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BRAF mutation status and survival after colorectal cancer diagnosis according to patient and tumor characteristics

Amanda I. Phipps[1], **Daniel D. Buchanan**[2], **Karen W. Makar**[1], **Andrea N. Burnett-Hartman**[1], **Anna E. Coghill**[1], **Michael N. Passarelli**[1], **John A. Baron**[3], **Dennis J. Ahnen**[4], **Aung Ko Win**[5], **John D. Potter**[1,6], and **Polly A. Newcomb**[1]

[1]Public Health Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA [2]Familial Cancer Laboratory, Queensland Institute for Medical Research, Brisbane, QLD, Australia [3]Department of Medicine, University of North Carolina, Chapel Hill, NC, USA [4]Denver Veterans Affairs Medical Center, University of Colorado School of Medicine, Denver, CO, USA [5]Centre for Molecular, Environmental, Genetic and Analytic Epidemiology, The University of Melbourne, Parkville, VIC, Australia [6]Centre for Public Health Research, Massey University, Wellington, New Zealand

Abstract

BACKGROUND—*BRAF* mutations in colorectal cancer (CRC) are disproportionately observed in tumors exhibiting microsatellite instability (MSI), and are associated with other prognostic factors. The independent association between *BRAF*-mutation status and CRC survival, however, remains unclear.

METHODS—We evaluated the association between the *BRAF* c.1799T>A (p.V600E) mutation and survival in individuals with incident invasive CRC diagnosed between 1997 and 2007 in Western Washington State. Tumor specimens were tested for this *BRAF* mutation and MSI status. We used Cox regression to estimate hazard ratios (HR) and 95% confidence intervals (CI) for the association between *BRAF*-mutation status and disease-specific and overall survival. Stratified analyses were conducted by age, sex, tumor site, stage, and MSI status.

RESULTS—Among 1,980 cases tested, 12% were *BRAF* c.1799T>A (p.V600E) mutation-positive (N=247). *BRAF*-mutated CRC was associated with poorer disease-specific survival adjusting for age, sex, time from diagnosis to enrollment, stage, and MSI status (HR=1.43, 95% CI: 1.05–1.95). This association was limited to cases diagnosed at ages <50 (HR=3.06, 95% CI: 1.70–5.52), and was not evident in cases with MSI-high tumors (HR=0.94, 95% CI: 0.44–2.03). Associations with overall survival were similar.

CONCLUSIONS—Our results show that the prevalence of *BRAF* mutations in CRC differs by patient and tumor characteristics, and suggest that the association between *BRAF* status and CRC survival may differ by some of these factors.

IMPACT—The presence of a *BRAF* c.1799T>A (p.V600E) mutation is associated with significantly poorer prognosis after CRC diagnosis among subgroups of patients.

Keywords

colorectal cancer; BRAF; survival; mortality; microsatellite instability

Corresponding author: Amanda Phipps, 1100 Fairview Ave. N., M4-B402, Seattle, WA 98109-1024, telephone: (206)667-7741, fax: (206)667-7850, aphipps@fhcrc.org.

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INTRODUCTION

Somatic mutations in *BRAF*, a proto-oncogene involved in the RAS-RAF-MAPK pathway, are observed in 10–20% of colorectal cancers (CRCs) (1–5). The *BRAF* c.1799T>A (p.V600E) mutation accounts for approximately 90% of such mutations (6, 7) and results in constitutive activation of BRAF kinase. Recent studies have suggested that this somatic mutation is associated with poorer survival after CRC diagnosis (1, 2, 8–13), and may impact response to certain treatment regimens (9, 14–16). Prior studies have also demonstrated that *BRAF*-mutation status is strongly associated with other CRC prognostic factors, most notably the presence of high microsatellite instability (MSI-H) (1, 2, 8–10, 12, 13, 17–19) as is mediated by the relationship between *BRAF*-mutation status and CpG island methylation (20).

Despite the fact that *BRAF* mutations are more common in MSI-H CRC, which is associated with better survival than CRC exhibiting microsatellite stability (MSS) (2, 8, 13, 21), *BRAF* mutations paradoxically appear to be associated with a poorer CRC prognosis. The few studies that have evaluated *BRAF*-mutation status and MSI status in combination have been limited by small numbers and inconsistent in their findings (1, 2, 8, 10–13). Ogino et al. recently reported that, in a clinical trial of stage III colon cancer, overall survival was similar in patients with *BRAF*-wildtype / MSS and *BRAF*-mutated / MSI-H disease, comparatively better in patients with *BRAF*-wildtype / MSI-H disease, and poorer in those with *BRAF*-mutated / MSS disease (12); however, associations with survival in this small study (n=506) did not attain statistical significance and require further evaluation.

