original report

Mutational Analysis of Patients With Colorectal Cancer in CALGB/SWOG 80405 Identifies New Roles of Microsatellite Instability and Tumor Mutational Burden for Patient Outcome

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abstract

PURPOSE CALGB/SWOG 80405 was a randomized phase III trial that found no statistically significant difference in overall survival (OS) in patients with first-line metastatic colorectal cancer treated with chemotherapy plus either bevacizumab or cetuximab. Primary tumor DNA from 843 patients has been used to discover genetic markers of OS.

PATIENTS AND METHODS Gene mutations were determined by polymerase chain reaction. Microsatellite status was determined by genotyping of microsatellites. Tumor mutational burden (TMB) was determined by next-generation sequencing. Cox proportional hazard models were used, with adjusting factors. Interaction of molecular alterations with either the bevacizumab or the cetuximab arms was tested.

RESULTS Patients with high TMB in their tumors had longer OS than did patients with low TMB (hazard ratio [HR], 0.73 [95% CI, 0.57 to 0.95]; $P = .02$). In patients with microsatellite instability–high (MSI-H) tumors, longer OS was observed in the bevacizumab arm than in the cetuximab arm (HR, 0.13 [95% CI, 0.06 to 0.30]; interaction $P < .001$ for interaction between microsatellite status and the two arms). Patients with *BRAF* mutant tumors had shorter OS than did patients with wild-type (WT) tumors (HR, 2.01 [95% CI, 1.49 to 2.71]; $P < .001$). Patients with extended *RAS* mutant tumors had shorter OS than did patients with WT tumors (HR, 1.52 [95% CI, 1.26 to 1.84]; $P < .001$). Patients with triple-negative tumors (WT for *NRAS*/*KRAS*/*BRAF*) had a median OS of 35.9 months (95% CI, 33.0 to 38.8 months) versus 22.2 months (95% CI, 19.6 to 24.4 months) in patients with at least one mutated gene in their tumors ($P < .001$).

CONCLUSION In patients with metastatic colorectal cancer treated in first line, low TMB, and *BRAF* and *RAS* mutations are negative prognostic factors. Patients with MSI-H tumors benefited more from bevacizumab than from cetuximab, and studies to confirm this effect of MSI-H are warranted.

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INTRODUCTION

Colorectal cancer (CRC) is the second-leading cause of cancer death in the United States.[1] Over the past two decades, 13 drugs, including antibodies targeting vascular endothelial growth factor (bevacizumab) and the epidermal growth factor receptor (EGFR; cetuximab and panitumumab), have been approved for the treatment of metastatic CRC (mCRC). However, the optimal combination and sequence of these drugs is likely dependent on many factors including the mutational status of the tumor cells.

Cancer and Leukemia Group B (CALGB)/SWOG 80405 was designed initially to test whether the addition of either cetuximab or bevacizumab or both to fluorouracil and leucovorin with either oxaliplatin (FOLFOX) or irinotecan leads to superior outcomes as first-line therapy in advanced CRC or mCRC. The primary end point of the trial was overall survival (OS). In 1,137 patients with *KRAS* wild-type (WT; codons 12 and 13), there was no statistically significant difference in OS between the bevacizumab arm and the cetuximab arm.[2] The regimens tested in CALGB/SWOG 80405 represent the current standard of care for first-line treatment in mCRC. CALGB is now part of the Alliance for Clinical Trials in Oncology (Alliance).

To individualize and optimize treatment, molecular analysis of tumors of patients, particularly those patients enrolled in clinical trials, is imperative. Outcome data and biospecimens collected from this relatively large study might provide an unprecedented opportunity for performing translational studies on molecular

ASSOCIATED CONTENT

Appendix

Author affiliations and support information (if applicable) appear at the end of this article.

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markers. We hypothesized that, in this context, novel somatic genetic alterations that drive tumor progression and/or resistance to therapy might affect patient outcome. Aside from our knowledge that the presence of any of a number of *RAS* mutations conveys resistance to EGFR inhibitors, and that microsatellite instability–high (MSI-H) tumors are more likely to respond to checkpoint inhibitors (which are under study in the first-line setting), there are no molecular markers to inform the selection of the most efficacious regimen for patients with mCRC.

