Microsatellite Instability and BRAF Mutation Testing in Colorectal Cancer Prognostication

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BRAF mutation in colorectal cancer is associated with microsatellite instability (MSI) through its relationship with high-level CpG island methylator phenotype (CIMP) and MLH1 promoter methylation. MSI and BRAF mutation analyses are routinely used for familial cancer risk assessment. To clarify clinical outcome associations of combined MSI/BRAF subgroups, we investigated survival in 1253 rectal and colon cancer patients within the Nurses’ Health Study and Health Professionals Follow-up Study with available data on clinical and other molecular features, including CIMP, LINE-1 hypomethylation, and KRAS and PIK3CA mutations. Compared with the majority subtype of microsatellite stable (MSS)/BRAF-wild-type, MSS/BRAF-mutant, MSI-high/BRAF-mutant, and MSI-high/BRAF-wild-type subtypes showed multivariable colorectal cancer-specific mortality hazard ratios of 1.60 (95% confidence interval [CI] = 1.12 to 2.28; \( P = .009 \)), 0.48 (95% CI = 0.27 to 0.87; \( P = .02 \)), and 0.25 (95% CI = 0.12 to 0.52; \( P < .001 \)), respectively. No evidence existed for a differential prognostic role of BRAF mutation by MSI status (\( P_{\text{interaction}} > .50 \)). Combined BRAF/MSI status in colorectal cancer is a tumor molecular biomarker for prognostic risk stratification.


High-level microsatellite instability (MSI-high) is present in approximately 15% of colorectal cancers and is associated with superior survival (1–9). BRAF mutation, present in 10% to 20% of colorectal cancers, is associated with MSI-high through its relationship to high-level CpG island methylator phenotype (CIMP) (10–14) and is generally associated with inferior prognosis (15–28). Because the presence of BRAF mutation in MSI-high colorectal cancer decreases the likelihood of Lynch syndrome, MSI and BRAF analyses have an established clinical utility (29–31). Clinicians are therefore increasingly availed of MSI/BRAF status in colorectal cancer (29–31); however, outcomes for combined MSI/BRAF subgroups have not been clearly defined. It remains uncertain whether the prognostic role of BRAF mutation depends on MSI status (15–18).

Using the database of two US nationwide prospective cohort studies, the Nurses’ Health Study and the Health Professionals Follow-up Study (32–34), we tested the hypothesis that combined MSI/BRAF status could serve as a prognostic molecular biomarker.

Rectal and colon cancer cases were identified through reporting by participants or next-of-kin and by searching the National Death Index for unreported lethal cases. The National Death Index was used to ascertain deaths (32–34). Cause of death was determined by study physicians. Informed consent was obtained from all study subjects. This study was approved by the Human Subjects Committees of Harvard School of Public Health and Brigham and Women’s Hospital.

DNA was extracted from formalin-fixed paraffin-embedded specimens, collected from hospitals across the United States where participants had undergone tumor resection or diagnostic biopsy (33). No statistically significant demographic differences existed between case subjects with and without available tissue (33). Tumor molecular biomarkers (including MSI, CIMP, LINE-1 hypomethylation, and KRAS, BRAF, and PIK3CA mutations) were analyzed as previously described (35–41) (details provided in Supplementary Methods, available online).

All statistical analyses were performed using SAS version 9.2; SAS Institute, Cary, NC). All statistical tests were two-sided. Survival time was assessed using the Kaplan–Meier and log-rank methods. Cox proportional hazards models were used to estimate mortality hazard ratios (HRs), adjusting for potential confounders (details provided in Supplementary Methods).

Characteristics of 1253 colorectal cancer case subjects are summarized in Supplementary Table 1 (available online). During follow-up (median = 8.2 years; interquartile range = 3.5–13.1 years), there were 608 deaths, including 361 colorectal cancer–specific deaths. We first analyzed BRAF mutation and MSI status as independent variables in survival analyses (Supplementary Figures 1 and 2, Supplementary Table 2, available online). In multivariable analyses, BRAF mutation was associated with statistically significantly higher colorectal cancer–specific mortality (multivariable HR = 1.64, 95% confidence interval [CI] = 1.18 to 2.27; \( P = .003 \)). MSI-high was associated with statistically significantly lower colorectal cancer–specific mortality (multivariable HR = 0.28, 95% CI = 0.17 to 0.46; \( P < .001 \)). MSI status was a confounder for BRAF mutation; when we simply adjusted for MSI status, the colorectal cancer–specific hazard ratio for BRAF mutation was 2.05 (compared with univariate HR estimate of 1.14).