We used data from two concurrent population-based studies of incident invasive CRC conducted in Western Washington State to further evaluate the relationship between *BRAF* c.1799T>A (p.V600E) mutation status and survival after CRC diagnosis, both overall and among subsets defined by other tumor and patient characteristics.

METHODS AND MATERIALS

Study Population

Details of the studies included here have been published elsewhere (22, 23). Briefly, eligible participants included men and women diagnosed with incident invasive CRC between January 1998 and June 2002 who, at the time of diagnosis, were aged 20–74 years and resided in King, Pierce, or Snohomish counties in Western Washington State. Over this same period, we recruited women diagnosed with invasive CRC between ages 50–74 residing in 10 additional surrounding counties. During a second phase of study recruitment, we identified eligible participants as individuals with invasive CRC in this broader ascertainment area (i.e., 13 Washington State counties) who were diagnosed at younger ages (i.e., 18–49 years) between April 2002 and July 2007. All cases were identified via the population-based Surveillance, Epidemiology, and End Results (SEER) cancer registry serving Western Washington State. Eligibility was limited to English speakers with publicly available telephone numbers. Of 3,585 individuals contacted and identified as eligible, 463 (13%) were deceased, 351 (10%) refused participation, 128 (4%) were lost to follow-up before interview, and 24 (0.7%) completed only a partial interview. Adequate tumor specimens were available for 78% (N=2120) of enrolled participants who completed the interview (N=2708).

All participants completed a structured telephone interview at enrollment. Interviews were conducted an average of 8.6 months after diagnosis (range=2.6–32.7 months). Participants were asked to provide detailed information on exposures occurring at least 2 years pre-

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diagnosis, including smoking history, alcohol consumption, family history of CRC, demographic factors, history of CRC screening, and use of selected medications.

Vital status was determined through linkage to SEER and the National Death Index. Through these sources, we obtained information on the date and cause of death, classified according to ICD-10 conventions (24). Disease-specific deaths included those with an underlying cause attributed to ICD-10 codes C18.0-C20.0 or C26.0. Vital status linkage was performed periodically, with the most recent linkage capturing deaths occurring through September 2010.

This study was approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center in accordance with assurances filed with and approved by the U.S. Department of Health and Human Services.

Tumor characteristics

DNA was extracted from paraffin-embedded formalin-fixed tumor tissue. Extracted DNA was tested for the c.1799T>A (p.V600E) *BRAF* mutation (N=2006) using a fluorescent allele-specific PCR assay as described previously (25). Cases for whom *BRAF*-mutation status was found to be equivocal (N=2) or for whom testing failed (N=24) were excluded from the analysis.

For most cases, MSI status was determined via testing on a 10-gene panel in tumor DNA and DNA from normal surrounding tissue (BAT25, BAT26, BAT40, MYCL, D5S346, D17S250, ACTC, D18S55, D10S197, and BAT34C4) as previously described (N=1430) (23, 26). Briefly, tumors were classified as MSI-H if instability was observed for ≥30% of markers, and as MSS if instability was observed in <30% of markers. For cases tested in later years of the study (N=470), MSI status was based on immunohistochemistry testing of four markers: MLH1, MSH2, MSH6, and PMS2 (27, 28). Cases whose tissue exhibited positive staining for all markers were considered MSS; cases negative for at least one marker were considered MSI-H. Cases for whom test results were equivocal or for whom testing was not completed (N=80) were classified as having unknown MSI status.

Tumor site and stage at diagnosis information was available from SEER. Tumors located in the cecum to splenic flexure were grouped together as proximal colon cancers (ICD-O-3 codes C180, C182-C185) (29). Tumors in the descending (C186) and sigmoid colon (C187) were classified as distal colon cancers, and tumors in the rectosigmoid junction (C199) and rectum (C209) were grouped together as rectal cancers. Stage at diagnosis was recorded according to SEER summary staging conventions (localized, regional, distant stage) (30).

Statistical analysis

We used Cox regression to evaluate the association between *BRAF* c.1799T>A (p.V600E) mutation status and survival after CRC diagnosis, where the time axis was defined as days since diagnosis. We conducted separate analyses for disease-specific and overall survival. In analyses of disease-specific survival, persons who died due to causes other than CRC were censored at the time of death. In all analyses, participants still alive at their last vital status assessment were censored at that date. We evaluated associations between *BRAF*-mutation status and survival outcomes in the full cohort and within strata defined by patient characteristics (age at diagnosis, sex) and tumor characteristics (tumor site, stage, MSI status). Proportional hazards assumptions were assessed by testing for a non-zero slope of the scaled Schoenfeld residuals on ranked failure times (31).

