Original Investigation

Prognostic Effect of *BRAF* and *KRAS* Mutations in Patients With Stage III Colon Cancer Treated With Leucovorin, Fluorouracil, and Oxaliplatin With or Without Cetuximab
A Post Hoc Analysis of the PETACC-8 Trial

Julien Taieb, MD, PhD; Aziz Zaanan, MD, PhD; Karine Le Malicot, MS; Catherine Julié, MD; Hélène Blons, MD, PhD; Laurent Mineur, MD; Jaafar Bennouna, MD, PhD; Josep Tabernero, MD, PhD; Enrico Mini, MD, PhD; Gunnar Folprecht, MD, PhD; Jean Luc Van Laethem, MD, PhD; Come Lepage, MD, PhD; Jean-François Emile, MD, PhD; Pierre Laurent-Puig, MD, PhD

IMPORTANCE The prognostic value of *BRAF* and *KRAS* mutations in patients who have undergone resection for colon cancer and have been treated with combination leucovorin, fluorouracil, and oxaliplatin (FOLFOX)-based adjuvant chemotherapy is controversial, possibly owing to a lack of stratification on mismatch repair status.

OBJECTIVE To examine the prognostic effect of *BRAF* and *KRAS* mutations in patients with stage III colon cancer treated with adjuvant FOLFOX with or without cetuximab.

DESIGN, SETTING, AND PARTICIPANTS This study included patients with available tumor blocks of resected stage III colon adenocarcinoma who participated between December 2005 and November 2009 in the PETACC-8 phase III randomized trial. Mismatch repair, *BRAF* V600E, and *KRAS* exon 2 mutational status were determined on prospectively collected tumor blocks from 2559 patients enrolled in the PETACC-8 trial. The data were analyzed in April 2015.

INTERVENTION Patients were randomly assigned to receive 6 months of FOLFOX4 or FOLFOX4 plus cetuximab after surgical resection for stage III colon cancer.

MAIN OUTCOMES AND MEASURES Associations between these biomarkers and disease-free survival (DFS) and overall survival (OS) were analyzed with Cox proportional hazards models. Multivariate models were adjusted for covariates (age, sex, tumor grade, T/N stage, tumor location, Eastern Cooperative Oncology Group performance status).

RESULTS Among the 2559 patients enrolled in the PETACC-8 trial (42.9% female; median [range] age, 60.0 [19.0-75.0] years), microsatellite instability (MSI) phenotype, *KRAS,* and *BRAF* V600E mutations were detected in, respectively, 9.9% (177 of 1791), 33.1% (588 of 1776), and 9.0% (148 of 1643) of cases. In multivariate analysis, MSI (hazard ratio [HR] for DFS: 1.10 [95% CI, 0.73-1.64], $P = .67$; HR for OS: 1.02 [95% CI, 0.61-1.69], $P = .94$) and *BRAF* V600E mutation (HR for DFS: 1.22 [95% CI, 0.81-1.85], $P = .34$; HR for OS: 1.13 [95% CI, 0.64-2.00], $P = .66$) were not prognostic, whereas *KRAS* mutation was significantly associated with shorter DFS (HR, 1.55 [95% CI, 1.23-1.95]; $P < .001$) and OS (HR, 1.56 [95% CI, 1.12-2.15]; $P = .008$). The subgroup analysis showed in patients with microsatellite-stable tumors that both *KRAS* (HR for DFS: 1.64 [95% CI, 1.29-2.08], $P < .001$; HR for OS: 1.71 [95% CI, 1.21-2.41], $P = .002$) and *BRAF* V600E mutation (HR for DFS: 1.74 [95% CI, 1.14-2.69], $P = .01$; HR for OS: 1.84 [95% CI, 1.01-3.36], $P = .046$) were independently associated with worse clinical outcomes. In patients with MSI tumors, *KRAS* status was not prognostic, whereas *BRAF* V600E mutation was associated with significantly longer DFS (HR, 0.23 [95% CI, 0.06-0.92]; $P = .04$) but not OS (HR, 0.19 [95% CI, 0.03-1.24]; $P = .08$).

CONCLUSIONS AND RELEVANCE *BRAF* V600E and *KRAS* mutations were significantly associated with shorter DFS and OS in patients with microsatellite-stable tumors but not in patients with MSI tumors. Future trials in the adjuvant setting will have to take into account mismatch repair, *BRAF,* and *KRAS* status for stratification.

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Author Affiliations: Author affiliations are listed at the end of this article.

Corresponding Author: Julien Taieb, MD, PhD, Department of Gastroenterology and Digestive Oncology, European Georges Pompidou Hospital, and Paris Descartes University, 20 rue Leblanc, 75015 Paris, France (julien.taieb@egp.aphp.fr).

Colorectal cancer (CRC) is the third most common cancer worldwide, causing more than 600 000 deaths each year.[1] Disease stage remains the strongest prognostic variable and is the key determinant of treatment. The majority of newly diagnosed cases of CRC are in patients with nonmetastatic disease that can potentially be cured by surgery, either alone or combined with adjuvant chemotherapy. However, there is considerable stage-independent prognostic variability, likely due to tumor characteristics. Colorectal cancer is a biologically heterogeneous disease that develops via 2 well-described pathways of colorectal carcinogenesis, including chromosomal instability and, less commonly, microsatellite instability (MSI), which occurs in approximately 15% of cases. Microsatellite instability is a consequence of deficient DNA mismatch repair (MMR) that results in the accumulation of insertion and/or deletion mutations within microsatellite DNA regions.[2] Deficient MMR can result from inheritance of a germline mutation in an MMR gene (*MLH1, MSH2, MSH6, PMS2*), causing Lynch syndrome,[3] or more commonly, from epigenetic inactivation of *MLH1,*[4] which is associated with hypermethylation of the promoter regions of cancer-specific genes, a situation known as the CpG island methylator phenotype (CIMP).[5] Sporadic MSI CRC tumors are enriched with the *BRAF*-activating somatic V600E mutation (*BRAF* V600E), which is absent from MSI tumors associated with Lynch syndrome.[6] The *BRAF* V600E mutation has an overall frequency of approximately 10% in all CRCs and is mutually exclusive of *KRAS* mutations, which are detected in 40% to 45% of cases.[7,8]

Although *KRAS* mutations are predictive of resistance to epidermal growth factor receptor inhibitors in metastatic CRC,[9-11] and although *BRAF* V600E is now recognized as a marker of poor prognosis in this setting,[8,12] the prognostic effect of these mutations in nonmetastatic CRC is controversial. *KRAS* mutations have been linked to disease recurrence and poorer overall survival in some studies but not in others, and there is some evidence that its role depends on the tumor site.[7,13-16] Consistent data indicate that *BRAF* V600E mutation is associated with poor outcomes after relapse,[14,17] but its direct effect on recurrence for patients in the adjuvant setting requires clarification.[7,14,18,19] Most studies have shown an association of MSI phenotype with a better survival in earlier tumor stage, whereas the effect in stage III tumors is more controversial, and data in patients treated by the current combination leucovorin, fluorouracil, and oxaliplatin (FOLFOX) standard are scarce.[19-21]