This study aimed to determine the tumor mutational profile of patients with mCRC in CALGB/SWOG 80405, to evaluate the prognostic value of DNA mutations, and to determine the differential treatment effects of bevacizumab versus cetuximab in patients with a specific mutational profile.

PATIENTS AND METHODS

The trial was designed to compare chemotherapies plus either cetuximab, bevacizumab, or cetuximab and bevacizumab as first-line treatment of advanced CRC and mCRC. Within 3 years, data suggesting that dual antibody combinations compromised outcomes and the discovery of the lack of efficacy of EGFR antibodies in *KRAS* mutant tumors led to a restriction of eligibility to patients with *KRAS* WT (codons 12 and 13; *KRAS* amendment) and to closure of the dual antibody group. A revised two-arm trial (chemotherapy plus either cetuximab or bevacizumab) became the primary cohort (Appendix Fig A1, online only), and the OS results have been reported.[2]

Eligible patients had pathology-documented, untreated, locally advanced CRC or mCRC. Patients were 18 years or older with an Eastern Cooperative Oncology Group performance status of 0 to 1 and normal hepatic, renal, and hematologic laboratory values. Random assignment was stratified by chemotherapy, prior adjuvant chemotherapy, and prior pelvic radiation.

The primary end point of OS was defined as time of random assignment until death. Patients without reported deaths were censored at their last known follow-up. Progression-free survival (PFS) was measured from time of random assignment until first documented progression or death. Patients alive without documented tumor progression were censored for PFS at the most recent disease assessment. Median follow-up is 66.5 months (95% CI, 64.3 to 69.8 months).

The numbers of patients enrolled in the trial and the specimens used for analyses are in Fig 1. Table 1 reports patient demographics and clinical characteristics. Institutional review board approval was required, and all participating patients provided written informed consent to the genetic analysis.

Genotyping of Mutation Hotspots

Tumor DNA was obtained from 843 formalin-fixed, paraffin-embedded tumor blocks (92% primary, 4% metastatic, 4% unknown). DNA was extracted by QIAamp DNA formalin-fixed, paraffin-embedded tissue kits (QIAGEN, Hilden, Germany). Allele-specific polymerase chain reaction has been used to genotype mutation hotspots in *AKT*, *APC*, *BRAF*, *CTNNB1*, *EGFR*, *FBXW7*, *HRAS*, *KRAS*, *NRAS*, *PIK3CA*, and *TP53*. The analysis was conducted at Genentech (South San Francisco, CA). Methods have been described previously.[3] The genotyped mutations and their frequency are in Appendix Table A1 (online only).

Tumor Mutational Burden

Each tumor DNA underwent next-generation sequencing using the FoundationOne platform and was conducted at Foundation Medicine (Cambridge, MA) following standard procedures. The gene list is reported in Appendix Table A2 (online only).

The tumor mutational burden (TMB) in each tumor has been calculated per standard criteria by Foundation Medicine. TMB is reported as the number of mutations divided by the Mb of the genomic region being sequenced.

Microsatellite Instability

The MSI Analysis System (Promega, Madison, WI) has five mononucleotide repeat markers (BAT-25, BAT-26, NR-21, NR-24, MONO-27) and two pentanucleotide markers for sample identification and contamination (Penta C, Penta D). Samples with two or more altered markers were classified as MSI-high (MSI-H), samples with one altered marker were classified as microsatellite instability–low, and samples without altered markers were classified as microsatellite stable (MSS). In the outcome analysis, microsatellite instability–low tumors were grouped with MSS tumors.

Statistical Analysis

The main analysis tested the effect of mutational status on OS in all patients for whom mutational analysis was available, which includes patients enrolled before (59.7% of patients in this study) and after the *KRAS* amendment. A subgroup analysis was conducted in the primary cohort, such as patients with *KRAS* WT before the *KRAS* amendment and all patients after the *KRAS* amendment, who received either bevacizumab or cetuximab (plus chemotherapy). The distribution of time-to-event end points was estimated by Kaplan-Meier curves. Associations with OS and PFS were tested using the log-rank test. A multivariable stratified Cox model was used to identify the association between a biomarker and time-to-event end points.

