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Differential Radiographic Appearance of *BRAF* V600E–Mutant Metastatic Colorectal Cancer in Patients Matched by Primary Tumor Location

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Abstract

Background—*BRAF*-mutant metastatic colorectal cancers (mCRCs) share many clinicopathologic features with right-sided colon tumors, including frequent peritoneal involvement. Because of the poorer outcomes associated with *BRAF* mutations, early enrollment in clinical trials has been encouraged. However, the use of standard eligibility and assessment criteria, such as measurable disease, has anecdotally impeded patient accrual and restricted appraisal of treatment response. We investigated whether the presence of a *BRAF*V600E mutation is differentially associated with sites and appearance of metastatic disease in patients matched by primary tumor location.

Methods—A total of 40 patients with *BRAF*-mutant mCRC were matched to 80 patients with *BRAF* wild-type mCRC by location of primary tumor (right or left colon; rectum), sex, and age. Associations between *BRAF* mutation status and clinicopathologic characteristics and metastatic sites were analyzed using proportion tests. Survival was summarized with Kaplan-Meier and Cox regression methods.

Results—The distribution of primary tumor locations was: 60% right colon, 30% left colon, and 10% rectum. Compared with *BRAF* wild-type tumors, *BRAF*-mutant tumors more commonly associated with peritoneal metastases (50% vs 31%; $P=.045$) and ascites (50% vs 24%; $P=.0038$). In patients with left colon primaries, *BRAF* mutations were associated with more frequent ascites (58% vs 12%; $P=.0038$) and less frequent liver metastases (42% vs 79%; $P=.024$). Among patients with right colon primaries, no significant difference in sites of disease by *BRAF* mutation status was observed. Disease was not measurable by RECIST 1.1 in 24% of patients with right-sided

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primary tumors, irrespective of *BRAF* mutation status. In the *BRAF*-mutated cohort, ascites correlated unfavorably with survival (hazard ratio, 2.35; 95% CI, 1.14, 4.83; $P=.02$).

Conclusions—Greater frequency of ascites and peritoneal metastases, which pose challenges for RECIST 1.1 interpretation of therapeutic outcomes, are seen with *BRAF*-mutant mCRC, even when patients are matched for primary tumor location.

Background

Missense mutation of *BRAF* occurs in 5% to 10% of metastatic colorectal cancers (mCRCs).^{1,2} *BRAF* encodes a protein kinase in the mitogen-activated protein kinase (MAPK) pathway that may be constitutively activated by substitution at valine 600 to glutamic acid (V600E). This mutation defines a unique molecular subtype of mCRC, commonly originating from a serrated adenoma, more often in the right colon, with associated microsatellite instability (MSI), hypermutation, and a high degree of CpG island methylation (CIMP-H).^{3–10} *BRAF* mutations are more frequent in women, older patients, and in whites compared with Asians or blacks with CRC.^{2,11,12} Importantly, *BRAF*V600E-mutated mCRC is associated with a poor prognosis in patients treated with conventional chemotherapy.^{13–22} In the TRIBE and FIRE-3 studies, patients with *BRAF* wild-type tumors treated with FOLFIRI plus bevacizumab had a median overall survival (mOS) of 2 or more years, whereas mOS of patients with *BRAF*-mutated tumors was approximately 1 year.^{21,22} The explanation for the poor survival of patients with *BRAF*-mutated mCRC is incompletely understood but may relate to inherent chemoresistance, aggressive disease biology and kinetics, and/or a distinct pattern of metastatic spread.^{19,23}

At diagnosis of mCRC, Yaeger et al²³ found that *BRAF*-mutated tumors were associated with more frequent peritoneal metastases (26% vs 14%; $P<.01$) and less frequent liver-limited metastases (41% vs 63%; $P<.01$). Over the course of disease, Tran et al¹⁹ observed significantly higher rates of peritoneal metastases (46% vs 24%; $P=.001$) and distant lymph node metastases (53% vs 38%; $P=.044$) and lower rates of lung metastases (35% vs 49%; $P=.049$) in *BRAF*-mutant tumors.¹⁹ Of note, two-thirds of *BRAF*-mutated tumors originated in the right versus the left colon (68% vs 32%); this ratio was reversed for *BRAF* wild-type tumors (35% vs 65%; $P<.001$).¹⁹