Prognostic evaluation of these biomarkers is hampered by the fact that published data often mix prospective and retrospective studies, colon and rectal cancer, stage I to III tumors, and patients who did or did not receive a variety of adjuvant regimens; also, tumors are often not uniformly analyzed for all these biological molecular markers together (MSI, *KRAS, BRAF*). In fact, the frequency of *KRAS* and *BRAF* V600E mutations differs according to MMR status, and this may have impaired our interpretation of the effect of these mutations on clinical outcomes. We therefore examined disease-free survival (DFS) and overall survival (OS) according to MMR, *KRAS,* and *BRAF* status, determined on stage III colon cancer specimens collected prospectively from patients who received adjuvant FOLFOX alone or combined with cetuximab in the PETACC-8 randomized clinical trial. We also examined the prognostic value of *KRAS* and *BRAF* V600E mutations according to MMR status.

Key Points

Question What is the prognostic value of *BRAF* and *KRAS* mutations in patients who have undergone resection for colon cancer and have been treated with combination leucovorin, fluorouracil, and oxaliplatin (FOLFOX)-based adjuvant chemotherapy?

Findings This post hoc analysis of patients with stage III colon cancer who participated in the PETACC-8 phase III randomized clinical trial found that in patients with microsatellite-stable tumors, both *KRAS* and *BRAF* V600E mutations were independently linked to shorter disease-free and overall survival. In patients with microsatellite-unstable tumors, *KRAS* status was not prognostic, whereas *BRAF* V600E mutation was associated with significantly longer disease-free but not overall survival.

Meaning Microsatellite, *KRAS,* and *BRAF* V600E status assessment should be mandatory to stratify adequately in future adjuvant trials and must be discussed in our daily practice.

Methods

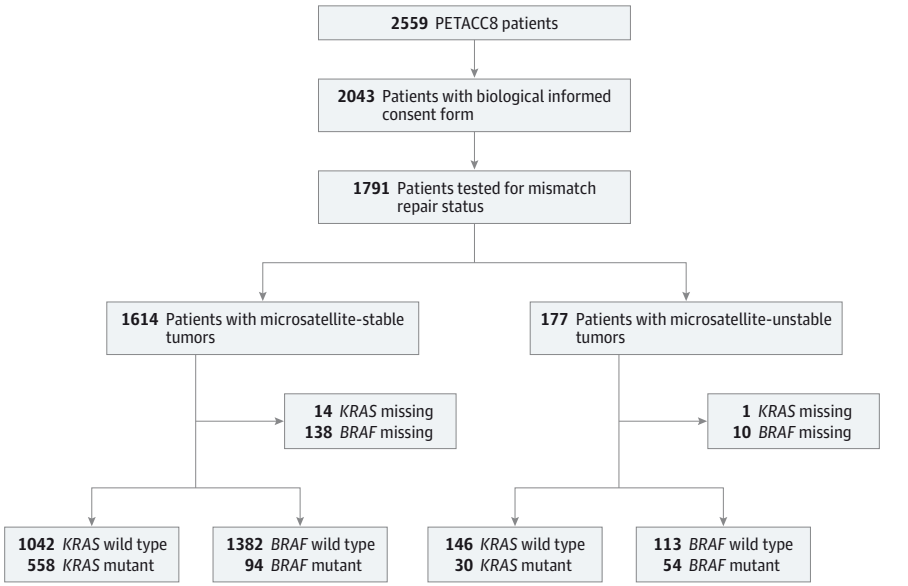
Study Population

This study included all patients with biological informed consent signed and available tumor blocks of resected stage III (any T, N1 or N2, M0) colon adenocarcinoma who have participated in the PETACC-8 phase III randomized trial.[22] Patients in this trial were randomized to receive 6 months of FOLFOX4: 85 mg/m^2 oxaliplatin (2-hour infusion) on day 1, and leucovorin 200 mg/m^2 followed by fluorouracil (bolus) 400 mg/m^2 and a 22-hour continuous infusion of fluorouracil 600 mg/m^2 for 2 consecutive days, or FOLFOX4 plus cetuximab. The PETACC-8 study included 2559 patients between December 2005 and November 2009 and was amended in June 2008 to restrict random assignment to patients whose tumors expressed wild-type *KRAS*. Among the 2096 patients randomized before amendment, 1881 (90%) were retrospectively screened for *KRAS* mutations. The PETACC-8 trial, which received ethical approval for the study protocol and the translational data research integration, demonstrates that the addition of cetuximab to FOLFOX4 did not improve DFS compared with FOLFOX4 alone in patients with *KRAS* exon 2 wild-type resected stage III colon cancer.

MMR Status Determination

Mismatch repair tumor status was determined by immunohistochemical analysis (IHC), or by MSI testing when IHC was indeterminate. Microsatellite instability phenotype tumors were defined as exhibiting the loss of expression of 1 or more MMR proteins by IHC or exhibiting high-level tumor DNA MSI on MSI testing. Microsatellite-stable (MSS) phenotype tumors were defined by normal MMR protein expression in IHC, or MSS or low-level MSI status on MSI testing.

Figure 1. Flowchart of PETACC-8 Trial Molecular Study Evaluating the Prognostic Impact of Mismatch Repair, *KRAS,* and *BRAF* Status



Immunohistochemical Analysis

Mismatch repair protein (MLH1, MSH2, MSH6, PMS2) expression was analyzed by IHC on tissue microarrays. Primary monoclonal antibodies against MLH1 (clone G168-728, diluted 1:100; BD Pharmingen), MSH2 (clone FE11, diluted 1:100; Oncogene Research Products), MSH6 (clone 44, diluted 1:100; BD Pharmingen), and PMS2 (clone A16-4, diluted 1:100; BD Pharmingen) were applied. Mismatch repair protein loss was defined as the absence of nuclear staining in neoplastic cells but positive nuclear staining in lymphocytes and normal adjacent colonic epithelium.[23]

MSI Testing

DNA was extracted from formalin-fixed paraffin-embedded (FFPE) tumor tissues for MSI analysis using 5 monomorphic mononucleotide markers (BAT-25, BAT-26, NR-21, NR-24, NR-27).[24] Specimens with at least 3 unstable markers were scored as highly unstable, and specimens with fewer than 3 unstable markers were scored as stable.

