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 prevalence of simultaneous 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 months 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.

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Introduction

The PIK3CA gene encodes the 110k 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.
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 c534 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 10 (E590K), 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
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 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...
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/C21, which was similar to stable disease/C21/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).

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

<table>
<thead>
<tr>
<th>Variable</th>
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<th>KRAS mutation (%)</th>
<th>WT KRAS (%)</th>
<th>P value</th>
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<td>99 (100)</td>
<td>Not applicable</td>
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<td>22 (24)</td>
<td>9 (9)</td>
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<tr>
<td>WT PIK3CA</td>
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<td>90 (91)</td>
<td>0.01</td>
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<tr>
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<td>16 (18)</td>
<td>6 (6)</td>
<td>0.48</td>
</tr>
<tr>
<td>WT or exon 20 PIK3CA mutations</td>
<td>168</td>
<td>74 (82)</td>
<td>93 (94)</td>
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<tr>
<td>PIK3CA exon 20 mutations</td>
<td>8</td>
<td>5 (6)</td>
<td>3 (3)</td>
<td></td>
</tr>
<tr>
<td>WT or exon 9 PIK3CA mutations</td>
<td>181</td>
<td>85 (94)</td>
<td>96 (97)</td>
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</tr>
</tbody>
</table>

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.
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 demonstrated by the superior outcomes with combined PI3K and 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; \( P = 0.44 \)) 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 \( PIK3CA \)/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 \( PIK3CA \)/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
F. Janku received commercial research grants from Novartis, Roche, Biocartis, Transgenomic, and Trovagene and is a consultant/advisory board member of Trovagene. S. Kopetz is a consultant/advisory board member of Roche, Sanofi, Agen, Bristol-Meyer Squibb, and Bayer. R. Kurzrock has research support from GlaxosmithKline, Novartis, Merck, and Bayer. No potential conflicts of interest were disclosed by the other authors.

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