“Sidedness,” differences between cancers originating in the left versus right colon, is an area of active research. Consensus molecular subtypes, mutations, and other genetic and epigenetic features vary in prevalence from the proximal to distal colon,^{3,5,24–31} possibly relating to the distinct embryologic origins of the right (from the midgut) and left (from the hindgut) colon. Patients with left-sided tumors experience superior OS compared with those with right-sided primaries.^{30–37} Right-sided tumors are associated with female sex, older age, and peritoneal metastases, whereas left-sided tumors are associated with more frequent liver and lung metastases.³² Although these differences cannot be explained by *BRAF* mutation alone, they may relate to enrichment of a “*BRAF* mutant–like” poor prognosis gene expression signature in right-sided *BRAF* wild-type tumors.³⁸ It remains undetermined whether *BRAF*-mutated tumors really have a distinct pattern of metastatic spread, or

whether the *BRAF* “phenotype” is representative of right-sided tumors, independent of mutation status.

Meanwhile, several clinical trials for *BRAF*-mutated mCRC are underway,^{39–46} and radiologic assessment of disease appearance is critical, both for patient management and for evaluating efficacy. Quantitative tumor burden assessments, such as RECIST 1.1, depend on finding “measurable disease.” Several findings suggestive of metastases that are known to be difficult to quantify in the RECIST 1.1 system are commonly observed with *BRAF*-mutated mCRC, including ascites, which is nonspecific and may be disproportionate to disease burden, and peritoneal metastases, which are often diffuse, mobile, band-like, or interdigitated with fat, and therefore difficult to quantify accurately with scan-to-scan comparisons.

As we work to translate our expanding knowledge of mCRC heterogeneity and the role of primary tumor location to improved clinical outcomes, we remain reliant on a handful of biomarkers (*KRAS*, *NRAS*, *BRAF*, *MSI*, and carcinoembryonic antigen)⁴⁷ and serial radiographic assessments. We propose that careful radiologic review may identify novel radiogenomic “biomarkers” for prediction and prognosis. By matching patients with *BRAF* V600E–mutant mCRC to their *BRAF* wild-type counterparts, we queried whether there is a differential radiographic appearance of *BRAF*-mutant mCRC and whether radiographic appearance has implications for treatment or survival.

Methods

Patients

Institutional Review Board approval was obtained for retrospective review of imaging and clinical data from patients with mCRC seen in an academic gastrointestinal oncology practice. All known cases of *BRAF*-mutant mCRC seen from January 2013 to September 2015 with available contrast-enhanced CT images were included in this analysis. Each patient with *BRAF*-mutant mCRC identified was matched to 2 patients with *BRAF* wild-type mCRC, according to location of primary tumor (right colon, left colon, rectum), sex, and age (<50 years, 50 years at diagnosis). If multiple matches were identified, patients were selected based on availability of expanded *RAS* mutation testing results and age proximity.

Image Analysis

All available cross-sectional imaging studies from date of diagnosis to December 2015 were reviewed by a radiologist with expertise in abdominal imaging who was blinded to patients’ clinical histories. Images were evaluated for presence of liver, lung, lymph node, and osseous metastases, and for peritoneal implants and ascites. Liver and lung metastases were defined as lesions measuring greater than 1 cm or lesions that were new or increasing in size from prior studies. Lymph node metastases were defined as nodes measuring greater than 1.5 cm in short axis. Both sclerotic and lytic osseous metastases were noted. Peritoneal or omental disease was considered present when soft tissue densities or nodular infiltration soft tissue were present that could not be attributed to a vessel, lymph nodes, or prior surgeries.

