Target-Based Therapeutic Matching in Early-Phase Clinical Trials in Patients with Advanced Colorectal Cancer and *PIK3CA* Mutations

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Abstract

Target-matched treatment with PI3K/AKT/mTOR pathway inhibitors in patients with diverse advanced cancers with *PIK3CA* mutations have shown promise. Tumors from patients with colorectal cancer were analyzed for *PIK3CA, KRAS*, and *BRAF* mutations. *PIK3CA*-mutated tumors were treated, whenever feasible, with agents targeting the PI3K/AKT/mTOR pathway. Of 194 patients analyzed, 31 (16%) had *PIK3CA* mutations and 189 (97%) were assessed for *KRAS* mutations. Patients with *PIK3CA* mutations had a higher prevalence of simultaneous *KRAS* mutations than patients with wild-type *PIK3CA* (71%, 22/31 vs. 43%, 68/158; $P = 0.006$). Of 31 patients with *PIK3CA* mutations, 17 (55%) were treated with protocols containing PI3K/AKT/mTOR pathway inhibitors [median age, 57 years; median number of prior therapies, 4; mTORC1 inhibitors (11), phosphoinositide 3-kinase (PI3K) inhibitors (5), or an AKT inhibitor (1)]. None (0/17) had a partial or complete response (PR/CR) and only 1 [6%, 95% confidence interval (CI), 0.01–0.27] had stable disease 6 month or more, which was not significantly different from a stable disease ≥ 6 month/PR/CR rate of 16% (11/67; 95% CI, 0.09–0.27) in patients with colorectal cancer without *PIK3CA* mutations treated with PI3K/AKT/mTOR pathway inhibitors ($P = 0.44$). Median progression-free survival was 1.9 months (95% CI, 1.5–2.3). In conclusion, our data provide preliminary evidence that in heavily pretreated patients with *PIK3CA*-mutant advanced colorectal cancer, protocols incorporating PI3K/AKT/mTOR inhibitors have minimal activity. *PIK3CA* mutations are associated with simultaneous *KRAS* mutations, possibly accounting for therapeutic resistance. *Mol Cancer Ther; 12(12); 2857–63. ©2013 AACR.*

Introduction

The *PIK3CA* gene encodes the 110α subunit of phosphoinositide 3-kinase (PI3K) and is commonly mutated in a myriad of human cancers (1). *PIK3CA* mutations activate the PI3K/AKT/mammalian target of rapamycin (mTOR) pathway, which leads to carcinogenesis and tumor progression (2–4). Preclinical and early clinical data suggest that *PIK3CA* mutations can render tumors sensitive to PI3K/AKT/mTOR pathway inhibition, whereas simultaneous *KRAS* mutations can drive therapeutic resistance (3, 5–9). Many of the latest advances in cancer medicine have occurred when tumor-specific molecular abnormalities were matched with appropriately selected targeted therapies (10–12). Examples in solid tumors include treatment with KIT inhibitors in gastrointestinal stromal tumors with *KIT* mutations (13), EGF receptor (EGFR) inhibitors in non–small cell lung cancer harboring *EGFR* mutations (14), and BRAF inhibitors in melanoma with *BRAF* mutations (15, 16). It is plausible that matching patients with colorectal cancer harboring *PIK3CA* mutations with therapies targeting the PI3K/AKT/mTOR pathway may lead to improved therapeutic benefit, as has been suggested in breast and gynecologic cancers (7, 8). *PIK3CA* mutations occur in approximately 17% of colorectal cancers; however, there are limited data on the outcomes of matched targeting of the PI3K/AKT/mTOR pathway in these patients (17–20). We investigated patients with colorectal cancer referred to the Clinical Center for Targeted Therapy at MD Anderson Cancer Center (MD Anderson) for the presence of *PIK3CA* mutations and analyzed their treatment outcomes.

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Materials and Methods

Patients

Patients with advanced colorectal cancer refractory to standard therapies referred for early clinical trials with targeted therapeutic agents to the Clinical Center for Targeted Therapy at MD Anderson were eligible for analysis provided they had adequate tissue available for mutation analysis. The registration of patients in the database, pathology assessment, and mutation analysis were performed at MD Anderson. All treatments and analyses were performed in accordance with MD Anderson IRB guidelines.

