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KRASness and PIK3CAanness in Patients with Advanced Colorectal Cancer: Outcome after Treatment with Early-Phase Trials with Targeted Pathway Inhibitors

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Abstract

Purpose: To evaluate clinicopathologic and molecular features of patients with metastatic colorectal cancer (mCRC) and their outcomes in early-phase trials using pathway-targeting agents.

Patients and Methods: We analyzed characteristics of 238 patients with mCRC referred to the phase 1 trials unit at MD Anderson Cancer Center. KRAS, PIK3CA and BRAF status were tested using PCR-based DNA sequencing.

Results: Fifty-one percent of patients harbored KRAS mutations; 15% had PIK3CA mutations. In the multivariate regression model for clinical characteristics KRAS mutations were associated with an increased incidence of lung and bone metastases and decreased incidence of adrenal metastases; PIK3CA mutations were marginally correlated with mucinous tumors (p = 0.05). In the univariate analysis, KRAS and PIK3CA mutations were strongly associated. Advanced Duke's stage (p < 0.0001) and KRAS mutations (p = 0.01) were the only significant independent predictors of poor survival (Cox proportional hazards model). Patients with PIK3CA mutations had a trend toward shorter progression-free survival when treated with anti-EGFR therapies (p = 0.07). Eighteen of 78 assessable patients (23%) treated with PI3K/Akt/mTOR axis inhibitors achieved stable disease (SD) ≥6 months or complete response/partial response (CR/PR), only one of whom were in the subgroup (N = 15) with PIK3CA mutations, perhaps because 10 of these 15 patients (67%) had coexisting KRAS mutations. No SD ≥6 months/CR/PR was observed in the 10 patients treated with mitogen-activating protein kinase (MAPK) pathway targeting drugs.

Conclusions: KRAS and PIK3CA mutations frequently coexist in patients with colorectal cancer, and are associated with clinical characteristics and outcome. Overcoming resistance may require targeting both pathways.

Introduction

There is increasing support for the concept that specific mutations predict the clinical manifestations and response to therapy of patients with cancer. In colorectal cancer, RAS mutations have received mounting scrutiny. In particular, activating mutations in KRAS drive resistance to anti-EGFR therapies in patients with metastatic disease. [1,2] However, a subset of individuals with KRAS mutations (p.G13D) might derive benefit from anti-EGFR therapies. [3] The role of PIK3CA mutations in predicting resistance to anti-EGFR therapies has been debated, with initial studies reaching opposing conclusions. [4,5] Recently, a multicenter retrospective study showed that PIK3CA mutations in exon 20 were involved in resistance to anti-EGFR therapies, whereas mutations in exon 9 were not. [6] Finally, our group demonstrated that activating mutations in PIK3CA may predict response to PI3K/Akt/mTOR inhibitors. [7] Here, we review the clinical and molecular characteristics of patients with metastatic colorectal cancer (mCRC) who were referred to our clinical trials unit. The purpose of the study was to identify clinical characteristics associated with KRAS and PIK3CA mutations, and outcomes on early clinical trials of PI3K/Akt/mTOR and MAPK inhibitors.

Results

Patient Characteristics

Patient and tumor clinicopathologic characteristics for the 238 study patients are listed in Table 1. Fifty-four percent of patients were men. Seventy-one percent of patients were over the age of...
Fifty. Most patients (69%) were Caucasians. The most common sites of metastases were liver, lymph nodes and lung, found in 93%, 75%, and 72% of patients, respectively. All 230 patients were tested for KRAS status. One-hundred-and-twenty-two patients (51%) had KRAS mutations. Of the 168 patients tested for PIK3CA mutations, 25 (15%) had a mutation. Of the 175 patients tested for BRAF mutations, 11 (6%) had a mutation.

**Frequency of KRAS Mutation Subtypes**

Next, we assessed the incidence of different types of KRAS mutations. The location of KRAS mutations was available in 108 out of 122 patients. The most frequent KRAS mutation was p.G12D (121 patients [99%]), followed by p.G12V (25 patients [21%]), p.G12A (14 patients [13%]) and p.G13D (13 patients [12%]). Other mutations occurred at the following low incidences: p.G12C (11 patients [10%]), p.G12S (8 patients [7%]), p.Q61H (5 patients [5%]) and p.G12R, p.G13C and p.G12F, one patient each (1%).

