Published in final edited form as:

Cell Rep. 2014 January 30; 6(2): 377–387. doi:10.1016/j.celrep.2013.12.035.

PIK3CA, and PTEN Aberrations in Early-Phase Trials with PI3K/AKT/mTOR Inhibitors: Experience with 1,656 Patients at MD Anderson Cancer Center

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Abstract

Despite a wealth of preclinical studies, it is unclear whether *PIK3CA* or PTEN gene aberrations are actionable in the clinical setting. Of 1,656 patients with advanced, refractory cancers tested for *PIK3CA* or PTEN abnormalities, *PIK3CA* mutations were found in 9% (146/1,589), and PTEN loss and/or mutation in 13% (149/1,157). In multicovariable analysis, treatment with a PI3K/AKT/mTOR inhibitor was the only independent factor predicting response to therapy in individuals harboring a *PIK3CA* or *PTEN* aberration. The rate of stable disease (SD) ≥6 months/partial response reached 45% in a subgroup of individuals with H1047R *PIK3CA* mutations. Aberrations in the PI3K/AKT/mTOR pathway are common and potentially actionable in patients with diverse advanced cancers. This work provides further important clinical validation for continued and accelerated use of biomarker-driven trials incorporating rational drug combinations.

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CONFLICT OF INTEREST

Filip Janku has research support from Novartis. Razelle Kurzrock has research support from GlaxoSmithKline, Novartis, Merck, and Bayer.

Keywords

PIK3CA; PTEN; KRAS; NRAS; BRAF; Cancer; Clinical trial

INTRODUCTION

The PI3K/AKT/mTOR pathway is frequently activated in many human cancers, often via molecular abnormalities such as *PIK3CA* mutations or loss of PTEN function.(Engelman, 2009; Hollander et al., 2011; Samuels et al., 2004) Preclinical models and early clinical data in several tumor types suggested that *PIK3CA* mutations and loss of PTEN function can result in increased sensitivity to therapies targeting the PI3K/AKT/mTOR signaling pathway.(Di Nicolantonio et al., 2010; Engelman et al., 2008; Ihle et al., 2009; Janku et al., 2011b; Moroney et al., 2011; Ni et al., 2012; Tsimberidou et al., 2012; Wee et al., 2008; Weigelt et al., 2011)

Patients with gynecological and breast tumors and *PIK3CA* mutations demonstrated a partial response (PR) rate of 30% in early phase clinical trials with PI3K/AKT/mTOR inhibitors compared to 10% in patients without *PIK3CA* mutations.(Janku et al., 2012b) It is conceivable that loss of PTEN function, which is a major negative regulator of the pathway, can be similarly predictive, whereas simultaneous mutations in the mitogen-activated protein kinase (MAPK) pathway may lead to therapeutic resistance.(Di Nicolantonio et al., 2010; Engelman et al., 2008; Ihle et al., 2009; Tsimberidou et al., 2012)

Identifying actionable molecular aberrations has been critical to several major therapeutic advances in cancer medicine. Examples include *BCR-ABL* fusion in chronic myeloid leukemia (CML), epidermal growth factor (*EGFR*) mutations and *EML4-ALK* fusion in non-small cell lung cancer, and *BRAF* mutations in melanoma.(Druker et al., 2001; Falchook et al., 2012; Flaherty et al., 2010; Lynch et al., 2004) Therefore, we investigated the relationship among *PIK3CA* mutations and PTEN aberrations and treatment outcomes in patients with advanced cancer who were referred to the Clinical Center for Targeted Therapy at The University of Texas MD Anderson Cancer Center (MD Anderson).

RESULTS

Patients

A total of 1,656 patients with diverse advanced cancers were analyzed for the presence of *PIK3CA* mutations and/or PTEN aberrations (Table 1). Their median age was 59 years (range, 13 to 92 years) and most patients 1,288 (77%) were White. The most common tumor types were colorectal cancer 298 (18%), ovarian cancer 184 (11%), and melanoma 126 (8%).

PIK3CA mutations and PTEN aberrations

Of the 1,656 patients, 1,589 were tested for *PIK3CA* mutations, 1,157 for PTEN aberrations, and 1,090 for both *PIK3CA* mutations and PTEN aberrations. *PIK3CA* mutations were detected in 9% (146/1,589) of patients; PTEN aberrations, in 13% (149/1,157); and

simultaneous *PIK3CA* mutations and PTEN aberrations, in 1% (14/1,090). When analyzing 1,090 patients, who were tested for both *PIK3CA* mutations and PTEN aberrations, 89 (8%) had *PIK3CA* mutations, 134 (12%) PTEN aberrations, and 14 (1%) had simultaneous *PIK3CA* mutations and PTEN aberrations (Figure 1).

In 160 patients with *PIK3CA* mutations, the most frequent mutation was E545K (1633G>A) in 32.5% of patients (52/160), followed by E542K (1624G>A) in 20% of patients (32/160), and H1047R (3140A>G) in 18% of patients (29/160) (Supplementary Table 1).

PIK3CA mutations were not associated with age or ethnicity.

There were 163 patients with PTEN aberrations. These aberrations include loss of staining on immunohistochemistry in 155 patients (1,123 tested for expression, but not for mutations), loss of staining on immunohistochemistry in the absence of *PTEN* mutations in 2 patients (25 tested for mutations and expression), loss of staining on immunohistochemistry in the presence of *PTEN* mutations in 3 patients (25 tested for mutations and expression), *PTEN* mutation in the presence of reduced staining on immunohistochemistry in 1 patient (25 tested for mutations and expression), or *PTEN* mutations in 2 patients who had no immunohistochemistry performed (9 tested for mutation only). *PTEN* mutations were most frequent in exon 5 (4/6, 75%).

PTEN aberrations were not associated with gender, age or ethnicity.

Mutations in mitogen-activated protein kinase pathway

Of the 1,656 patients 1,238 were tested for *KRAS* mutations and 18% (229/1,238) were found to have mutations. The most prevalent was the G12D mutation (35G>A) present in 31% of patients (72/229), G12V mutation (35G>T) in 22% (50/229), G13D mutation (38G>A) in 10% (23/229), G12C (34G>T) in 9% (21/229), and G12A mutation (35G>C) in 8% of patients (18/229).

Of the 1,656 patients 618 were tested for *NRAS* and 5% (32/618) were found to have mutations. The most prevalent was the Q61K mutation (181C>A) in 25% of patients (8/32), and a Q61L mutation (182_183AA>TG) in 12.5% of patients (4/32).

Of the 1,656 patients, 1,175 were tested for *BRAF* and 6% (70/1,175) had mutations. The most prevalent was the V600E mutation (1799T>A) in 76% (53/70) of patients, and a V600K mutation (1798_1799GT>AA) in 14% (10/70) of patients.

Mutations in *KRAS*, *NRAS*, and *BRAF* were mutually exclusive with the exception of two patients with malignant melanoma who had simultaneous *BRAF* and *NRAS* mutations.

Associations among PIK3CA mutations, PTEN aberrations and MAPK mutations

PIK3CA mutations were more prevalent in patients with *KRAS* mutations than wild-type (wt) *KRAS* (42/225, 19% vs. 89/975, 9%; $p<0.001$; Figure 2).

PIK3CA mutations were not associated with *NRAS* or *BRAF* mutations.

Patients with PIK3CA mutations or PTEN aberrations treated with PI3K/AKT/mTOR inhibitors

Response rate—Of the 309 patients with *PIK3CA* mutations alone (n=146), PTEN aberrations (n=149) and simultaneous *PIK3CA* mutations and PTEN aberrations (n=14), 136 (44%) patients (*PIK3CA* mutations, n=76; PTEN aberrations, n=51; *PIK3CA* mutation and PTEN aberration, n=9) were enrolled in studies that included PI3K/AKT/mTOR inhibitors (Figure 3); 67 of the 309 patients (22%) received other protocol-based experimental therapies, often because *PIK3CA*/PTEN status was not available at the time of decision making; 106 (34%) were not treated, usually due to ineligibility or patient/doctor preference.