All studies were assessed for whether the bulk of disease was measurable according to RECIST 1.1. Followup was extended to August 2016 to evaluate time to ascites (details in supplementary eAppendix 1, available with this article at JNCCN.org).

Statistical Analysis

Univariate analyses of *BRAF* mutation status, clinicopathologic characteristics, sites of metastases, and measurable disease were examined using frequencies and proportions for categorical data and *t* tests for continuous variables. OS, stratified by *BRAF* mutation status and ascites (within *BRAF* mutation status), was summarized with Kaplan-Meier curves and Cox proportional hazards models after ensuring that the hazard assumption was met. For multivariate analysis, backward deletion was applied to capture negatively confounded predictors. Cumulative incidence function was used to estimate the probability of developing ascites from the date of diagnosis with mCRC in patients who did not have ascites at initial presentation. Death without ascites was treated as a competing event.

Results

Clinicopathologic Characteristics

A cohort of 40 patients with *BRAF*-mutant mCRC was identified. Of these 40 patients, 24 (60%) were women, 9 (23%) were younger than 50 years at diagnosis, 24 (60%) had right-sided colon primaries, 12 (30%) had left-sided colon primaries, and 4 (10%) had rectal primaries (Table 1 and supplemental eAppendix 2). Female sex was associated with right-sided tumors ($P=.013$); there was no statistically significant association between age and primary tumor location. A total of 60% of patients with *BRAF*-mutated tumors had received treatment including a *BRAF* inhibitor on a clinical trial.^{40,41,43}

Compared with matched patients with *BRAF* wild-type tumors, a significantly lower proportion of patients with *BRAF*-mutant tumors underwent surgical resection of metastases (Table 1; $P=.013$), and when metastasectomy was performed, it was typically for palliation. Among patients originally diagnosed with stage II or III CRC, the time to radiographic detection of metastatic disease was shorter with *BRAF*-mutant as opposed to *BRAF* wild-type tumors (median, 9 vs 16 months). All other clinico-pathologic characteristics were balanced.

Sites of Metastatic Disease

We observed no significant differences in distribution of sites of metastases by sex or age. MSI-high/mismatch repair-deficient tumors exhibited less frequent lung metastases and more frequent lymph node metastases (supplemental eAppendix 3 and 4). In the cohort as a whole, a significantly higher frequency of ascites ($P=.0038$) occurred in patients with *BRAF*-mutant tumors (eAppendix 3). The mean number of sites of metastatic disease was 2.7 ± 1.4 with *BRAF*-mutant and 2.3 ± 1.2 with *BRAF* wild-type tumors ($P=.13$). Comparing *BRAF*- and *KRAS*-mutated tumors, *BRAF* mutation was again associated with a higher proportion of patients with ascites ($P=.01$). Among *BRAF* wild-type tumors, *RAS* mutations were overrepresented in right- versus left-sided tumors: 72% versus 37.5% ($P=.0045$).

In patients with right colon primaries, we observed no significant difference in metastatic disease sites by *BRAF* mutation status. In patients with left colon primaries, *BRAF* mutations were associated with less frequent liver metastases (42% vs 79%; $P=.024$) and more frequent ascites (58% vs 12%; $P=.0038$) (Table 2). If rectal tumors were grouped with left-sided primaries, *BRAF* mutations remained associated with ascites (56% vs 9%; $P=.0004$).

In our initial analysis, tumors in the proximal transverse colon (1 *BRAF*-mutated; 3 wild-type) were classified as right-sided, and tumors in the distal transverse colon (3 *BRAF*-mutated) or unspecified location in transverse colon (2 *BRAF*-mutated) were classified as left-sided. Our observations were similar after exclusion of patients with primary tumors in the transverse colon (supplemental eAppendix 5).