Increased colorectal cancer–specific mortality appeared to be associated with BRAF mutation in both MSS (multivariable HR = 1.60, 95% CI = 1.12 to 2.28; \( P = .009 \)) and MSI-high tumor strata (multivariable HR = 1.90, 95% CI = 0.79 to 4.57; \( P = .15 \)) (Supplementary Table 3, available online). Lower colorectal cancer–specific mortality
was associated with MSI-high in both 
BRAF–wild-type (multivariable HR = 0.25, 
95% CI = 0.12 to 0.52; P < .001) and BRAF-
mutant strata (multivariable HR = 0.30, 
95% CI = 0.16 to 0.58; P < .001).

For combined MSI/BRAF subgroups, 
5-year colorectal cancer–specific sur-
vival was 46% for MSS/BRAF-mutant, 
65% for MSS/BRAF–wild-type, 73% for 
MSI-high/BRAF-mutant, and 79% for 
MSI-high/BRAF–wild-type (log-rank 
P < .001) (Figure 1). In multivariable 
analyses (Table 1), compared with the 
majority subtype of MSS/BRAF–wild-type, 
MSS/BRAF-mutant, MSI-high/BRAF–mutant and MSI-high/BRAF–wild-type subtypes showed colorectal cancer-specific mortality hazard ratios of 1.60 (95% CI = 1.12 to 2.28; P = .009), 
0.48 (95% CI = 0.27 to 0.87; P = .02), and 
0.25 (95% CI = 0.12 to 0.52; P < .001), respectively. We found no evidence of 
interaction between MSI and 
BRAF status 
in survival models (all 
P_{interaction} > .50).

Tumor molecular classification has 
become crucial for clinical, translational, 
and epidemiologic research (42–49) because 
of uniqueness of each tumor and the con-
tinuum of colorectal biogeography influen-
ting tumor characteristics (50–52). Despite 
their frequent coexistence as a result of their 
associations with high-level CIMP (CIMP-
high) (53–58), we found MSI-high and 
BRAF mutation in colorectal cancer to have 
divergent associations with patient survival. 
Our findings are compatible with previous

![Graph A](https://example.com/graphA.png)

![Graph B](https://example.com/graphB.png)

**Figure 1.** Kaplan–Meier survival plots for colorectal cancer according to combined MSI/BRAF subgroup. A) Colorectal cancer–specific survival. B) Overall survival. Multi-group log-rank P values demonstrate statistically significant deviation of any one of the survival curves from the null hypothesis. MSI = microsatellite instability; MSS = microsatellite stable.
Table 1. Colorectal cancer–specific and overall mortality according to combined microsatellite instability (MSI)/BRAF subgroup*

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>No. of cases (%)</th>
<th>No. of events</th>
<th>Univariate HR (95% CI)</th>
<th>Multivariable HR (95% CI)</th>
<th>P</th>
<th>No. of events</th>
<th>Univariate HR (95% CI)</th>
<th>Multivariable HR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSS/BRAF–wild-type</td>
<td>979 (78)</td>
<td>299</td>
<td>1 (referent)</td>
<td>1 (referent)</td>
<td></td>
<td>485</td>
<td>1 (referent)</td>
<td>1 (referent)</td>
<td></td>
</tr>
<tr>
<td>MSS/BRAF–mutant</td>
<td>81 (6.5)</td>
<td>40</td>
<td>2.10 (1.51 to 2.93)</td>
<td>&lt;.001</td>
<td></td>
<td>49</td>
<td>1.53 (1.14 to 2.06)</td>
<td>.005</td>
<td></td>
</tr>
<tr>
<td>MSI-high/BRAF–mutant</td>
<td>101 (8.1)</td>
<td>14</td>
<td>0.44 (0.26 to 0.75)</td>
<td>.003</td>
<td></td>
<td>42</td>
<td>0.86 (0.63 to 1.18)</td>
<td>.36</td>
<td></td>
</tr>
<tr>
<td>MSI-high BRAF–wild-type</td>
<td>92 (73)</td>
<td>8</td>
<td>0.26 (0.13 to 0.52)</td>
<td>&lt;.001</td>
<td></td>
<td>32</td>
<td>0.63 (0.44 to 0.90)</td>
<td>.01</td>
<td></td>
</tr>
<tr>
<td>P interaction between MSI and BRAF</td>
<td>.67</td>
<td>.72</td>
<td>.70</td>
<td>.83</td>
<td></td>
<td></td>
<td></td>
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</table>

