [base 1 and 2]), exon 18 (F909L) and exon...
20 (Y1021 [base 1 and 2], T1025 [base 1], M1043I, M1043V, A1046V, H1047Y, H1047, 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 1-2.20 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, Carpinteria, CA, USA) 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 1). 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 MD Anderson and reviewed in the Department of Investigational Cancer Therapeutics tumor measurement clinic. Responses were categorized per RECIST 1.0 criteria.21 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 PR nor sufficient increase to qualify for PD.

**Statistical analysis**

Two-way contingency tables were employed to summarize the relationship between two categorical variables. Fisher’s exact test was used to assess the association among categorical variables and PIK3CA mutation status. A Wilcoxon rank-sum test was applied to assess the association among continuous variables and PIK3CA 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 two-sided, and P values less than 0.05 were considered statistically significant. All statistical analyses were carried out using SPSS 17 computer software (SPSS Chicago, IL).
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 to 91 years) and 796 (79%) were Caucasian, 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%) 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 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 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).

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
Interestingly, PIK3CA mutations in exon 9 compared to 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.

Of the 1,012 patients, 586 were tested for PTEN expression and 88 (15%) demonstrated 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 of 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 three prior therapies (range, 1 to 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) (Figure 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 1). Single-agent therapies were given to 28 (42%) patients and 38 (58%) received combination
therapy. Overall, 11 (17%, 95% CI 0.10-0.27) patients achieved a PR and an additional 4 (6%, 95% CI 0.02-0.15) had SD≥6 months (rate of SD≥6 months/PR 23%, 15/66, 95% CI 0.14-0.34).

Patients with a PIK3CA H1047R mutation compared to patients with other PIK3CA mutations had a higher PR rate (6/16, 38% vs. 5/50, 10%; p=0.018), and a higher rate of SD≥6 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 a not significantly different rate of SD≥6 months/PR (5/18, 28% vs. 10/48, 21%; p=0.53) compared to patients with 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 no significantly different rate of SD≥6 months/PR (1/11, 9% vs. 14/55, 25%; p=0.43) compared to patients with 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 to patients with wt PIK3CA had a higher PR rate (6/16, 38% vs. 23/174, 13%; p=0.02), but not SD≥6 months/PR rate (7/16, 44% vs. 49/174, 28%; p=0.25). Characteristics of patients with H1047R vs. 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. Of the 16 patients with PIK3CA and simultaneous KRAS mutations in codon 12 or 13 none had a PR compared to 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≥6 months/PR compared to 13 SD≥6 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≥6 months (colorectal cancer, n=1).
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 five patients had had SD ranging from 3.7 months to 4.4 months (n=2) or PD (n=3).

Of 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≥6 months/PR (14/38, 37%, vs. 1/28, 4%; p=0.002) than patients treated with PI3K/AKT/mTOR inhibitor monotherapies. Also, 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≥6 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≥6 months/PR (12/38, 32% vs. 3/28, 11%; p=0.07) in patients *PIK3CA* mutations with up to 3 prior therapies compared to patients with 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≥6 months/PR (10/39, 26% vs. 5/27, 19%; p=0.56) compared to patients with mutation analysis done in tissue from metastatic sites.

Since most patients (52/66, 79%) received treatment with rapalogs, we performed a separate analysis for this group and found that patients with a H1047R mutation compared to 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≥6 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≥6 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 (<3 vs. >3), histology (colorectal vs. others), PIK3CA mutation type (H1047R vs. others), type of therapy (combination vs. monotherapy), demonstrated 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 (<3 vs. >3), histology (colorectal vs. others), KRAS mutation (codons 12 and 13 vs. others), PIK3CA mutation type (H1047R vs. others), type of therapy (combination vs. monotherapy), demonstrated that the PIK3CA H1047R mutation was the only factor trending towards 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 PIK3CA mutations treated with PI3K/AKT/mTOR inhibitors was 2 months (95% CI 1.4-2.6). Patients with PIK3CA H1047R mutations compared to patients with other PIK3CA mutations trended toward having a longer median PFS (5.7 months vs. 2 months; p=0.06). Patients with a PIK3CA E545K mutation did not have a significantly different median PFS compared to patients with other PIK3CA mutations (3.1 months vs. 1.9 months; p=0.54). Patients with a PIK3CA E542K mutation compared to patients with other PIK3CA mutations had a trend toward having a shorter median PFS (1.8 months vs. 2.6 months; p=0.06). 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 to patients with wt PIK3CA had a similar median PFS (5.7 months 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 to patients with wt KRAS or codon 61 mutations (1.8 months vs. 2.6 months; p=0.046).
Patients with PIK3CA mutations treated with combination therapies had a longer median PFS than patients treated with monotherapies (3.1 months 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 months vs. 2.5 months; p=0.11). There was no difference in median PFS between patients with up to 3 prior therapies compared to patients with 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 to other PIK3CA mutations had a longer median PFS (8.2 months vs. 2 months; p=0.023). Patients treated with rapalogs in combination with other therapies had a longer median PFS (2.2 months vs. 1.7 months; p=0.0005) than patients treated with rapalog monotherapies.