**Evaluation of “KRASness”**

**Clinical Features.** Patients with KRAS mutations (N = 122) had a higher incidence of lung (79% [96/122] vs. 66% [76/116], p = 0.03) and bone metastases (20% [24/122] vs. 9% [11/116], p = 0.03) compared to those with wild-type KRAS (N = 116).

To further assess the association between KRAS mutations and different clinical features in patients with colorectal cancer, we fitted univariate and multivariate logistic regression models. The univariate models suggested that patients with KRAS mutations had a higher probability of having lung (p = 0.02) and bone metastases (p = 0.03) (Table 2). We fitted a multivariate model including those variables with univariate p-values <0.5 in the full model. After backward model selection, the multivariate model showed that increased lung and bone metastases and decreased adrenal metastases are significantly associated with KRAS mutations (Table 2).

**Molecular Characteristics.** One-hundred-and-sixty-eight patients had testing for both KRAS and PIK3CA mutations. One-half of the patients tested (N = 84) had a KRAS mutation and one-half (N = 84) did not. Compared to KRAS wild-type patients, patients harboring KRAS mutations more frequently had PIK3CA mutations (21% [18/84] vs. 8% [7/84], p = 0.03). As previously reported, BRAF and KRAS mutations were mutually exclusive (173 patients tested for both) (Table 1). [6]

PIK3CA mutations were significantly associated with KRAS mutations in univariate models (p = 0.03). Of interest, when we introduced molecular features into the clinical multivariate model (i.e., PIK3CA status), the only variable associated with KRAS mutations was PIK3CA (data not shown).

**KRAS Mutations Were Associated With a Shorter OS**

We conducted a univariate analysis of survival for different patient characteristics, including age, gender, race, tumor type (mucinous vs. not), Duke’s stage at diagnosis, site of primary tumor, KRAS, BRAF and PIK3CA mutations. In the multivariate analysis, Duke’s stage at diagnosis and KRAS status were significant predictors of survival (KRAS HR 1.71, 95% CI 1.11–2.62) (Table 3). The median OS from time of diagnosis for patients with KRAS mutations was 57.5 months (95% CI: 50.0–64.3 months), whereas for KRAS wild-type patients, the median OS was 95.5 months (95% CI 63.3–120.1 months) (log-rank p = 0.007) (Figure 1).

We also assessed whether different types of KRAS mutations were associated with survival. We found no differences in OS across different types of KRAS mutations. For example, the median OS for patients with a codon 12 mutation was 57.5 months (95% CI 50–62.2 months) and the median OS for patients with a codon 13 mutation was 56.8 months (95% CI 28.3–not estimable) (log-rank p-value = 0.52). We further assessed OS, as defined by the specific type of amino acid involved in the different mutations. For this analysis we kept only subgroups with at least 10 observations (Table S1). There was no difference in OS. However, the number of patients in each subtype group was small, ranging from 11 to 31, precluding definitive conclusions.

**Evaluation of “PIK3CAAness”**

**Clinical Features.** To assess the association between PIK3CA mutations and different clinical features in patients with colorectal cancer, we tried to fit univariate and multiple logistic regression models. The univariate model suggested that patients with PIK3CA mutations more frequently had mucinous tumors (p = 0.04). They also had a trend to have less frequent liver metastases compared to patients with PIK3CA wild-type tumors (OR 0.40, p = 0.07) (Table 4). In the multivariate analysis, mucinous tumors were marginally significant (p-value = 0.05), with patients having mucinous tumors more frequently having PIK3CA mutations (OR 2.61; 95% CI 0.99–6.87). In addition, age showed a week trend (p-value = 0.15), with older patients less frequently having PIK3CA mutations (OR 0.52; 95% CI 0.22–1.26). As mentioned previously, PIK3CA status was not an independent variable predicting survival in multivariate analysis.