Models were adjusted for age, treatment arm, sex, ethnicity, synchronous versus metachronous metastases, number of metastatic sites (0, 1, 2, 3, greater than or equal to 3), primary tumor location (right and transverse *v* left), MSI-H versus MSS, *BRAF* (mutant *v* WT), and extended *RAS* (mutant *v* WT) while stratifying for prior adjuvant chemotherapy and prior radiation. The proportional hazard

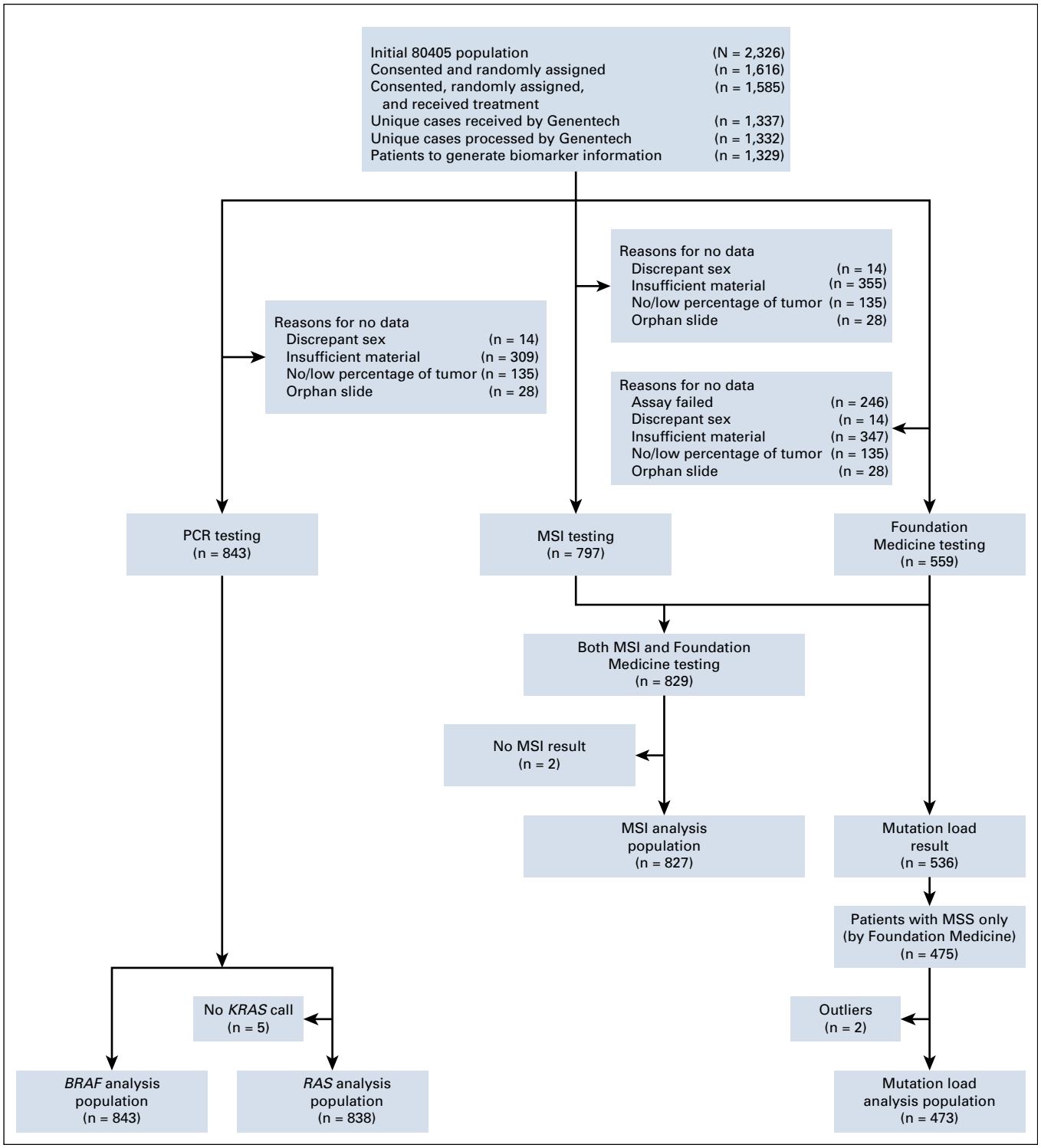

FIG 1. CONSORT diagram. MSI, microsatellite instability; MSS, microsatellite stable; PCR, polymerase chain reaction.

assumption was examined by the Kolmogorov-type supremum test and graphical method using weighted residuals (Appendix).