A multivariable Cox regression model, which included, histology (colorectal vs. others), PIK3CA mutation type (H1047R vs. others and E542K vs. others), type of therapy (combination vs. monotherapy), demonstrated prolonged PFS in patients treated with combination therapies (HR 0.51, 95% CI 0.27-0.94; p=0.03). A separate multivariable 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 (H1047R vs. others and E542K vs. others), 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). Also there was no difference in a median OS between combinations vs. monotherapies and in patients with up to 3 prior treatment regimens compared to patients with more than 3.
prior treatment regimens. However, patients with colorectal cancer compared to other histologies had a shorter median OS (5.4 months 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 months vs. 8.9 months. P=0.96). Patients treated with rapalogs in combination with other therapies had a longer median OS (10.0 months 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), histology (colorectal vs. others) demonstrated 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>6 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 demonstrated 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 to wt PIK3CA have a higher prevalence of simultaneous KRAS mutations (36% vs. 17%; \(p<0.001\)). That was mainly due to the prevalence of simultaneous KRAS mutations in patients with PIK3CA exon 9 compared to 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.\(^{23}\)

In the current study, we also analyzed treatment outcomes (PR, PFS, OS) for patients with the most frequent PIK3CA mutations such as E545K, E542K in exon 9 coding for the helical domain and H1047R in exon 20 coding for the kinase domain, and we found that patients with a PIK3CA H1047R mutation compared to patients with other PIK3CA mutations treated on the same protocols had a higher PR rate (38% vs. 10%; \(p=0.018\)) and a trend towards having a longer median PFS (5.7 months vs. 2 months; \(p=0.06\)). Similarly, patients with a PIK3CA
H1047R mutation compared to 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 to patients with 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≥6 months (colorectal cancer, n=1). Patients with PIK3CA mutations and codon 12 and 13 KRAS mutations had a shorter median PFS compared to patients with 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 to 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. It is also 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. 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 Further, preclinical models demonstrated 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 Also, 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, though 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, though the relatively small number of patients might preclude definitive conclusions.

In summary, we have demonstrated 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.
ACKNOWLEDGEMENTS

We thank Ms. Joann Aaron for scientific review and editing of this article. This study was supported in part by Grant Number RR024148 from the National Center for Research Resources, a component of the NIH Roadmap for Medical Research (http://nihroadmap.nih.gov/clinicalresearch/overview-translational.asp).
REFERENCES


Table 1: Patient characteristics (n=1,012)