Regression models included adjustment terms for age (five-year categories), time from diagnosis to interview (<6, 6–9, >9 months), and sex. We also assessed confounding by a

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series of patient and tumor characteristics: cigarette smoking (never, former, current), body mass index (BMI) (<25.0, 25.0–29.9, ≥30.0 kilograms/meters^2), tumor site (proximal colon, distal colon/rectum), stage (localized, regional, distant), MSI status (MSS, MSI-H). Of these additional factors, only stage and MSI status were retained in our final analytic model as adjustment for other variables had minimal impact on effect estimates (<5% change).

To account for missing MSI data in cases with known *BRAF*-mutation status, we used an iterative multiple imputation model for the prediction of unknown MSI status. Our imputation model included all covariate variables from the multivariate model, as well as family history of CRC, tumor site, BMI, smoking history, race, survival time, and the survival outcome of interest (32–34). All analyses were conducted in STATA SE version 12.0 (College Park, Texas).

RESULTS

Over study follow-up (median=7.4 years, range=0.4–13.8 years), 38% of enrolled cases died (i.e., 62% overall survival), of whom 62% died due to CRC. Characteristics of the study population are presented by *BRAF*-mutation status in Table 1. *BRAF* c.1799T>A (p.V600E) mutations were evident in 12% of cases (i.e., *BRAF*-mutated). Cases with *BRAF*-mutated CRC tended to be older at diagnosis than cases without a *BRAF* c.1799T>A (p.V600E) mutation (i.e., *BRAF*-wildtype) and were more likely to be female, to have MSI-H tumors, and to have tumors located in the proximal colon (p<0.001). The prevalence of *BRAF* mutations increased across subsites from the rectum (2%) to ascending colon (30%). *BRAF*-mutated cases were also less likely to have distant-stage disease at diagnosis (p=0.008).

In unadjusted analyses, there was no difference in disease-specific or overall survival for *BRAF*-mutated vs. wildtype cases (Table 2). However, after multivariable-adjustment, the presence of a *BRAF* c.1799T>A (p.V600E) mutation was associated with statistically significantly poorer disease-specific survival (HR=1.43, 95% CI: 1.05–1.95); adjustment for stage and MSI status had the greatest impact on point estimates. Stratified analyses indicated statistically significant heterogeneity in the association between *BRAF*-mutation status and survival by age at diagnosis ($p_{interaction}$<0.001 and 0.04 for disease-specific and overall survival, respectively). The adjusted association between *BRAF*-mutation status and survival was strongest in cases aged <50 at diagnosis (HR=3.06, 95% CI: 1.70–5.52 and HR=2.12, 95% CI: 1.20–3.76, for disease-specific and overall survival, respectively), with little evidence of an association in cases diagnosed at ages ≥50. The adjusted association with *BRAF*-mutation status also appeared stronger in cases with regional or distant-stage CRC, particularly in analyses of disease-specific survival ($p_{interaction}$=0.07). There was no heterogeneity in associations by sex or tumor site. Interaction by MSI status was not statistically significant ($p_{interaction}$=0.17 and 0.85 for disease-specific and overall survival, respectively); however, the presence of a *BRAF* c.1799T>A (p.V600E) mutation was associated with significantly poorer disease-specific survival for cases with MSS disease (HR=1.62, 95% CI: 1.16–2.26) but not for cases with MSI-H disease (HR=0.94, 95% CI: 0.44–2.03).

When we evaluated the association between joint *BRAF* / MSI status and survival we found that, relative to cases with *BRAF*-wildtype / MSS disease, cases *BRAF*-mutated / MSS CRC experienced the poorest disease-specific survival (HR=1.60, 95% CI: 1.14–2.23) (Table 3). Cases with MSI-H disease experienced more favorable disease-specific survival, regardless of *BRAF* c.1799T>A (p.V600E) mutation status. This pattern was attenuated in analyses of overall survival, but results continued to suggest that cases with *BRAF*-mutated / MSS disease experienced the poorest survival. Analyses excluding cases with unknown MSI

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status (i.e., not using multiple imputation to account for unknown MSI status) yielded almost identical results (Supplementary Tables 1–2).