Tissue samples and mutation analyses

PIK3CA and *KRAS* mutations were investigated in archival formalin-fixed, paraffin-embedded tissue blocks or material from fine needle aspiration biopsy obtained from diagnostic and/or therapeutic procedures. All histologies were centrally reviewed at MD Anderson. *PIK3CA* and *KRAS* mutation testing was done at the Clinical Laboratory Improvement Amendment (CLIA)–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 further studied using a PCR-based DNA sequencing method for *PIK3CA* mutations in codons c532 to c554 of exon 9 (helical domain) and c1011 to c1062 of exon 20 (kinase domain), which included the mutation hotspot region of the *PIK3CA* protooncogene by Sanger sequencing after amplification of 276- and 198-bp amplicons, respectively, using primers designed by the MD Anderson Molecular Diagnostic Laboratory. After January 2011, the assay used was 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, and 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, and G1049R]. The mutations identified during the initial screening were confirmed by Sanger sequencing assay. The lower limit of detection is approximately 10%. In addition, whenever possible, mutation analyses for *KRAS*, *NRAS* codons 12 and 13, and 61 mutations of exons 2–3 and *BRAF* mutations in exon 15 were carried out using PCR-based DNA sequencing mutation, as previously described (21).

Treatment and evaluation

Consecutive patients with underlying *PIK3CA* mutations were offered, whenever possible, a clinical trial, which included an inhibitor of the PI3K/AKT/mTOR pathway. Treatment continued until disease progression or the occurrence of unacceptable toxicity. Treatment was carried out according to the 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 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 Response Evaluation Criteria in Solid Tumors (RECIST) 1.0 (22). 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 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 was defined as neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for progressive disease.

Statistical analysis

Two-way contingency tables were used to summarize the relationship between two categorical variables. A Fisher exact test was used to assess the association among categorical variables and *PIK3CA* mutation status. Progression-free survival (PFS), estimated by the Kaplan–Meier method, 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. All statistical analyses were carried out using SPSS 19 computer software (SPSS).

Results

Patients' characteristics

A total of 194 patients with advanced colorectal cancer were screened for the presence of *PIK3CA* mutations, starting January 2009. Their median age was 58 years (range, 25 to 81 years); 140 (72%) were White, 28 (14%) African American, 19 (10%) Hispanic, and 7 (4%) Asian. Detailed patient characteristics are listed in Table 1.

PIK3CA mutations

PIK3CA mutations were detected in 31 (16%) of the 194 patients, with 22 mutations in the kinase domain in exon 9, and 8 mutations in the helical domain in exon 20. The most frequent point mutation was E545K (1633G>A) in 11 (35%) patients, followed by E542K (1624G>A) in 8 patients (26%), and H1047L (3140A>T) in 3 (10%) patients (Table 2). No association between *PIK3CA* mutation and age or ethnicity or tumor site was identified (Table 1).

KRAS and BRAF mutations

Of the 194 patients, 189 patients were tested for *KRAS* mutations and 90 (47%) had a mutation. Among patients

Table 1. Baseline patient characteristics

Variable	Number	*PIK3CA* mutation (%)	Wild-type *PIK3CA* (%)	*P* value
All	194	31 (100)	163 (100)	Not applicable
Median age, y	58	56	59	0.21
	range, 25–81	range, 36–71	range, 25–81	
Ethnicity				
White	140	21 (68)	119 (73)	0.58
African American	28	6 (19)	22 (13.5)	
Hispanic	19	2 (6.5)	17 (10.5)	
Asian	7	2 (6.5)	5 (3)	
Tumor site				
Colon	156	28 (90)	128 (79)	0.15
Rectum	38	3 (10)	35 (21)	
KRAS (n = 189)[a]				
KRAS mutation	84	22 (71)	68 (43)	0.006
KRAS wild-type	91	9 (29)	90 (57)	

[a]Only patients tested for *KRAS* mutations are included in the analysis.

with *KRAS* mutations, the most prevalent ones were G12D (35G>A) in 30% (27/90), G12V (35G>T) in 20% (18/90), G13D (38G>A) in 11% (10/90), and G12A (35G>C) in 11% (10/90) of patients. Patients with *PIK3CA* mutations were more likely to have simultaneous *KRAS* mutations compared with patients with wild-type (WT) *PIK3CA* (71%, 22/31 vs. 43%, 68/158; *P* = 0.006). *KRAS* mutations compared with WT *KRAS* were associated with *PIK3CA* mutations in exon 9 (18%, 16/90 vs. 6%, 6/99; *P* = 0.01), but not in exon 20 (6%. 5/90 vs. 3%, 3/99; *P* = 0.48; Table 3).