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<td></td>
<td></td>
<td>0.26&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>58; range, 13-91</td>
<td></td>
<td>56; range, 16-81</td>
<td>59; range,13-91</td>
<td></td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Caucasian</td>
<td>796 (79)</td>
<td>81 (77)</td>
<td>715 (79)</td>
<td>0.24&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>African-American</td>
<td>96 (9)</td>
<td>13 (12)</td>
<td>83 (9)</td>
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</tr>
<tr>
<td>Hispanic</td>
<td>74 (7)</td>
<td>4 (4)</td>
<td>70 (8)</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>46 (5)</td>
<td>7 (7)</td>
<td>39 (4)</td>
<td></td>
</tr>
<tr>
<td><strong>Site of biopsy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary tumor</td>
<td>514 (51)</td>
<td>59 (56)</td>
<td>455 (50)</td>
<td>0.26&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Metastatic tumor</td>
<td>498 (49)</td>
<td>46 (44)</td>
<td>452 (50)</td>
<td></td>
</tr>
<tr>
<td><strong>Tumor type</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colorectal</td>
<td>195 (19)</td>
<td>32 (30)</td>
<td>163 (18)</td>
<td>NA</td>
</tr>
<tr>
<td>Ovarian</td>
<td>112 (11)</td>
<td>11 (10)</td>
<td>101 (11)</td>
<td></td>
</tr>
<tr>
<td>Melanoma</td>
<td>88 (9)</td>
<td>2 (2)</td>
<td>86 (9)</td>
<td></td>
</tr>
<tr>
<td>Non-small cell lung</td>
<td>58 (6)</td>
<td>5 (5)</td>
<td>53 (6)</td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>54 (5)</td>
<td>15 (14)</td>
<td>39 (4)</td>
<td></td>
</tr>
<tr>
<td>Head &amp; neck: squamous</td>
<td>53 (5)</td>
<td>8 (8)</td>
<td>45 (5)</td>
<td></td>
</tr>
<tr>
<td>Endometrial</td>
<td>46 (5)</td>
<td>12 (11)</td>
<td>34 (4)</td>
<td></td>
</tr>
<tr>
<td>Thyroid</td>
<td>40 (4)</td>
<td>2 (2)</td>
<td>38 (4)</td>
<td></td>
</tr>
<tr>
<td>Soft tissue sarcomas</td>
<td>37 (4)</td>
<td>0 (0)</td>
<td>37 (4)</td>
<td></td>
</tr>
<tr>
<td>Renal cell</td>
<td>29 (3)</td>
<td>2 (2)</td>
<td>27 (3)</td>
<td></td>
</tr>
<tr>
<td>Pancreatic</td>
<td>26 (3)</td>
<td>1 (1)</td>
<td>25 (3)</td>
<td></td>
</tr>
<tr>
<td>Gastric</td>
<td>25 (2)</td>
<td>1 (1)</td>
<td>24 (3)</td>
<td></td>
</tr>
<tr>
<td>Prostate</td>
<td>24 (2)</td>
<td>0 (0)</td>
<td>24 (3)</td>
<td></td>
</tr>
<tr>
<td>Neuroendocrine</td>
<td>24 (2)</td>
<td>1 (1)</td>
<td>23 (3)</td>
<td></td>
</tr>
<tr>
<td>Cervical: squamous</td>
<td>20 (2)</td>
<td>6 (6)</td>
<td>14 (2)</td>
<td></td>
</tr>
<tr>
<td>Biliary tract</td>
<td>20 (2)</td>
<td>0 (0)</td>
<td>20 (2)</td>
<td></td>
</tr>
<tr>
<td>Salivary gland</td>
<td>17 (2)</td>
<td>0 (0)</td>
<td>17 (2)</td>
<td></td>
</tr>
<tr>
<td>Head and neck: non-squamous</td>
<td>15 (1)</td>
<td>2 (2)</td>
<td>13 (1)</td>
<td></td>
</tr>
<tr>
<td>Hepatocellular</td>
<td>12 (1)</td>
<td>0 (0)</td>
<td>12 (1)</td>
<td></td>
</tr>
<tr>
<td>Cervical: adenocarcinoma</td>
<td>10 (1)</td>
<td>0 (0)</td>
<td>10 (1)</td>
<td></td>
</tr>
<tr>
<td>Ewing sarcoma</td>
<td>10 (1)</td>
<td>0 (0)</td>
<td>10 (1)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>97 (10)</td>
<td>5 (5)</td>
<td>92 (10)</td>
<td></td>
</tr>
<tr>
<td><strong>KRAS mutations – total tested</strong></td>
<td>717 (100)</td>
<td>86 (100)</td>
<td>631 (100)</td>
<td>&lt;0.001&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>KRAS mutated&lt;sup&gt;e&lt;/sup&gt;</td>
<td>137 (19)</td>
<td>31 (36)</td>
<td>106 (17)</td>
<td></td>
</tr>
<tr>
<td>KRAS wild-type&lt;sup&gt;e&lt;/sup&gt;</td>
<td>580 (81)</td>
<td>55 (64)</td>
<td>525 (83)</td>
<td></td>
</tr>
</tbody>
</table>

NA: not applicable

<sup>a</sup> PIK3CA mutations were more prevalent in women than in men.
<sup>b</sup> No difference in prevalence of PIK3CA mutations and age.
<sup>c</sup> No difference in prevalence of PIK3CA mutations among different ethnic groups.
<sup>d</sup> No difference in prevalence of PIK3CA mutations and site of biopsy.
<sup>e</sup> KRAS mutations were more prevalent in patients with PIK3CA mutations.
<sup>f</sup> Tested for KRAS, n=717 (PIK3CA mutation, n=86; wild-type PIK3CA, n=631).
Table 2: Types of PIK3CA and KRAS mutations