When examining the 136 patients with *PIK3CA* and/or PTEN aberrations treated with PI3K/AKT/mTOR axis inhibitors, we found that these patients were refractory to a median of 3 prior therapies (range, 1 to 12). Of these 136 patients, 25 (18%) had colorectal cancer, 21 (15%) breast cancer, 18 (13%) endometrial cancer, 14 (10%) ovarian cancer, 11 (8%) squamous cell head and neck cancer, 8 (6%) squamous cell cervical cancer, 7 (5%) renal cancer, 4 (3%) salivary gland cancer, 4 (3%) non-small cell lung cancer, 3 (2%) sarcoma, and 21 (15%) other cancers (adenoid cystic head and neck cancer, adrenocortical carcinoma, anal squamous cell cancer, appendiceal carcinoma, carcinoma of unknown primary, cervical adenocarcinoma, gastric cancer, hepatocellular carcinoma, melanoma, Merkel cell carcinoma, neuroendocrine cancer, pancreatic cancer, papillary thyroid cancer, urothelial carcinoma, and small intestine cancer). Most patients (104, 76%) received mTORC1 inhibitor (rapalog) -based therapy; 20 (15%), PI3K inhibitor-based therapy; 6 (4.5%), dual PI3K and mTOR kinase inhibitor-based therapy; and 6 (4.5%), AKT inhibitor-based therapy (Figure 3). Single-agent therapies were given to 41 (30%) of patients and 95 (70%) received combination therapy (Supplementary Table 2). Combination therapies that included chemotherapy were administered to 49 patients (36%) and a combination of targeted therapies to 46 patients (34%). Of note, 7 (5%) patients received combinations simultaneously targeting the PI3K/AKT/mTOR and MAPK pathways. Overall, 25 (18%, 95% CI 0.13–0.26, Supplementary Table 3) patients achieved a PR defined on line 79(Figure 4) and an additional 9 (7%, 95% CI 0.04–0.12) had stable disease (SD) ≥6 months (rate of SD ≥6 months/PR 25%, 34/136, 95% CI 0.18–0.33). The observed PR rate compared favorably to a complete response [CR]/PR rate of 6% (26/458; 95%CI 0.04–0.08; $p<0.001$) in 458 patients without known *PIK3CA* mutations and/or PTEN aberrations treated on the same PI3K/AKT/mTOR protocols (Supplementary Table 4).

Of the 67 patients with *PIK3CA* mutations and/or PTEN aberrations who received protocol-based experimental therapies other than PI3K/AKT/mTOR inhibitors, only 3 (4%, 95% CI 0.02–0.12) attained a PR, which was significantly inferior compared to 25 (18%) PRs in 136 patients treated with PI3K/AKT/mTOR inhibitors (p=0.008) (Supplementary Table 4). An additional analysis, which excluded patients with colorectal cancer, showed that of the 52 patients with *PIK3CA* mutations and/or PTEN aberrations who received protocol-based experimental therapies other than PI3K/AKT/mTOR inhibitors, only 3 (6%, 95% CI 0.02–0.16) attained a PR, which was significantly inferior compared to 25 (23%, 95% CI 0.16–0.31) PRs in 111 patients treated with PI3K/AKT/mTOR inhibitors (p=0.007). In addition, we have shown that patients with breast and gynecological malignancies and PIK3CA

mutations can benefit from therapies that include PI3K/AKT/mTOR inhibitors (Janku et al., 2012b) Therefore, we performed an analysis, which excluded patients with gynecological (ovarian, cervical and uterine), breast cancers, and colorectal cancers, and demonstrated that of the 45 patients with *PIK3CA* mutations and/or PTEN aberrations who received protocol-based experimental therapies other than PI3K/AKT/mTOR inhibitors, 3 (7%, 95% CI 0.02–0.18) attained a PR compared to 8 (17%, 95% CI 0.09–0.30) PRs in 47 patients treated with PI3K/AKT/mTOR inhibitors (p=0.20). Furthermore, we performed an additional analysis on 104 patients with *PIK3CA* mutations and/or PTEN aberrations, who received treatment with mTORC1 inhibitors (rapalogs). Of these 104 patients, 22 (21%, 95%CI 0.14–0.30) had a PR compared to 3 (4%, 95% CI 0.02–0.12) PRs in 67 patients with *PIK3CA* mutations and/or PTEN aberrations who received protocol-based experimental therapies other than PI3K/AKT/mTOR inhibitors (p=0.003).

There was no difference among patients with *PIK3CA* mutations, PTEN aberrations, or both *PIK3CA* mutations and PTEN aberrations in the rate of PR (14/76, 18% vs. 10/51, 20% vs. 1/9, 11%; p=0.83) and SD ≥6 months/PR (19/76, 25% vs. 14/51, 27% vs. 1/9, 11%; p=0.58) on PI3K/AKT/mTOR inhibitors.

Of 136 patients with *PIK3CA* mutations and/or PTEN aberrations treated with PI3K/AKT/mTOR inhibitors, there was no PR and only 1 (4%) SD ≥6 months/PR in 25 patients with colorectal cancer compared to 25 (23%, p=0.008) PRs and 33 (30%, p=0.005) SD ≥6 months/PR in 111 patients with other cancers. Also, patients treated with single-agent therapies had lower PR rates (2/41, 5% vs. 23/95, 24%; p=0.007) and lower rates of SD ≥6 months/PR (4/41, 10% vs. 30/95, 32%; p=0.009) than patients treated with combinations. There was no difference in PR rate in patients with 3 or fewer prior therapies compared to more than 3 prior therapies (18/80, 23% vs. 7/56, 13%; p=0.18); however, patients with 3 or fewer therapies had a higher SD≥6 months/PR rate (25/80, 31% vs. 9/56, 14%; p=0.047).

Of the 136 treated patients with *PIK3CA* mutations or PTEN aberrations, 109 (80%) had available tissue for *KRAS* mutation testing. Of the 26 patients with *PIK3CA* mutations and/or PTEN aberrations and simultaneous *KRAS* mutations in codon 12 or 13, only 1 (4%) had a PR compared to 20 (24%) PRs in 83 patients with *PIK3CA* mutations and/or PTEN aberrations without *KRAS* codon 12 or 13 mutations (p=0.023). Similarly, only 1 (4%) patient with *PIK3CA* mutations and/or PTEN aberrations and simultaneous *KRAS* mutations in codon 12 or 13 had SD≥6 months/PR compared to 27 (33%) SD≥6 months/PR in 83 patients with *PIK3CA* mutations and/or PTEN aberrations without *KRAS* codon 12 or 13 mutations (p=0.004).

In 85 patients with *PIK3CA* mutations treated with PI3K/AKT/mTOR inhibitors, there were 7 (35%) PRs and 9 (45%) SD≥6 months/PR in 20 patients with H1047R mutations compared to 8 (12%, p=0.039) PRs and 11 (17%) SD≥6 months/PR in 65 patients with other *PIK3CA* mutations (p=0.016).

Multicovariate analysis: We created a multicovariate logistic regression model for 203 patients with *PIK3CA* mutations and/or PTEN aberrations, which included 136 patients treated with PI3K/AKT/mTOR inhibitors and 67 patients treated with other protocol-based

therapies. This model included histology (colorectal vs. others), type of therapy (combination vs. single agent), and treatment with PI3K/AKT/mTOR inhibitors (yes vs. no), which were significant factors identified on univariate analysis (data not shown). Treatment with PI3K/AKT/mTOR inhibitors was the only independent factor predicting a PR (odds ratio [OR] 4.34, 95% CI 1.23–15.24; p=0.02; Table 2).

In addition, a multicovariable logistic regression model within the subgroup of patients with *PIK3CA* mutations or PTEN aberrations treated with PI3K/AKT/mTOR inhibitors, which included histology (colorectal vs. others), type of therapy (combination vs. single agent), and prior therapies (up to 3 vs. more than 3) demonstrated that treatment with combination therapies was the only independent factor predicting a PR with PI3K/AKT/mTOR inhibitors (OR 5.31, 95% CI 1.16–24.25; p=0.03) and SD≥6 months/PR (OR 4.99, 95% CI 1.39–17.89; p=0.01; Table 3). A separate multicovariable logistic regression model with 109 patients having *PIK3CA* mutations or PTEN aberrations treated with PI3K/AKT/mTOR inhibitors, who were tested for *KRAS* mutations, which included histology (colorectal vs. others), *KRAS* mutation (codons 12 or 13 vs. others), type of therapy (combination vs. single agent) and prior therapies (up to 3 vs. more than 3) showed a trend for combination therapies (OR 4.33, 95% CI 0.90–20.71; p=0.07) predicting a PR and a trend for combination therapies (OR 3.78, 95% CI 0.99–14.32; p=0.05) and absence of *KRAS* mutation (OR 0.15, 95% CI 0.02–1.27; p=0.08) predicting SD≥6 months/PR.