Of the 194 patients, 167 patients were tested for *BRAF* mutations and 11 (7%) had mutated *BRAF* (10 patients had a V600E mutation and 1 patient had a D594G mutation). All patients with a *BRAF* mutation were negative for *KRAS* mutations. Of these 11 patients with *BRAF* mutations, 2 (18%) had coexistent *PIK3CA* mutations. There

Table 2. Types of *PIK3CA* mutations identified

Mutation type	N (%)
PIK3CA mutations	31
N345K	1 (<5)
E542K	8 (26)
E545A	1 (<5)
E545K	11 (35)
Q546P	1 (<5)
Q546R	1 (<5)
R1023Q	1 (<5)
M1043V	1 (<5)
H1047R	2 (6)
H1047L	3 (10)
G1049R	1 (<5)

was no difference in the incidence of *BRAF* mutations among those with or without *PIK3CA* mutations (7%, 2/27 vs. 6%, 9/140; *P* = 0.69).

Response rate to PI3K/AKT/mTOR–directed therapies

Of the 31 patients with *PIK3CA* mutations, 17 (55%) were treated in clinical trials including a PI3K/AKT/mTOR pathway inhibitor. These patients had been refractory to a median of four prior therapies (range, 2–7). Most patients (11/17, 65%) received mTORC1 inhibitor (rapalog)-based therapy; 5 of 17 (29%) received PI3K inhibitor-based therapy, and 1 (6%) received an AKT inhibitor-based therapy (Supplementary Table S1). Most patients (13/17, 76%) received PI3K/AKT/mTOR inhibitors at 100% of the maximum tolerated dose/recommended phase II dose/FDA (U.S. Food and Drug Administration)-approved dose (range, 30%–100%). Of the 11 patients treated with mTORC1 inhibitors, 10 (91%) received the maximum tolerated dose/recommended phase II dose/FDA-approved dose. In contrast, only 1 (20%) of 5 patients treated with PI3K inhibitors was treated with the maximum tolerated dose (Supplementary Table S1). Of the 17 patients, none achieved PRs or CRs and only 1 (6%; 95% CI, 0.01–0.27) patient had stable disease for more than 6 months (stable disease ≥6; Fig. 1). This was similar to the stable disease ≥6/PR/CR rate of 16% (11/67; 95% CI, 0.09–0.27) in patients with colorectal cancer without *PIK3CA* mutations treated on the same protocols targeting the PI3K/AKT/mTOR pathway (*P* = 0.44).

We also reviewed 14 of 31 patients (45%) with *PIK3CA* mutations who were not treated with PI3K/AKT/mTOR inhibitors. Of these 14 patients, 5 were not treated because of ineligibility or patient/doctor preference and 9 received

Table 3. Coexistence of *PIK3CA* and *KRAS* mutations

| Variable	Number	*KRAS* mutation (%)	WT *KRAS* (%)	*P* value
Tested	189	90 (100)	99 (100)	Not applicable
PIK3CA mutations (any)	31	22 (24)	9 (9)	0.006
WT *PIK3CA*	158	68 (76)	90 (91)	
PIK3CA exon 9 mutations	21	16 (18)	6 (6)	0.01
WT or exon 20 *PIK3CA* mutations	168	74 (82)	93 (94)	
PIK3CA exon 20 mutations	8	5 (6)	3 (3)	0.48
WT or exon 9 *PIK3CA* mutations	181	85 (94)	96 (97)	

other experimental therapies, often because *PIK3CA* status was not available at the time of decision making. None (0%; 95% CI, 0.00–0.29) of these 9 patients attained a PR or stable disease ≥6, which was similar to stable disease ≥6/PR/CR rate of 6% (1/17; 95% CI, 0.01–0.27) in the remaining 17 patients treated with PI3K/AKT/mTOR inhibitors (*P* = 1.00).