<table>
<thead>
<tr>
<th>Mutation type</th>
<th>N (%)</th>
</tr>
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<tbody>
<tr>
<td><strong>PIK3CA mutations</strong>*</td>
<td></td>
</tr>
<tr>
<td>N345K</td>
<td>1 (&lt;1)</td>
</tr>
<tr>
<td>E542K</td>
<td>19 (18)</td>
</tr>
<tr>
<td>E542V</td>
<td>1 (&lt;1)</td>
</tr>
<tr>
<td>E545K</td>
<td>35 (33)</td>
</tr>
<tr>
<td>E545A</td>
<td>1 (&lt;1)</td>
</tr>
<tr>
<td>E545G</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Q546K</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Q546P</td>
<td>1 (&lt;1)</td>
</tr>
<tr>
<td>Q546R</td>
<td>1 (&lt;1)</td>
</tr>
<tr>
<td>S553N</td>
<td>1 (&lt;1)</td>
</tr>
<tr>
<td>P539R, E545A</td>
<td>1 (&lt;1)</td>
</tr>
<tr>
<td>E545K, D549H</td>
<td>1 (&lt;1)</td>
</tr>
<tr>
<td>Exon 9 deletion</td>
<td>1 (&lt;1)</td>
</tr>
<tr>
<td>E545A, H1047Y</td>
<td>1 (&lt;1)</td>
</tr>
<tr>
<td>Y1021C</td>
<td>1 (&lt;1)</td>
</tr>
<tr>
<td>R1023Q</td>
<td>1 (&lt;1)</td>
</tr>
<tr>
<td>T1025A</td>
<td>2 (2)</td>
</tr>
<tr>
<td>M1043I</td>
<td>2 (2)</td>
</tr>
<tr>
<td>M1043V</td>
<td>2 (2)</td>
</tr>
<tr>
<td>N1444K</td>
<td>1 (&lt;1)</td>
</tr>
<tr>
<td>D1045N</td>
<td>1 (&lt;1)</td>
</tr>
<tr>
<td>H1047L</td>
<td>4 (4)</td>
</tr>
<tr>
<td>H1047R</td>
<td>20 (19)</td>
</tr>
<tr>
<td>G1049R</td>
<td>3 (3)</td>
</tr>
<tr>
<td><strong>KRAS</strong> mutations **</td>
<td></td>
</tr>
<tr>
<td>G12A</td>
<td>12 (9)</td>
</tr>
<tr>
<td>G12C</td>
<td>12 (9)</td>
</tr>
<tr>
<td>G12D</td>
<td>41 (30)</td>
</tr>
<tr>
<td>G12F</td>
<td>1 (&lt;1)</td>
</tr>
<tr>
<td>G12R</td>
<td>5 (4)</td>
</tr>
<tr>
<td>G12S</td>
<td>5 (4)</td>
</tr>
<tr>
<td>G12V</td>
<td>26 (19)</td>
</tr>
<tr>
<td>G13C</td>
<td>1 (&lt;1)</td>
</tr>
<tr>
<td>G13D</td>
<td>18 (13)</td>
</tr>
<tr>
<td>Q61H</td>
<td>5 (4)</td>
</tr>
<tr>
<td>Q61L</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Not specified</td>
<td>9 (7)</td>
</tr>
</tbody>
</table>

* Tested for PIK3CA, n=1,012
** Tested for KRAS, n=717
Table 3: Characteristics of patients treated with PI3K/AKT/mTOR inhibitors according to the presence of H1047R mutation

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

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 (one patient died of unrelated causes prior to 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 (marked with *) had a PR rate of 37.5% (6/16 – denominator includes the patient, who is not depicted), whereas none of the patients with KRAS mutations in codons 12 and 13 (marked with #) had a PR. Two patients with KRAS mutation Q61H had a SD > 6 months and a PR, respectively (marked with ‡).

Figure 2: Kaplan-Meier plot for progression-free survival (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) demonstrated a trend toward having a longer median PFS compared to patients with other PIK3CA mutations (blue) (5.7 months vs. 2 months; p=0.06). B. Patients with a PIK3CA E545K mutation (yellow) did not have a significantly different median PFS compared to patients with other PIK3CA mutations (blue) (3.1 months vs. 1.9 months; p=0.54). C. Patients with a PIK3CA E542K mutation (yellow) compared to patients with other PIK3CA mutations (blue) had a trend toward having a shorter median PFS (1.8 months 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 to patients with PIK3CA mutations and wt KRAS or codon 61 mutations (yellow) (1.8 months vs. 2.6 months; p=0.046).
Figure 2

A

B

C

D

Progression-free survival %

Time (months)

Progression-free survival %

Time (months)

Progression-free survival %

Time (months)

Progression-free survival %

Time (months)
PIK3CA mutation H1047R is associated with response to PI3K/AKT/mTOR signaling pathway inhibitors in early phase clinical trials

Filip Janku, Jennifer J. Wheler, Aung Naing, et al.

Cancer Res  Published OnlineFirst October 12, 2012.