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## Mutational Analysis and Clinical Correlation of Metastatic Colorectal Cancer

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### Abstract

**BACKGROUND**—Early identification of mutations may guide patients with metastatic colorectal cancer toward targeted therapies that may be life prolonging. The authors assessed tumor genotype correlations with clinical characteristics to determine whether mutational profiling can account for clinical similarities, differences, and outcomes.

**METHODS**—Under Institutional Review Board approval, 222 patients with metastatic colon adenocarcinoma (n = 158) and rectal adenocarcinoma (n = 64) who underwent clinical tumor genotyping were reviewed. Multiplexed tumor genotyping screened for >150 mutations across 15 commonly mutated cancer genes. The chi-square test was used to assess genotype frequency by tumor site and additional clinical characteristics. Cox multivariate analysis was used to assess the impact of genotype on overall survival.

**RESULTS**—Broad-based tumor genotyping revealed clinical and anatomic differences that could be linked to gene mutations. *NRAS* mutations were associated with rectal cancer versus colon cancer (12.5% vs 0.6%;  $P < .001$ ) and with age  $\geq 56$  years (7% vs 0.9%;  $P = .02$ ). Conversely, v-raf murine sarcoma viral oncogene homolog B (*BRAF*) mutations were associated with colon cancer (13% vs 3%;  $P = .024$ ) and older age (15.8% vs 4.6%;  $P = .006$ ). *TP53* mutations were

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associated with rectal cancer (30% vs 18%;  $P = .048$ ), younger age (14% vs 28.7%;  $P = .007$ ), and men (26.4% vs 14%;  $P = .03$ ). Lung metastases were associated with *PIK3CA* mutations (23% vs 8.7%;  $P = .004$ ). Only mutations in *BRAF* were independently associated with decreased overall survival (hazard ratio, 2.4; 95% confidence interval, 1.09–5.27;  $P = .029$ ).

**CONCLUSIONS**—The current study suggests that underlying molecular profiles can differ between colon and rectal cancers. Further investigation is warranted to assess whether the differences identified are important in determining the optimal treatment course for these patients.

### Keywords

colorectal cancer; mutation; clinicopathologic; *NRAS*; *BRAF*

## INTRODUCTION

Colorectal cancer (CRC) is the third most common form of cancer in the United States, with 143,460 estimated cases diagnosed in 2012 and 51,690 deaths.<sup>1</sup> Colon and rectal cancers are often described as a single entity, but they differ in natural history and management. Local recurrence is significantly higher in rectal cancer,<sup>2</sup> and rectal cancers often metastasize first to lung, whereas colon cancers metastasize first to liver.<sup>3</sup> Treatment for locally advanced colon cancer often involves surgery followed by adjuvant chemotherapy; whereas, for rectal cancer, treatment involves preoperative chemoradiation followed by surgery and adjuvant chemotherapy. Whether these differences can be explained by the underlying biology, embryologic derivation, anatomy, and/or surgical technique is unclear.

It has been demonstrated that specific molecular alterations in tumors correlate significantly with patient and tumor characteristics. In lung adenocarcinoma, activating epidermal growth factor receptor (EGFR) mutations are identified most commonly in Asian female nonsmokers.<sup>4</sup> In CRCs, v-raf murine sarcoma viral oncogene homolog B (*BRAF*) mutations have been associated with right-sided high-grade tumors in older patients.<sup>5</sup> Tumor genotyping is rapidly being integrated into routine clinical care to gain a better understanding of the patient's prognosis and to define the most appropriate treatment. Our institution has been performing clinical mutational profiling on patients with CRC since 2009. This testing represents a subset of patients with advanced metastatic disease for whom testing would assist in identifying targeted therapies for that individual. We retrospectively evaluated mutational signatures for correlation with clinical characteristics, anatomic site, and patient outcomes in the lower gastrointestinal tract.

## MATERIALS AND METHODS

### Patient Population

Under Institutional Review Board approval, we reviewed all patients ( $n = 222$ ) with metastatic adenocarcinoma of the colon ( $n = 158$ ) or rectum ( $n = 64$ ) who received mutational profiling as part of their standard care between April 2009 and July 2011. Patients were chosen to undergo testing at the discretion of their treating physician. Patients with follow-up <6 months were excluded to provide adequate time for assessment of patient outcomes. Patients were initially diagnosed between January 1980 and February 2011 and

were diagnosed with metastatic disease between January 1999 and February 2011. The distinction of colon versus rectal cancer was made by the location proximal or distal to the rectosigmoid junction, as determined by the patient's treating physician.