Measurable Disease

The presence of peritoneal disease and ascites were each associated with nonmeasurable disease by RECIST 1.1 ($P=.001$ and $P=.0032$, respectively) (Figure 1). Conversely, liver, lung, and lymph node metastases were associated with measurable disease by RECIST 1.1 ($P<.0001$, $P=.0023$, and $P=.0003$, respectively). The association between nonmeasurable disease and right-sided tumors had a P value of 0.12. No association was observed between *BRAF* mutation status and measurable disease, even on further stratification by location of primary tumor. Overall, 20% of patients with *BRAF*-mutated tumors and 17.5% of *BRAF* wild-type tumors had nonmeasurable disease by RECIST 1.1 (eAppendix 3).

Survival

The mOS of patients with *BRAF*-mutant mCRC was 24 months compared with 45 months with *BRAF* wild-type mCRC ($P<.001$) (Figure 2A). In patients with *BRAF*-mutated tumors, the presence of ascites was negatively correlated with survival (hazard ratio [HR], 2.35; 95% CI, 1.14, 4.83; $P=.02$) (Figure 2B). Variables positively correlated with survival were female sex (HR, 0.37; 95% CI, 0.18, 0.76; $P=.009$) and having the primary tumor resected (HR, 0.30; 95% CI, 0.13, 0.70; $P=.009$). There was a trend toward improved survival among clinical trial participants, with an mOS of 18 versus 27 months (HR, 0.5; 95% CI, 0.23, 1.05; $P=.07$) (supplemental eAppendix 6). A trend toward inferior survival with left-sided colon primaries was observed, with an mOS of 18 months for left versus 25 months for right-sided primaries (HR, 0.47; 95% CI, 0.21, 1.04; $P=.07$). No statistically significant differences in mOS by age or stage at diagnosis, tumor grade, other sites of metastases, metastasectomy, or presence of RECIST-measurable disease were detected (eAppendix 6).

Multivariate Analysis

The best model fit for the association of radiographic detection of ascites and survival in patients with *BRAF*-mutated tumors followed adjustment for sex, age at diagnosis, primary tumor location, and peritoneal metastases. Adjustment for these confounders greatly strengthened the negative association between ascites and survival (HR, 6.81; 95% CI, 2.41, 19.22; likelihood ratio, $P<.0001$) (Figure 2C, eAppendix 6). The interaction product term for ascites and peritoneal metastases had a P value of 0.98.

Time to Ascites

With extended follow-up, no additional patients with *BRAF*-mutant mCRC developed ascites; 3 patients with *BRAF* wild-type mCRC developed ascites. The proportion of patients who developed ascites within 3 months of mCRC diagnosis versus within 3 months of death did not differ by *BRAF*-mutation status ($P=.56$) (supplemental eAppendix 7). The cumulative incidence of ascites was higher among patients with *BRAF*-mutant disease (2-year cumulative incidence of 35% vs 15%; $P=.018$) (Figure 3).

Discussion

A cohort of patients with *BRAF*-mutated mCRC was matched 1:2 to patients with *BRAF* wild-type mCRC to determine whether *BRAF*-mutated tumors have a differential radiographic appearance after controlling for sex, age, and primary tumor location. In the matched cohort, ascites was the radiographic feature most strongly associated with the presence of a *BRAF* mutation.

Among patients with right-sided tumors, no statistically significant differences in metastatic sites were observed, although ascites and peritoneal disease were more common in patients whose tumors harbored a *BRAF* mutation. Among patients with left-sided colon primaries, ascites was associated with the presence of a *BRAF* mutation and peritoneal disease, which is often seen in conjunction with ascites, was also more frequent. Conversely, liver metastases were significantly more common in *BRAF* wild-type left-sided colon primaries.

We hypothesized that the association between ascites and *BRAF* mutation, significant in the cohort as a whole and the subset of patients with left- but not right-sided primaries, might be attributable to tumors in the transverse colon classified as originating from the left colon (5 *BRAF*-mutated, no *BRAF* wild-type). However, even after exclusion of patients with transverse colon primaries, the association between ascites and *BRAF*-mutant tumors persisted. Furthermore, with longer-follow up we did not find evidence that the difference in the frequency of ascites between *BRAF*-mutant and wild-type mCRC cases was due to the compressed course of disease for *BRAF*-mutant mCRC.