Progression-free survival—The median progression-free survival (PFS) for all patients with *PIK3CA* mutations and/or PTEN aberrations treated with PI3K/AKT/mTOR inhibitors was 2.5 months (95% CI 1.8–3.2). There was no significant difference among patients with *PIK3CA* mutations (n=76), PTEN aberrations (n=51), or both *PIK3CA* mutations and PTEN aberrations (n=9) in median PFS (2.3 months, 95% CI 1.7–2.9 vs. 3.5 months, 95% CI 1.5–5.5 vs. 2.8 months, 95% CI 0–5.7; p=0.83).

Patients (n=67) with *PIK3CA* mutations and/or PTEN aberrations who received protocol-based experimental therapies other than PI3K/AKT/mTOR inhibitors, had a similar median PFS as patients (n=136) with *PIK3CA* mutations and/or PTEN aberrations treated with PI3K/AKT/mTOR-based therapies (1.9 months, 95% CI 0.9–2.9 vs. 2.5 months, 95% CI 1.8–3.2; p=0.70). In addition, an analysis that excluded patients with colorectal cancers showed that patients (n=52) with *PIK3CA* mutations and/or PTEN aberrations who received protocol-based experimental therapies other than PI3K/AKT/mTOR inhibitors, had a similar median PFS as patients (n=111) with *PIK3CA* mutations and/or PTEN aberrations treated with PI3K/AKT/mTOR-based therapies (2.7 months, 95% CI 0.8–4.5 vs. 2.8 months, 95% CI 1.8–3.8; p=0.93).

Patients (n=25) with *PIK3CA* mutations and/or PTEN aberrations and colorectal cancer treated with PI3K/AKT/mTOR inhibitors had a shorter median PFS than patients (n=111) with *PIK3CA* mutations and/or PTEN aberrations and other histologies (1.8 months, 95% CI 1.5–2.1 vs. 2.8 months, 95% CI 1.8–3.8; p=0.003). Patients (n=95) with *PIK3CA* mutations and/or PTEN aberrations treated with combination therapies that included PI3K/AKT/mTOR inhibitors had a longer median PFS than patients (n=41) treated with single-agent PI3K/AKT/mTOR-based therapies (3.0 months, 95% CI 2.0–4.0 vs. 1.8 months, 95% CI

1.6–2.0; p<0.001; Figure 5A). There was no difference in median PFS in patients (n=80) with 3 or fewer prior therapies compared to patients (n=56) with more than 3 prior therapies (2.5 months, 95% CI 1.8–3.2 vs. 2.6 months, 95% CI 1.6–3.6; p=0.40).

Of the 109 treated patients with *PIK3CA* mutations and/or PTEN aberrations who had available tissue for *KRAS* mutation testing, 26 patients with *PIK3CA* mutations and/or PTEN aberrations and simultaneous *KRAS* mutations in codon 12 or 13 had a shorter median PFS compared to 83 patients without *KRAS* mutations in codon 12 or 13 (1.8 months, 95% CI 1.6–2.0 vs. 2.9 months, 95% CI 1.9–3.9; p=0.004) when treated with PI3K/AKT/mTOR inhibitors (Figure 5B).

In 85 patients with *PIK3CA* mutations treated with PI3K/AKT/mTOR inhibitors, 20 patients with a H1047R mutation compared to patients with other *PIK3CA* mutations had a longer median PFS (4.6 months, 95% CI 0.6–8.6 vs. 2 months, 1.6–2.4; p=0.03; Figure 5C).

Multicovariate analysis: Similarly, we created a multicovariate Cox regression model for 203 patients with *PIK3CA* mutations and/or PTEN aberrations, which included 136 patients treated with PI3K/AKT/mTOR inhibitors and 67 patients treated with other protocol-based therapies. This model included number of prior therapies (up to 3 vs. more than 3), histology (colorectal vs. others), type of therapy (combination vs. single agent), and treatment with PI3K/AKT/mTOR inhibitors (yes vs. no) that were either significant factors on univariate analysis (data not shown) or were anticipated to be important. Treatment with combinations predicted longer PFS (hazard ratio [HR] 0.70, 95% CI 0.51–0.97; p=0.03), while patients with colorectal cancer had a shorter PFS (HR 1.83, 95% CI 1.25–2.68; p=0.002; Table 2).

In addition, a multicovariable Cox regression model in patients with *PIK3CA* mutations or PTEN aberrations treated with PI3K/AKT/mTOR inhibitors, which included histology (colorectal vs. others), type of therapy (combination vs. single agent), demonstrated longer PFS in patients treated with combinations (HR 0.54, 95% CI 0.35–0.82; p=0.004) and patients with colorectal cancer had a trend to shorter PFS (HR 1.59, 95% CI 0.98–2.59; p=0.06;Table 3). A separate multicovariable Cox regression model with 109 patients with *PIK3CA* mutations or PTEN aberrations treated with PI3K/AKT/mTOR inhibitors tested for *KRAS* mutations, which included histology (colorectal vs. others), *KRAS* mutation (codons 12 and 13 vs. others), and type of therapy (combination vs. single agent), showed a strong trend to longer PFS for patients treated with combinations (HR 0.62, 95% CI 0.39–1.00; p=0.05).

Overall survival—The median overall survival (OS) for all 136 patients with *PIK3CA* mutations and/or PTEN aberrations treated with PI3K/AKT/mTOR inhibitors was 7.7 months (95% CI 5.6–9.8). There was no difference among patients with *PIK3CA* mutations, PTEN aberrations, or both *PIK3CA* mutations and PTEN aberrations in median OS (7.5 months, 95% CI 4.3–10.7 vs. 7.7 months, 95% CI 6.0–9.4 vs. 14.9 months, 95% CI 6.3–23.5; p=0.56).

Patients (n=67) with *PIK3CA* mutations and/or PTEN aberrations who received protocol-based experimental therapies other than PI3K/AKT/mTOR inhibitors, had a trend to longer

median OS compared to patients (n=136) with *PIK3CA* mutations and/or PTEN aberrations treated with PI3K/AKT/mTOR-based therapies (8.9 months, 95% CI 2.7–15.1 vs. 7.7 months, 95% CI 5.6–9.8; p=0.06). In addition, an analysis excluding patients with colorectal cancer demonstrated that patients (n=52) with *PIK3CA* mutations and/or PTEN aberrations who received protocol-based experimental therapies other than PI3K/AKT/mTOR inhibitors had a similar median OS as patients (n=111) with *PIK3CA* mutations and/or PTEN aberrations treated with PI3K/AKT/mTOR-based therapies (7.1 months, 95% CI 2.3–11.9 vs. 7.7 months, 95% CI 5.5–9.9; p=0.17).

Also, there was no difference in median OS between patients (n=25) with *PIK3CA* mutations and/or PTEN aberrations and colorectal cancer treated with PI3K/AKT/mTOR inhibitors compared to patients (n=111) with other histologies (8.9 months, 95% CI 4.3–13.5 vs. 7.7 months, 95% CI 5.4–9.9; p=0.18). Patients (n=95) with *PIK3CA* mutations and/or PTEN aberrations treated with combination therapies that included PI3K/AKT/mTOR inhibitors had a similar median OS as patients (n=41) treated with single-agent PI3K/AKT/mTOR inhibitor-based therapies (8.0 months, 95% CI 5.7–10.2 vs. 7.5 months, 95% CI 4.1–10.9; p=0.17). Finally, patients (n=80) with *PIK3CA* mutations and/or PTEN aberrations who received up to 3 prior therapies had a similar median OS as patients (n=56) who received more than 3 therapies (7.4 months, 95% CI 4.7–10.1 vs. 8.2 months, 95% CI 5.4–11.0; p=0.98) prior to treatment with PI3K/AKT/mTOR inhibitors.