### Mutational Analysis

Clinical tumor genotyping was performed on all 222 patients using nucleic acids extracted from diagnostic formalin-fixed, paraffin-embedded tumor tissue using a modified Agencourt FormaPure method automated on a Beckman Coulter NX<sup>P</sup> workstation (Beckman Coulter, Pasadena, Calif). Mutational profiling simultaneously queried over 150 previously described hotspot mutations across 15 cancer genes, including v-akt murine thymoma viral oncogene homolog 1 (*AKT1*); adenomatous polyposis coli (*APC*); *BRAF*; catenin (cadherin-associated protein)  $\beta$ 1, 77 kDa (*CTNNB1*); *EGFR*; v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2 (*ERBB2*); isocitrate dehydrogenase 1 (NAPD+), soluble (*IDH1*); v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog (*KIT*); Kirsten rat sarcoma viral oncogene homolog (*KRAS*); mitogen-activated protein kinase kinase 1 (*MAP2K1*); notch 1 (*NOTCH1*); neuroblastoma RAS viral oncogene homolog (*NRAS*); phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit  $\alpha$  (*PIK3CA*); phosphatase and tensin homolog (*PTEN*); and tumor protein 53 (*TP53*).<sup>6</sup> This was performed using a custom-modified ABI PRISM SNaPshot Multiplex System (Applied Biosystems/Life Technologies Corporation, Carlsbad, Calif), as previously described.<sup>7</sup> An earlier version of this assay, which was used on 74 of the specimens, did not test for *IDH2* or *MAP2K1* mutations. Specific mutations that were assessed using SNaP-shot are listed in Supporting Table 1 (see online supporting information). Testing of the tumor suppressor genes *TP53*, *APC*, and *PTEN* was limited to only the most common mutation sites (limiting coverage to 29%, 15%, and 15%, respectively, of all known somatic mutations).<sup>7</sup>

### Statistical Analysis

The chi-square test was used to compare genotype frequency by tumor site, age at diagnosis (either *greater than or equal to* or *less than* the median age of 56 years), sex, family history of gastrointestinal cancer, history of polyps, stage at initial presentation, smoking status (former/current vs none), and site of first metastasis. A secondary exploratory analysis was performed to assess the impact of genotype mutation on overall survival (OS) and relapse-free survival (RFS) using Cox regression univariate and multivariate analyses and adjusting for age, sex, disease location (colon vs rectal), smoking history, and stage at presentation. The Kaplan-Meier method was used to estimate OS and RFS, and the results were compared using the log-rank test. OS was defined as the time from initial diagnosis to the date of death. Patients not known to have died were censored at date of last follow-up. RFS was calculated for those with stage I through III disease at diagnosis from the date of initial diagnosis to the date of first relapse. Because specific *KRAS* codons have been associated with inferior outcomes,<sup>8,9</sup> each *KRAS* codon was independently assessed for OS and RFS using the log-rank test and was compared using patients with wild-type *KRAS/BRAF* (n = 118); patients who had concurrent *BRAF* mutations were excluded from analysis of the *KRAS* mutant patients, because *BRAF* mutations are known to be associated with poor outcome.<sup>5,10</sup>

## RESULTS

### Patient Characteristics

Patient characteristics are listed in Table 1. The median age at initial diagnosis was 56 years, and 58% of patients were men. Forty-three percent of patients had localized disease, and 56.3% had metastatic disease at diagnosis. Mutational profiling revealed that 9 patients (4%) had an *NRAS* mutation, 18 (8%) had an *APC* mutation, 23 (10%) had a *BRAF* mutation, 28 (13%) had a *PIK3CA* mutation, 47 (21%) had a *TP53* mutation, and 81 (36%) had a *KRAS* mutation. No mutations were identified in *AKT*, *CTNNB1*, *EGFR*, *FLT3*, *IDH1*, *IDH2*, *JAK2*, *KIT*, or *NOTCH1*. Only 1 patient had a *PTEN* mutation; therefore, *PTEN* was not evaluated further in the correlative analysis. The 81 patients who had *KRAS* mutations included mutations in the following codons: glycine (G) to cysteine (C) substitution in codon 12 (G12C) (n = 8), G to aspartic acid (D) substitution in codon 12 (G12D) (n = 28), G to valine (V) substitution in codon 12 (G12V) (n = 14); G to D substitution in codon 13 (G13D) (n = 19), G to serine (S) substitution in codon 12 (G12S) (n = 5), G to alanine (A) substitution in codon 12 (G12A) (n = 6), and G12V/G12C (n = 1). In total, 47 patients had more than 1 mutation, including 38 patients with 2 mutations and 9 patients with 3 mutations. Specifically, these combinations included 11 *KRAS/TP53* mutations, 7 *KRAS/APC* mutations, 6 *KRAS/PIK3CA* mutations, 6 *TP53/BRAF* mutations, 3 *KRAS/TP53/PIK3CA* mutations, 3 *KRAS/APC/PIK3CA* mutations, 3 *PIK3CA/BRAF* mutations, 1 *NRAS/TP53* mutation, 1 *NRAS/TP53/PIK3CA* mutation, 1 *KRAS/PIK3CA/PTEN* mutation, 1 *PIK3CA/APC* mutation, 1 *APC/PIK3CA/BRAF* mutation, 1 *TP53/APC* mutation, 1 *TP53/PIK3CA* mutation, and 1 *APC/BRAF* mutation.

