Predictive and Prognostic Roles of BRAF Mutation in Stage III Colon Cancer: Results from Intergroup Trial CALGB 89803

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Colon Cancer: Results from Intergroup Trial CALGB 89803

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Abstract

Purpose: Alterations in the RAS-RAF-MAP2K (MEK)-MAPK signaling pathway are major drivers in colorectal carcinogenesis. In colorectal cancer, BRAF mutation is associated with microsatellite instability (MSI), and typically predicts inferior prognosis. We examined the effect of BRAF mutation on survival and treatment efficacy in patients with stage III colon cancer.

Methods: We assessed status of BRAF c.1799T>A (p.V600E) mutation and MSI in 506 stage III colon cancer patients enrolled in a randomized adjuvant chemotherapy trial [5-fluorouracil and leucovorin (FU/LV) vs. irinotecan (CPT11)]. FU and LV (IFL; CALGB 89803). Cox proportional hazards model was used to assess the prognostic role of BRAF mutation, adjusting for clinical features, adjuvant chemotherapy arm, and MSI status.

Results: Compared with 431 BRAF wild-type patients, 75 BRAF-mutated patients experienced significantly worse overall survival [OS; log-rank P = 0.015; multivariate HR = 1.66; 95% CI: 1.05–2.63]. By assessing combined status of BRAF and MSI, it seemed that BRAF-mutated MSS (microsatellite stable) tumor was an unfavorable subtype, whereas BRAF wild-type MSI-high tumor was a favorable subtype, and BRAF-mutated MSI-high tumor and BRAF wild-type MSS tumor were intermediate subtypes. Among patients with BRAF-mutated tumors, a nonsignificant trend toward improved OS was observed for IFL versus FU/LV arm (multivariate HR = 0.52; 95% CI: 0.25–1.10). Among patients with BRAF wild-type cancer, IFL conferred no suggestion of benefit beyond FU/LV alone (multivariate HR = 1.02; 95% CI: 0.72–1.46).

Conclusions: BRAF mutation is associated with inferior survival in stage III colon cancer. Additional studies are necessary to assess whether there is any predictive role of BRAF mutation for irinotecan-based therapy. Clin Cancer Res; 18(3); 890–900. ©2011 AACR.

Introduction

BRAF is a part of the RAS-RAF-MAP2K (MEK)-MAPK signaling pathway. BRAF mutations are observed in 10% to 20% of colon cancers in population-based studies (1–9). In colon cancer, BRAF mutation is associated with proximal tumor location and microsatellite instability (MSI; refs. 1, 3, 10–13), and with significantly worse patient survival in most (1, 6, 14–22), though not all studies (2). In contrast, MSI-high colon cancers have been associated with a significantly improved survival (1, 2, 6, 16, 23), and several studies have suggested the prognostic impact of BRAF mutation status may vary according to the concurrent presence or absence of MSI-high (1, 14, 21). Thus, investigation of the prognostic impact of BRAF mutation or MSI in colon cancer may be most informative when these markers are simultaneously assessed.

The predictive role of BRAF mutation in colon cancer remains less clear. Few studies have examined the impact of BRAF mutation on the efficacy of available chemotherapy regimens (24, 25). A recent analysis of stage III colon cancer patients enrolled in a randomized trial comparing 5-fluorouracil (5-FU) and leucovorin (FU/LV) to irinotecan (CPT11), 5-FU and leucovorin (IFL; CALGB 89803) suggested that, among patients with MSI-high cancer, IFL conferred a superior disease-free survival when compared with FU/LV (23). In light of the association between BRAF...
Translational Relevance

*BRAF* mutation is associated with microsatellite instability (MSI) in colon cancer. Thus, the prognostic role of *BRAF* mutation or MSI in colon cancer can only be properly assessed when these markers are simultaneously determined. We examined *BRAF* mutation status in stage III colon cancer patients who enrolled in a phase III trial CALGB 89803, which randomized patients to either a combination of irinotecan, 5-fluorouracil, and leucovorin (IFL) or 5-fluorouracil, and leucovorin (FU/LV). We found that *BRAF* mutation was independently associated with inferior overall survival. We also observed a nonsignificant trend toward an improved overall survival of patients randomized to IFL (vs. FU/LV) among *BRAF*-mutated patients, but not among *BRAF* wild-type patients. Our findings provide important data on the prognostic role of *BRAF* mutation. Whether *BRAF* status has any predictive role for irinotecan-based chemotherapy needs to be examined by additional studies.

mutation and MSI, we hypothesized that *BRAF* mutation in colon cancer may similarly influence the efficacy of irinotecan-based chemotherapy in this setting.