**PFS on PI3K/AKT/mTOR therapies**

The median PFS for these 17 patients with a *PIK3CA* mutation on target-matched therapy with PI3K/AKT/mTOR inhibitors was 1.9 months (95% CI; 1.5–2.3). There was no difference in median PFS in 11 patients with *KRAS* mutations versus 6 patients with WT *KRAS* (1.8 months; 95%CI, 1.4–2.2 vs. 1.9 months; 95%CI, 1.1–2.7; *P* = 0.59). The same 17 patients had a median PFS of 3.1 months (95%

CI; 0.4–5.8) on their last FDA-approved therapy before referral to the Clinical Center for Targeted Therapy (*P* = 0.1; Fig. 2). Patients (*n* = 67) without *PIK3CA* mutations treated with the same therapies with PI3K/AKT/mTOR inhibitors had a similar median PFS (2.3 months; 95%CI, 2.1–2.6) as did patients with *PIK3CA* mutations treated with PI3K/AKT/mTOR inhibitors (1.9 months; 95% CI, 1.5–2.3; *P* = 0.44; Fig. 2).

In addition, we reviewed PFS in 9 patients with colorectal cancer and *PIK3CA* mutations who were treated with experimental therapies other than PI3K/AKT/mTOR inhibitors. The median PFS for those 9 patients was 1.9 months (95%CI, 1.0–2.7), which was similar to the median PFS of 1.9 months (95%CI 1.5–2.3) in 17 patients with *PIK3CA* mutations treated with matched therapy (*P* = 0.77; Fig. 2).