**Molecular Characteristics.** 168 (71%) patients were tested for PIK3CA mutations. Of the 143 patients with wild-type PIK3CA, 66 (46%) had KRAS mutations. Of the 25 (15%) patients with PIK3CA mutations, 18 (72%) had KRAS mutations (p = 0.03) (Table 1). Of the 147 patients tested for both PIK3CA and BRAF, one patient (0.7%) had both mutations. A recent report showed that PIK3CA mutations in exon 20 were involved in resistance to anti-EGFR therapies, whereas mutations in exon 9 were not. [6] Further, although KRAS mutations are believed to predict resistance to EGFR inhibitor treatment, a subset of individuals with KRAS p.G13D mutations may still derive benefit from anti-EGFR therapies. [3] We, therefore, analyzed coexistent PIK3CA mutations (exon 9 and 20) in patients with KRAS mutations. We found that patients with KRAS p.G13D mutations had the lowest incidence of PIK3CA mutations (10%) compared to patients with KRAS p.G12A (36% had PIK3CA mutations), p.G12C (33%), p.G12D (22%) and p.G12V (12%). Moreover, none of the nine patients with KRAS p.G13D mutations had a PIK3CA mutation in exon 20, whereas PIK3CA mutations in exon 20 were found in all other types of KRAS mutations (Table S2). However, the small numbers of patients precludes drawing any statistically significant conclusions.

**PFS on anti-EGFR treatment.** We analyzed PFS in patients treated with anti-EGFR therapies (cetuximab or panitumumab). Patients with KRAS mutant mCRC treated with anti-EGFR therapies (N = 24) had shorter PFS compared to patients with wild-type KRAS (N = 90) (15 vs. 22 weeks, p = 0.01). Within the group of patients tested for PIK3CA mutations who received anti-EGFR therapies, patients with PIK3CA mutations (N = 9) had a trend toward a shorter PFS than PIK3CA wild-type patients (N = 82) (17 vs. 22 weeks, p = 0.07) (Figure S1).
Table 1. Patient characteristics.

| VARIABLES                | Overall, N = 238 | KRAS wild-type N = 116 | KRAS mutation N = 122 | p-value |
|--------------------------|------------------|------------------------|-----------------------|---------|
| Gender                   |                  |                        |                       |         |
| Male                     | 128 (54%)        | 63 (54.3%)             | 65 (53.3%)            | 0.90    |
| Female                   | 110 (46%)        | 53 (45.7%)             | 57 (46.7%)            |         |
| Age (years)              |                  |                        |                       |         |
| ≤50                      | 68 (29%)         | 33 (28.5%)             | 35 (28.7%)            | 0.94    |
| >50                      | 170 (71%)        | 83 (71.5%)             | 87 (71.3%)            |         |
| Race                     |                  |                        |                       |         |
| White                    | 165 (69%)        | 82 (70.7%)             | 83 (68.0%)            | 0.25    |
| Hispanic                 | 21 (9%)          | 13 (11.2%)             | 8 (6.6%)              |         |
| African-American         | 41 (17%)         | 15 (12.9%)             | 26 (21.3%)            |         |
| Other                    | 11 (5%)          | 6 (5.2%)               | 5 (4.1%)              |         |
| Histology                |                  |                        |                       |         |
| Non-mucinous             | 197(17%)         | 99 (85.3%)             | 98 (80.3%)            | 0.39    |
| Mucinous                 | 41 (83%)         | 17 (14.7%)             | 24 (19.7%)            |         |
| Site                     |                  |                        |                       |         |
| Ascending                | 56 (24%)         | 21 (18.6%)             | 35 (28.9%)            | 0.28    |
| Transverse               | 15 (6%)          | 7 (6.2%)               | 8 (6.6%)              |         |
| Descending/sigmoid       | 95 (41%)         | 48 (42.5%)             | 47 (38.8%)            |         |
| Rectum                   | 68 (29%)         | 37 (32.7%)             | 31 (25.6%)            |         |
| Dukes stage*             |                  |                        |                       |         |
| A                        | 1 (0.4%)         | 0 (0%)                 | 1 (0.8%)              | 0.51    |
| B                        | 19 (8%)          | 10 (8.8%)              | 9 (7.4%)              |         |
| C                        | 79 (33%)         | 42 (36.8%)             | 37 (30.3%)            |         |
| D                        | 137 (58%)        | 62 (54.4%)             | 75 (61.5%)            |         |
| BRAF*                    |                  |                        |                       |         |
| wild-type                | 162 (94%)        | 83 (88.3%)             | 79 (100%)             | 0.002   |
| mutated                  | 11 (6%)          | 11 (11.7%)             | 0 (0%)                |         |
| PIK3CA**                 |                  |                        |                       |         |
| wild-type                | 143 (85%)        | 77 (91.7%)             | 66 (78.6%)            | 0.03    |
| mutated                  | 25 (15%)         | 7 (8.3%)               | 18 (21.4%)            |         |
| Adjuvant chemo           |                  |                        |                       |         |
| None                     | 17 (7%)          | 7 (13.5%)              | 10 (20.8%)            | 0.43    |
| Yes                      | 83 (93%)         | 45 (86.5%)             | 38 (79.2%)            |         |
| Anti-EGFR therapy        |                  |                        |                       |         |
| No                       | 109 (46%)        | 15 (12.9%)             | 94 (77.0%)            | 0.0001  |
| Yes                      | 129 (54%)        | 101 (87.1%)            | 28 (23.0%)            |         |
| Metastases               |                  |                        |                       |         |
| Liver                    | 198 (83%)        | 94 (81.0%)             | 104 (85.2%)           | 0.39    |
| Lung                     | 172 (72%)        | 76 (65.5%)             | 96 (78.7%)            | 0.03    |
| Adrenal gland            | 31 (13%)         | 19 (16.4%)             | 12 (9.8%)             | 0.18    |
| Brain                    | 9 (4%)           | 4 (3.4%)               | 5 (4.1%)              | 1.00    |
| Peritoneum               | 81 (34%)         | 36 (31.0%)             | 45 (36.9%)            | 0.41    |
| Lymph nodes              | 179 (75%)        | 85 (73.3%)             | 94 (77.0%)            | 0.55    |
| Ovarian                  | 14 (6%)          | 8 (6.9%)               | 6 (4.9%)              | 0.59    |
| Bone                     | 35 (15%)         | 11 (9.5%)              | 24 (19.7%)            | 0.03    |