Univariable, two-group comparisons were performed using Wilcoxon rank-sum, Kruskal-Wallis, and χ^2 tests. The two groups are those in Table 1. For the TMB analysis, an optimized cutoff was selected using a previous method that was based on maximizing the log-rank statistics for comparing two groups.[4] Interactions between the molecular alteration and treatment arms were also tested by the Wald

TABLE 1. Clinical Characteristics and Demographics of Patients

Characteristic	Population Without Mutational Analysis (n = 1,483)	Population With Mutational Analysis (n = 843)	Total (N = 2,326)	*P*
Age, years				< .001*
Median	58.4	60.4	59.1	
Range	21.8-89.5	20.8-84.3	20.8-89.5	
Arm				.3311†
Chemotherapy and bevacizumab	582 (39.2)	315 (37.4)	897 (38.6)	
Chemotherapy and cetuximab	576 (38.8)	321 (38.1)	897 (38.6)	
Chemotherapy, bevacizumab, and cetuximab	325 (21.9)	207 (24.6)	532 (22.9)	
Chemotherapy				.1637†
FOLFOX	1,158 (78.1)	637 (75.6)	1,795 (77.2)	
FOLFIRI	325 (21.9)	206 (24.4)	531 (22.8)	
Prior adjuvant chemotherapy				.7758†
No	1,273 (85.8)	720 (85.4)	1,993 (85.7)	
Yes	210 (14.2)	123 (14.6)	333 (14.3)	
Prior pelvic radiation				.0381†
No	1,340 (90.4)	783 (92.9)	2,123 (91.3)	
Yes	143 (9.6)	60 (7.1)	203 (8.7)	
Sex				.5453†
Male	857 (57.8)	498 (59.1)	1,355 (58.3)	
Female	626 (42.2)	345 (40.9)	971 (41.7)	
Ethnicity				< .001†
Missing	2	4	6	
Unknown	48 (3.2)	6 (0.7)	54 (2.3)	
White	1,168 (78.9)	728 (86.8)	1,896 (81.7)	
Black	193 (13.0)	85 (10.1)	278 (12.0)	
Asian	60 (4.1)	13 (1.5)	73 (3.1)	
Native Hawaiian or Pacific Islander	6 (0.4)	1 (0.1)	7 (0.3)	
American Indian or Alaska Native	6 (0.4)	5 (0.6)	11 (0.5)	
Not reported	0 (0.0)	1 (0.1)	1 (0.0)	
ECOG PS				.3508†
0	860 (58.0)	501 (59.4)	1,361 (58.5)	
1	620 (41.8)	342 (40.6)	962 (41.4)	
2	3 (0.2)	0 (0.0)	3 (0.1)	
No. of metastatic sites				< .001†
Missing	39	4	43	
1	590 (40.9)	449 (53.5)	1,039 (45.5)	
2	564 (39.1)	275 (32.8)	839 (36.7)	
≥ 3	290 (20.1)	115 (13.7)	405 (17.7)	
Tumor location				< .001†
Left	854 (57.6)	439 (52.1)	1,293 (55.6)	
Right	325 (21.9)	278 (33.0)	603 (25.9)	
Transverse	104 (7.0)	61 (7.2)	165 (7.1)	
Multiple	3 (0.2)	6 (0.7)	9 (0.4)	
Unknown	197 (13.3)	59 (7.0)	256 (11.0)	

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TABLE 1. Clinical Characteristics and Demographics of Patients (continued)

Characteristic	Population Without Mutational Analysis (n = 1,483)	Population With Mutational Analysis (n = 843)	Total (N = 2,326)	*P*
Liver metastases only				< .001†
Missing	40	4	44	
No	1,048 (72.6)	542 (64.6)	1,590 (69.7)	
Yes	395 (27.4)	297 (35.4)	692 (30.3)	
Overall survival, months				.0043‡
Median (95% CI)	25.2 (24.0 to 26.4)	29.0 (26.8 to 30.9)	26.3 (25.4 to 27.4)	
Progression-free survival, months				.0124‡
Median (95% CI)	10.2 (9.8 to 10.8)	10.9 (10.0 to 11.3)	10.5 (10.0 to 10.8)	

NOTE. Data are presented as No. (%) unless indicated otherwise. Comparisons are made between the population with the mutational analysis and the population without it.