Of the 109 patients with *PIK3CA* mutations and/or PTEN aberrations treated with PI3K/AKT/mTOR inhibitors who had tissue available for *KRAS* mutation testing, 26 patients with *PIK3CA* mutations and/or PTEN aberrations and simultaneous *KRAS* mutations in codon 12 or 13 had a similar median OS compared to 83 patients with *PIK3CA* mutations and/or PTEN aberrations without *KRAS* mutations in codon 12 or 13 (7.5 months, 95% CI 3.7–11.3 vs. 8.2 months, 95% CI 4.5–11.9; p=0.25).

In 85 patients with *PIK3CA* mutations treated with PI3K/AKT/mTOR inhibitors, 20 patients with a H1047R mutation compared to patients with other *PIK3CA* mutations had a trend to a longer median OS (10.0 months, 95% CI 1.9–18.1 vs. 8.2 months, 4.2–12.2; p=0.15).

Multicovariate analysis: We created a multicovariate Cox regression model for 203 patients with *PIK3CA* mutations and/or PTEN aberrations, which included 136 patients treated with PI3K/AKT/mTOR inhibitors and 67 patients treated with other protocol-based therapies. This model included number of prior therapies (up to 3 vs. more than 3), histology (colorectal vs. others), type of therapy (combination vs. single agent), and treatment with PI3K/AKT/mTOR inhibitors (yes vs. no), which were either close to significance on univariate analysis (data not shown) or were anticipated to be important. None of the factors independently predicted OS; however, patients treated with experimental therapies other than PI3K/AKT/mTOR inhibitors had a trend toward a longer OS (HR 0.69, 95% CI 0.47–1.01; p=0.06; Table 2).

In addition, a multicovariable Cox regression model, which included, histology (colorectal vs. others), type of therapy (combination vs. single agent), demonstrated that none of the tested variables predicted survival (Table 3). Similarly, in a separate multicovariable Cox

regression model with 109 patients tested for *KRAS* mutations, which included histology (colorectal vs. others), *KRAS* mutation (codons 12 and 13 vs. others) and type of therapy (combination vs. single agent), none of the tested variables predicted survival.

DISCUSSION

In our study, we observed that *PIK3CA* mutations and/or PTEN aberrations can be detected in approximately 20% of patients with diverse advanced cancers (Figure 1). In agreement with previous reports, the most frequent *PIK3CA* mutations were E545K (32.5%), E542K (20%) in the helical domain and H1047R (18%) in the kinase domain.(Forbes et al., 2011; Janku et al., 2012a) PTEN aberrations were mostly determined by loss of staining on immunohistochemistry (95% of patients with PTEN aberration) as only 5% of patients were tested for *PTEN* mutations. Anecdotally, we noticed that *PTEN* mutations could occasionally be detected without the loss of staining on immunohistochemistry, which is in agreement with previous publications.(Cheung et al., 2011)

Our group and others showed that, in colorectal and gynecological cancers, *PIK3CA* mutations often coexist with mutations in the MAPK pathway such as *KRAS* and *BRAF* mutations, which can abrogate response to PI3K/AKT/mTOR pathway inhibitors.(De Roock et al., 2010; Di Nicolantonio et al., 2010; Engelman et al., 2008; Ihle et al., 2009; Janku et al., 2011a; Janku et al., 2012b) The current study confirms preclinical findings demonstrating that mutations in the MAPK pathway are associated with an attenuated response rate to PI3K/AKT/mTOR inhibitors.(Di Nicolantonio et al., 2010; Engelman et al., 2008; Ihle et al., 2009) Furthermore, aberrations in the PI3K/AKT/mTOR pathway often coexist with aberrations in the MAPK pathway.(De Roock et al., 2010; Janku et al., 2011a) Indeed, *PIK3CA* mutations compared to wt *PIK3CA* were associated with an increased prevalence of coexisting *KRAS* (19% vs. 9%; $p<0.001$; Figure 2). Interestingly, PTEN aberrations were not associated with *KRAS* mutations; however, when we grouped all tested MAPK mutations (*KRAS*, *NRAS*, *BRAF*) together, patients with PTEN aberrations were more likely to have coexisting MAPK mutations than patients without PTEN aberrations (18% vs. 11%; $p=0.047$).

Overall, 44% (136/309) of heavily pretreated patients with *PIK3CA* mutations or PTEN aberrations were treated with therapies that included PI3K/AKT/mTOR inhibitors, which consisted of rapalog-based regimens in 76% of them. The overall PR rate was 18% (in addition, 7% achieved SD≥6 months; Figure 4) and this response rate compared favorably to a CR/PR rate of 6% in patients without known *PIK3CA* mutations or PTEN aberrations, who received treatment on the same protocols ($p<0.001$), and also to a PR rate of 4% in patients with *PIK3CA* mutations and/or PTEN aberrations, who received experimental therapies without PI3K/AKT/mTOR inhibitors ($p=0.008$). In addition, treatment with PI3K/AKT/mTOR inhibitors was found, in multicovariate analysis, to be an independent predictive factor for a PR in patients (n=203) with *PIK3CA* mutations and/or PTEN aberrations treated with PI3K/AKT/mTOR or other protocol-based therapies (OR 4.34, 95% CI 1.23–15.24; $p=0.02$; Table 2) although it did not translate to prolonged PFS and OS.

There was no difference in PR rate (18% vs. 20% vs. 11%; p=0.83), PFS (2.3 months vs. 3.5 months vs. 2.8 months; p=0.83) and OS (7.5 months vs. 7.7 months vs. 14.9 months; p=0.56) on therapies with PI3K/AKT/mTOR inhibitors between patients with *PIK3CA* mutations, PTEN aberrations or both, respectively. None of the patients with *PIK3CA* mutations and/or PTEN aberrations and colorectal cancer attained a PR on therapies with PI3K/AKT/mTOR inhibitors compared to 23% of patients with other histologies (p=0.008). Additionally, patients with colorectal cancer demonstrated a shorter PFS compared to other histologies treated with PI3K/AKT/mTOR inhibitors (1.8 months vs. 2.8 months; p=0.003), which suggests that specific molecular aberrations can have different biological and therapeutic consequences in different disease types. Alternatively, it is plausible that aberrations in the PI3K/AKT/mTOR axis more frequently coexist with MAPK aberrations in colorectal cancer than in other histologies.(Janku et al., 2011a) Interestingly, Dienstmann et al. demonstrated that only 1 (2%) of 42 patients with colorectal cancer and *PIK3CA* mutations (n=10) or PTEN loss (n=32) responded to PI3K pathway inhibitors.(Dienstmann et al., 2012) Another example showing how the same mutation can have diverse implications in different contexts is the *BRAF* V600E mutation, which is highly predictive of response, PFS and OS to BRAF inhibitors in melanoma but not in colorectal cancer.(El-Osta et al., 2011; Flaherty et al., 2010; Kopetz et al., 2010) In addition, *HER2* amplification or overexpression predicts PFS and OS when HER2 targeting therapies are used for treatment in breast and gastric cancers, but not necessarily in other cancers.(Bang et al., 2010; Galsky et al., 2012) On the other hand, for many malignancies, the presence of molecular aberrations predicts response across several histologies, with *BRAF* mutations predicting response to BRAF inhibitors in melanoma, papillary thyroid cancer and hairy cell leukemia.(Dietrich et al., 2012; Falchook et al., 2012) Similarly, in our study, in patients with *PIK3CA* mutations and/or PTEN aberrations, responses to PI3K/AKT/mTOR inhibitors were seen across all histologies except for colorectal cancer.