Site at diagnosis was unknown for four patients,

*Dukes stage* at diagnosis was unknown for two patients,

*BRAF status was known in 173 patients,

**PIK3CA status was known in 168 patients

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Table 2. Univariate and multivariate logistic regression model for clinical characteristics associated with KRAS mutations in colorectal cancer.

| Variable                                      | Coefficient | SE   | P-value | Odds Ratio  | 95% Confidence Interval |
|-----------------------------------------------|-------------|------|---------|-------------|-------------------------|
| Age at diagnose ≥50 (vs. ≤50)                 | −0.01       | 0.29 | 0.97    | 0.99        | 0.56 - 1.74             |
| Male (vs. female)                             | −0.04       | 0.26 | 0.87    | 0.96        | 0.58 - 1.60             |
| Mucinous (vs. non-mucinous)                   | 0.35        | 0.35 | 0.31    | 1.42        | 0.72 - 2.82             |
| Dukes = a, b, c (vs. d)                       | −0.29       | 0.26 | 0.27    | 0.75        | 0.45 - 1.26             |
| Liver metastases = yes (vs. no)               | 0.3         | 0.35 | 0.39    | 1.35        | 0.68 - 2.68             |
| Lung metastases = yes (vs. no)                | 0.66        | 0.3  | **0.02**| 1.94        | 1.09 - 3.46             |
| Adrenal = yes (vs. no)                        | −0.59       | 0.39 | 0.14    | 0.56        | 0.26 - 1.21             |
| Brain metastases = yes (vs. no)               | 0.18        | 0.68 | 0.79    | 1.20        | 0.31 - 4.57             |
| Peritoneal metastases = yes (vs. no)          | 0.26        | 0.27 | 0.34    | 1.30        | 0.76 - 2.23             |
| Lymph node metastases = yes (vs. no)          | 0.2         | 0.3  | 0.50    | 1.22        | 0.68 - 2.21             |
| Ovarian metastases = yes (vs. no)             | −0.36       | 0.56 | 0.52    | 0.70        | 0.24 - 2.08             |
| Bone metastases = yes (vs. no)                | 0.85        | 0.39 | **0.03**| 2.34        | 1.09 - 5.02             |