Abbreviations: ECOG, Eastern Cooperative Oncology Group; FOLFIRI, fluorouracil, leucovorin, and irinotecan; FOLFOX, fluorouracil, leucovorin, and oxaliplatin; PS, performance status.

*Kruskal-Wallis test.

†χ^2 test.

‡Log-rank test.

test. The reported *P* values (interaction *P*) in the text are from interaction tests between the molecular alteration and two arms (bevacizumab *v* cetuximab) in the primary cohort.

A two-sided *P* value of < .05 was considered statistically significant, with the exception of the interaction test, in which *P* values < .1 were considered statistically significant. No adjustments were made for multiple comparisons, because some of the analyses (eg, TMB) were neither preplanned nor included in the protocol, and because the clinical study was not powered to detect associations with molecular markers. Data collection and statistical analyses were conducted by the Alliance Statistics and Data Center. All analyses were performed using SAS software version 9.4 (SAS Institute, Cary, NC).

RESULTS

BRAF mutations (12% [100 of 843]) were almost exclusively V600E (98%). Patients with *BRAF* mutated tumors had a median OS of 13.5 months (95% CI, 11.2 to 18.5 months), and patients with *BRAF* WT tumors had a median OS of 30.6 months (95% CI, 29.0 to 33.5 months; hazard ratio [HR], 2.01 [95% CI, 1.49 to 2.71]; *P* < .001; Fig 2 [top]; Table 2). To evaluate the contribution of tumor location to the effect of *BRAF* mutations, tumor location was removed from the statistical model, and the resulting HR was 2.26 (95% CI, 1.70 to 3.01; *P* < .001; Table 2). No difference in OS was observed between the two treatment arms (primary cohort) on the basis of *BRAF* status (interaction *P* = .293; Appendix Table A3, online only).

Patients with extended *RAS* mutant tumors (32% [266 of 838]) had a median OS of 25.0 months (95% CI, 22.6 to 28.1 months), and patients with *RAS* WT tumors had a median OS of 32.1 months (95% CI, 28.9 to 35.5 months; HR, 1.52 [95% CI, 1.26 to 1.84]; *P* < .001; Fig 2 [bottom]; Table 2). Removing tumor location from the model does not affect this result (Table 2). No difference in OS was observed between the two treatment arms (primary cohort) on the basis of the *RAS* status (interaction *P* = .602; Appendix Table A3).

Patients with triple-negative tumors (WT for *NRAS*/*KRAS*/*BRAF*) had a median OS of 35.9 months (95% CI, 33.0 to 38.8 months) versus 22.2 months in patients with at least one mutated gene in their tumors (95% CI, 19.6 to 24.4 months; *P* < .001). In the primary cohort, this difference is even more pronounced (36.4 *v* 19.4 months; *P* < .001). The presence or absence of a *PIK3CA* mutation did not change the results of quadruple-negative (WT for *NRAS*/*KRAS*/*BRAF*/*PIK3CA*) compared with triple-negative tumors (results not shown).

In patients whose tumors were MSI-H (6% [52 of 827]) versus MSS, the HR was 0.87 (95% CI, 0.60 to 1.28; *P* = .491). Removal of tumor location from the model does not affect this result (Table 2).

In patients with MSI-H tumors, longer OS was observed in the bevacizumab arm than in the cetuximab arm (HR, 0.13 [95% CI, 0.06 to 0.30]; *P* < .001; Table 2). In the bevacizumab arm, median OS was 30.0 months (95% CI, 23.6 months to NE) versus 11.9 (95% CI, 10.3 to 24.6 months) in the cetuximab arm (Fig 3). In patients with MSS tumors, no difference was observed between the two arms (*P* = .539; Table 2). There is a differential treatment effect across microsatellite instability status (interaction *P* < .001, primary cohort).