Patients who had *NRAS* and *BRAF* mutations were further characterized. Mutations in *NRAS* were identified in codon 12 (n = 5), codon 61 (n = 3), and codon 13 (n = 1). These mutations included 5 from pretreatment primary tumors, 3 from sites of metastatic disease, and 1 from a post-treatment primary site. Eight patients with rectal cancer and 1 patient with colon cancer had *NRAS* mutations. The tumor in the patient with colon cancer was located in the descending colon, and he presented with metastatic disease at diagnosis. Among the 23 *BRAF* mutations, 12 were from pretreatment primary sites, 7 were from metastatic sites, 2 were from locally recurrent sites, and 2 were from unspecified sites. Twenty-one mutations were from patients with colon cancer. The 2 patients with rectal cancer who had *BRAF* mutations were both men and carried the common V600E mutation. One patient had stage III disease, and 1 had stage IV disease at diagnosis.

### Mutational Profiles by Anatomic Site

Figure 1 demonstrates the frequency of mutations identified by tumor site. There were significant differences between colon tumors and rectal tumors with regard to both *NRAS* and *BRAF*. Patients who had rectal cancer had a higher incidence of *NRAS* mutations compared with those who had colon cancer (12.5% vs 0.6%;  $P < .001$ ). Conversely, *BRAF* mutations were more prevalent in patients who had colon cancer compared with those who had rectal cancer (13.3% vs 3.1%;  $P = .024$ ). *TP53* mutations were significantly more common in patients who had rectal cancer compared with those who had colon cancer

(29.7% vs 17.7%;  $P = .048$ ). There was no difference in the frequency of *KRAS*, *APC*, or *PIK3CA* mutations between patients with colon cancer versus rectal cancer.

### Exploratory Analysis of Genotype and Clinical Characteristics

An exploratory analysis of additional clinical characteristics by genotype also was assessed (Table 2). *NRAS* mutations were associated with patients aged  $\geq 56$  years at diagnosis (7% vs 0.9%;  $P = .02$ ) and with local recurrence as the first site of relapse (12.5% vs 3%;  $P = .03$ ). *BRAF* mutations were associated with patients aged  $\geq 56$  years (15.8% vs 4.6%;  $P = .006$ ) and with distant lymph node disease as the first site of relapse (26.2% vs 6.7%;  $P < .001$ ), and they trended toward an association with women (15.1% vs 7%;  $P = .05$ ) and an association with a personal history of polyps (19.4% vs 8.9%;  $P = .08$ ). *APC* mutations were associated with nonsmokers (13.3% vs 2.8%;  $P = .004$ ). *PIK3CA* mutations were associated with lung as the first site of relapse (23% vs 8.7%;  $P = .004$ ) and trended toward an association in patients without polyps (14.1% vs 3.2%;  $P = .09$ ). *TP53* mutations were associated with men (26.4% vs 14%;  $P = .03$ ) and patients aged  $< 56$  years (28.7% vs 14%;  $P = .007$ ), and they trended toward an association in patients without a family history of gastrointestinal cancers (25% vs 15.6%;  $P = .09$ ). Sites of first relapse in bone, liver, peritoneum, or other locations were not associated with any genotypes (data not shown), nor was stage at initial presentation. Genotypes and clinical correlations are summarized in Table 3.

### Overall Survival and Relapse-Free Survival by Genotype

After a median follow-up of 91.2 months (range, 12.4–176.5 months), there were 154 deaths. Having a *BRAF* mutation was associated with a significantly inferior OS (hazard ratio, 3.13; 95% confidence interval, 1.56–6.29;  $P = .001$ ). The estimated 5-year OS was 45.3% in *BRAF*-negative patients compared with 6.3% in *BRAF*-positive patients ( $P = .003$ ) (Fig. 2). After adjusting for age, disease site, sex, smoking history, and stage at presentation, *BRAF* mutation remained a significant predictor of decreased OS (hazard ratio, 2.4; 95% confidence interval, 1.09–5.27;  $P = .029$ ). No other genotypes were associated with OS. In the 97 patients who had localized disease at presentation, no mutations were associated with decreased RFS (Table 4).

### Subgroup Analysis of *KRAS* Mutant Patients

When we compared the outcomes of patients who had specific *KRAS* mutations with the outcomes of patients who had *KRAS/BRAF* wild-type tumors, only the G13D mutation trended toward worse OS ( $P = .063$ ).

## DISCUSSION

This study profiles the mutational and clinical differences in patients with metastatic colon and rectal adenocarcinoma. Most importantly, of the 222 patients analyzed, a significant difference in the frequency of *NRAS* mutations was noted between patients with rectal cancer (12.5%) and those with colon cancer (0.6%). There were also significant differences in the rates of *BRAF* mutations and the subset of *TP53* mutations screened for in colon cancers versus rectal cancers (13.3% vs 3.1% and 17.7% vs 29.7%, respectively). A clinical

profile associated with certain mutations also was noted. *NRAS* mutations were identified in older patients with rectal cancer, whereas *BRAF* mutations were identified in older women patients with colon cancer. *APC* mutations were observed more commonly in nonsmokers. *TP53* mutations were identified in younger men with rectal cancer.