Enrolled cases for whom *BRAF*-mutation status was unknown (N=728) were younger at diagnosis than cases with known mutation status, and more likely to have distant-stage disease, and to have rectal cancer; however, survival did not differ in enrolled cases with unknown versus known *BRAF*-mutation status (HR=0.99, 95% CI: 0.65–1.51 and HR=0.92, 95% CI: 0.65–1.31 for disease-specific and overall survival, respectively) (data not shown).

DISCUSSION

In this cohort of men and women with incident invasive CRC, we found that individuals with tumors exhibiting the *BRAF* c.1799T>A (p.V600E) mutation were significantly more likely to die from their disease than individuals without this mutation. This association was most evident in individuals diagnosed before age 50. Although there was no significant interaction by MSI status, the association between *BRAF*-mutation status and disease-specific survival was evident only among those with MSS CRC. Cases with *BRAF*-mutated / MSS CRC had the poorest prognosis across case groups defined by joint *BRAF*-mutation / MSI status. Associations with overall survival were more modest than associations with disease-specific survival.

Previous studies have similarly reported that *BRAF*-mutated CRC is associated with poorer prognosis than *BRAF*-wildtype disease (1, 2, 10–13, 35). Most recently, Kalady et al. reported that the presence of a somatic *BRAF* mutation was associated with a 1.79-fold (95% CI: 1.05–3.05) increased risk of all-cause mortality in patients with stage I-III CRC (13). Other studies have noted even stronger associations between *BRAF*-mutation status and survival (1, 10–12, 18, 35), particularly with respect to disease-specific survival (1, 2, 35).

Given the association between *BRAF*-mutation status and MSI (1, 2, 8, 10–13, 17, 19), and the well-established prognostic value of MSI status (21), it is important to consider MSI when evaluating the relationship between *BRAF* status and survival. Here, we found that the adverse association between the presence of a *BRAF* c.1799T>A (p.V600E) mutation and CRC survival was evident only in individuals with MSS CRC, although interaction by MSI status was not significant. Some previous, small studies have noted that the association between *BRAF*-mutation status and survival is more pronounced in, if not restricted to, patients with MSS CRC (10, 11, 18, 35). Using data from a phase III clinical trial of stage III colon cancer, Ogino et al. recently reported that patients with *BRAF*-mutated / MSS tumors had the poorest recurrence-free, disease-free, and overall survival, whereas survival was most favorable in patients with *BRAF*-wildtype / MSI-H disease, and intermediate in patients with *BRAF*-wildtype / MSS or *BRAF*-mutated / MSI-H disease (12). Studies in patients with MSS CRC have also reported that the *BRAF* c.1799T>A (p.V600E) mutation is independently associated with poorer survival (1, 36). In a recent analysis of patients with proximal colon cancers exhibiting proficient DNA mismatch repair (i.e., MSS), Pai et al. noted that the presence of a *BRAF*-mutation was associated with distinct clinical, pathologic, and molecular features, including: more frequent lymphatic invasion, lymph node metastasis, mucinous histology, signet ring histology, and high tumor budding. These aggressive features could contribute to a poorer prognosis. In contrast, other studies have reported that *BRAF*-mutation status is more informative of CRC prognosis in MSI-H cases (2, 8, 13). The basis for such inconsistencies is unclear, but may be related to sample size limitations. Given that testing for MSI and *BRAF*-mutation status is becoming increasingly routine clinical practice for distinguishing Lynch Syndrome and sporadic cases, and for

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guiding treatment approaches (37), it is important to understand the relationship between these markers and CRC prognosis.

Although the presence of a somatic *BRAF* c.1799T>A (p.V600E) mutation appears to be independently associated with shorter disease-specific survival, several characteristics typical of *BRAF*-mutated CRC are also associated with prognosis. In particular, *BRAF* mutations are more prevalent among CRC patients diagnosed at an advanced age (12, 13, 18) and patients with proximal colon cancer (11–13, 17–19). With respect to tumor site, Yamauchi et al. recently demonstrated an increase in the frequency of *BRAF* mutations along colorectum subsites from the rectum to ascending colon (5); this pattern was evident in our data, lending support to the theory of a CRC continuum (38). The frequency of MSI-H follows a similar pattern and is highly correlated with the presence of a *BRAF* mutation (2, 10–13, 17, 18, 39). Although the age and tumor site distribution associated with *BRAF*-mutated CRC may be expected to confer poorer overall and disease-specific survival, respectively, the fact that patients with *BRAF*-mutated CRC are more likely to have MSI-H tumors could be considered prognostically favorable. Consistent with some degree of balancing out of these potentially prognostic attributes, we observed that *BRAF* c.1799T>A (p.V600E) mutation status was not associated with survival in the absence of multivariate adjustment. Instead, the association between *BRAF*-mutation status and poorer disease-specific survival appeared to be most pronounced among those groups of cases among whom *BRAF* mutations are less common (i.e., cases aged <50 at diagnosis or with MSS). These results highlight the need to consider the association between *BRAF*-mutation status and CRC survival in the context of potential modifying factors. Our findings also support the argument that mutated *BRAF* is on the causal pathway to poorer survival, inasmuch as it is not only directly associated with poorer outcomes, but also has greater impact when it occurs in the absence of its usual biologic and patient-characteristic correlates.