Patients with *PIK3CA* mutations and/or PTEN aberrations treated with combination therapies that included PI3K/AKT/mTOR inhibitors had higher PR rates (24% vs. 5%; p=0.007) and longer PFS (3.0 months vs. 1.8 months; p<0.001; Figure 5A) than patients treated with single-agent PI3K/AKT/mTOR inhibitors. Combinations were also used frequently in the wt *PIK3CA* group, and the PR/CR rate was significantly lower, suggesting that factors other than the use of combinations mediates response. In addition, the higher PR rate with combinations is not unexpected as combinations have shown more benefit in multiple preclinical models and clinical studies.(Engelman et al., 2008; Janku et al., 2012b; Wee et al., 2009) Single-agent inhibition of the PI3K/AKT/mTOR pathway is often cytostatic rather than cytotoxic, and activation of compensatory pathways by other molecular aberrations can lead to therapeutic resistance.(Faber et al., 2009; Wee et al., 2009) Alternatively, sensitivity to single-agent inhibition can be dependent on BIM (a pro-apoptotic Bcl-2 family protein) levels; low levels of BIM preclude cancer cells from undergoing apoptosis in response to targeted therapy.(Faber et al., 2011; Ng et al., 2012) In addition, the efficacy of single-agent therapies can be compromised because of underlying tumor heterogeneity, which can potentially be overcome with combination therapies. (Gerlinger et al., 2012)

In agreement with the hypothesis that *KRAS* mutations can induce resistance to PI3K/AKT/mTOR pathway inhibitors, we observed that patients with *PIK3CA* mutations and/or PTEN aberrations and simultaneous *KRAS* mutations in codon 12 or 13 compared to patients with *PIK3CA* mutations and without *KRAS* mutations in codon 12 and 13 had a significantly lower PR rate (4% vs. 24%; p=0.023) and shorter median PFS (1.8 months vs. 2.9 months; p=0.004; Figure 5B); however, these findings should be interpreted with caution since the presence of *KRAS* mutations did not reach significance as an independent factor predicting response or lack thereof in multicovariate analysis.

Preclinical data and our preliminary clinical data suggested that the *PIK3CA* H1047R mutation compared to others can be a stronger driver for tumor development and can be associated with better efficacy in PI3K targeting.(Bader et al., 2006; Janku et al., 2013; Matthews et al., 2011; Ross et al., 2012) We observed that patients with a H1047R mutation compared to patients with other *PIK3CA* mutations had a higher PR rate (35% vs. 12%; p=0.039), higher SD≥6 months/PR rate (45% vs. 17%; p=0.016) and longer PFS (4.6 months vs. 2 months; p=0.03; Figure 5C).

Our study has several important limitations. First, although multicovariate analysis showed that the only independent factor predicting response in patients with tumors and *PIK3CA* mutations and/or PTEN aberrations was treatment with PI3K/AKT/mTOR inhibitors, our analysis was performed retrospectively and it was not randomized. Second, we included diverse cancers; however, the latter could suggest that the conclusions are generalizable across histologies. Third, molecular analysis was usually performed on archived tumor tissue, which was obtained at a variety of time points in relationship to administration of treatment. This study should therefore be considered hypothesis generating, and prospective validation of key findings will be needed.

In conclusion, we have demonstrated that screening for *PIK3CA* mutations, PTEN aberrations and MAPK mutations can identify a subset of patients with advanced, heavily pretreated cancers who respond to therapeutic targeting with PI3K/AKT/mTOR pathway inhibitors. Patients with H1047R mutations did especially well with a SD≥6 months/PR rate of 75%, albeit with only a small number of patients treated (n = 20). The observed PR rate and even more so PFS falls short compared to some other targeted therapies such as EGFR inhibitors in *EGFR*-mutant NSCLC, BRAF inhibitors in *BRAF*-mutant melanoma, or imatinib in *BCR-ABL* rearranged CML.(Druker et al., 2001; Falchook et al., 2012; Flaherty et al., 2010; Lynch et al., 2004) This can be partially explained by the presence of simultaneous *KRAS* mutations; however, other factors such as insufficient target inhibition, activating feedback loops, pathway circumvention, or alternate mechanism of pathway activation can be involved. Importantly, in the case of CML, treatment early in the disease was key to improving PFS and OS; when imatinib is given to patients with blast transformation, a disease stage that can be viewed as analogous to metastatic disease in solid tumors, only a minority of patients respond and survival benefit is measured in months rather than years (Westin and Kurzrock, 2012). However, even with these limitations drugs targeting the PI3K/AKT/mTOR pathway still make an impact, with a PR rate tripled (18% vs. 6%) in patients with PIK3CA mutations or PTEN aberrations compared to patients with no aberrations in PIK3CA or PTEN. Nevertheless, the treatment with a PI3K/AKT/mTOR

pathway inhibitor may not be sufficient and, therefore, the improvement in the PR rate does not translate to prolonged PFS. Collectively, these observations warrant further prospective investigation, especially since many PI3K/AKT/mTOR inhibitors are now entering the clinical arena.

METHODS

Patients

PIK3CA mutations and PTEN aberrations were retrospectively investigated in consecutive patients with advanced tumors and available tissue referred to the Clinical Center for Targeted Therapy 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 were conducted in accordance with MD Anderson Institutional Review Board guidelines.

Tumor tissue analyses

PIK3CA mutations and PTEN aberrations were investigated in archival formalin-fixed, paraffin-embedded tissue blocks obtained from diagnostic and/or therapeutic procedures from primary or metastatic sites. 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 analysis encompassed the mutation hot spot regions 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, H1047R, H1047L, G1049R). The mutations identified during the initial screening were confirmed by Sanger sequencing assay. The lower limit of detection is approximately 10%. *PTEN* mutations were detected in exons 1–9 using PCR-based DNA sequencing and the lower limit of detection was approximately 20%. PTEN expression was tested with immunohistochemistry using the monoclonal mouse anti-human antibody clone 6H2.1 (Dako ®, Carpinteria, CA, USA) and complete loss of staining was classified as PTEN loss. Whenever possible, additional MAPK mutation analyses for *KRAS*, *NRAS* codons 12, 13, and 61 mutations of exons 1–2 and *BRAF* mutations in exon 15 were carried out using PCR-based DNA sequencing.(Zuo et al., 2009) The lower limit of detection was approximately 20%.

Treatment and evaluation

Assignment to a clinical trial was determined after clinical, laboratory, and pathologic data from all available patient records were reviewed. Consecutive patients who had tumor tissue that could be tested or had been tested with underlying *PIK3CA* mutations and/or a PTEN aberration were enrolled, whenever possible, in clinical trials that included inhibitors of the PI3K/AKT/mTOR pathway. 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 approximately every 2 cycles (6–8 weeks). All radiographs were read in the Department of Radiology and reviewed in the Department of Investigational Cancer Therapeutics tumor measurement clinic at MD Anderson. Responses were categorized per RECIST criteria and were reported as best response.(Therasse et al., 2000) In brief, a 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

Statistics were verified by our statistician (JJL). Two-way contingency tables were formed to summarize the relationship between two categorical variables. Fisher's exact test was used to assess the association among categorical variables and mutation status. Wilcoxon rank-sum test was applied to assess the association among continuous variables and mutation status. Multicovariable logistic regression analysis was applied to identify the multiple predictors associated with the response outcomes and number of prior therapies, histology, type of therapy, *PIK3CA* mutations, PTEN aberrations and MAPK (*KRAS, NRAS, BRAF)* mutations, and others. 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.(Kaplan, 1958) Cox proportional hazards regression models were fit to assess the association between patient characteristics and PFS or OS.(Cox, 1972) All tests were two-sided, and P values less than 0.05 were considered statistically significant. All statistical analyses were carried out using SPSS 19 computer software (SPSS Chicago, IL).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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).

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).

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Highlights

- *PIK3CA* mutations and/or PTEN aberrations are frequent in diverse advanced cancers
- Testing for *PIK3CA* and PTEN aberrations can predict benefit of PI3K/mTOR inhibitors
- This work further supports accelerated use of biomarker-driven trials in cancer