We therefore examined prognostic and predictive roles of *BRAF* mutation among stage III colon cancer patients enrolled in this National Cancer Institute (NCI)-sponsored randomized clinical trial comparing postoperative adjuvant FU/LV to IFL (CALGB 89803; ref. 26). Because data on pathologic stage, performance status, postoperative treatment, follow-up, and tumor molecular features such as *KRAS* and MSI status were carefully recorded in this trial, the simultaneous impact of disease characteristics and the use of adjuvant therapy could be assessed to control for potential confounding. Moreover, the simultaneous impact of *BRAF* mutational status and MSI on patient outcome could be explored.

Materials and Methods

Study population

Patients in this study were participants in the NCI-sponsored Cancer and Leukemia Group B (CALGB) adjuvant therapy trial for stage III colon cancer comparing therapy with the weekly Roswell Park regimen of 5-FU and leucovorin (FU/LV) to weekly bolus regimen of irinotecan, 5-FU, and leucovorin (IFL; CALGB 89803; ref. 26). Between April 1999 and May 2001, 1,264 patients were enrolled on the treatment trial. Patients in the treatment trial (and thus this companion study) were eligible if they underwent a complete surgical resection of the primary tumor within 56 days prior to study entry, and had regional lymph node metastases (stage III colon cancer) but no evidence of distant metastases. Moreover, patients were required to have a baseline Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2 (ambulatory) and have adequate bone marrow, renal and hepatic function. Data on family history of colorectal cancer in first-degree relatives were obtained by questionnaire at diagnosis (26). The current analysis was limited to 506 patients for whom archived formalin-fixed paraffin-embedded tumor tissue and *BRAF* sequencing data were available. All patients signed informed consent, approved by each site’s Institutional Review Board.

We compared baseline characteristics of the patients who were included in this study (with available *BRAF* data, *N* = 506) with those who were excluded from this study due to unavailability of tissue data (*N* = 758). We did not detect any significant or substantial difference between these 2 groups in terms of age, sex, body mass index (BMI), family history, tumor location, pT stage, pN stage, performance status, bowel perforation, bowel obstruction, or treatment arm (all *P > 0.08*). In addition, recurrence-free and disease-free survival did not significantly differ in subjects with available *BRAF* data as compared with those without *BRAF* data (multivariate HR = 0.96; 95% CI: 0.79–1.18; and multivariate HR = 0.95; 95% CI: 0.78–1.15, respectively).

As part of the quality assurance program of the CALGB, members of the Audit Committee visit all participating institutions at least once every 3 years to review source documents. The auditors verify compliance with federal regulations and protocol requirements, including those pertaining to eligibility, treatment, adverse events, tumor response, and outcome in a sample of protocols at each institution. Such on-site review of medical records was carried out for a subgroup of 328 patients (26%) of the 1,264 patients included in this study.

Definitions of study endpoints

The study endpoints were; (i) recurrence-free survival (RFS), defined as the time from the study enrollment to tumor recurrence or occurrence of a new primary colon tumor; (ii) disease-free survival (DFS), defined as time from the study enrollment to tumor recurrence, occurrence of a new primary colon tumor, or death from any cause; and (iii) overall survival (OS), defined as the time from the study enrollment to death from any cause. For RFS, patients who died without known tumor recurrence were censored at last documented evaluation by a treating provider.