BRAF and *KRAS* Gene Mutations

BRAF and *KRAS* mutation status was determined on genomic DNA extracted from FFPE tissues, using the QIAamp DNA Mini Kit (Qiagen). Molecular analysis was centralized and carried out retrospectively for patients included before trial amendment and prospectively for all other patients. Testing for 7 *KRAS* exon 2 mutations and the *BRAF* V600E hotspot exon 15 mutation (c.1799T>A/p.V600E) was based on real-time polymerase chain reaction using TaqMan probes (Applied Biosystems). Each assay was validated to detect 10% of mutated alleles.[25]

Statistical Analysis

Biomarker status was analyzed by investigators blinded to patient outcomes, and then transmitted for survival analyses to the data center. Disease-free survival was defined as the time between randomization and local or metastatic recurrence, diagnosis of a second colon or rectal cancer, or death, whichever occurred first. Overall survival was measured from randomization until death from any cause. For comparisons of baseline characteristics, categorical outcomes were analyzed with χ^2 tests, and continuous outcomes, with standard parametric or nonparametric tests. Continuous variables are presented as the mean (SD) and median with interquartile range.

Disease-free and overall survival curves were estimated with the Kaplan-Meier method. Differences between groups of patients were analyzed with unstratified log-rank tests. Patients in the 2 treatment arms were combined because no difference was found between the 2 arms for efficacy analyses. An unstratified Cox regression model was used to estimate hazard ratios (HRs), 95% confidence intervals, and *P* values for candidate prognostic factors. Multivariate analyses were adjusted for stratification factors (nodal category, T stage, and obstruction or perforation status), sex, age, Eastern Cooperative Oncology Group performance status, tumor grade, primary tumor location, vascular invasion or lymphatic infiltration (VELI), and MMR, *KRAS,* and *BRAF* status.

Analyses were carried out with a 2-sided significance level of 5%. Results were unadjusted for multiple comparisons. All statistical analyses were performed with the SAS statistical software package, version 9.4.

Results

Patients

Among the 2559 patients included in the PETACC-8 phase III trial, 2043 signed the biological informed consent form, including 1791 patients with available FFPE specimen and no

Table 1. Clinical and Pathological Characteristics According to Mismatch Repair (MMR), *KRAS*, and *BRAF* Status in the Present Study Population

Characteristic	Status, No. (%)								
	MMR			*KRAS*			*BRAF*		
	MSS (n = 1614)	MSI (n = 177)	*P* Value	Wild Type (n = 1188)	Mutant (n = 588)	*P* Value	Wild Type (n = 1495)	Mutant (n = 148)	*P* Value
Treatment arm, No.	1614	177	.14	1188	588	.46	1495	148	.26
FOLFOX	799 (49.5)	98 (55.4)		590 (49.7)	303 (51.5)		760 (50.8)	68 (45.9)	
FOLFOX + cetuximab	815 (50.5)	79 (44.6)		598 (50.3)	285 (48.5)		735 (49.2)	80 (54.1)	
Sex, No.	1614	177	.03	1188	588	.23	1495	148	.006
Male	937 (58.1)	88 (49.7)		690 (58.1)	324 (55.1)		872 (58.3)	69 (46.6)	
Female	677 (41.9)	89 (5.3)		498 (41.9)	264 (44.9)		623 (41.7)	79 (53.4)	
Age, No.	1614	177	.60	1188	588	.18	1495	148	.91
≤70 y	1452 (90.0)	157 (88.7)		1075 (90.5)	520 (88.4)		1339 (89.6)	133 (89.9)	
>70 y	162 (10.0)	20 (11.3)		113 (9.5)	68 (11.6)		156 (10.4)	15 (10.1)	
ECOG PS, No.	1557	169	.71	1147	565	.99	1442	141	.12
0	1266 (81.3)	139 (82.2)		934 (81.4)	461 (81.6)		1174 (81.4)	106 (75.2)	
1	285 (18.3)	30 (17.8)		209 (18.2)	102 (18.1)		262 (18.2)	35 (24.8)	
2-3	6 (0.4)	0		4 (0.3)	2 (0.4)		6 (0.4)	0	
Tumor site, No.	1589	173	<.001	1178	571	.02	1468	147	<.001
Distal	1040 (65.4)	34 (19.7)		740 (62.8)	325 (56.9)		939 (64.0)	38 (25.9)	
Proximal	549 (34.6)	139 (8.3)		438 (37.2)	246 (43.1)		529 (36.0)	109 (74.1)	
Tumor grade, No.	1596	172	<.001	1173	581	.34	1477	146	.04
G1-G2	1337 (83.8)	98 (57.0)		947 (80.7)	480 (82.6)		1226 (83.0)	90 (61.6)	
G3-G4	259 (16.2)	74 (43.0)		226 (19.3)	101 (17.4)		251 (17.0)	56 (38.4)	
Lymph node status, No.	1614	177	.82	1188	588	.29	1495	148	.02
pN1	1008 (62.5)	109 (61.6)		733 (61.7)	378 (64.3)		946 (63.3)	79 (53.4)	
pN2	606 (37.5)	68 (38.4)		455 (38.3)	210 (35.7)		549 (36.7)	69 (46.6)	
T stage, No.	1614	177	.19	1188	588	.07	1495	148	.32
pT1/pT2/pTis	158 (9.8)	10 (5.6)		122 (10.3)	46 (7.8)		147 (9.8)	9 (6.1)	
pT3	1122 (69.5)	24 (70.1)		832 (70.0)	404 (68.7)		1035 (69.2)	108 (73.0)	
pT4	333 (20.6)	43 (24.3)		234 (19.7)	138 (23.5)		313 (20.9)	31 (20.9)	
pTx	1 (0.1)	0		0	0		0	0	
Bowel obstruction and/or perforation, No.	1614	177	.08	1188	588	.22	1495	148	.25
Yes	317 (19.6)	25 (14.1)		216 (18.2)	121 (20.6)		279 (18.7)	22 (14.9)	
No	1297 (80.4)	152 (85.9)		972 (81.8)	467 (79.4)		1216 (81.3)	126 (85.1)	
Vascular invasion and/or lymphatic infiltration, No.	1614	177	.41	1188	588	.007	1495	148	.04
Yes	903 (55.9)	104 (58.8)		699 (58.8)	300 (51.0)		827 (55.3)	94 (63.5)	
No	476 (29.5)	44 (24.9)		321 (27.0)	193 (32.8)		452 (30.2)	30 (20.3)	
KRAS, No.	1600	176	<.001				1492	148	<.001
Wild type	1042 (65.1)	146 (83.0)					959 (64.3)	144 (97.3)	
Mutated	558 (34.9)	30 (17.0)					533 (35.7)	4 (2.7)	
BRAF, No.	1476	167	<.001	1103	537	<.001			
Wild type	1382 (93.6)	113 (67.7)		959 (86.9)	533 (99.3)				
Mutated	94 (6.4)	54 (32.3)		144 (13.1)	4 (0.7)				

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status; FOLFOX, leucovorin, fluorouracil, and oxaliplatin; MMR, mismatch repair; MSI, microsatellite instability; MSS, microsatellite stability; NA, not applicable; OS, overall survival.

technical failure for the MMR status analysis. One hundred forty-eight (8.3%) and 15 (0.8%) of these patients were respectively excluded from *BRAF* V600E and *KRAS* exon 2 mutation analysis because of a technical issue (insufficient material or test failure) (**Figure 1**).