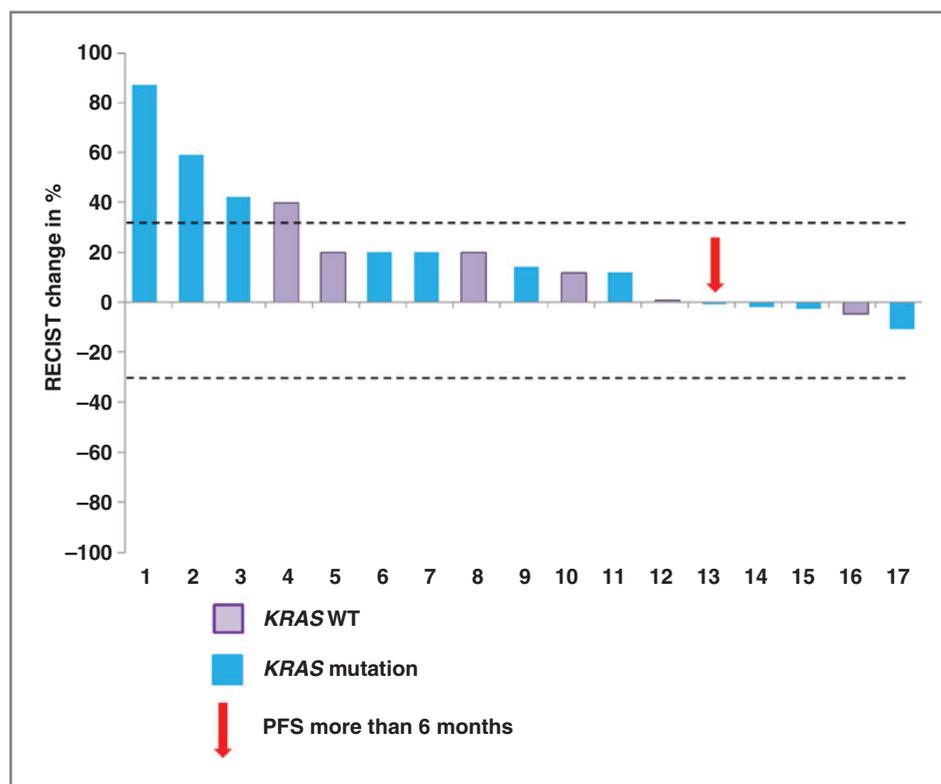
Figure 1. Waterfall plot describing responses per RECIST in 17 patients with metastatic colorectal cancer and *PIK3CA* mutations treated with PI3K/AKT/mTOR pathway inhibitors.
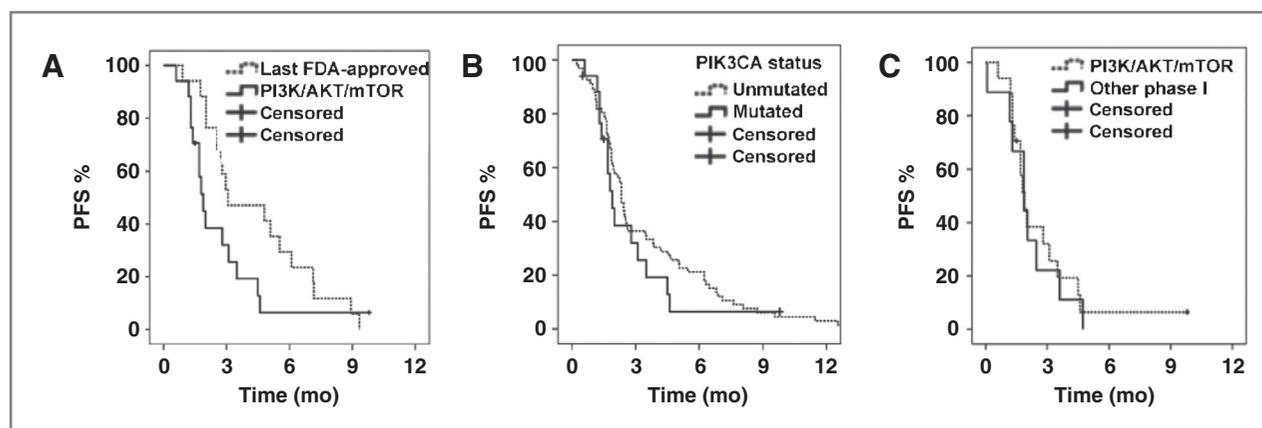
Figure 2. A, PFS in 17 patients with metastatic colorectal cancer and *PIK3CA* mutations treated with PI3K/AKT/mTOR pathway inhibitors compared with median PFS in the same patients treated with the last FDA-approved therapy for metastatic disease (median PFS of 1.9 months vs. 3.1 months; *P* = 0.1). B, PFS in patients with *PIK3CA* mutations who received PI3K/AKT/mTOR-directed therapy (*n* = 17) compared with patients without *PIK3CA* mutations (*n* = 67) treated with same therapies (median PFS of 1.9 months vs. 2.3 months; *P* = 0.44). C, PFS in patients with *PIK3CA* mutations who received PI3K/AKT/mTOR-directed therapy (*n* = 17) compared with patients (*n* = 9) treated with other phase I therapies (median PFS of 1.9 months vs. 1.9 months; *P* = 0.77).