The finding that *NRAS* mutations were identified almost exclusively in patients with rectal cancer is noteworthy. To our knowledge, this is the first study to identify *NRAS* as a predominantly rectal cancer mutation. In a study of 225 colorectal cancers, *NRAS* was detected in 5 patients (2.2%) in codons 12 and 61.<sup>11</sup> It is noteworthy that no *NRAS* mutations were located in tumors of the proximal colon, whereas 4 mutations were located in tumors of the distal colon, and 1 mutation was located in a tumor of the rectum. In our study, 8 of 9 *NRAS* mutations were identified in rectal adenocarcinomas, whereas a single mutation was identified in a tumor of the descending colon. We also observed that *NRAS* mutations were more common in older patients. To our knowledge, this has not been described previously in CRC; however, it is noteworthy that this association has been observed in melanoma.<sup>12</sup>

Mutations in *BRAF* were identified most commonly in older women with colon cancer. These findings are concordant with prior studies.<sup>5,13</sup> Although downstream pathway mitogen-activated protein kinase and extracellular signal-regulated kinase inhibitors are being actively investigated in early phase clinical trials, either alone or in combinations with other agents, it will be of interest to determine the impact of *BRAF* and *NRAS* mutations in predicting response.

*TP53* mutations were identified more commonly in younger men with rectal cancer, consistent with reports that patients aged < 50 years are more likely to harbor *TP53* mutations.<sup>14</sup> This has been suggested as 1 of the earlier mutations once an adenoma has transitioned to cancer.<sup>15</sup> Others have also observed that distal rather than proximal colon cancers have a higher incidence of *TP53* mutations.<sup>16</sup> The presence of an *APC* mutation was more common in nonsmokers than in smokers (13.3% vs 2.8%;  $P = .004$ ). At least 2 prior studies reported no association with smoking and *APC* mutation in CRC, suggesting that this more likely is a sporadic mutation<sup>17,18</sup> and is 1 of the earliest mutations in colon cancer progression.<sup>19</sup>

There are conflicting data regarding the similarities and differences between colon and rectal cancers at the molecular level. Different single nucleotide polymorphism profiles have been reported; and it has been demonstrated that protein expression of the cluster differentiation 44 standard isoform (CD44s), CD44 variant 6 (CD44v6), nuclear  $\beta$ -catenin, and CD68 can discriminate between left-sided and right-sided tumors.<sup>20–22</sup> However, a gene expression study of microsatellite-stable colorectal tumors and a recent analysis of *The Cancer Genome Atlas* (TCGA) demonstrated only minor or no differences in colon cancers versus rectal cancers.<sup>23,24</sup> In addition, this lack of difference in TCGA was only in the nonhypermethylated specimens, in which copy number, methylation status, messenger RNA, and micro-RNA levels were assessed. Both colon and rectal cancers were analyzed together for somatic mutations, and the frequency of *NRAS* mutations among colon versus rectal adenocarcinomas was not described for that study. Furthermore, the high sensitivity of our

SNaPshot assay relative to the depth of sequencing used in the next-generation studies may also contribute to these discrepancies.

Sites of first relapse by genotype also were analyzed. *PIK3CA* mutations were associated with lung, *BRAF* mutations were associated with distant lymph nodes, and *NRAS* mutations were associated with local recurrence as the first site of relapse. Tie et al reported a similar finding for *PIK3CA* mutations and lung metastases on univariate analysis, which trended toward significance on multivariate analysis.<sup>25</sup> This raises the possibility that, although sites of first metastasis are more likely driven by anatomy, because the colon drains to the portal vein and the rectum drains to the vena cava through the inferior rectal vein,<sup>2</sup> genotype may also play a role and should be further investigated.

We also assessed the association of genotype with clinical outcomes. *BRAF* was the only mutation associated with decreased OS. This has been demonstrated in multiple studies; and, in a recent meta-analysis of >11,000 patients with CRC, the presence of a *BRAF* mutation increased the risk of mortality greater than 2 times.<sup>5,10,26</sup> When specific *KRAS* codons were analyzed, the G13D *KRAS* mutation trended toward significance for decreased OS compared with patients who had wild-type *KRAS/BRAF*. The G13D mutation has been associated with decreased OS in patients with CRC who received treatment with folinic acid (leucovorin), fluorouracil, and oxaliplatin (FOLFOX) plus panitumumab in 1 study<sup>8</sup> but with improved survival compared with codon 12 mutations in a meta-analysis of patients who received cetuximab either alone or in combination with chemotherapy.<sup>27</sup> Others have observed that the G12V mutation was associated with higher CRC-specific mortality,<sup>9</sup> and the G12A mutation was associated with inferior OS in a pooled analysis.<sup>8</sup> Our small numbers of individual codon mutations provided only limited insight.