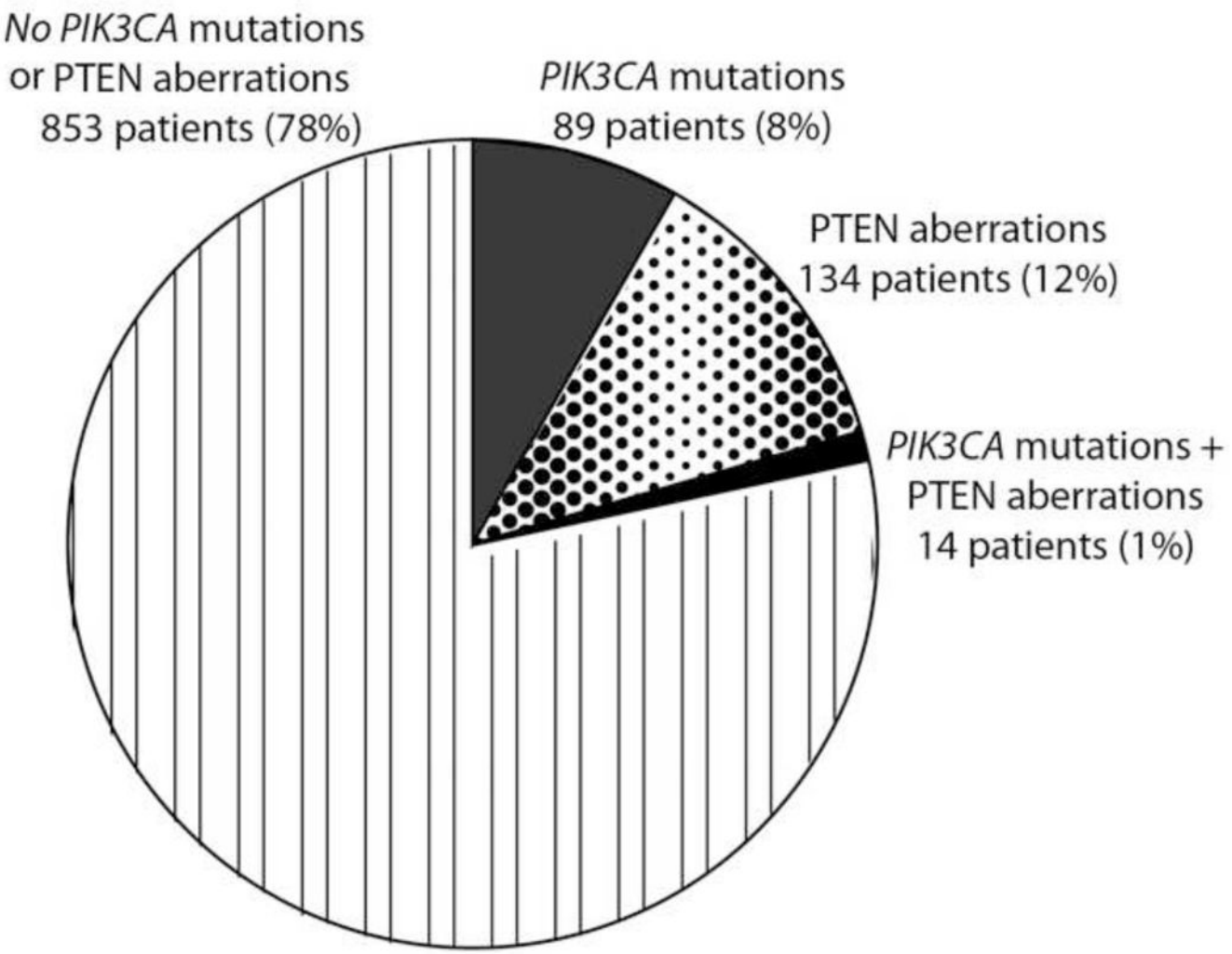

Figure 1.
Proportion of *PIK3CA* mutations and PTEN aberrations in 1,090 patients who had both *PIK3CA* and PTEN testing.

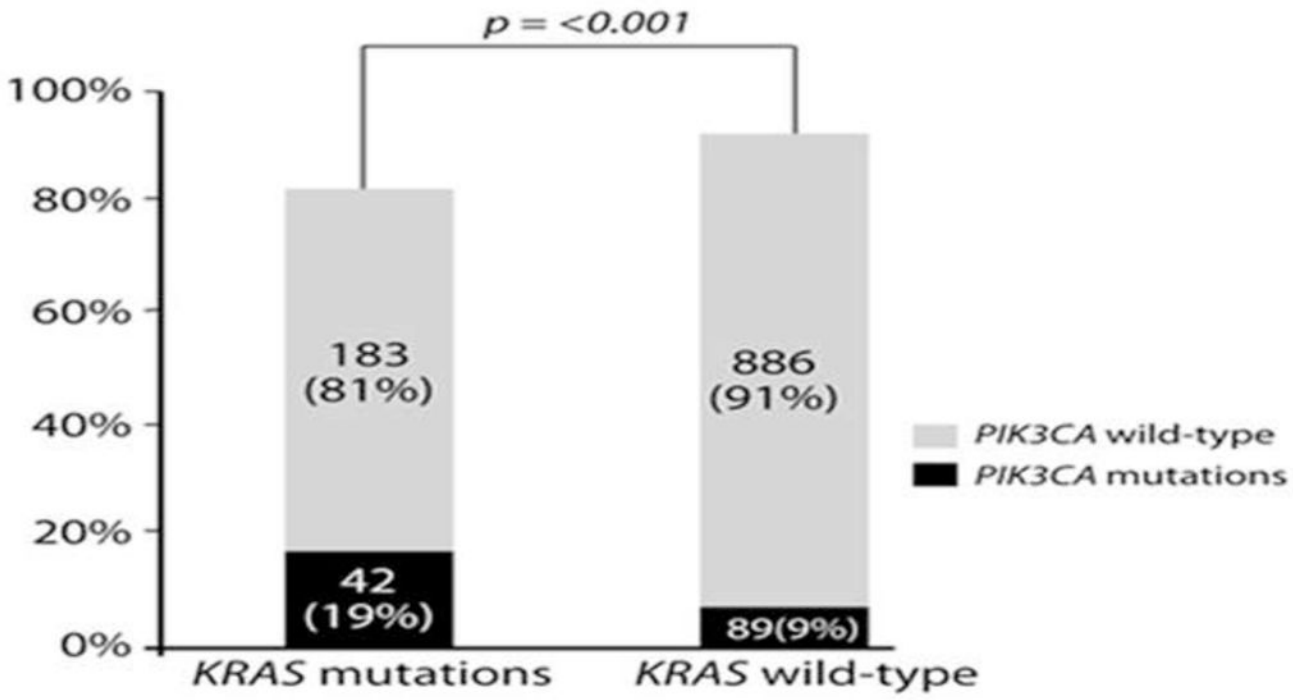

Figure 2.

PIK3CA mutations are more frequent in tumors with simultaneous *KRAS* mutations (42/225, 19% vs. 89/975, 9%; p<0.001).