Characteristics of the study population are presented in **Table 1**. No noteworthy difference was observed between the present study population and the entire PETACC-8 trial population in terms of sex (male, 57.2% vs 57.1%), age (≤70 years, 89.8% vs 89.4%), and other clinical features, as well as pathological characteristics (eTable 1 in the Supplement). In the present study population, MSI phenotype and *KRAS* and *BRAF* V600E mutations were detected in 9.9% (177 of 1791), 33.1% (588 of 1776), and 9.0% (148 of 1643) of cases, respectively (Figure 1).

Table 2. Univariate Cox Proportional Hazards Regression Models for Disease-Free Survival (DFS) and Overall Survival (OS)

Parameter	Patients, No.	DFS			OS		
		Events, No.	HR (95% CI)	*P* Value	Events, No.	HR (95% CI)	*P* Value
Treatment arm, FOLFOX + cetuximab vs FOLFOX	1791	472	1.14 (0.95-1.36)	.17	240	1.13 (0.88-1.46)	.34
Male vs female sex	1791	472	1.13 (0.94-1.36)	.20	240	1.19 (0.92-1.54)	.19
Age, ≤70 vs >70 y	1791	472	1.01 (0.75-1.36)	.94	240	0.78 (0.53-1.13)	.19
Tumor grade, G3-G4 vs G1-G2	1768	463	1.46 (1.18-1.80)	<.001	233	1.89 (1.43-2.51)	<.001
Tumor site, distal vs proximal	1762	467	0.93 (0.77-1.12)	.43	237	0.61 (0.48-0.79)	<.001
pT stage	1791	471			239		
pT2 vs pT1			1.22 (0.40-3.75)	.72		0.72 (0.13-3.94)	.71
pT3 vs pT1			2.84 (1.06-7.62)	.04		2.52 (0.62-10.16)	.19
pT4 vs pT1			6.42 (2.38-17.31)	<.001		6.33 (1.56-25.71)	.01
pN stage, pN2 vs pN1	1791	472	2.29 (1.91-2.75)	<.001	240	2.56 (1.98-3.31)	<.001
ECOG PS, 1-2 vs 0	1726	451	1.31 (1.05-1.64)	.02	229	1.70 (1.27-2.28)	<.001
Bowel obstruction and/or perforation, yes vs no	1791	472	1.51 (1.23-1.86)	<.001	240	1.57 (1.18-2.10)	.002
Vascular invasion and/or lymphatic infiltration, yes vs no	1527	406	1.28 (1.03-1.59)	.02	207	1.21 (0.90-1.63)	.21
MMR status, MSS vs MSI	1791	472	1.12 (0.81-1.54)	.49	240	0.79 (0.53-1.17)	.23
Mutated vs wild type							
KRAS status	1776	465	1.41 (1.17-1.69)	<.001	235	1.25 (0.97-1.63)	.09
BRAF status	1643	427	1.07 (0.77-1.49)	.68	219	1.48 (0.97-2.25)	.07
In MSS Patients							
Mutated vs wild type							
KRAS status	1600	425	1.47 (1.21-1.77)	<.001	208	1.37 (1.04-1.80)	.02
BRAF status	1476	388	1.41 (0.97-2.04)	.07	193	1.88 (1.16-3.06)	.01
In MSI Patients							
Mutated vs wild type							
KRAS status	176	40	0.65 (0.25-1.65)	.36	27	0.50 (0.15-1.65)	.25
BRAF status	167	39	0.54 (0.25-1.17)	.12	26	0.67 (0.27-1.67)	.39

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status; FOLFOX, leucovorin, fluorouracil, and oxaliplatin; HR, hazard ratio; MMR, mismatch repair; MSS, microsatellite stability; MSI, microsatellite instability.

Among the 1791 patients tested for MMR status, all tumors were tested by IHC assay, except for 105 cases that were also tested by MSI assay because of indeterminate IHC results. Among the 177 tumors with MSI phenotype, 130 (73.4%) had lost MLH1 protein expression, 23 (13.0%) MSH2 expression, 6 (3.4%) MSH6 expression, and 12 (6.8%) PMS2 expression. The remaining 6 MSI tumors were considered indeterminate by means of IHC assay but positive by means of MSI testing. As expected, MSI compared with MSS phenotype was significantly associated with proximal tumors, high grade, and female sex (Table 1).[19-21,26] Mutated vs nonmutated *KRAS* tumor was significantly associated with proximal site and VELI-positive status, whereas mutated vs nonmutated *BRAF* tumor was significantly associated with female sex, proximal site, higher N and T stage, higher grade, and VELI-positive status (Table 1). *KRAS* and *BRAF* V600E mutations were mutually exclusive except in 4 patients (Table 1). Even if the co-occurrence of *KRAS* and *BRAF* V600E mutations is rare in CRC, this event has already been described with a frequency of approximately 0.2%, which is in line with our results.[27,28] The prevalence of *KRAS* mutations was higher in patients with MSS tumors (34.9%) than in patients with MSI tumors (17.0%) ($P < .001$).[7,19] In contrast, *BRAF* V600E mutation was significantly more frequent in patients with MSI tumors (32.3%) than in those with MSS tumors (6.4%) ($P < .001$)[7,19] (Table 1).

Prognostic Factors in the Overall Population

With an overall median follow-up of 3.52 years (95% CI, 3.46-3.64 years), higher T and N stage were independently associated with shorter DFS, whereas proximal site, higher N stage, and higher tumor grade were independently associated with shorter OS (**Tables 2** and **3**). In the biomarker analysis, no interaction was found between treatment (with or without cetuximab) and MMR status in terms of DFS or OS, but an interaction was found between both *BRAF* V600E and *KRAS* mutation and MMR status in terms of DFS and OS. Furthermore, no interaction was found between treatment (FOLFOX vs FOLFOX plus cetuximab) and *KRAS* status in terms of DFS ($P = .82$) and OS ($P = .73$); and the treatment administered significantly influenced neither DFS (HR, 0.88 [95% CI, 0.66-1.16]; $P = .36$) nor OS (HR, 0.98 [95% CI, 0.66-1.45]; $P = .91$) in patients with *KRAS*-mutated tumors.

The 3-year DFS rates were 77.9% and 73.9% among patients with MSI and MSS tumors, respectively (**Figure 2**A). In multivariate analysis, MSI phenotype was not significantly associated with DFS (HR, 1.10 [95% CI, 0.73-1.64]; $P = .67$) or OS (HR, 1.02 [95% CI, 0.61-1.69]; $P = .94$) (Table 3).