There are additional points that merit further discussion. This was a retrospective, exploratory study and, thus, was strictly hypothesis-generating. Furthermore, because of the retrospective nature, there is likely selection bias among our patient cohort, and our results are only applicable to patients with metastatic disease, because these are the patients who are routinely offered mutation profiling at our institution. Only a portion of the known mutations in the tumor suppressor genes *APC*, *TP53*, *NOTCH1*, and *PTEN* were evaluated in our assay. Because of the limited coverage, the mutation rate of *TP53* was only 21%, however it is well established that the mutation rate in CRC is closer to 40% to 50%.<sup>28</sup> Therefore, other inactivating mutations in these genes probably were missed using this technique. In addition, the current study included patients who had genomic analysis performed on a mix of pretreatment and post-treatment primary tumors (as detailed in Table 1), sites of metastatic disease, and sites of local recurrences. It is possible that the mutational profile could change in the metastatic or post-treatment setting; however, it has been demonstrated that there is a high concordance of *NRAS*, *KRAS*, *PIK3CA*, *BRAF*, and *TP53* mutations between primary and metastatic sites in CRC.<sup>25,29</sup> Others have observed high concordance between pre-FOLFOX and post-FOLFOX treated CRCs when assessing *KRAS*, *NRAS*, *BRAF*, and *PIK3CA*.<sup>30</sup> Data from those studies support the notion that sites of both primary and metastatic disease, as well as pretreatment and post-treatment tumors, may be valid sources for assessing mutational status. Finally, we did not have microsatellite instability information for the majority of patients with *BRAF* mutations, because we did not

routinely test for this at our institution during the time these patients were evaluated; this can also provide important prognostic information.