DNA extraction from tumor, *BRAF* and *KRAS* sequencing, and MSI, MLH1, and MSH2 analyses

Tumor molecular analyses were carried out blinded to patient and outcome data. DNA was extracted from paraffin-embedded colon cancer tissue (27). We marked tumor areas on H&E slide, and dissected tumor tissue by a sterile needle. PCR and Pyrosequencing spanning *BRAF* codon 600 (28), and *KRAS* codons 12 and 13 were carried out as previously described (27) in the laboratory at the Dana-Farber Cancer Institute. Our previous study (27) has shown that Pyrosequencing assay is more sensitive than Sanger sequencing (29), and can detect approximately 5% to 10%
of mutant allele among a mixture of mutant and normal alleles. MSI was assessed by PCR for 10 markers, and MLH1 and MSH2 expression was examined by immunohistochemistry (IHC) as previously described (23). Tumors with instability in ≥50% of the loci were classified as MSI-high, and those with instability in 0% to 40% of the loci as microsatellite stable (MSS), and the concordance between MSI testing and IHC for MLH1 or MSH2 loss was 97% (23). For 28 cases without PCR MSI results, those with loss of MLH1 or MSH2 were classified as MSI-high, and those with intact expression of MLH1 and MSH2 as MSS. All tumor tissue analyses were carried out completely blinded to patient identity, clinical, and outcome data.

Statistical analyses
The goal of this correlative study was to determine whether tumor BRAF mutation status was associated with clinical outcome for patients with stage III colon cancer. Patient registration and clinical data collection were managed by the CALGB Statistical Center, and analyses were conducted collaboratively between the CALGB Statistical Center and Dana-Farber Cancer Institute. All analyses were based on the study database frozen on November 9, 2009, except for the tumor BRAF data. All analyses used SAS version 9.2 (SAS Institute) and all P values were 2-sided.

The Kaplan–Meier method was used to estimate the distribution of survival time according to BRAF status, and the log-rank test was used to compare survival between subgroups. We used the multivariable Cox proportional hazards model to estimate survival HR by tumor BRAF status. The following variables were considered in the multivariable analysis: age at study entry (continuous), sex, baseline BMI (≥30 vs. <30 kg/m²), family history of colorectal cancer in first-degree relatives (present vs. absent), baseline perforation status (0 vs. 1–2), presence of bowel perforation or obstruction at time of surgery, treatment arm, tumor location (proximal vs. distal), pT stage (pT1-2 vs. pT3 vs. pT4 vs. unknown), pN stage (pN1 vs. pN2), KRAS (wild-type vs. codon 12 mutation vs. codon 13 mutation), and MSI status (high vs. MSS). A backward stepwise elimination with a threshold of P = 0.20 was conducted to select covariates in the final model. pT stage was used as a stratifying variable using the strata option in the SAS "proc phreg" command. No collinearity was evident among the variables studied. Although KRAS and BRAF mutations were almost mutually exclusive (Table 1) and KRAS mutation overall did not influence outcome in this dataset (30), we included KRAS codon 12 and 13 mutations separately in the model, to examine codon-specific effects of KRAS mutation. The proportionality of hazards assumption was assessed using standard survival plots and by evaluating a time-dependent variable, which was the cross-product of BRAF and survival time (P = 0.011 for RFS; P = 0.22 for DFS; P = 0.26 for OS). Data were missing on family history in 1% of patients, tumor location in 1% of patients, pN stage in 0.6% of patients, perforation status in 1.8% of patients, obstruction status in 0.6% of patients, and MSI status in 0.2% of patients; those were included in a majority category in multivariable Cox models to maximize the efficiency of multivariable analyses. To assess the potential differential effect of treatment arm according to BRAF status (or combined BRAF and MSI status), we carried out a single multivariate Cox regression analysis, in which we could estimate the effect of treatment arm simultaneously in 2 strata of BRAF status (or in 4 strata of combined BRAF and MSI status) using a reparameterization of the interaction term(s) (3). Interaction was also assessed by including the cross-product of BRAF and another variable of interest (without data-missing cases) in a multivariate model, using the Wald test.