The 3-year DFS rates were 69.4% vs 77.1% among patients with mutated vs wild-type *KRAS* tumors (Figure 2B), and 73.8% vs 74.7% among patients with mutated vs wild-type *BRAF* tumors (Figure 2C). In multivariate analysis, *KRAS* mutation was

Table 3. Multivariate Cox Proportional Hazards Regression Models for Disease-Free Survival (DFS) and Overall Survival (OS)

	DFS		OS	
Parameter	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Treatment arm, FOLFOX + cetuximab vs FOLFOX	1.10 (0.89-1.36)	.38	1.03 (0.77-1.40)	.83
Male vs female sex	1.21 (0.97-1.50)	.10	1.28 (0.93-1.74)	.13
Age, ≤70 vs >70 y	0.98 (0.70-1.36)	.88	0.81 (0.53-1.25)	.34
Tumor grade, G3-G4 vs G1-G2	1.26 (0.97-1.64)	.08	1.55 (1.10-2.19)	.01
Tumor site, distal vs proximal	1.05 (0.83-1.33)	.69	0.66 (0.48-0.91)	.01
pT stage				
pT2 vs pT1	0.97 (0.30-3.09)	.95	0.66 (0.12-3.63)	.63
pT3 vs pT1	1.85 (0.69-5.02)	.22	1.37 (0.33-5.63)	.66
pT4 vs pT1	3.66 (1.34-10.01)	.01	2.99 (0.72-12.41)	.13
pN stage, pN2 vs pN1	2.05 (1.65-2.56)	<.001	2.08 (1.51-2.84)	<.001
ECOG PS, 1-2 vs 0	1.20 (0.93-1.55)	.16	1.72 (1.24-2.40)	.001
Bowel obstruction and/or perforation, yes vs no	1.04 (0.80-1.35)	.77	1.02 (0.70-1.49)	.90
Vascular invasion and/or lymphatic infiltration, yes vs no	1.00 (0.79-1.27)	.99	0.93 (0.67-1.30)	.66
MMR status, MSS vs MSI	1.10 (0.73-1.64)	.67	1.02 (0.61-1.69)	.94
Mutated vs wild type				
KRAS status	1.55 (1.23-1.95)	<.001	1.56 (1.12-2.15)	.008
BRAF status	1.22 (0.81-1.85)	.34	1.13 (0.64-2.00)	.66
In MSS Patients				
Mutated vs wild type				
KRAS status	1.64 (1.29-2.08)	<.001	1.71 (1.21-2.41)	.002
BRAF status	1.74 (1.14-2.69)	.01	1.84 (1.01-3.36)	.046
In MSI Patients				
Mutated vs wild type				
KRAS status	0.94 (0.32-2.74)	.91	0.90 (0.23-3.45)	.88
BRAF status	0.23 (0.06-0.92)	.04	0.19 (0.03-1.24)	.08

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status; FOLFOX, leucovorin, fluorouracil, and oxaliplatin; HR, hazard ratio; MMR, mismatch repair; MSS, microsatellite stability; MSI, microsatellite instability.

significantly associated with shorter DFS (HR, 1.55 [95% CI, 1.23-1.95]; *P* < .001) and shorter OS (HR, 1.56 [95% CI, 1.12-2.15]; *P* = .008), whereas *BRAF* V600E mutation influenced neither outcome (Tables 2 and 3).

Prognostic Effect of *KRAS* and *BRAF* V600E Mutations in Patients With MSS Tumors

The 3-year DFS rates were 68.7% and 77.1%, respectively, among MSS patients with mutated and wild-type *KRAS* tumors (**Figure 3**A). In multivariate analysis, MSS patients with mutated vs wild-type *KRAS* tumors had significantly shorter DFS (HR, 1.64 [95% CI, 1.29-2.08]; *P* < .001) and shorter OS (HR, 1.71 [95% CI, 1.21-2.41]; *P* = .002) (Table 3). A similar negative effect was observed for *BRAF* V600E mutation. The 3-year DFS rates were 67.0% and 74.7%, respectively, among MSS patients with mutated and wild-type *BRAF* tumors (Figure 3B). In multivariate analysis, *BRAF* V600E mutation in patients with MSS tumors remained significantly associated with shorter DFS (HR, 1.74 [95% CI, 1.14-2.69]; *P* = .01) and shorter OS (HR, 1.84 [95% CI, 1.01-3.36]; *P* = .046) (Table 3).

Prognostic Effect of *KRAS* and *BRAF* V600E Mutations in Patients With MSI Tumors

The 3-year DFS rates were 82.8% and 77.5%, respectively, among MSI patients with mutated and wild-type *KRAS* tumors (Figure 3C). In multivariate analysis, *KRAS* status in patients with MSI tumors was not significantly associated with DFS (HR, 0.94 [95% CI, 0.32-2.74]; *P* = .91) or OS (HR, 0.90 [95% CI, 0.23-3.45]; *P* = .88) (Table 3). As observed in patients with MSS tumors, *BRAF* V600E mutation was again associated with clinical outcome in patients with MSI tumors, but the prognostic effect was in the opposite direction. Indeed, the 3-year DFS rates were 85.2% and 74.3%, respectively, among MSI patients with mutated and wild-type *BRAF* tumors (Figure 3D). In multivariate analysis, MSI tumors harboring *BRAF* V600E mutation were associated with longer DFS (HR, 0.23 [95% CI, 0.06-0.92]; *P* = .04) but not longer OS (HR, 0.19 [95% CI, 0.03-1.24]; *P* = .08) (Table 3).

Discussion

The aim of this study was to determine the prognostic value of MMR, *KRAS*, and *BRAF* status determined on prospectively collected stage III colon cancer specimens from patients receiving FOLFOX with or without cetuximab in a randomized trial of adjuvant therapy. We found that MMR status was not predictive of either DFS or OS. Most previous studies have shown a favorable prognostic effect of the MSI phenotype,[20,21,26,29-32] but others showed no significant difference in clinical outcome between patients with MSI and MSS tumors.[33-35] This dis-