## Conclusions

In summary, this was an exploratory analysis of clinical correlations by genotype in patients with metastatic colon and rectal cancer. Notably, *NRAS* was identified predominantly in older patients with rectal cancer. This novel finding is important because, whereas the frequency in this study was only 12.5%, with over 40,000 rectal cancers per year in the United States, approximately 4800 patients may have *NRAS* mutations. Although we did not demonstrate inferior outcomes for patients with *NRAS* mutations, our numbers were small, and these patients warrant further investigation. Our study also confirmed the association of *BRAF* mutations with older patients who had colon cancer and with decreased survival. *TP53* was correlated with young men who had rectal cancer, and *APC* was associated with non-smokers. Whereas some of these findings have been reported previously in single-institution studies, to our knowledge, no studies to date have provided a comprehensive assessment of these clinical correlates. These findings suggest there may be a genetic difference in colon and rectal adenocarcinomas. Furthermore, clinical profiles by genotype may aid clinicians in earlier identification of patients who harbor specific mutations and who may benefit from enrollment in clinical trials of targeted therapy in the metastatic setting. The long-term utility of this strategy is unknown and will depend on finding effective treatments for certain genotypes.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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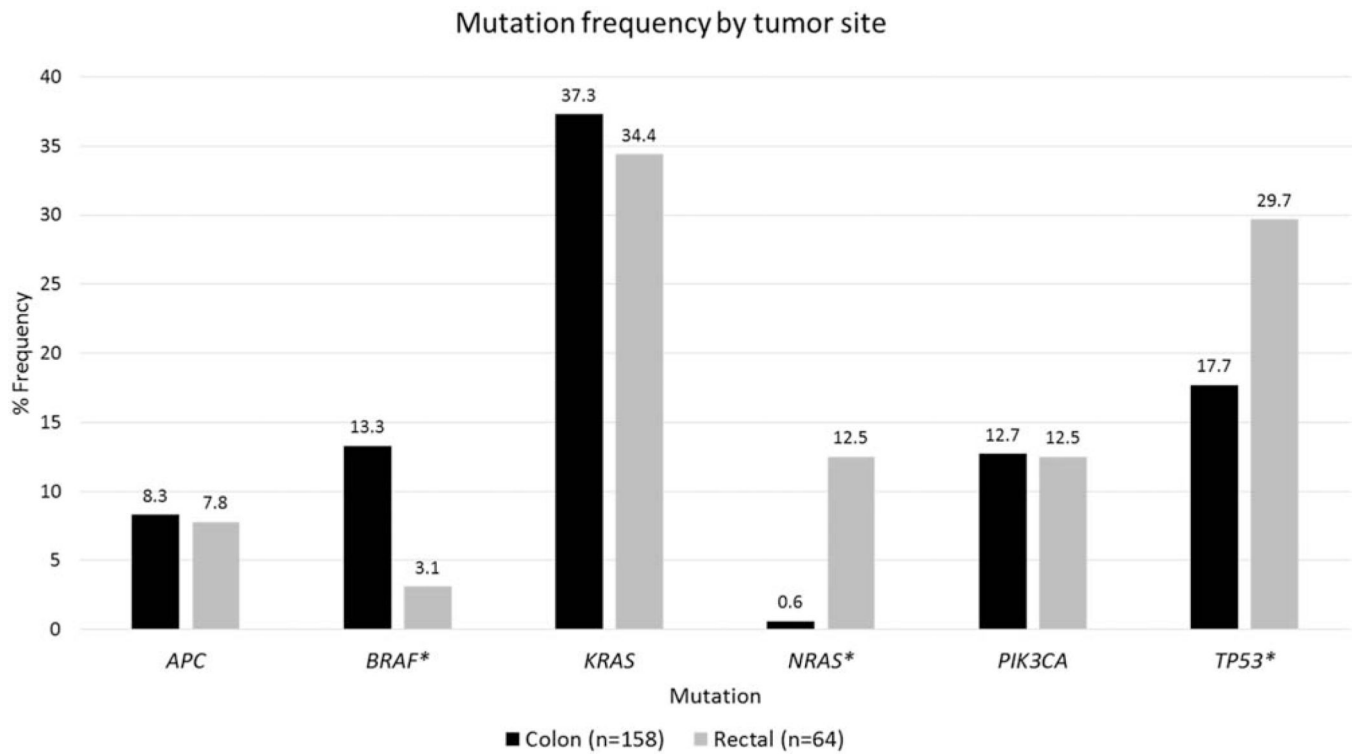
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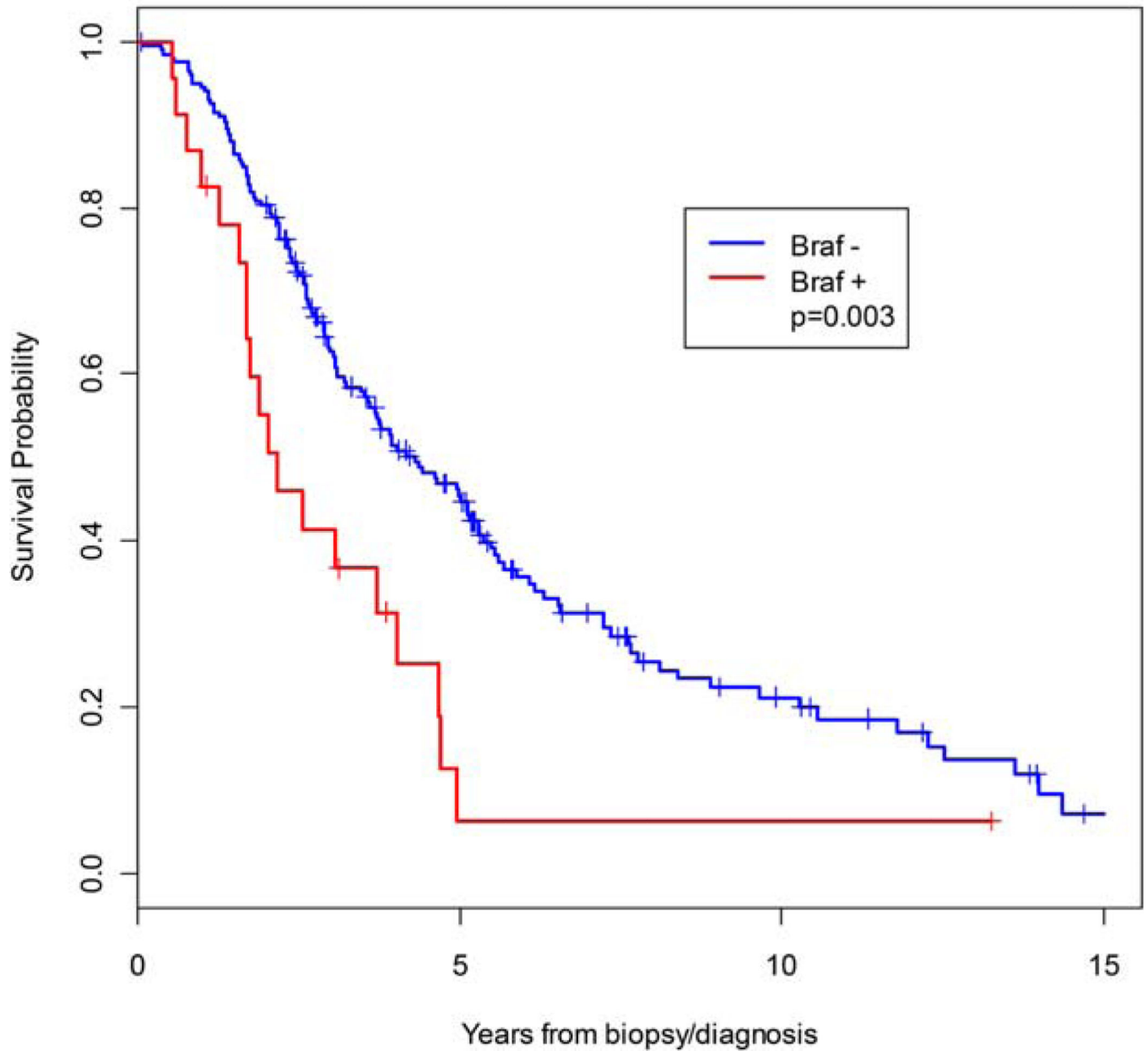
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**Figure 1.** Mutation frequency is illustrated by tumor site. An asterisk denotes statistical significance ( $P < .05$ ). The percentage frequency is listed above each column. *APC* indicates adenomatous polyposis coli; *BRAF\**, v-raf murine sarcoma viral oncogene homolog B; *KRAS*, Kirsten rat sarcoma viral oncogene homolog; *NRAS\**, neuroblastoma RAS viral oncogene homolog; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit  $\alpha$ ; *TP53\**, tumor protein p53.