Results

BRAF mutation in stage III colon cancer
Study participants were drawn from a multicenter study of postoperative adjuvant chemotherapy in stage III colon cancer patients who underwent a curative-intent surgical resection (CALGB 89803 protocol; ref. 26). We included 506 cases in the current study based on availability of tumor tissue for BRAF sequencing, which detected c.1799T>A (p.V600E) mutation in 75 (15%) patients. This BRAF mutation frequency is comparable with data in the previous large population-based studies in the United States (1, 16). Table 1 summarizes baseline characteristics according to BRAF mutation status. BRAF mutation was significantly associated with female sex, older age, proximal tumor location, MSI-high, and wild-type KRAS (all P < 0.0045; a P value for significance was adjusted to P = 0.0045 by Bonferroni correction).

Prognostic role of BRAF mutation
With median follow-up of 7.6 years among survivors, there were 183 events for RFS analysis, 202 events for DFS analysis, and 160 events for OS analysis. In a Kaplan–Meier analysis (Fig. 1), BRAF-mutated cases experienced a non-significant trend toward inferior RFS and DFS. For BRAF-mutated versus wild-type cases, 5-year RFS was 60% versus 65%, and 5-year DFS was 55% versus 64%, respectively. BRAF mutation was associated a statistically significant reduction in OS (5-year OS: 63% in BRAF-mutant vs. 75% in BRAF wild-type; log-rank P = 0.015).

In multivariate Cox regression analysis, we examined the prognostic association of BRAF mutation adjusting for other predictors of patient survival (Table 2). Compared with BRAF wild-type cases, BRAF-mutated cases experienced a significantly worse OS (multivariable HR = 1.66; 95% CI: 1.05–2.63), adjusting for other factors including MSI and KRAS mutational status. For RFS and DFS analyses, trends were similar in direction, but not statistically significant.

We also examined the associations of MSI and KRAS mutation with patient outcome. Although MSI-high tumors were independently associated with an improved OS (multivariable HR = 0.61; 95% CI: 0.38–0.97), adjusting for other factors including BRAF and KRAS mutational status, KRAS
## Table 1. Baseline characteristics according to BRAF mutational status in stage III colon cancer