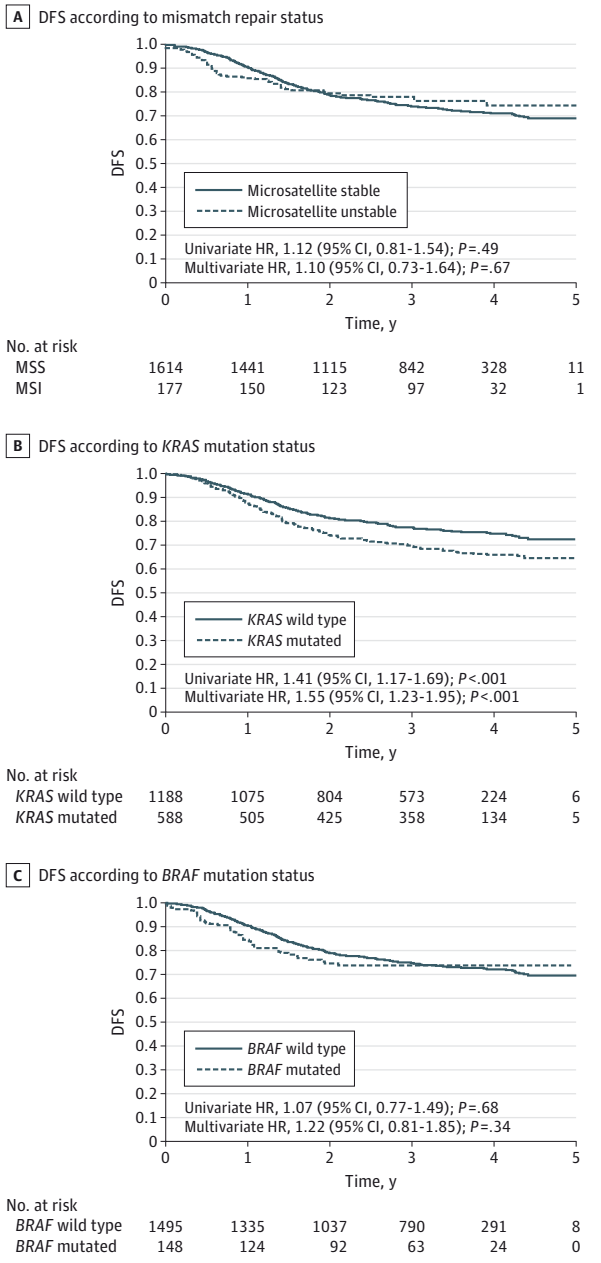
crepancy might be explained by a lack of adjustment for *BRAF* and *KRAS* status, tumor stage, or the adjuvant treatment regimen. Indeed, although our cohort was composed only of patients with stage III colon cancer, studies showing longer survival among patients with MSI vs MSS tumors generally combined stage II and III tumors, and the favorable prognostic effect seemed to be stronger in stage II disease.[21,36] In the NCCTG N0147 study, which had a design similar to that of the PETACC-08 trial, analysis of the 2580 patients with stage III colon cancer participating in this trial showed that MMR status had no prognostic value.[19] Furthermore, in vitro studies have shown that oxaliplatin,[37] contrary to fluorouracil,[38] is active on CRC cell lines independently of MMR status, suggesting that the clinical effect of MMR status might be attenuated in patients receiving FOLFOX-based adjuvant chemotherapy.

We found that *BRAF* V600E mutation influenced neither DFS nor OS in the overall study population, whereas a negative effect on DFS and OS was observed in the MSS subgroup. Recently, data from the NCCTG N0147 trial showed that *BRAF* V600E mutation was significantly associated with shorter DFS in multivariate analysis (HR, 1.37 [95% CI, 1.08-1.70]; P = .009).[19] However, the adverse effect of *BRAF* V600E mutation was limited to patients with MSS tumors after stratification on MMR status.[19] It seems important to adjust the *BRAF* prognostic value to the MMR status to identify more precisely the patients with poor clinical outcomes in stage III colon cancer. Three retrospective analyses of randomized adjuvant trials suggested that *BRAF* V600E mutation was independently associated with shorter OS but not with disease-free or recurrence-free survival[7,14,18] (eTable 2 in the Supplement). However, when MMR status was taken into account in these studies, the worse prognostic value of *BRAF* V600E mutation was attenuated in patients with MSI tumors. Indeed, MSI/*BRAF* wild-type patients had the best prognosis, whereas the MSS/*BRAF* V600E mutation subgroup had the worst prognosis. Patients with MSS/*BRAF* wild-type or MSI/*BRAF* V600E-mutated tumors had intermediate survival.[14,18]

Here we found that *BRAF* V600E mutation was significantly associated with longer DFS but not OS in patients with MSI tumors. This suggests that the prognostic effect of *BRAF* V600E mutation in MSI patients treated with FOLFOX with or without cetuximab adjuvant chemotherapy may be indirectly related to the CIMP phenotype. Indeed, there is considerable overlap among tumors characterized as MSI, mutated *BRAF* V600E, and CIMP.[5] Tumors with *BRAF* V600E mutation and MSI phenotype occur almost exclusively as a consequence of CIMP, associated with methylation of the *MLH1* promoter. The prognostic value of the CIMP phenotype in patients receiving fluorouracil-based adjuvant chemotherapy is controversial.[39-41] To our knowledge, only 1 retrospective study has evaluated the prognostic effect of both MMR and CIMP status in patients with stage III colon cancer receiving FOLFOX-based adjuvant chemotherapy, showing that MSI/CIMP-positive tumor status was associated with poorer DFS than MSI/CIMP-negative tumor status.[42] However, in the MSI/CIMP-positive subgroup analysis, patients with *MLH1* methylation had a longer DFS than those with methylation at other loci.[42]

We found that *KRAS* mutations were linked to survival defined by a shorter DFS and OS in the overall study population.