Compared with the study by Tran et al,¹⁹ in which the pattern of metastatic spread was analyzed in an unmatched cohort of *BRAF*-mutant and wild-type mCRC (68% vs 35% right-sided primaries, respectively), the association of *BRAF* mutations with peritoneal metastases was consistent; however, we did not recapitulate the weaker correlations between *BRAF*-mutant tumors and lymph node metastases or *BRAF* wild-type tumors and lung metastases that they observed. We did, however, consistently find more frequent lymph node metastases and less frequent lung metastases in *BRAF*-mutated tumors, so it is possible that these associations could become significant with enhanced sample size.

Because ascites and peritoneal disease are more common with *BRAF*-mutant tumors, we hypothesized that more of these patients would have disease that is nonmeasurable by RECIST 1.1. Instead, in our matched cohort, we found similar, and quite high, frequencies of RECIST nonmeasurable disease with or without a *BRAF* mutation, particularly in mCRC originating from a right-sided primary tumor (24%). This has implications for clinical trial

design, wherein response and progression-free survival end points rely on RECIST assessments, and also for standard clinical care, wherein contrast-enhanced CT imaging is the gold standard for therapeutic decision-making.

Survival of patients with *BRAF*-mutated tumors was longer than that reported previously with standard therapies, even in clinical trial populations.^{13–22} Here, 60% of patients with *BRAF*-mutated mCRC were treated on a clinical trial including a *BRAF*-targeted inhibitor^{40,41,43}; the mOS of these patients was 27 months, comparable to the survival of patients with *BRAF*-wild-type mCRC, and double that of patients with *BRAF*-mutated mCRC who received first-line FOLFIRI plus bevacizumab on 2 recent studies.^{21,22} In the *BRAF*-mutated cohort, ascites was associated with shorter survival, and this correlation strengthened in an exploratory multivariate model. Unexpectedly, among patients with *BRAF*-mutated mCRC, female sex associated favorably with mOS, and there was also a trend toward longer survival with right-sided colon primaries. If reiterated in other data sets, this could represent a real biological difference from mCRC overall, wherein right-sided tumors are associated with inferior survival^{30,32–35}; however, it could also be that typical presentation of *BRAF*-mutated mCRC (female sex with a right colon primary)¹² resulted in earlier mutation testing and referral for clinical trial participation.

Limitations of this study include the relative rarity of *BRAF*-mutated mCRC, which limits the ability to establish a large cohort for research purposes at any single institution. Thus, our study may have been underpowered for detection of clinicopathologic characteristics associated with *BRAF* mutation. In addition, we were unable to report on the frequency of brain metastases because a minority of patients underwent neuroimaging as part of routine care (eAppendix 3). Finally, due to the retrospective nature of this analysis, treatments administered for management of mCRC varied among patients in both the *BRAF*-mutant and *BRAF*-wild type cohorts; therefore, the survival analysis does not account for different treatments delivered.

With this analysis, we demonstrate for the first time the importance of using a matched cohort to characterize the effects of a mutation, *BRAF*V600E, which is differentially distributed in tumors arising in the proximal to distal colon and rectum. We conclude that ascites is the radiographic feature most robustly correlated with *BRAF*-mutant mCRC. *BRAF* mutation testing should be strongly considered with a left-sided colon primary if ascites is present; however, *BRAF* testing should not be limited to patients with this clinical presentation. Ascites correlated with inferior survival among patients with *BRAF*-mutated tumors. Moreover, a quarter of patients with mCRC with right colon primaries, with or without a *BRAF* mutation, had disease that was not measurable by RECIST 1.1.