<table>
<thead>
<tr>
<th>Clinical or molecular feature</th>
<th>No. of cases</th>
<th>BRAF</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Wild type</td>
<td>Mutant (c.1799T&gt;A, p.V600E)</td>
</tr>
<tr>
<td>Total N</td>
<td>506</td>
<td>431</td>
<td>75</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>274 (54%)</td>
<td>245 (57%)</td>
<td>29 (39%)</td>
</tr>
<tr>
<td>Female</td>
<td>232 (46%)</td>
<td>186 (43%)</td>
<td>46 (61%)</td>
</tr>
<tr>
<td>Age (y)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;50</td>
<td>99 (20%)</td>
<td>97 (23%)</td>
<td>2 (2.7%)</td>
</tr>
<tr>
<td>50–59</td>
<td>131 (26%)</td>
<td>121 (28%)</td>
<td>10 (13%)</td>
</tr>
<tr>
<td>60–69</td>
<td>156 (31%)</td>
<td>125 (29%)</td>
<td>31 (41%)</td>
</tr>
<tr>
<td>≥70</td>
<td>120 (24%)</td>
<td>88 (20%)</td>
<td>32 (43%)</td>
</tr>
<tr>
<td>Mean age ± SD</td>
<td>59.7 ± 11.5</td>
<td>58.6 ± 11.7</td>
<td>66.5 ± 8.0</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>164 (32%)</td>
<td>137 (32%)</td>
<td>27 (36%)</td>
</tr>
<tr>
<td>25–29</td>
<td>185 (37%)</td>
<td>159 (37%)</td>
<td>26 (35%)</td>
</tr>
<tr>
<td>≥30</td>
<td>157 (31%)</td>
<td>135 (31%)</td>
<td>22 (29%)</td>
</tr>
<tr>
<td>Family history of colorectal cancer in any first-degree relative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(−)</td>
<td>419 (84%)</td>
<td>361 (85%)</td>
<td>58 (77%)</td>
</tr>
<tr>
<td>(+)</td>
<td>82 (16%)</td>
<td>65 (15%)</td>
<td>17 (23%)</td>
</tr>
<tr>
<td>Tumor location</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal (cecum to transverse colon)</td>
<td>287 (57%)</td>
<td>219 (51%)</td>
<td>68 (92%)</td>
</tr>
<tr>
<td>Distal (splenic flexure to sigmoid)</td>
<td>214 (43%)</td>
<td>208 (49%)</td>
<td>6 (8.1%)</td>
</tr>
<tr>
<td>pT stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pT1–pT2</td>
<td>58 (12%)</td>
<td>50 (12%)</td>
<td>8 (11%)</td>
</tr>
<tr>
<td>pT3</td>
<td>409 (82%)</td>
<td>351 (82%)</td>
<td>58 (78%)</td>
</tr>
<tr>
<td>pT4</td>
<td>33 (6.6%)</td>
<td>25 (5.9%)</td>
<td>8 (11%)</td>
</tr>
<tr>
<td>pN stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pN1</td>
<td>318 (63%)</td>
<td>277 (65%)</td>
<td>41 (55%)</td>
</tr>
<tr>
<td>pN2</td>
<td>185 (37%)</td>
<td>152 (35%)</td>
<td>33 (45%)</td>
</tr>
<tr>
<td>AJCC tumor stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IIIA</td>
<td>48 (9%)</td>
<td>42 (10%)</td>
<td>6 (8%)</td>
</tr>
<tr>
<td>IIIB</td>
<td>268 (53%)</td>
<td>233 (54%)</td>
<td>35 (47%)</td>
</tr>
<tr>
<td>IIIC</td>
<td>185 (36%)</td>
<td>152 (35%)</td>
<td>33 (44%)</td>
</tr>
<tr>
<td>III, unknown substage</td>
<td>5 (1%)</td>
<td>4 (1%)</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Performance status score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>387 (76%)</td>
<td>335 (78%)</td>
<td>52 (69%)</td>
</tr>
<tr>
<td>1–2</td>
<td>119 (24%)</td>
<td>96 (22%)</td>
<td>23 (31%)</td>
</tr>
<tr>
<td>Clinical bowel perforation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(−)</td>
<td>475 (96%)</td>
<td>407 (96%)</td>
<td>68 (92%)</td>
</tr>
<tr>
<td>(+)</td>
<td>22 (4%)</td>
<td>16 (4%)</td>
<td>6 (8%)</td>
</tr>
<tr>
<td>Clinical bowel obstruction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(−)</td>
<td>390 (78%)</td>
<td>333 (78%)</td>
<td>57 (77%)</td>
</tr>
<tr>
<td>(+)</td>
<td>113 (22%)</td>
<td>96 (22%)</td>
<td>17 (23%)</td>
</tr>
<tr>
<td>MSI status*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSS</td>
<td>428 (85%)</td>
<td>387 (90%)</td>
<td>41 (55%)</td>
</tr>
<tr>
<td>MSI-high</td>
<td>77 (15%)</td>
<td>43 (10%)</td>
<td>34 (45%)</td>
</tr>
<tr>
<td>KRAS mutation status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td>330 (65%)</td>
<td>256 (69%)</td>
<td>74 (99%)</td>
</tr>
<tr>
<td>Mutant</td>
<td>176 (35%)</td>
<td>175 (41%)</td>
<td>1 (1.3%)</td>
</tr>
</tbody>
</table>

(Continued on the following page)
Table 1. Baseline characteristics according to BRAF mutational status in stage III colon cancer (Cont’d)

<table>
<thead>
<tr>
<th>Clinical or molecular feature</th>
<th>No. of cases</th>
<th>BRAF</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Wild type</td>
<td>Mutant (c.1799T&gt;A, p.V600E)</td>
</tr>
<tr>
<td>Treatment arm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FU/LV</td>
<td>267 (53%)</td>
<td>234 (54%)</td>
<td>33 (44%)</td>
</tr>
<tr>
<td>IFL</td>
<td>239 (47%)</td>
<td>197 (46%)</td>
<td>42 (56%)</td>
</tr>
</tbody>
</table>

NOTE: (%) indicates the proportion of tumors with a specific clinical or molecular feature in BRAF wild-type tumors (or BRAF-mutated tumors). There were cases with missing value/status for some of the variables. ^For 28 cases without MSI results by PCR, those with loss of MLH1 or MSH2 were classified as MSI-high, and those with intact expression of MLH1 and MSH2 as MSS, because concordance between MSI PCR and IHC for MLH1 and MSH2 was very high (97%) among cases with both results available (23). Abbreviation: AJCC, American Joint Committee on Cancer.

