Research Article

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BRAF Mutation Status and Survival after Colorectal Cancer Diagnosis According to Patient and Tumor Characteristics

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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 HRs 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. *Cancer Epidemiol Biomarkers Prev; 21(10); 1792–8. ©2012 AACR.*

Introduction

Somatic mutations in *BRAF*, a proto-oncogene involved in the RAS/RAF/MAPK pathway, are observed in 10% to 20% of colorectal cancers (CRC; refs. 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 shown that *BRAF* mutation status is strongly associated with other CRC prognostic factors, most notably the presence of high microsatellite instability (MSI-H; refs. 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; refs. 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 and colleagues recently reported that in a clinical trial of stage III colon cancer, overall survival was similar in patients with *BRAF*-wild-type/MSS and *BRAF*-mutated/MSI-H disease, comparatively better in patients with *BRAF*-wild-type/MSI-H disease and poorer in those with *BRAF*-mutated/MSS disease (12); however, associations

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with survival in this small study ($n = 506$) did not attain statistical significance and require further evaluation.

We used data from 2 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.

Materials and Methods

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 to 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 and 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% ($n = 2{,}120$) of enrolled participants who completed the interview ($n = 2{,}708$).

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-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 conducted 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 (Seattle, WA) 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 ($n = 2{,}006$) 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 ($n = 1{,}430$; refs. 23, 26). Briefly, tumors were classified as MSI-H if instability was observed for $\geq$30% of markers and as MSS if instability was observed in <30% of markers. For cases tested in later years of the study ($n = 470$), MSI status was based on immunohistochemical testing of 4 markers: MLH1, MSH2, MSH6, and PMS2 (27, 28). Cases whose tissue exhibited positive staining for all markers were considered MSS; cases negative for at 1 one marker were considered MSI-H. Cases for whom test results were equivocal or for whom testing was not completed ($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; ref. 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; ref. 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 because of 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 (5-year categories), time from diagnosis to interview (<6, 6–9, >9 months), and sex. We also assessed confounding

by a series of patient and tumor characteristics: cigarette smoking (never, former, current), body mass index (<25.0, 25.0–29.9, ≥30.0 kg/m^2), tumor site (proximal colon, distal colon/rectum), stage (localized, regional, distant), and 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, body mass index, smoking history, race, survival time, and the survival outcome of interest (32–34). All analyses were conducted in STATA SE version 12.0.

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 because of 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*-wild-type) 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 versus wild-type 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% confidence interval (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*-wild-type/MSS disease, cases with

Table 1. Study population characteristics by *BRAF* mutation status

	***BRAF*-wild-type ($n = 1,733$)**	***BRAF*-mutated ($n = 247$)**	***P*[a]**
Age at diagnosis, y			
<50	480 (28)	29 (12)	<0.001
50–59	415 (24)	31 (13)	
60–69	514 (30)	105 (43)	
70–74	324 (19)	82 (33)	
Sex			
Male	832 (48)	68 (28)	<0.001
Female	901 (52)	179 (72)	
Vital status			
Alive	1,078 (62)	145 (59)	0.29
Deceased	655 (38)	102 (41)	
Tumor site			
Proximal colon	592 (35)	192 (80)	<0.001[c]
Cecum	240 (14)	70 (29)	<0.001[d]
Ascending colon	161 (10)	69 (28)	
Hepatic flexure	48 (3)	20 (8)	
Transverse colon	106 (6)	27 (11)	
Splenic flexure	37 (2)	6 (2)	
Distal colon	495 (29)	31 (13)	
Descending colon	67 (4)	6 (2)	
Sigmoid colon	428 (25)	25 (10)	
Rectal	604 (36)	18 (7)	
Rectosigmoid junction	151 (19)	7 (3)	
Rectum	453 (27)	11 (5)	
Stage at diagnosis[b]			
Localized	690 (40)	95 (39)	0.008
Regional	804 (47)	133 (55)	
Distant	211 (12)	15 (6)	
Unknown	28	4	
MSI status[b]			
MSS	1,494 (90)	109 (46)	<0.001
MSI-H	171 (10)	126 (54)	
Unknown	68	12	

[a]*P* for χ^2.
[b]Percent distribution excludes cases with unknown value of characteristic.
[c]*P* for χ^2 of proximal/distal/rectal tumor site distribution.
[d]*P* for χ^2 of tumor subsite distribution (e.g., cecum, ascending colon, hepatic flexure).

Table 2. *BRAF* mutation status and survival after CRC 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)	413/1,733	53/247	0.95 (0.72–1.27)		655/1,733	102/247	1.11 (0.90–1.38)	
Overall (adjusted)	413/1,733	53/247	1.43 (1.05–1.95)		655/1,733	102/247	1.21 (0.96–1.54)	
By age at diagnosis								
<50 y	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 y	309/1,253	39/218	1.23 (0.85–1.77)		523/1,253	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	254/1,099	14/49	1.76 (1.00–3.10)		395/1,099	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	375/1,494	38/109	1.62 (1.16–2.26)	0.17	581/1,494	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*-wild-type case group.

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 status (i.e., not using multiple imputation to account for unknown MSI status) yielded almost identical results (Supplementary Tables S1 and S2).

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 CRCs, 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 the age of 50. Although there was no significant interaction by MSI status, the

Table 3. Joint *BRAF*/MSI status and survival after CRC diagnosis

	Disease-specific survival		Overall survival	
	Deaths/cases	**HR (95% CI)[a]**	**Deaths/cases**	**HR (95% CI)[a]**
BRAF-wild-type/MSS	375/1,494	1.00 (ref.)	581/1,494	1.00 (ref.)
BRAF-wild-type/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.

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*-wild-type disease (1, 2, 10–13, 35). Most recently, Kalady and colleagues 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 and colleagues 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*-wild-type/MSI-H disease and intermediate in patients with *BRAF*-wild-type/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 and colleagues 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 (36). 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 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 CRCs are also associated with prognosis. In particular, *BRAF* mutations are more prevalent among patients with CRC diagnosed at an advanced age (12, 13, 18) and patients with proximal colon cancer (11–13, 17–19). With respect to tumor site, Yamauchi and colleagues recently showed 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 CRCs may be expected to confer poorer overall and disease-specific survival, respectively, the fact that patients with *BRAF*-mutated CRCs 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 EGF receptor (EGFR) inhibitors is more favorable in individuals with *BRAF*-wild-type CRCs (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 about such mutations would alter our findings. In addition, *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 CRCs 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.

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 the 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 CRCs that may contribute to disease progression and prognosis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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