## OS from diagnosis by Braf mutation



**Figure 2.** Overall survival is illustrated for patients with (+) and without (–) v-raf murine sarcoma viral oncogene homolog B (*BRAF*) mutations. The Kaplan-Meier method was used to estimate overall survival. Curves are compared using the log-rank test.

TABLE 1

## Patient Characteristics

Characteristic	No. of Patients (%)
Age: Median [range], y	56 [29–84]
Sex	
Men	129 (58)
Women	93 (42)
Anatomic site	
Rectal	64 (29)
Colon	158 (71)
Initial stage at diagnosis	
I	10 (4.5)
II	22 (9.9)
III	65 (29)
IV	125 (56.3)
History of polyps	30 (14)
Positive family history of GI cancer	90 (41)
Smoking status	
None	112 (50)
Former	88 (40)
Current	22 (10)
Specimen type for mutational analysis	
Pretreatment primary	108 (49)
Post-treatment primary	11 (5)
Metastatic site	76 (34)
Primary recurrent disease	7 (3)
Unspecified	20 (9)
Overall frequency of mutations	
<i>NRAS</i>	9 (4)
<i>KRAS</i>	81 (36)
<i>TP53</i>	47 (21)
<i>APC</i>	18 (8)
<i>PIK3CA</i>	28 (13)
<i>BRAF</i>	23 (10)
<i>PTEN</i>	1 (0.5)

Abbreviations: APC, adenomatosis polyposis coli; BRAF, v-raf murine sarcoma viral oncogene homolog B; GI, gastrointestinal; KRAS, Kirsten rat sarcoma viral oncogene homolog; NRAS, neuroblastoma RAS viral oncogene homolog; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit a; PTEN, phosphatase and tensin homolog; TP53, tumor protein p53.

TABLE 2

## Chi-Square Analysis of Mutations and Clinical Characteristics

Characteristic	Genotype: Proportion of Patients (No.) <sup>a</sup>						
	NRAS	KRAS	TP53	APC	PIK3CA	BRAF	
Rectal cancer (64) vs colon cancer (158)	<b>12.5% (8) vs 0.6% (1)</b>	34.4% (22) vs 37.3% (59)	<b>29.7% (19) vs 17.7% (28)</b>	7.8% (5) vs 8.2% (13)	12.5% (8) vs 12.7% (20)	<b>3.1% (2) vs 13.3% (21)</b>	
<i>P</i>	<.001	.7	<b>.048</b>	.9	.9	<b>.02</b>	
Family history of GI cancer: Yes (90) vs no (132)	3.3% (3) vs 4.5% (6)	33.3% (30) vs 38.6% (51)	15.6% (14) vs 25% (33)	5.6% (5) vs 9.8% (13)	15.6% (14) vs 10.6% (14)	11.1% (10) vs 9.8% (13)	
<i>P</i>	.7	.4	.09	.3	.3	.8	
Men (129) vs women (93)	4.7% (6) vs 3.2% (3)	35.7% (46) vs 37.6% (35)	<b>26.4% (34) vs 14% (13)</b>	6.2% (8) vs 10.8% (10)	14.7% (19) vs 9.7% (9)	7% (9) vs 15.1% (14)	
<i>P</i>	.6	.8	<b>.03</b>	.2	.3	.05	
History of polyps: Yes (31) vs no (191)	3.2% (1) vs 4.2% (8)	41.9% (13) vs 35.6% (68)	22.6% (7) vs 20.9% (40)	9.7% (3) vs 7.9% (15)	3.2% (1) vs 14.1% (27)	19.4% (6) vs 8.9% (17)	
<i>P</i>	.8	.5	.8	.7	.09	.08	
Smoking history: Yes (109) vs no (113)	5.5% (6) vs 2.7% (3)	31.2% (34) vs 41.6% (47)	22% (24) vs 20.4% (23)	<b>2.8% (3) vs 13.3% (15)</b>	10.1% (11) vs 15% (17)	9.2% (10) vs 11.5% (13)	
<i>P</i>	.3	.1	.8	<b>.004</b>	.3	.6	
Stage at presentation: I (n = 10) vs II (n = 22) vs III (n = 64) vs IV (n = 125)	10% (1) vs 4.5% (1) vs 3.1% (2) vs 3.2% (4)	20% (2) vs 31.8% (7) vs 34.4% (22) vs 40% (50)	10% (1) vs 27.3% (6) vs 17.2% (11) vs 23.2% (29)	0 vs 9.1% (2) vs 10.9% (7) vs 7.2% (9)	0 vs 22.7% (5) vs 12.5% (8) vs 12% (15)	0 vs 9.1% (2) vs 15.6% (10) vs 8.8% (11)	
<i>P</i>	.7	.5	.5	.6	.3	.3	
Age at diagnosis: <56 y (108)	<b>7% (8) vs 0.9% (1)</b>	34.2% (39) vs 38.9% (42)	<b>14% (16) vs 28.7% (31)</b>	5.3% (6) vs 11.1% (12)	13.2% (15) vs 12% (13)	<b>15.8% (18) vs 4.6% (5)</b>	
<i>P</i>	<b>.02</b>	.5	<b>.007</b>	.1	.8	<b>.006</b>	
Site of first recurrence Distant lymph node: Yes (42) vs no (180)	0% (0) vs 5% (9)	23.8% (10) vs 39.4% (71)	26.2% (11) vs 20% (36)	7.1% (3) vs 8.3% (15)	14.3% (6) vs 12.2% (22)	<b>26.2% (11) vs 6.7% (12)</b>	
<i>P</i>	.14	.06	.04	.8	.7	<.001	
Local recurrence (24 vs 198)	<b>12.5% (3) vs 3% (6)</b>	25% (6) vs 37.9% (75)	16.7% (4) vs 21.7% (43)	8.3% (2) vs 8.1% (16)	12.5% (3) vs 12.6% (25)	20.8% (5) vs 9.1% (18)	
<i>P</i>	<b>.03</b>	.2	.6	.9	.9	.08	
Lung (61 vs 161)	4.9% (3) vs 3.7% (6)	45.9% (28) vs 32.9% (53)	21.3% (13) vs 21.1% (34)	8.2% (5) vs 8.1% (13)	<b>23% (14) vs 8.7% (14)</b>	8.2% (5) vs 11.2% (18)	
<i>P</i>	.7	.07	.9	.9	<b>.004</b>	.5	