Figure 1. BRAF mutation and clinical outcome in colon cancer. A–C, Kaplan–Meier curves according to BRAF mutation in 506 stage III colon cancers for RFS (A), DFS (B), and OS (C). The y axis indicates the survival probability. D–F, Kaplan–Meier curves for RFS (D), DFS (E), and OS (F) according to treatment arm and BRAF mutation status. G, proposed strategy for prognostication of colon cancer by MSI and BRAF tests. DFS, disease-free survival; FU/LV, 5-fluorouracil and leucovorin; IFL, irinotecan, 5-fluorouracil, and leucovorin; MSI, microsatellite instability; MSS, microsatellite stable; Mut, mutant; OS, overall survival; RFS, recurrence-free survival; WT, wild-type.
mutations in either codon 12 or codon 13 were not associated with patient outcome.

**Combined BRAF and MSI status and prognosis**

We further categorized patients according to both BRAF and MSI status to assess the joint effect on patient outcome (Table 3). Compared with patients whose tumors were both BRAF wild-type and MSS, those with BRAF-mutated and MSS tumors experienced a trend toward an inferior OS (multivariate HR = 1.61; 95% CI: 0.96–2.63). In contrast, compared with BRAF wild-type MSS patients, those with BRAF wild-type MSI-high tumors showed consistent trends toward superior RFS, DFS, and OS. Finally, patients with BRAF-mutated MSI-high cancers experienced no significant difference in outcome when compared with BRAF wild-type MSS patients (multivariate HR = 1.02; 95% CI: 0.54–1.93), suggesting opposing prognostic effects of BRAF mutation and MSI high.

**Predictive role of BRAF mutation for irinotecan-based therapy**

We assessed the prognostic role of BRAF mutation within each treatment arm and the effect of treatment according to BRAF status. Among patients treated with FU/LV, the presence of BRAF mutation was associated with a significantly reduced DFS and OS (multivariate OS HR = 2.43; 95% CI: 1.34–4.40) when compared with BRAF wild-type tumors (Table 4). In contrast, among subjects treated with IFL, BRAF mutation was not significantly associated with patient outcome (multivariate OS HR = 1.24; 95% CI: 0.67–2.31; vs. BRAF wild type).

Among patients with BRAF-mutated tumors, we observed a nonsignificant trend toward improved RFS, DFS, and OS for subjects treated with IFL when compared with FU/LV (Table 4); however, statistical power was limited and results should be interpreted with caution. Among patients with BRAF wild-type cancer, IFL was associated with no benefit when compared with FU/LV alone.

In a Kaplan–Meier analysis by treatment arm and BRAF status (Fig. 1), BRAF-mutated cases treated with FU/LV experienced a significantly worse OS compared with BRAF-mutated cases treated with IFL or to BRAF wild-type cases in either treatment arm (log-rank $P = 0.030$).

**Predictive role of combined BRAF and MSI subtyping for irinotecan-based therapy**

We examined the predictive role of combined BRAF and MSI status on adjuvant treatment efficacy (Table 5). Among
subjects with either $BRAF$ wild-type MSS tumors or $BRAF$-mutated MSI-high tumors, IFL was not associated with any improvement in patient outcome. Although statistical power was limited, among patients with either $BRAF$ wild-type MSI-high tumors or $BRAF$-mutated MSS tumors, IFL seemed to confer a consistent trend toward improved RFS, DFS, and OS when compared with FU/LV-treated subjects. In contrast, there seemed to be no appreciable benefit of IFL (compared with FU/LV) among $BRAF$-mutated MSI-high or $BRAF$ wild-type MSS patients.