Findings presented here should be interpreted in the context of study limitations. In the absence of treatment information, we were unable to assess possible treatment interactions with *BRAF*-mutation status; however, although there is some suggestion that response to EGFR inhibitors is more favorable in individuals with *BRAF*-wildtype CRC (9), studies have noted no significant differences in response to standard chemotherapy regimens by *BRAF*-mutation status (12, 19, 40). We also did not test for *BRAF* mutations other than c.1799T>A (p.V600E). It is plausible that other *BRAF* mutations with effects on BRAF kinase activity could be associated with CRC survival. In light of the rarity of other *BRAF* mutations, however, it is unlikely that information regarding such mutations would alter our findings. Additionally, *BRAF* c.1799T>A (p.V600E) mutation status was not determined in 27% of enrolled cases, nor was it determined in cases who were eligible for the study but were not enrolled. It is plausible that the distribution of *BRAF*-mutation status could differ among cases excluded from the present analysis due to missing data. In particular, if *BRAF*-mutated CRC is truly associated with poorer prognosis, one might expect the prevalence of *BRAF* mutations to have been higher in cases who died before they could be enrolled. Exclusion of such cases could have attenuated our effect estimates, although the extent and impact of survivor bias is unknowable.. We also lacked information on the CpG island methylation phenotype (CIMP) status of tumors, which is highly correlated with *BRAF*-mutation status (1, 2, 35, 39, 41). Promoter methylation of *MLH1*, as part of CIMP, is a principal cause of MSI in sporadic CRC, thus constituting a link between *BRAF*-mutation status and MSI (42–45). The presence of MSI in the absence of CIMP and a *BRAF* mutation may be indicative of Lynch Syndrome-associated CRC (43), which has been associated with better prognosis (46). *BRAF*-mutated / MSI-H and *BRAF*-mutated / MSS CRC are both thought to develop along the so-called serrated pathway (42, 43) with epigenetic inactivation of a different panel of genes. Although we did not have information on CIMP, our results are suggestive of a poorer prognosis associated with the *BRAF*-mutated / MSS phenotype.

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There are also several important strengths of this analysis. The population-based design of the cohort contributes to the generalizability of our results. Well-annotated, existing cohorts such as the one used here represent an important resource for informing cancer research (47, 48). The availability of detailed information on tumor and patient characteristics allowed us to efficiently evaluate of potential sources of heterogeneity in the association between *BRAF*-mutation status and survival.

In conclusion, in this large prospective study, the presence of a somatic *BRAF* c.1799T>A (p.V600E) mutation was independently associated with poorer CRC survival. Our data are consistent with previous reports that the prevalence of *BRAF* mutations in CRC differs by age at diagnosis, tumor site, and MSI status, and suggest that the association between *BRAF* status and survival may differ according to some of these characteristics. Future studies should explore the potential mechanisms responsible for these observed associations, and further describe the features of *BRAF*-mutated CRC that may contribute to disease progression and prognosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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TABLE 1