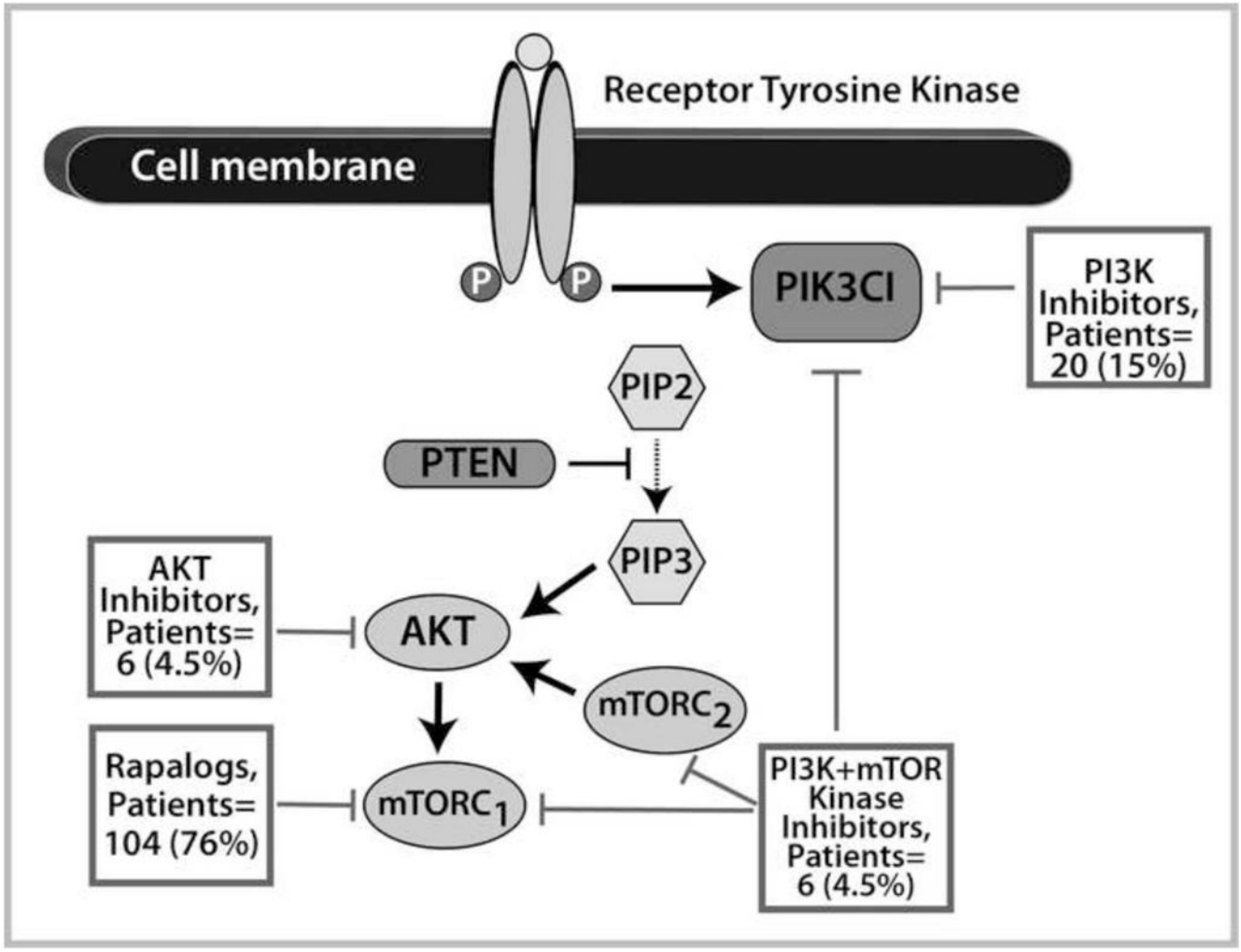

Figure 3.

Therapies targeting the PI3K/AKT/mTOR pathway. Most patients (104, 76%) received mTORC1 inhibitor (rapalog)-based therapy, 20 (15%) PI3K inhibitor-based therapy, 6 (4.5%) dual PI3K and mTOR kinase inhibitor-based therapy, and 6 (4.5%) AKT inhibitor-based therapy.

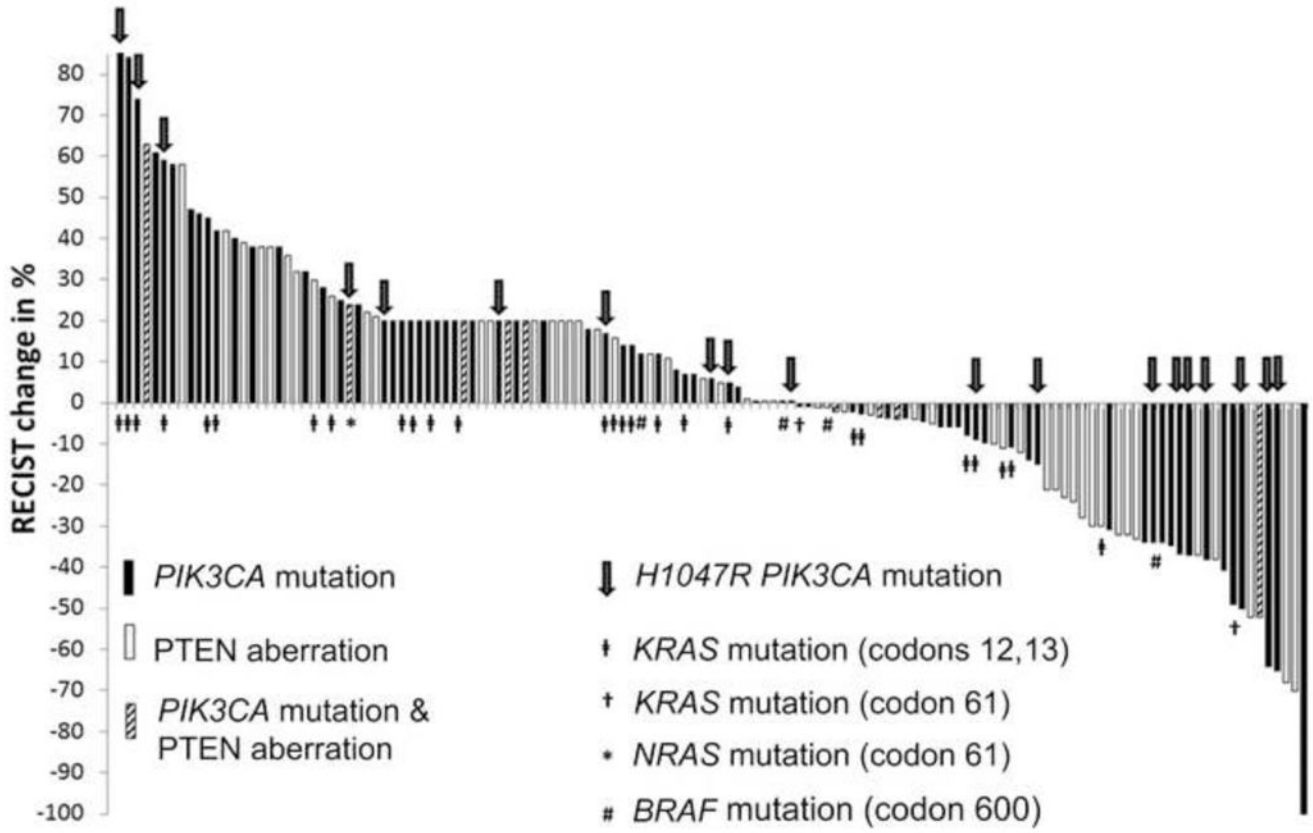

Figure 4.

Waterfall plot shows best response for patients with *PIK3CA* mutations or PTEN aberrations treated with PI3K/AKT/mTOR inhibitors. Of the 136 treated patients, 135 are depicted in the waterfall plot (one patient died of unrelated causes prior to her first restaging). A total of 25 PRs and 33 minor regressions less than PR were observed. The overall PR rate was 18%.

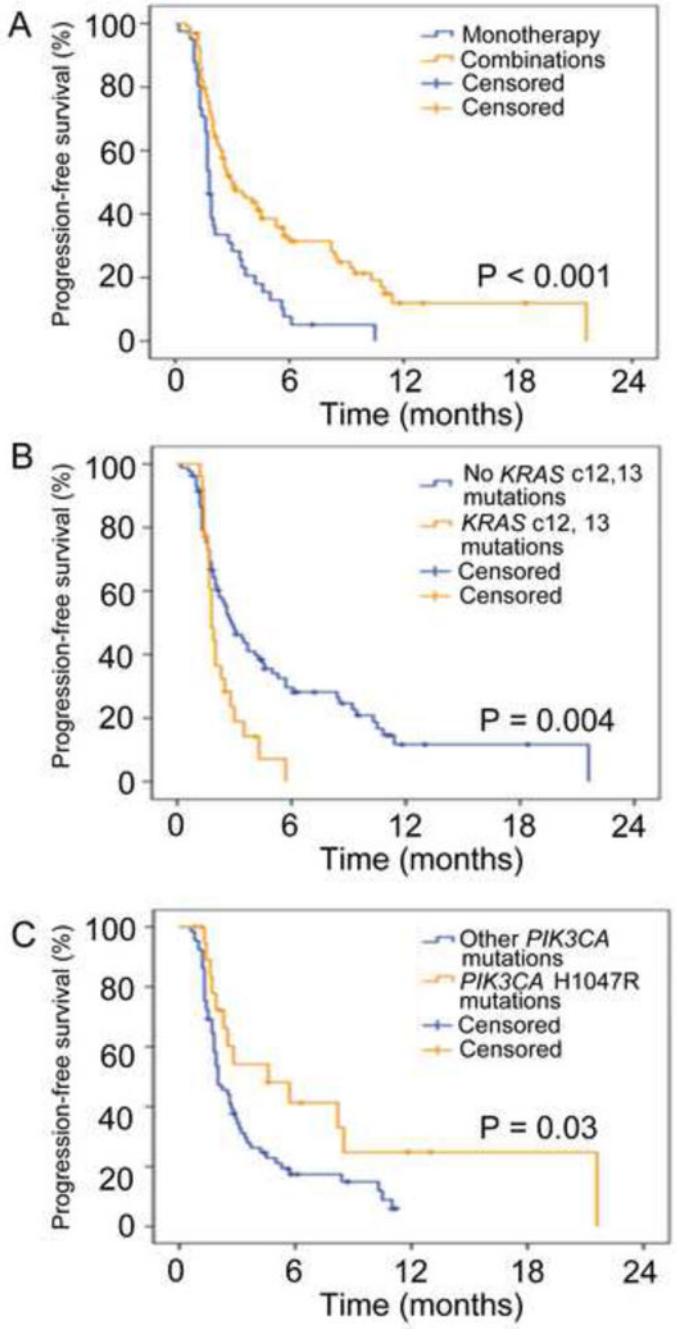

Figure 5.