We also carried out analyses for response to IFL (vs. FU/LV) according to MSI status (Supplementary Table S1, Supplementary Fig. S2) in the current dataset. There might be a possible beneficial effect of IFL in MSI-high patients, similar to the previous analysis in the CALGB 89803 trial (23).

### Table 4. Stage III colon cancer and clinical outcome according to treatment arm and $BRAF$ mutation status

<table>
<thead>
<tr>
<th>No.</th>
<th>RFS</th>
<th>DFS</th>
<th>OS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Five-year survival probability</td>
<td>Multivariate HR (95% CI)</td>
<td>Five-year survival probability</td>
</tr>
<tr>
<td>FU/LV</td>
<td>BRAF wild type</td>
<td>234</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>BRAF mutant</td>
<td>33</td>
<td>0.54</td>
</tr>
<tr>
<td>IFL</td>
<td>BRAF wild type</td>
<td>197</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td>BRAF mutant</td>
<td>42</td>
<td>0.64</td>
</tr>
<tr>
<td>BRAF wild type</td>
<td>FU/LV</td>
<td>234</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>IFL</td>
<td>197</td>
<td>0.63</td>
</tr>
<tr>
<td>BRAF mutant</td>
<td>FU/LV</td>
<td>33</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>IFL</td>
<td>42</td>
<td>0.64</td>
</tr>
</tbody>
</table>

NOTE: The multivariate Cox regression model included the same set of covariates which were selected in the final models of the analyses in Table 2.

### Table 5. Effect of treatment arm on stage III colon cancer outcome, according to combined $BRAF$ and MSI status

<table>
<thead>
<tr>
<th>No.</th>
<th>RFS</th>
<th>DFS</th>
<th>OS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Five-year survival probability</td>
<td>Multivariate HR (95% CI)</td>
<td>Five-year survival probability</td>
</tr>
<tr>
<td>BRAF wild-type MSS</td>
<td>FU/LV</td>
<td>212</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>IFL</td>
<td>175</td>
<td>0.60</td>
</tr>
<tr>
<td>BRAF wild-type MSI-high</td>
<td>FU/LV</td>
<td>22</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>IFL</td>
<td>21</td>
<td>0.91</td>
</tr>
<tr>
<td>BRAF-mutant MSS</td>
<td>FU/LV</td>
<td>16</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>IFL</td>
<td>25</td>
<td>0.59</td>
</tr>
<tr>
<td>BRAF-mutant MSI-high</td>
<td>FU/LV</td>
<td>17</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>IFL</td>
<td>17</td>
<td>0.71</td>
</tr>
</tbody>
</table>

NOTE: The multivariate Cox regression model included the same set of covariates which were selected in the final models of the analyses in Table 2.
Finally, we examined treatment effects according to status of BRAF mutation and MLH1 and MSH2 by IHC with available IHC data. There were 4 cases with MSH2 loss, and all those 4 cases were BRAF wild-type and likely Lynch syndrome cases. There were 37 cases of MLH1 loss. Among those 37 cases, 17 cases were BRAF wild-type and included Lynch syndrome cases. Among the 4 cases with MSH2 loss, 2 cases received IFL with no RFS, DFS, or OS event (follow-time, 8.1 and 8.5 years). Among the other 2 cases with MSH2 loss in the FU/LV arm, 1 case experienced a RFS/DFS/OS event at 3.5 years, and the other case was censored at 6.7 years. We analyzed the effects of IFL (vs. FU/LV) in the 17 cases with MLH1 loss and wild-type BRAF, and multivariate HR (with 95% CI) for IFL treatment (vs. FU/LV) was 0.11 (0.011–1.07) for DFS; 0.11 (0.011–1.08) for OS. These data were suggestive of good response of Lynch syndrome cases to IFL (vs. FU/LV), although statistical power was limited.