Study population characteristics by *BRAF* mutation status

	***BRAF*-wildtype (N=1733)**	***BRAF*-mutated (N=247)**	**p-value[a]**
Age at diagnosis	480 (28)	29 (12)	<0.001
<50	415 (24)	31 (13)	
50–59	514 (30)	105 (43)	
60–69	324 (19)	82 (33)	
70–74			
Sex	832 (48)	68 (28)	<0.001
Male	901 (52)	179 (72)	
Female			
Vital status	1078 (62)	145 (59)	0.29
Alive	655 (38)	102 (41)	
Deceased			
Tumor site	592 (35)	192 (80)	<0.001[c]
Proximal colon:	240 (14)	70 (29)	<0.001[d]
Cecum	161 (10)	69 (28)	
Ascending colon	48 (3)	20 (8)	
Hepatic flexure	106 (6)	27 (11)	
Transverse colon	37 (2)	6 (2)	
Splenic flexure	495 (29)	31 (13)	
Distal colon:	67 (4)	6 (2)	
Descending colon	428 (25)	25 (10)	
Sigmoid colon	604 (36)	18 (7)	
Rectal:	151 (19)	7 (3)	
Rectosigmoid junction	453 (27)	11 (5)	
Rectum			
Stage at diagnosis[b]	690 (40)	95 (39)	0.008
Localized	804 (47)	133 (55)	
Regional	211 (12)	15 (6)	
Distant	28	4	
Unknown			
MSI status[b]	1494 (90)	109 (46)	<0.001
MSS	171 (10)	126 (54)	
MSI-H	68	12	
Unknown			

[a] p-value for chi-square

[b] % distribution excludes cases with unknown value of characteristic

[c] p-value for chi-square of proximal / distal / rectal tumor site distribution

[d] p-value for chi-square of tumor subsite distribution (e.g., cecum, ascending colon, hepatic flexure etc)

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TABLE 2

BRAF mutation status and survival after colorectal cancer diagnosis by patient and tumor characteristics

		Disease-Specific Survival				Overall Survival			
		***BRAF*-wt Deaths / Cases**	***BRAF*-mut Deaths / Cases**	**HR (95% CI)[a]**	***p-interaction***	***BRAF*-wt Deaths / Cases**	***BRAF*-mut Deaths / Cases**	**HR (95% CI)[a]**	***p-interaction***
Overall (unadjusted)		413/1733	53/247	0.95 (0.72–1.27)		655/1733	102/247	1.11 (0.90–1.38)	
Overall (adjusted)		413/1733	53/247	1.43 (1.05–1.95)		655/1733	102/247	1.21 (0.96–1.54)	
By age at diagnosis:	<50 years	104/480	14/29	3.06 (1.70–5.52)	<0.001	132/480	14/29	2.12 (1.20–3.76)	0.04
	≥50 years	309/1253	39/218	1.23 (0.85–1.77)		523/1253	88/218	1.12 (0.86–1.46)	
By sex:	Male	200/832	16/68	1.34 (0.79–2.27)	0.99	335/832	32/68	1.27 (0.86–1.89)	0.43
	Female	213/901	37/179	1.50 (1.01–2.22)		320/901	70/179	1.23 (0.91–1.67)	
By tumor site:	Proximal	152/592	38/192	1.27 (0.86–1.88)	0.36	243/592	78/192	1.22 (0.91–1.64)	0.97
	Distal/rectal	254/1099	14/49	1.76 (1.00–3.10)		395/1099	20/49	1.25 (0.78–2.00)	
By stage at diagnosis:	Localized	46/690	1/95	0.20 (0.03–1.58)		165/690	24/95	0.75 (0.44–1.25)	
	Regional	204/804	39/133	1.55 (1.07–2.27)	0.07	305/804	61/133	1.31 (0.96–1.79)	0.23
	Distant	161/211	13/15	1.72 (0.92–3.24)		173/211	14/15	1.57 (0.85–2.91)	
By MSI:	MSS	375/1494	38/109	1.62 (1.16–2.26)	0.17	581/1494	49/109	1.23 (0.91–1.65)	0.85
	MSI-H	22/171	14/126	0.94 (0.44–2.03)		49/171	51/126	1.17 (0.75–1.81)	

[a]Adjusted for age at diagnosis, sex, time from diagnosis to enrollment, stage, and MSI status unless otherwise noted. All associations are relative to *BRAF*-wildtype case group.

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TABLE 3

Joint *BRAF* / MSI status and survival after colorectal cancer diagnosis

	Disease-Specific Survival		Overall Survival	
	Deaths / Cases	**HR (95% CI)[a]**	**Deaths / Cases**	**HR (95% CI)[a]**
BRAF-wildtype / MSS	375/1494	1.00 (ref)	581/1494	1.00 (ref)
BRAF-wildtype / MSI-H	22/171	0.60 (0.39–0.93)	49/171	0.84 (0.62–1.12)
BRAF-mutated / MSS	38/109	1.60 (1.14–2.23)	49/109	1.24 (0.92–1.66)
BRAF-mutated / MSI-H	14/126	0.57 (0.33–0.98)	51/126	0.99 (0.73–1.33)

[a]Adjusted for age at diagnosis, sex, time from diagnosis to enrollment, and stage.