Multivariate Regression Model (N = 238)

| Variable                                      | Coefficient | SE   | P-value | Odds Ratio  | 95% Confidence Interval |
|-----------------------------------------------|-------------|------|---------|-------------|-------------------------|
| Intercept                                     | −0.40       | 0.25 | 0.12    | -           | -                       |
| Lung mets = yes (vs. no)                      | 0.61        | 0.31 | 0.05    | 1.85        | 1.01 - 3.36             |
| Bone mets = yes (vs. no)                      | 0.83        | 0.41 | 0.05    | 2.29        | 1.02 - 5.14             |
| Adrenal = yes (vs. no)                        | −0.86       | 0.42 | 0.04    | 0.42        | 0.19 - 0.96             |

Figure 1. Kaplan-Meier plot of overall survival (OS) and KRAS status. Patients with KRAS wild-type mCRC had longer OS compared to KRAS mutant patients (tick marks represent patients still alive at time of last follow up).

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Response to PI3K/Akt/mTOR Inhibitors or MAP Kinase Pathway Inhibitors: impact of coexistence of PIK3CA and KRAS mutations

Eighty patients were treated on phase 1 protocols containing PI3K/Akt/mTOR inhibitors (NCT00454090, NCT00554268, NCT00610493, NCT00687622, NCT00726583, NCT00731263, NCT00756847, NCT00761644, NCT00770731, NCT00880321, NCT00920257, NCT00940381, NCT00972686, NCT01054313, NCT01072175, NCT01087554, NCT01138085, NCT01155453, NCT01263145) (www.clinicaltrials.gov). Seventy-eight patients were assessable for response by RECIST (Figure 2).

Of these 78 patients, 43 had a KRAS mutation in their tumors and 35 were KRAS wild-type. Of the 43 patients with KRAS-mutant disease, 9 (21%) attained SD $\geq 6$ months/CR/PR; of the 33 patients with KRAS wild-type, 9 (27%) had SD $\geq 6$ months/CR/PR (Fisher’s exact test p = 0.59).

Fifteen patients with PIK3CA mutations were treated with PI3K/Akt/mTOR inhibitors, and one (7%) had SD $\geq 6$ months/CR/PR; 10 of those patients (67%) had a concomitant KRAS mutation and none had a BRAF mutation. Sixty-three patients with wild-type or unknown PIK3CA status were treated with PI3K/Akt/mTOR inhibitors, and 17 (27%) had SD $\geq 6$ months/CR/PR (Fisher’s exact test for SD $\geq 6$ months/CR/PR in PIK3CA mutant vs. wild-type p = 0.17); 33 of these patients (52%) had a KRAS mutation.

A total of 10 patients were treated with MAPK pathway inhibitors (generally BRAF or MEK inhibitors). Of those patients, four had BRAF and five had KRAS mutations. None had PIK3CA mutations.

**Table 3.** Univariate Analysis and Multivariate Cox Model for Overall Survival.

| Covariate                      | HR      | 95% CI     | P-value | N death | N* |
|--------------------------------|---------|------------|---------|---------|----|
| male (vs. female)              | 1.15    | 0.76–1.75  | 0.50    | 94      | 238|
| mucinous (vs. non-mucinous)    | 1.59    | 0.96–2.56  | 0.07    | 94      | 238|
| Dukes = a, b, c (vs. d)        | 0.38    | 0.25–0.60  | $<0.0001$ | 93      | 236|
| BRAF = yes (vs. no)            | 1.28    | 0.46–3.55  | 0.63    | 64      | 173|
| PIK3CA = yes (vs. no)          | 1.15    | 0.61–2.17  | 0.67    | 63      | 168|
| KRAS = yes (vs. no)            | 1.77    | 1.16–2.70  | 0.01    | 94      | 238|

**Multivariate Cox proportional hazards model for overall survival (n = 236; death = 93)**

| Covariate                      | HR      | 95% CI     | P-value | N death | N* |
|--------------------------------|---------|------------|---------|---------|----|
| Dukes = a, b, c (vs. d)        | 0.39    | 0.25–0.60  | $<0.0001$ | 93      | 236|
| KRAS = yes (vs. no)            | 1.71    | 1.11–2.62  | 0.01    | 94      | 238|

**Table 4.** Univariate and multivariate logistic regression model for clinical characteristics associated with PIK3CA mutations in colorectal cancer.