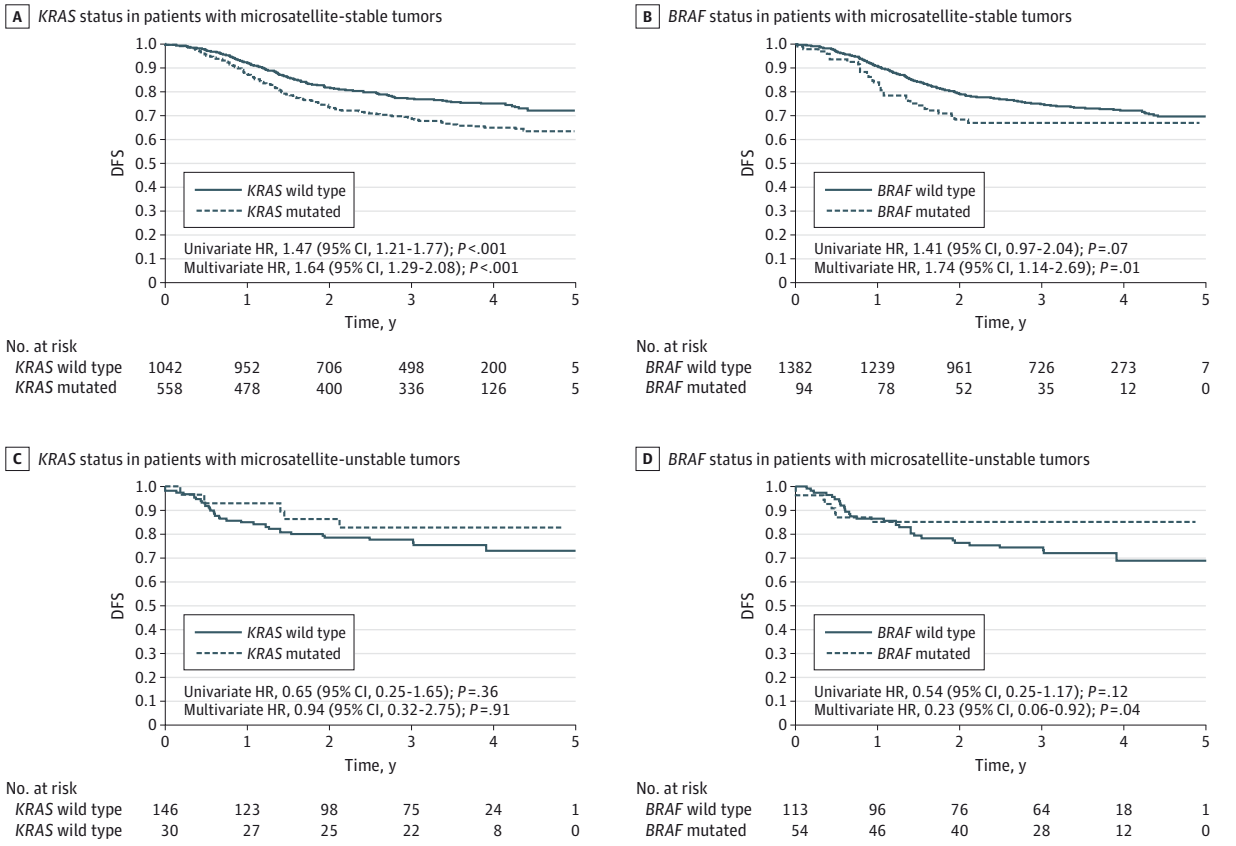
Figure 2. Kaplan-Meier Curves for Disease-Free Survival According to Mismatch Repair, *KRAS*, and *BRAF* Status



HR indicates hazard ratio.

Stratification on MMR status showed that this effect was restricted to the MSS subgroup whereas no effect of *KRAS* status was seen in the MSI subgroup. Large population-based cohorts and retrospective analyses of randomized adjuvant trials have reported conflicting results concerning the prognostic value of *KRAS* exon 2 mutations[7,13,43-45] (eTable 2 in the Supplement). Retrospective analyses of 3 randomized adjuvant trials (CKVO 90-11, CALGB 89803, and PETACC-3) failed to demonstrate any association between *KRAS* codon 12 and 13 mutations and recurrence-free survival or OS in patients with stage II and III colon cancer.[7,44,45] In contrast, multivariate analysis of data from

Figure 3. Effect of *KRAS* and *BRAF* Status on Disease-Free Survival (DFS) in Patients With Microsatellite-Stable and Microsatellite-Unstable Tumors



HR indicates hazard ratio.

patients with stage III colon cancer included in the NCCTG N0147 study showed that *KRAS* mutations in either codon 12 or 13 were independently associated with poorer DFS.[15] More recently, data on patients enrolled in the PETACC-8 trial showed that both *KRAS* codon 12 and 13 mutations were related to prognosis in patients with distal tumors only.[16] We report here that *KRAS* mutations were associated with a poor outcome in patients with MSS tumors, which represent approximately 90% of all stage III colon cancer. This is in accordance with our previous report[16] because MSS tumors are more frequently distal, whereas MSI tumors are mainly located in the proximal site. A lack of stratification on MMR tumor status may, at least in part, explain discrepancies concerning the prognostic value of *KRAS* mutation among previously published studies.

In randomized studies including patients with *KRAS*-mutated metastatic CRC, oxaliplatin-based chemotherapy in combination with epidermal growth factor receptor inhibitors (cetuximab and panitumumab) is associated with worse survival than oxaliplatin-based chemotherapy alone,[46,47] which is not the case for irinotecan-based chemotherapy.[48] In our study, cetuximab in combination with oxaliplatin-based chemotherapy did not worsen the clinical outcomes of patients with stage III colon cancer with *KRAS*-mutated tumors. Further analysis based on the complete assessment of *KRAS/NRAS* exons 2, 3, and 4, as well as survival after recurrence and treatments received after recurrence, is needed to better elucidate the real predictive effect of *KRAS* mutations in the adjuvant setting.

Strengths of our study include biomarker analysis in a prospective collection of tumor blocks from patients with stage III colon cancer treated in a randomized trial with the current standard FOLFOX-based adjuvant chemotherapy. The present study population was representative of the entire PETACC-8 population because no statistically significant difference was observed in terms of clinical and pathological characteristics (see eTable 1 in the Supplement). This large study allowed us to analyze the prognostic effect of *BRAF* V600E and *KRAS* mutations according to MMR status. Study limitations include the lack of patients treated with fluorouracil alone, making it difficult to analyze the predictive value of MMR status for the response to oxaliplatin-based adjuvant chemotherapy. The percentages of patients with mutated *KRAS* tumors were lower than expected in the MSI and MSS groups,[7] following amendment of the PETACC-8 trial eligibility criteria to restrict random assignment to patients with *KRAS* wild-type tumors. We also recognize the inherent limitations related to the lack of assessment of activating hotspot mutations in *KRAS/NRAS* exons 2, 3, and 4 on clinical outcomes of patients with colon cancer treated with FOLFOX with or without cetuximab in the adjuvant setting. Indeed,

recent data from randomized studies demonstrated that patients with metastatic CRC who have *RAS* wild-type tumors derived a significant survival benefit from the addition of epidermal growth factor receptor inhibitors (panitumumab or cetuximab) to chemotherapy, whereas patients with *RAS* tumor mutations did not.[47,49] Finally, our hypothesis suggesting that CIMP status may play a role in the good prognostic effect of *BRAF* V600E mutation in patients with MSI tumors should be interpreted with caution because of the small number of patients and the lack of CIMP profiling of the tumors. These results need to be confirmed on pooled data from large phase III adjuvant trials using FOLFOX.

Conclusions

This large analysis of patients with stage III colon cancer receiving FOLFOX-based adjuvant chemotherapy shows that MMR status should be taken into account in future prognostic studies involving *KRAS* and *BRAF* V600E mutations. *BRAF* V600E and *KRAS* exon 2 mutations are independently linked to shorter DFS and OS in patients with MSS tumors. In contrast, *KRAS* mutations have no prognostic value in patients with MSI tumors, whereas the *BRAF* V600E mutation could be associated with longer survival.