Discussion

We identified *PIK3CA* mutations in 16% of the 194 patients with colorectal cancer referred to the Clinical Center for Targeted Therapy. The most common types of *PIK3CA* mutations associated with colorectal cancer in our patients were E545K and E542K mutations in exon 9. These results are similar to those in published studies (18, 19). Earlier studies suggested that H1047R mutations may be more sensitive to PI3K/AKT/mTOR pathway inhibition, but in our study, only 2 patients (6%) had this particular mutation and that can potentially account for their lack of therapeutic response (8).

We found a strong association between *KRAS* mutations and *PIK3CA* mutations in our patients that supports previously published data (18, 19, 23–25). We found that *KRAS* mutations were more frequently associated with exon 9 than exon 20 *PIK3CA* mutations. A similar association was noted by De Roock and colleagues in a study of 743 patients with metastatic colorectal cancer, but a larger study of 1,170 patients with predominantly localized colorectal cancer did not find a similar association (18, 19).

The paradigm for matching targeted therapy based on specific molecular defects identified in the cancer cell was established by the remarkable success of imatinib in chronic myeloid leukemia (26). This model has been successfully replicated in solid tumors such as *BRAF* V600E mutated melanoma and non–small cell lung cancer with *ALK* rearrangement (15, 16, 27). In the context of targeting *PIK3CA*-mutated cancers with PI3K/AKT/mTOR inhibitors, we have observed encouraging responses in heavily pretreated patients with breast and gynecologic malignancies and *PIK3CA* mutations treated with PI3K/AKT/mTOR pathway inhibitors (9, 28, 29). In the current study, 17 heavily pretreated patients with colorectal cancer and *PIK3CA* mutations treated with PI3K/AKT/mTOR inhibitors did not achieve better out-

comes compared with patients with colorectal cancer without *PIK3CA* mutations treated with identical therapies (stable disease ≥6/PR/CR rate 6% vs.16%; *P* = 0.44) and their outcomes were strikingly similar response rates of 4% to 11% previously reported from early-phase clinical trials in an unselected population, including a 1.4% response rate and 9% rate of stable disease at 6 months for unselected patients with colorectal cancer from our center (30–33). In addition, the median PFS in patients with *PIK3CA* mutations treated with PI3K/AKT/mTOR inhibitors demonstrated a trend toward inferiority to the median PFS achieved by the same patients on their last FDA-approved therapy (1.9 months vs. 3.1 months; *P* = 0.1). Our observations are in line with the experience from a major European cancer center reporting lack of therapeutic benefit with therapeutic matching (including *PIK3CA*) in colorectal cancer (20). Reasons why these patients do not derive similar benefits from PI3K/AKT/mTOR-targeted therapies as patients do with *PIK3CA* mutations and breast or gynecologic cancers remains unknown. Preclinical models demonstrated that simultaneous mutations in the mitogen-activated protein kinase (MAPK) pathway (*RAS*, *RAF*, or *MEK*) can negate the effect of mTOR inhibitors in patients with *PIK3CA* mutations (5, 6). Therefore, it is plausible that this strong association between *PIK3CA* and *KRAS* mutations in colorectal cancer can contribute to therapeutic resistance as, in our series, 71% of patients with colorectal cancer and *PIK3CA* mutations had simultaneous *KRAS* mutations. In contrast, in patients with breast and gynecologic malignancies with *PIK3CA* mutations, only 23% had coexisting *KRAS* mutations, which were often in different exons than in colorectal cancer (9). Furthermore, Shimizu and colleagues analyzed phase I therapies targeting PI3K and MAPK pathways in diverse advanced cancers and demonstrated superior outcomes with combined PI3K and

MAPK inhibition in patients with cancers having alterations in both pathways (20, 34).

The majority of patients in our study were treated with rapalogs and it is possible that the outcomes with these inhibitors may be inferior due to feedback activation of AKT, as has been previously described *in vitro* and in patients (35, 36). Other biomarkers besides activating mutations in *PIK3CA* may be required to select for inhibitors of the PI3K pathway, including loss of the tumor suppressor PTEN or gene amplification of members the insulin growth factor family. Ongoing clinical efforts in *PIK3CA*-mutant colorectal cancer are using AKT or PI3K inhibitors and excluding concurrent KRAS mutations in an effort to isolate a responsive population.

Our study has several important limitations. First, there were only a small number of patients with *PIK3CA* mutations who were treated with PI3K/AKT/mTOR inhibitors; the majority of patients (65%) received mTORC1-targeting agents. Second, the analysis was retrospective. Third, patients received drugs that impacted different parts of the pathway (mTORC1 inhibitors, 11 patients; PI3K inhibitors, 5 patients; AKT inhibitor, 1 patient), although the latter might also imply that conclusions are not confined to one type of drug. Fourth, doses were variable. For instance, only 76% of patients received a maximum tolerated dose/recommended phase II dose/FDA-approved dose of a PI3K/AKT/mTOR inhibitor. Of interest, 91% of patients treated with mTORC1 inhibitors received a maximum tolerated dose/recommended phase II dose/FDA-approved dose compared with only 20% of patients treated with a PI3K inhibitor, which precludes firm conclusions about the latter class of drugs (32, 37). Fifth, none of the patients in our analysis received combined PI3K and MAPK-targeted therapy.

In conclusion, our data, although preliminary, suggest that targeted matched therapy with PI3K/AKT/mTOR pathway inhibitors (in particular mTORC1 inhibitors) in patients with metastatic colorectal cancer harboring *PIK3CA* mutations may be associated with minimal activity. The lack of benefit can conceivably be explained, in part, by the high prevalence of coexisting *KRAS* mutations driving therapeutic resistance.

Disclosure of Potential Conflicts of Interest

Authors' Contributions

Acknowledgments

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