**Univariate regression model (N = 168)**

| variable                      | Coefficient | SE   | P-value | Odds Ratio | 95% Confidence Interval |
|-------------------------------|-------------|------|---------|------------|-------------------------|
| Age at diagnose >50 (vs. \leq 50) | −0.70       | 0.44 | 0.11    | 0.49       | 0.21–1.18               |
| male (vs. female)             | 0.54        | 0.46 | 0.24    | 1.72       | 0.70–4.25               |
| mucinous (vs. non-mucinous)   | 1.01        | 0.49 | 0.04    | 2.75       | 1.05–7.14               |
| Dukes = a, b, c (vs. d)       | 0.57        | 0.44 | 0.19    | 1.77       | 0.75–4.15               |
| Liver metastases = yes (vs. no) | −0.93      | 0.51 | 0.07    | 0.39       | 0.15–1.07               |
| lung metastases = yes (vs. no) | −0.19       | 0.47 | 0.68    | 0.83       | 0.33–2.06               |
| Adrenal = yes (vs. no)        | 0.41        | 0.61 | 0.50    | 1.51       | 0.46–4.96               |
| Brain metastases = yes (vs. no) | 1.11       | 0.89 | 0.22    | 3.03       | 0.52–17.46              |
| Peritoneal metastases = yes (vs. no) | 0.47     | 0.44 | 0.28    | 1.60       | 0.68–3.81               |
| LN metastases = yes (vs. no)  | 0.90        | 0.64 | 0.16    | 2.46       | 0.70–8.73               |
| Ovarian metastases = yes (vs. no) | −0.79     | 1.06 | 0.46    | 0.45       | 0.06–3.66               |
| Bone metastases = yes (vs. no) | 0.10        | 0.59 | 0.86    | 1.11       | 0.35–3.55               |

**Multivariate regression model (N = 168)**

| variable                      | Coefficient | SE   | P-value | Odds Ratio | 95% Confidence Interval |
|-------------------------------|-------------|------|---------|------------|-------------------------|
| Intercept                     | −1.54       | 0.38 | $<0.0001$ | -          | -                       |
| mucinous (vs. non-mucinous)   | 0.96        | 0.49 | 0.05    | 2.61       | 0.99–6.87               |
| Age at Dx >50 (vs. \leq 50)   | −0.65       | 0.45 | 0.15    | 0.52       | 0.22–1.26               |

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mutations. None of those patients had SD ≥6 months/CR/PR (figure 2).

Discussion

In 238 patients with colorectal cancer referred to our clinical trials unit, we found that 122 (51%) had KRAS mutations. The most frequent KRAS mutation subtypes were p.G12D (31 patients [25%]), followed by p.G12V (23 patients [19%]), p.G12A (14 patients [11%]) and p.G13D (13 patients [11%]). This incidence of KRAS subtypes was consistent with that reported in the Catalogue of Somatic Mutations in Cancer (COSMIC) database (www.sanger.ac.uk/genetics/CGP/cosmic/accessed on June 17th, 2011).

In the multivariate analysis of clinical characteristics, KRAS mutations were associated with increased incidence of lung and bone metastases (p = 0.05 for each variable) and decreased incidence of adrenal metastases (p = 0.04) compared to KRAS wild-type patients. Previous studies found a higher probability of lung metastases in patients with mCRC with KRAS mutations. [8,9] At the molecular level, patients harboring KRAS mutations more frequently had concomitant PIK3CA mutations compared to individuals with wild-type KRAS (21% vs. 8%, [p = 0.03]). Further, 72% of patients with PIK3CA mutations also had a KRAS mutation while only 46% of patients with wild-type PIK3CA had a KRAS mutation (p = 0.03). PIK3CA mutations were significantly associated with KRAS mutations in both univariate and multivariate models.

The median OS from time of diagnosis for patients with KRAS mutations was shorter than for those with wild-type KRAS (57.5 vs 89.5 months; p = 0.007) (Figure 1). Furthermore, in multivariate analysis, Duke’s stage at diagnosis and KRAS status were significant independent predictors of survival. BRAF mutations have recently been found to be prognostic for shorter OS in patients with mCRC. [10,11] However, the role of KRAS status as a predictor of OS is controversial. A previous retrospective study including 3,439 patients in a multivariate survival analysis found that only p.G12V mutations were predictive of poor survival. Interestingly, the incidence of p.G12V mutations in that study was considerably lower than the one reported in the COSMIC database (8% vs. 23%). A recent study showed increased OS in patients with KRAS wild-type mCRC treated with best supportive care compared to patients with KRAS-mutant mCRC. [1] However, after progressing on supportive care, patients with KRAS wild-type were allowed to cross over to panitumumab; therefore, treatment effect cannot be completely ruled out. It is unclear why different studies show conflicting data regarding the prognostic effect of KRAS, though it is conceivable that this is related to therapy given and/or other selection factors.