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 *PIK3CA* mutations and/or PTEN aberrations treated with combination therapies (yellow, n=95) compared to patients treated with single-agent therapies (blue, n=41) had a longer median PFS than (3.0 months, 95% CI 2.0–4.0 vs. 1.8 months, 95% CI 1.6–2.0; p<0.001). B. Patients with *PIK3CA* mutations and/or PTEN aberrations and simultaneous *KRAS* mutations in codon 12 or 13 (yellow) compared to patients without *KRAS* mutations in

codon 12 or 13 (blue) had a shorter median PFS compared to 81 (1.8 months, 95% CI 1.6–2.0 vs. 2.9 months, 95% CI 1.9–3.9; p=0.004). C. Patients with a H1047R mutation (yellow) compared to patients with other *PIK3CA* mutations (blue) had a longer median PFS (4.6 months, 95% CI 0.6–8.6 vs. 2 months, 1.6–2.4; p=0.03).

Table 1

Patients characteristics (n=1,656)

Variable	*PIK3CA* mutation (%)	wild-type *PIK3CA* (%)	P value	PTEN aberration (%)	PTEN intact (%)	P value
All	160 (100)[a,b]	1,429 (100)[a]	NA	163 (100)[b,c]	994 (100)[c]	NA
Gender						
Men	62 (39)	683 (48)	0.03	86 (53)	478 (48)	0.27
Women	98 (61)	746 (52)		77 (47)	516 (52)	
Median age, range (years)	56, 16–83	59, 13–92	0.16	59, 20–83	59, 14–90	0.74
Ethnicity						
White	126 (79)	1,115 (78)	0.97	133 (82)	768 (77)	0.34
African-American	14 (9)	127 (9)		13 (8)	86 (9)	
Hispanic	11 (7)	112 (8)		7 (4)	90 (9)	
Asian	7 (4)	52 (3.5)		7 (4)	34 (3)	
Other	2 (1)	23 (1.5)		3 (2)	16 (2)	
Tumor type						
Colorectal	46 (29)	236 (17)	NA	28 (17)	174 (18)	NA
Ovarian	16 (10)	163 (11)		5 (3)	125 (13)	
Melanoma	2 (1)	120 (8)		12 (7)	61 (6)	
Head & neck: squamous	13 (8)	82 (6)		10 (6)	67 (7)	
Soft tissue sarcomas	2 (1)	97 (7)		5 (3)	74 (7)	
Non-small cell lung	6 (4)	83 (6)		15 (9)	49 (5)	
Breast	21 (13)	57 (4)		9 (6)	43 (4)	
Uterine	16 (10)	50 (3)		15 (9)	34 (3)	
Thyroid	3 (2)	41 (3)		3 (2)	24 (2)	
Pancreatic	1 (<1)	39 (3)		5 (3)	23 (2)	
Gastric	2 (1)	37 (3)		1 (<1)	28 (3)	
Neuroendocrine	2 (1)	36 (3)		2 (1)	30 (3)	
Prostate	1 (<1)	35 (2)		8 (5)	29 (3)	
Renal	3 (2)	33 (2)		10 (6)	13 (1)	

Variable	*PIK3CA* mutation (%)	wild-type *PIK3CA* (%)	P value	PTEN aberration (%)	PTEN intact (%)	P value
Salivary gland	1 (<1)	33 (2)		3 (2)	26 (3)	
Cervical: squamous	10 (6)	23 (2)		3 (2)	21 (2)	
Biliary tract	0 (0)	27 (2)		2 (1)	20 (2)	
Hepatocellular	0 (0)	27 (2)		6 (4)	16 (2)	
Bladder and urothelial	3 (2)	16 (1)		3 (2)	9 (<1)	
Head and neck: non-squamous	3 (2)	16 (1)		0 (0)	13 (1)	
Cervical: adenocarcinoma	1 (<1)	18 (1)		3 (2)	9 (<1)	
Unknown primary	2 (1)	16 (1)		2 (1)	13 (1)	
Ewing	0 (0)	15 (1)		1 <1)	11 (1)	
Small cell lung	0 (0)	15 (1)		3 (2)	7 (<1)	
Esophageal: adenocarcinoma	0 (0)	13 (<1)		2 (1)	10 (1)	
Other	6 (4)	101 (7)		7 (4)	65 (7)	

NA: not applicable

[a] *PIK3CA* mutations were tested in 1,589 patients.

[b] Patients with simultaneous *PIK3CA* mutations and PTEN aberrations are included.

[c] PTEN aberrations were tested in 1,157 patients

Table 2

Multicovariate model for response per RECIST (logistic regression) , progression-free survival (Cox regression), and overall survival (Cox regression) in patients (n=203) with *PIK3CA* mutations and/or PTEN aberrations treated with PI3K/AKT/mTOR inhibitors (n=136) or other systemic protocol-based therapies (n=67).

Outcome measure	Variable	Odds ratio or Hazard Ratio[1]	95% Confidence interval	P value
Partial response (RECIST)[2]	Colorectal cancer vs. other cancers	Not calculated[3]	Not calculated[2]	
	Combination therapies vs. single agents	2.85	0.92–8.84	0.07
	PI3K/AKT/mTOR vs. other protocols	4.34	1.23–15.24	**0.02**
Progression- free survival	> 3 prior vs. ≤ 3 prior therapies	1.22	0.88–1.71	0.24
	Colorectal cancer vs. other cancers	1.83	1.25–2.68	**0.002**
	Combination therapies vs. single agents	0.70	0.51–0.97	**0.03**
	PI3K/AKT/mTOR vs. other protocols	1.06	0.76–1.48	0.75
Overall survival	> 3 vs. ≤ 3 prior therapies	1.12	0.78–1.59	0.54
	Colorectal cancer vs. other cancers	1.21	0.78–1.87	0.40
	Combination therapies vs. single agents	0.85	0.59–1.21	0.36
	PI3K/AKT/mTOR vs. other protocols	1.45	0.99–2.12	0.06

[1] Odds ratio was calculated for response. Higher odds ratio indicates greater chance of response. Hazard ratio was calculated for progression-free and overall survival. Higher hazard ratio indicates greater chance of progression or death.

[2] RECIST, Response Evaluation Criteria in Solid Tumors

[3] Odds ratio and 95% confidence interval were not calculated because none of the patients with colorectal cancer attained a partial response

Table 3

Multicovariate model for response/prolonged stable disease per RECIST (logistic regression), progression-free survival (Cox regression), and overall survival (Cox regression) in patients (n=136) with *PIK3CA* mutations and/or PTEN aberrations treated with PI3K/AKT/mTOR inhibitors.

Outcome measure	Variable	Odds ratio/hazard ratio[1]	95% Confidence interval	P value
Partial response (RECIST)[2]	Colorectal cancer vs. other cancers	Not calculated[3]	Not calculated[3]	
	Combination therapies vs. single agents	5.31	1.16–24.25	**0.03**
	> 3 vs. ≤ 3 prior therapies	0.67	0.25–1.82	0.43
Partial response and stable disease ≥ 6 months (RECIST) [2]	Colorectal cancer vs. other cancers	0.13	0.02–1.07	0.06
	Combination therapies vs. single agents	4.99	1.39–17.89	**0.01**
	> 3 vs. ≤ 3 prior therapies	0.47	0.18–1.19	0.11
Progression-free survival	Colorectal cancer vs. other cancers	1.59	0.98–2.59	0.06
	Combination therapies vs. single agents	0.54	0.35–0.82	**0.004**
Overall survival	Colorectal cancer vs. other cancers	1.34	0.79–2.26	0.28
	Combination therapies vs. single agents	0.78	0.50–1.19	0.25

[1] Odds ratio was calculated for a partial response and stable disease ≥ 6. Hazard ratio was calculated for progression-free and overall survival.

[2] RECIST, Response Evaluation Criteria in Solid Tumors.

[3] Odds ratio and 95% confidence interval were not calculated because none of the patients with colorectal cancer attained a partial response.