Our future research directions will include the development of an alternative to RECIST 1.1 to characterize the burden of peritoneal-predominant disease and biomarkers that may be used in conjunction with CTs when disease is difficult to follow radiographically (eg, cell-free DNA). Given the limitations of single-institution analyses in this rare subset of mCRC, future multicenter clinical trials in which treatments are standardized should include correlative studies to validate these findings. Finally, we are encouraged by the survival of

patients with *BRAF*-mutated tumors in our cohort, suggesting that our research efforts may be positively impacting individuals with this especially aggressive form of cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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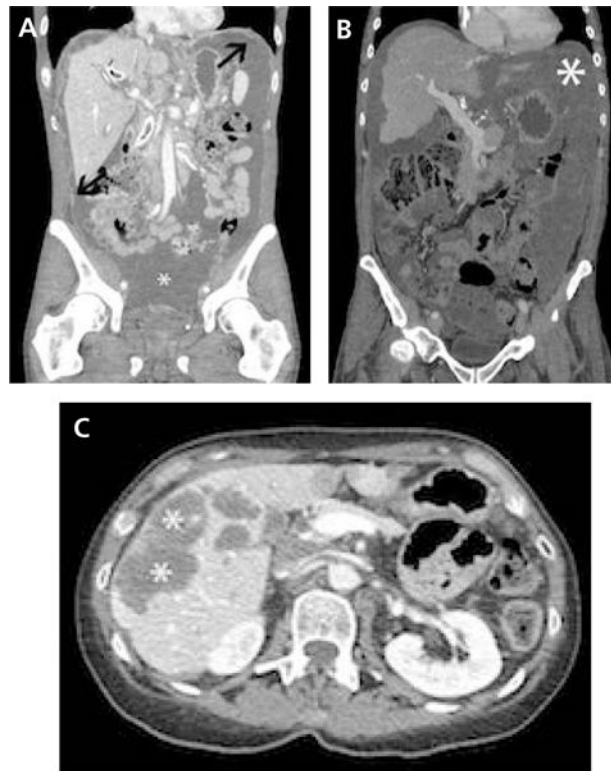


Figure 1.

Representative CT images from patients with *BRAF*-mutated tumors. (A) Coronal CT image showing extensive ascites (*) with multiple nodular soft tissue implants (arrows) in a patient with *BRAF*-mutated metastatic colorectal cancer (mCRC). No other measurable disease was found. (B) Coronal CT image showing extensive mixed low attenuating peritoneal implants and ascites (*) in a patient with *BRAF*-mutated mCRC. No other measurable disease was found. (C) Axial CT image showing predominantly low attenuating liver metastases (*) due to the high level of mucin, another radiographic feature of *BRAF*-mutated mCRC.