Abbreviations: *APC*, adenomatosis polyposis coli; *BRAF*, v-raf murine sarcoma viral oncogene homolog B; *GI*, gastrointestinal; *KRAS*, Kirsten rat sarcoma viral oncogene homolog; *NRAS*, neuroblastoma RAS viral oncogene homolog; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit  $\alpha$ ; *PTEEN*, phosphatase and tensin homolog; *TP53*, tumor protein p53.

<sup>a</sup> Boldface values indicate statistically significant differences.

TABLE 3

## Summary of Genotype and Clinical Correlations

Clinical Variable	Genotype				
	<i>NRAS</i>	<i>TP53</i>	<i>BRAF</i>	<i>APC</i>	<i>PIK3CA</i>
Site	Rectal	Rectal	Colon	NS	NS
Age	Older	Younger	Older	NS	NS
Sex	NS	Men	Women	NS	NS
Family history	NS	Negative <sup>d</sup>	NS	NS	NS
Polyps	NS	NS	Present <sup>d</sup>	NS	Not present <sup>d</sup>
Smoking status	NS	NS	NS	Nonsmokers	NS
Site of first recurrence	Local	NS	Distant lymph node	NS	Lung

Abbreviations: *APC*, adenomatosis polyposis coli; *BRAF*, v-raf murine sarcoma viral oncogene homolog B; *NRAS*, neuroblastoma RAS viral oncogene homolog; NS, no statistical significance; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit  $\alpha$ ; *TP53*, tumor protein p53.

<sup>d</sup>These findings only trended toward significance; all other factors had *P* values < .05.



**TABLE 4**

Estimated 5-Year Overall Survival and Relapse-Free Survival by Genotype

Genotype	Survival Rate (95% CI), %				Log-Rank <i>P</i>	Log-Rank <i>P</i>
	5-Year OS, n = 222		5-Year RFS, n = 97			
	Mutation-Positive	Mutation-Negative	Mutation-Positive	Mutation-Negative		
<i>NRAS</i>	52 (16–79)	41 (34–48)	60 (13–88)	4 (1–10)	.92	.12
<i>KRAS</i>	37 (25–49)	44 (35–52)	3 (0.2–14)	11 (5–19)	.47	.10
<i>TP53</i>	39 (24–54)	42 (34–50)	17 (4–37)	5 (2–12)	.89	.17
<i>APC</i>	55 (27–76)	40 (33–48)	11 (0.6–39)	8 (4–15)	.40	.83
<i>PIK3CA</i>	45 (25–63)	41 (34–49)	8 (0.5–29)	7 (3–14)	.42	.69
<b><i>BRAF</i></b>	<b>6 (0.4–24)</b>	<b>45 (38–53)</b>	8 (0.5–31)	7 (3–14)	<b>.003<sup>a</sup></b>	.56

Abbreviations: *APC*, adenomatosis polyposis coli; *BRAF*, v-raf murine sarcoma viral oncogene homolog B; CI, confidence interval; *KRAS*, Kirsten rat sarcoma viral oncogene homolog; *NRAS*, neuroblastoma RAS viral oncogene homolog; OS, overall survival; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit  $\alpha$ ; RFS, relapse-free survival; *TP53*, tumor protein p53.

<sup>a</sup>Values in boldface indicate statistically significant differences.