* The multivariable Cox regression models were stage-stratified. In addition to MSV/BRAF subgroup, covariables initially included: age at diagnosis (continuous), sex, year of diagnosis (continuous), body mass index (≥30 vs <30 kg/m²), tumor location (proximal vs distal colorectum), tumor differentiation (poor vs well-moderately differentiated), family history of colorectal cancer in any first degree relative (present vs absent), CIMP status (CIMP-high vs CIMP-low/0), LINE-1 methylation (continuous), and KRAS and PIK3CA mutations (present vs absent). A backward elimination with threshold of P equal to .10 was used to select covariables. Age, year of diagnosis, body mass index, tumor differentiation, and LINE-1 methylation remained in the colorectal cancer–specific survival model. The same covariables, with the exception of LINE-1 methylation, remained in the overall survival model. CI = confidence interval; HR = hazard ratio; MSS = microsatellite stable.
studies that have found MSI-high to be associated with favorable outcome (2–8,15,17) and BRAF mutation to be associated with poor survival (16–28) [except for (59)]. MSI status is an established prognostic biomarker and is associated with host–tumor immune response (60–65).

Concordant with several other studies (16–20,66,67) [except for (15)], MSS/BRAF-mutant tumors were associated with the highest mortality. Patients with MSI-high/BRAF–wild-type tumors experienced the lowest mortality, consistent with a number of previous reports (15–20,67). Although we found MSI-high/BRAF-mutant tumors to be associated with favorable prognosis (vs MSS/BRAF–wild-type), confirmation in other populations is required.

Although some studies (17–19,68) suggest that the adverse prognostic association of BRAF mutation is limited to MSS tumors, other studies (15,16) and our analysis suggest that BRAF mutation remains prognostic among MSI-high cancers. We found no evidence for a differential prognostic role of BRAF mutation according to MSI status, consistent with a large population-based study (18). Taking into account existing literature, our data justify stratifying patients into poor (MSS/BRAF-mutant), intermediate (MSS/BRAF–wild-type), and favorable (MSI-high/BRAF–wild-type) prognostic groups (Supplementary Figure 4, available online).

Limitations of our study include its observational nature and lack of treatment data, and thus unknown bias, including differential treatment assignment, might confound results. Nevertheless, our regression analyses were adjusted for disease stage, on which treatment decisions are largely based, and our findings are consistent with data from independent clinical trials of colon cancer patients (15,16).

Strengths of our study include use of a molecular pathological epidemiology (69–79) database containing more than 1200 colorectal cancer cases characterized for key tumor molecular features. MSI-high and BRAF–mutant tumors represent a minority of colorectal cancers. The size and comprehensiveness of this population-based, molecular pathological epidemiology database enabled us to estimate an effect size for each tumor subtype while controlling for multiple potential confounders, including disease stage, age at diagnosis, body mass index, tumor differentiation, and tumor LINE-1 methylation level.

In conclusion, our data support a prognostic role for combined MSI/BRAF testing in colorectal cancer. Future studies should examine the predictive role of MSI/BRAF classification for response to therapeutic and lifestyle interventions.

References


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Notes
P. Lochhead, A. Kuchiba, Y. Imamura, and X. Liao contributed equally. C.S. Fuchs and S. Ogino contributed equally.

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