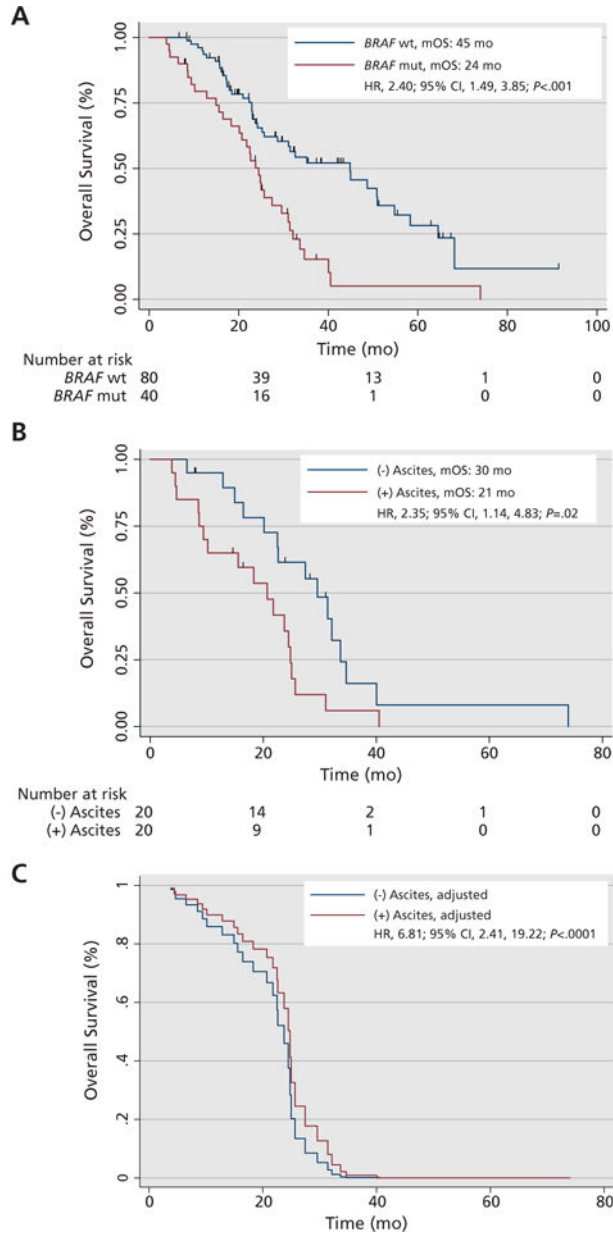


Figure 2. Survival by *BRAF* mutation status and presence of ascites. (A) Kaplan-Meier curves for median overall survival (mOS) from diagnosis with metastatic colorectal cancer by *BRAF* mutation status. Median follow-up among 40 censored patients with *BRAF* wild-type (wt) colorectal cancer was 29 months (range, 6–91 months). Median follow-up among 7 censored patients with *BRAF*-mutated (mut) colorectal cancer was 25 months (range, 8–37 months). (B) Kaplan-Meier curves for mOS of patients with *BRAF*-mutated tumors by absence or presence of ascites on CT imaging. (C) Cox regression for radiographic detection of ascites after adjusting for sex, primary tumor location (left or right colon), age, and peritoneal metastases patients with *BRAF*-mutated tumors. Abbreviation: HR, hazard ratio.

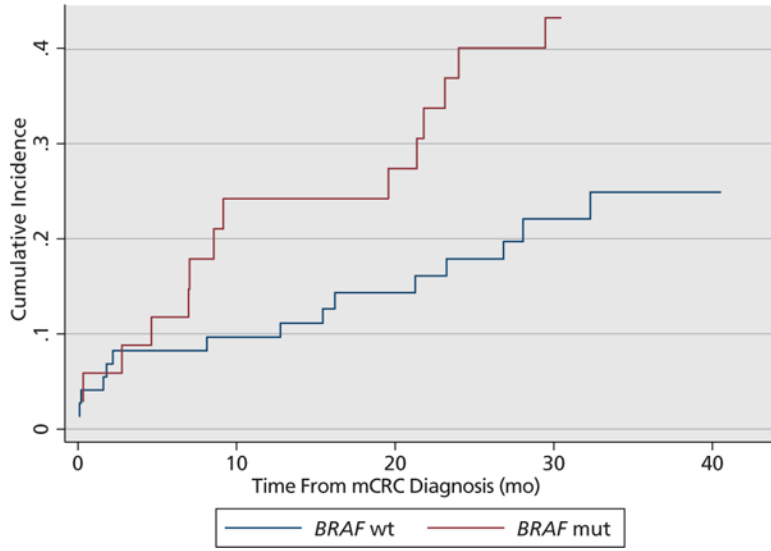


Figure 3. Time to ascites. Cumulative incidence of ascites in patients who did not have radiographic evidence of ascites at the time of initial presentation with metastatic colorectal cancer (mCRC). *BRAF*-mutated (mut): 14 patients developed ascites after diagnosis out of 34 patients total (6 patients with ascites at diagnosis excluded). *BRAF*wild-type (wt): 15 patients developed ascites after diagnosis out of 73 patients total (7 patients with ascites at diagnosis excluded).