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Author Affiliations: Paris Descartes University, Department of Digestive Oncology, European Georges Pompidou Hospital, Assistance Publique-Hôpitaux de Paris, France (Taieb, Zaanan); Centre de Recherché UMR-S 1147, Médecine Personnalisée, Pharmacogénomique, Optimisation Thérapeutique, Institut National de la Santé et de la Recherche Médicale, Paris, France (Zaanan, Blons, Laurent-Puig); Department of Statistics, Fédération Francophone de Cancérologie Digestive, Dijon, France (Le Malicot); Department of Pathology, Ambroise Paré Hospital, Assistance Publique–Hôpitaux de Paris, Boulogne-Billancourt, France (Julié, Emile); Versailles Saint-Quentin-en-Yvelines University, Boulogne-Billancourt, France (Julié, Emile); Paris Descartes University, Department of Biology, European Georges Pompidou Hospital, Assistance Publique–Hôpitaux de Paris, France (Blons, Laurent-Puig); Department of Oncology, Sainte Catherine Institute, Avignon, France (Mineur); Department of Oncology, Cancérologie de l'Ouest Institute, Nantes, France (Bennouna); Medical Oncology Department, Vall d'Hebron University Hospital and Institute of Oncology, Barcelona, Spain (Tabernero); Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy (Mini); First Medical Department, University Hospital Carl Gustav Carus, Dresden, Germany (Folprecht); Department of Gastroenterology, Erasme Hospital University, Brussels, Belgium (Van Laethem); Department of Hepato-Gastroenterology, Dijon University Hospital, Dijon, France (Lepage); Centre de Recherche UMR 866, Lipides, Nutrition, Cancer, Institut National de la Santé et de la Recherche Médicale, Dijon, France (Lepage).

Author Contributions: Dr Taieb and Ms Le Malicot had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Drs Taieb and Zaanan and Ms Le Malicot contributed equally to this work as first authors. Drs Emile and Laurent-Puig contributed equally to this work as senior authors.
Study concept and design: Taieb, Zaanan, Tabernero, Mini, Folprecht, Laurent-Puig.
Acquisition, analysis, or interpretation of data: All authors.
Drafting of the manuscript: Taieb, Zaanan, Le Malicot, Julié, Blons, Tabernero, Lepage, Laurent-Puig.
Critical revision of the manuscript for important intellectual content: Taieb, Zaanan, Le Malicot, Julié, Mineur, Bennouna, Tabernero, Mini, Folprecht, Van Laethem, Emile, Laurent-Puig.
Statistical analysis: Le Malicot, Tabernero, Laurent-Puig.
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Study supervision: Taieb, Zaanan, Laurent-Puig.

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Invited Commentary

Microsatellite Instability and *BRAF* and *KRAS* Mutations in Stage III Colon Cancer

Requirements for Accurate Prognosis Assessment

Dirk Klingbiel, PhD; Sabine Tejpar, MD, PhD

Microsatellite instability (MSI) and mutations of *BRAF* and *KRAS* have been investigated as candidate biomarkers of "independent" prognostic value in patients with colorectal cancer for some time.[1,2] Depending on tumor stage and treatment, their prognostic value seems to vary, but even in trials with similar populations and treatment the prognostic value is still controversial. Whether and how these 3 biomarkers are associated with outcome in patients with stage III colon cancer treated with combination leucovorin-calcium, fluorouracil, and oxaliplatin (FOLFOX)-based chemotherapy and how they influence each other are relevant research questions.

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Most of the current evidence supports the notion that stage-adjusted prognosis is more favorable for MSI-high than for microsatellite-stable tumors, with larger advantage for patients with stage II than stage III disease.[3] A putative predictive role of MSI for fluorouracil-based adjuvant chemotherapy has been a more contentious issue with conflicting evidence. Many studies have investigated the prognostic value of *KRAS* and *BRAF* tumor mutations on retrospectively collected cohorts of patients with colorectal cancer of different MSI status, also with conflicting results—some reporting no prognostic value, others finding prognostic value either alone or concomitantly with mutated *TP53* or *PIK3CA*. *KRAS* tumor mutation status is consequently now widely recognized as a predictive marker of resistance to epidermal growth factor receptor–targeted antibodies in colorectal cancer.

In this issue of *JAMA Oncology*, Taieb and colleagues[4] report a post hoc analysis of the PETACC-8 trial. For some impressive 1800 patients they assessed the association of MSI, *BRAF*, and *KRAS* status and more classic prognostic factors such as age, tumor grade, T/N stage, and location with outcome in terms of disease-free survival and overall survival. The authors are to be commended for studying such an important question in a large trial population and for emphasizing the results of multivariable models that adjust for potential confounding between variables. Their most important finding is a significant interaction of *BRAF* and *KRAS* mutations and MSI status, which we had observed and reported, albeit from a different angle, in the PETACC-3 trial as well.[4]

That being said, we still want to point out a number of issues. All analyses in the *BRAF*-mutant tumors (10%) and in the MSI-high subgroup have low power to detect presence or absence of effects reliably. While often done correctly, high *P* values are sometimes interpreted negligently.

For instance, they state that *KRAS* status was not prognostic in patients with MSI-high tumors. This would have been better presented as "there was no evidence for a prognostic effect" or the like. With a confidence interval from approximately 0.3 to 3 for both disease-free survival and overall survival, we cannot draw conclusions from these data, due to the lack of events and hence power as mentioned.

Also, it might have been more advisable to compare *BRAF*-mutated and *KRAS*-mutated with double wild-type tumors instead of *BRAF*/*KRAS*-nonmutated tumors, respectively. Also, importantly, because the *RAS* status is incompletely assessed in these samples, and as acknowledged by the authors, *KRAS* mutations beyond exon 2 might change the results. The absence of these data limits the immediate clinical interpretation.

Most questionably, the authors do not stratify for the treatment arms because "no difference was found between the 2 arms for efficacy analyses" and because no interaction was found between treatment and *KRAS* status. But interaction tests have low power with a high false-negative rate[5] and, again, just because we do not find a significant interaction does not mean that it is not there. Importantly, the interaction of *KRAS* status and cetuximab therapy is well known and established a priori from pathophysiological principles; hence, stratified subgroup analyses are justified and should be presented with similarities to or differences from the unstratified analyses.

We second the conclusion of Taieb and colleagues[4] that in future adjuvant trials MSI, *BRAF*, and *KRAS* status will need to be considered for stratification. This could also include tumor site (see their previous report[6] on this trial indicating that *KRAS* mutation status was only prognostic in distal tumors, a finding that the authors now suggest is explained by the MSI status). With this many highly correlated factors to assess, a pooled analysis with a carefully designed statistical analysis plan of individual patient data (full *RAS* status, MSI status, sidedness, and so forth) on stage II and III colon cancer from as many trials as possible would be timely and relevant. A first question to answer on existing adjuvant trial data sets would be whether there is any interaction of these subgroups with therapy.

Any markers that can help guide individualized toxicity and/or efficacy evaluations in this setting are welcome. Further stratification of the disease may also help assess more precisely the effect of drugs that failed in the adjuvant setting.

In terms of identification of purely prognostic subgroups in the disease, it will be interesting to confront these findings with gene expression–based prognostic stratifications of stage II and III colorectal cancer.