In univariate analysis, patients with PIK3CA mutations more frequently had mucinous tumors (p = 0.04), and tended to less frequently have liver metastases compared to patients with PIK3CA wild-type tumors. Only the association with mucinous tumor was (marginally) significant in the multivariate analysis (p = 0.05). PIK3CA status was not an independent variable predicting survival in multivariate analysis. At the molecular level, 72% of patients with PIK3CA mutations had coexisting KRAS mutations. Of possible interest, patients with mutated PIK3CA had a trend to a shorter PFS when treated with anti-EGFR therapies than those with wild-type PIK3CA (median = 22 weeks vs. 17 weeks, p = 0.07). Other reports also suggest that patients with PIK3CA mutations are less sensitive to anti-EGFR therapies. [4] However, a larger multicenter analysis recently found that only PIK3CA mutations in exon 20 were associated with a worse outcome after treatment with cetuximab. [6] Although several lines of evidence indicate that patients with colorectal cancer and KRAS mutations do poorly with EGFR inhibitors, recent data suggests that those individuals with KRAS mutation p.G13D may benefit from EGFR inhibitors. [3] We, therefore, analyzed patients with p.G13D KRAS mutations for exon 20 PIK3CA mutations and found none, whereas these
mutations were found in other KRAS mutant subtypes. However, the small number of patients assessed precludes definitive conclusions. It may be warranted to evaluate, in prospective studies, whether or not co-existence of exon 20 mutated PIK3CA in certain subtypes of KRAS mutant disease could contribute to EGFR inhibitor resistance.

Eighty patients were treated on protocols containing PI3K/AKT/mTOR inhibitors of whom 78 (98%) were assessable. 23% achieved SD ≥6 months/CR/PR. There was no significant difference in the rate of SD ≥6 months/CR/PR between those with wild-type or mutant KRAS. Of interest, only 1 patient with a PIK3CA mutation achieved SD ≥6 months/CR/PR when treated with a PI3K/Akt/mTOR inhibitor. Most of these patients (67%) had co-existing KRAS mutations, which may explain their resistance. Indeed, activation of the MAPK pathway has recently been proposed as a mechanism of resistance to PI3K inhibitors. [12] Even so, it may be of interest that among the 13 patients that had some kind of tumor reduction, 7 had KRAS mutation. The latter suggests that though the rates of SD ≥6 months/CR/PR were low, and patients with co-existing KRAS and PIK3CA mutations did not respond, the presence of a KRAS mutation alone is not an absolute indicator of complete resistance to PI3K/Akt/mTOR inhibitor-based therapy. We recently reported that coexistence of PIK3CA and KRAS mutations was not predictive of resistance to PI3K/Akt/mTOR in patients with ovarian cancer. [13] Certainly, the disease-type context plays a role and additional mutations not included in this analysis may impact the response to these therapies. A recent work has shown that activation of a complex network of feed-back loops may for instance explain resistance to BRAF inhibitors in patients with colorectal cancer with BRAF mutations. [14]

Unexpectedly, 27% of patients with wild-type or unknown PIK3CA status treated with a PI3K/Akt/mTOR inhibitor attained SD ≥6 months/CR/PR (p = 0.09). It is possible that a molecular aberration in the PI3K/Akt/mTOR pathway existed in these patients but was not recognized, and that such an aberration may not have the same propensity to co-exist with KRAS or BRAF mutations. Indeed, loss of PTEN expression, which often indicates resistance to PI3K/Akt/mTOR in patients with ovarian cancer. [13] Certainly, the disease-type context plays a role and additional mutations not included in this analysis may impact the response to these therapies. A recent work has shown that activation of a complex network of feed-back loops may for instance explain resistance to BRAF inhibitors in patients with colorectal cancer with BRAF mutations. [14]

A total of 10 patients (nine of whom had KRAS or BRAF mutations and none of whom had PIK3CA mutations) were treated with MAPK pathway inhibitors (generally BRAF or MEK inhibitors). Although the numbers of patients are small, none achieved SD ≥6 months/CR/PR.