Discussion

In this study of patients with stage III colon cancer participating in the randomized trial comparing postoperative IFL to FU/LV, somatic mutations in BRAF were associated with a statistically significant reduction in OS, with a nonsignificant trend toward an inferior RFS and DFS. These results persisted in multivariate analyses that adjusted for other predictors for patient outcome, supporting BRAF mutation as an independent prognostic marker in colon cancer. Furthermore, combined BRAF and MSI subtyping analysis suggests that BRAF-mutated MSS tumors is an unfavorable subtype, whereas BRAF wild-type MSI-high tumor is a favorable subtype, and BRAF-mutated MSI-high and BRAF wild-type MSS tumors are intermediate subtypes (Fig. 1G). The independent, opposing prognostic effects of BRAF mutation and MSI observed in the current study is also consistent with several previous studies (6, 16–20, 22).

Interestingly, the prognostic association of BRAF mutation seemed to be somewhat attenuated among patients treated with IFL, whereas BRAF mutation was associated with a significant increase in mortality among subjects treated with FU/LV. Among patients with BRAF-mutated colon cancer, IFL might be associated with a nonsignificant trend toward improved RFS, DFS, and OS compared with FU/LV, whereas there was no apparent benefit by IFL among BRAF wild-type cases. However, statistical power was quite limited and caution must be taken to interpret the results. Additional studies are needed to examine the predictive role of BRAF mutation in colon cancer.

Although a number of studies (31–34) have assessed potential predictive roles of various genetic or tumor biomarkers for irinotecan therapy [e.g., APTX expression (31), ABCB1 polymorphism (32), EGFR and ERCC1 mRNA expression (33)], none of these markers has yet been proven to be clinically useful. A previous analysis of patients in this clinical trial suggested that MSI-high might predict an improved patient outcome for treatment with IFL relative to FU/LV (23), although this finding was not observed in a concurrent trial conducted in Europe (35). Possibly, mismatch repair deficiency may cause DNA repair gene muta-

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In addition, detailed information on other prognostic variables was routinely collected at study entry. We recognize that patients who enroll in randomized trials may differ from the population-at-large. To participate, patients must meet eligibility criteria, be selected as an appropriate candidate, and be motivated to participate. In addition, patients were particularly selected for this study on the basis of availability of colon cancer tissue specimens. Nonetheless, demographic data of the patients in this study did not suggest considerable selection bias. Moreover, because the study included patients from both community and academic centers across North America, our findings should reflect the general population of stage III patients in North America. In addition, although data on BRAF mutational status were available on a subset of patients enrolled in the trial, baseline characteristics and patient survival did not substantially differ for patients with and without available archived tumor tissue in this trial. Finally, because BRAF status was not available on all patients, statistical power was attenuated. As such, confirmation of our findings is clearly needed.

In conclusion, we found that BRAF mutation was associated with an inferior prognosis in stage III colon cancer patients, supporting tumor BRAF mutation as an independent prognostic biomarker in colon cancer. Although BRAF mutation in stage III colon cancer may possibly predict improved response to irinotecan-based chemotherapy, the predictive role of BRAF mutation testing remains uncertain at this time, and additional trial studies are needed.

Disclosure of Potential Conflicts of Interest

I.B. Saltz is a consultant to Genomic Health, Genzyme, Asuragen. R. Whittom received honorarium from speakers’ bureau, Hoffmann-La Roche; and is a consultant to Eli-Lilly, Amgen, Novartis, Pfizer, Boehringer Ingelheim. A. Hanel is a member of Foundation Medicine Advisory Board. A.B. Benson received research funding from Pfizer, Imclone, Bristol-Myer Squibb, Amgen, Sanofi-Aventis. A. Hanel received research funding from AbbVie, Amgen, Bayer, Pfizer, Genentech, Myriad, Sanofi-Aventis. R.M. Goldberg received research funding from AbbVie, Amgen, Sanofi-Aventis. A. Hanel received research funding from AbbVie, Amgen, Sanofi-Aventis. C.S. Fuchs is a consultant to Sanofi-Aventis, Pfizer, Genentech, Roche, Bristol-Myer Squibb, Amgen. The sponsors did not participate in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Cancer Institute.

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BRAF Mutation in Colon Cancer

References


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