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Table 1Clinicopathologic Features by *BRAF* Mutation Status

Variable	<i>BRAF</i> V600E (N=40)	<i>BRAF</i> Wild-Type (N=80)
Sex (female)	24 (60%)	48 (60%)
Median age at diagnosis (range), y	56 (28–88)	58 (20–77)
Race, n (%)		
Asian ^a	7 (18%)	14 (20%)
White	31 (82%)	49 (71%)
Black	0 (0%)	6 (9%)
Hispanic, n (%)	2 (5%)	5 (7%)
Primary tumor location, n (%) ^b		
Right colon	24 (60%)	48 (60%)
Left colon	12 (30%)	24 (30%)
Rectum	4 (10%)	8 (10%)
Primary resected, n (%)	30 (74%)	71 (89%)
Metastases resected, n (%)	7 (17.5%)	32 (40%) ^c
Stage at diagnosis		
II	3 (7.5%)	8 (10%)
III	10 (25%)	22 (27.5%)
IV	27 (67.5%)	50 (62.5%)
Median time to metastasis (range), mo		
Stage II	12 (9–13)	38 (2–93)
Stage III	9 (2–92)	14 (2–100)
Stage II/III combined	9 (2–92)	16 (2–100)
Tumor grade (high), n (%) ^d	11 (32%)	16 (22%)
Mucinous features (present), n (%) ^e	6 (15%)	5 (6%)
MSI/MMR, n (%)		
Present	6 (15%)	4 (5%)
Absent	15 (37.5%)	53 (66%)
Unknown	19 (47.5%)	23 (29%)
<i>RAS</i> mutated, n (%) ^f	0 (0%)	47 (59%)

Abbreviations: MMR, mismatch repair (present=deficient); MSI, microsatellite instability (present=high).

^aNative Hawaiian or other Pacific Islander were grouped with Asian (2 *BRAF*-wild-type).

^bRight colon: cecum, ascending colon, hepatic flexure, and proximal transverse colon. Left colon: distal transverse colon, splenic flexure, descending colon, and sigmoid colon. Unspecified location in transverse colon was coded as left colon.

^c $P=0.013$; all other P values insignificant.

^dIntermediate-high grade was included with high-grade.

^eSignet-ring adenocarcinoma was grouped with mucinous features present.

^fOf patients without a known *RAS* or *BRAF* mutation, 15/33 (45%) lacked full expanded RAS testing (KRAS and NRAS codons 12, 13, 61, 117 and 146). The following variables contain missing values: Race (2 *BRAF*-mutant; 11 wild-type); Hispanic (3 *BRAF*-mutant; 9 wild-type); grade (6 *BRAF*-mutant; 8 wild-type).

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Table 2

Patterns of Metastasis by *BRAF* Mutation Status and Location of CRC Primary

Metastatic Sites	Entire Cohort			Right Colon Primaries			Left Colon Primaries		
	Mutant (N=40)	Wild-Type (N=80)	P Value	Mutant (N=24)	Wild-Type (N=48)	P Value	Mutant (N=12)	Wild-Type (N=24)	P Value
Liver	21 (53%)	54 (68%)	.11	14 (58%)	31 (65%)	.61	5 (42%)	19 (79%)	.024
Lung	12 (30%)	33 (41%)	.23	7 (29%)	16 (33%)	.72	4 (33%)	11 (46%)	.47
Bone	4 (10%)	7 (9%)	.82	3 (12%)	2 (4%)	.19	1 (8%)	4 (17%)	.49
Lymph node	21 (54%)	37 (46%)	.44	11 (50%)	23 (48%)	.87	8 (73%)	12 (50%)	.21
Peritoneal	20 (50%)	25 (31%)	.045	14 (58%)	20 (42%)	.18	5 (42%)	4 (17%)	.10
Ascites	20 (50%)	19 (24%)	.0038	11 (46%)	16 (33%)	.30	7 (58%)	3 (12%)	.0038
RECIST measurable	32 (80%)	66 (83%)	.74	18 (75%)	37 (77%)	.84	10 (83%)	22 (92%)	.45

Using the false discovery method of Benjamini and Hochberg (1995)⁴⁸ as implemented by Glickman et al (2014),⁴⁹ the *P* values related to frequency of ascites in *BRAF* mutant versus wild-type CRC remain statistically significant, whereas the *P* value of .045 for peritoneal metastases in the entire cohort no longer meets the criteria for statistical significance.

Abbreviation: CRC, colorectal cancer.