Our work has several limitations. First, this is a retrospective study in a single institution with a relatively small number of patients. Second, we could not validate response rate to anti-EGFR therapies as this information was not available for many of the patients who were treated in institutions other than MD Anderson prior to being referred to our unit; hence, the only available clinical outcome for these patients was PFS. Third, all mutational analyses could not be completed for all patients included in the study because of limited amounts of available tissue.

In conclusion, we show that KRAS and PIK3CA mutations are frequently associated in patients with colorectal cancer. In the context of early clinical trials, drugs targeting the PI3K/Akt/mTOR pathway had limited activity in these patients, even in the presence of PIK3CA mutations, possibly because of the frequent coexistence of activating mutations in the MAPK pathway.

**Methods**

**Patients**

We retrospectively reviewed the clinicopathologic characteristics and clinical outcomes of 238 consecutive patients with mCRC who were seen in the Phase 1 Clinic (Clinical Center for Targeted Therapy) at The University of Texas MD Anderson Cancer Center beginning in October 2008, and for whom KRAS status was known. Data were collected from transcribed notes and radiology reports in the electronic database of these patients. The sites of metastatic disease were collected from the last available radiology report for each patient. Pathology was reviewed by an MD Anderson pathologist in all cases. The MD Anderson Cancer Center Institutional Review Board has approved the study. Written consent was given by the patients for their information to be stored in the hospital database and used for research.

**KRAS, BRAF, PIK3CA Mutation Testing**

Mutation testing was done 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 micro-dissected paraffin-embedded tumor and analyzed by a polymerase chain reaction (PCR)-based DNA sequencing method to examine codons 12, 13 and 61 of the KRAS proto-oncogene. The sensitivity of detection of this assay is approximately 1 in 10 mutation-bearing cells in the microdissected area. Whenever possible, analysis was done for PIK3CA mutations in codons [c] 532–554 of exon 9 (helical domain) and c1011–1062 of exon 20 (kinase domain) and BRAF c595–600 mutations of exon 15 by pyrosequencing as previously described. [16]

**Statistical Methods**

Statistical analysis was performed and validated by our statistician (XW). Patient characteristics are summarized using descriptive statistics. The association between KRAS or PIK3CA mutation status and patient characteristics was assessed using Fisher’s exact test. Overall survival (OS) is defined as the time interval between date of diagnosis and death. Patients who were alive were censored at the last follow-up date. Progression-free survival (PFS) was defined as the time from treatment initiation to detection of progressive disease or death. Patients who did not progress while on treatment were censored at the last date of follow up. The probabilities of OS and PFS were estimated using the method of Kaplan and Meier and were compared among subgroups of patients using the log-rank test. [17,18] Cox proportional hazards regression models were fit to assess the association between OS and patient characteristics and KRAS mutation status. [19] Univariate and multiple logistic regression models were fit to assess the association between KRAS and PIK3CA mutation and patient clinical characteristics. Initially, univariate logistic regression models were fit and variables with p-values less than 0.5 were included in the multiple logistic regression model. We then performed backward model selection and kept only those variables with p-values less than 0.05. All statistical analyses were conducted using SAS software (version9.2) and Splus (version 8.0).

**Supporting Information**

Figure S1 Kaplan-Meier plot of progression-free survival (PFS) and PIK3CA status on patients with mCRC treated with regimens including anti-EGFR therapies.
Patients with mCRC/\(\text{PIK3CA}\) mutant had a trend toward a shorter PFS compared to \(\text{PIK3CA}\) wild-type patients.

| Table S1 | Median overall survival (OS) for each group of \(\text{KRAS}\) mutations. |
| (DOCX) |

| Table S2 | Type of \(\text{PIK3CA}\) mutations found in patients with \(\text{KRAS}\) mutations*.
| (DOCX) |

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### Author Contributions
Conceived and designed the experiments: IGL DSH FJ XW RK. Performed the experiments: IGL RL. Analyzed the data: IGL DSH FJ XW RK. Contributed reagents/materials/analysis tools: IGL DSH FJ LMN GSF JJW RL AN XW RK. Wrote the paper: IGL DSH FJ XW RK.

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