TMB was available from 536 patients. In patients with MSI-H tumors (n = 35), the median TMB was 52 mutations/Mb (range, 11 to 208 mutations/Mb), and in patients with MSS tumors (n = 475), the median TMB was six mutations/Mb (range, 0 to 361 mutations/Mb; Appendix Fig A2). Two

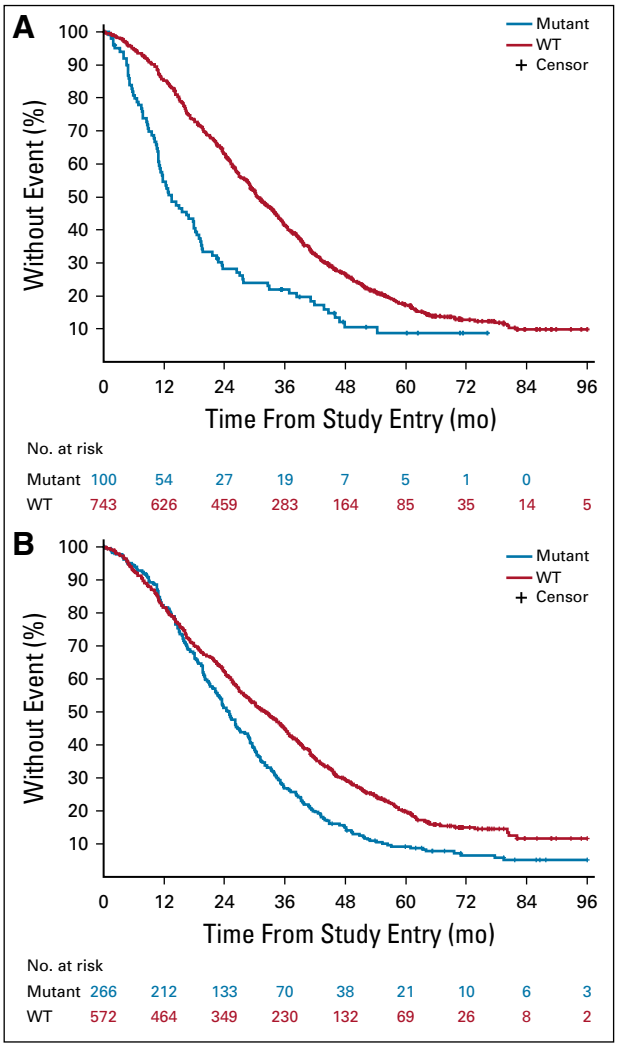

FIG 2. Kaplan-Meier plots of the effect of *BRAF* mutations (top) and extended *RAS* mutations (bottom) on overall survival. Log-rank *P* values are reported from an unadjusted analysis. The results refer to all patients for whom mutational analysis was available, which includes pre-*KRAS* amendment and post-*KRAS* amendment patients. WT, wild type.

patients in the MSS group had a high TMB (93 and 361 mutations/Mb) and were excluded from the analysis. In patients with MSS tumors, on the basis of an optimized TMB cutoff of eight mutations/Mb, there were 366 patients with eight or fewer mutations/Mb in their tumors who were classified as TMB low (77%), and the other 107 patients with more than eight mutations/Mb in their tumors were classified as TMB high (23%). No major differences in patient and tumor characteristics were noted between patients with TMB-high and TMB-low tumors (Appendix Table A4, online only). Patients with TMB-high tumors had a median OS of 33.8 months (95% CI, 30.1 to 43.1 months), and patients with TMB-low tumors had a median OS of 28.1 months (95% CI, 24.9 to 31.8 months; HR, 0.73 [95% CI, 0.57 to 0.95]; $P = .020$; Fig 4). Removing tumor location from the model does not affect this result (Table 2). No difference in OS was observed between the two treatment arms (primary cohort) on the basis of TMB status (interaction $P = .848$; Appendix Table A3). An inverse correlation was observed between TMB and HRs (Appendix Fig A3). The results of these associations with OS and PFS when including only patients in the primary cohort are reported in Appendix Table A3. Mutations in *APC, PIK3CA*, and *TP53* (mutations in *FBXW7* were not included because of their low prevalence) were not associated with outcome ($P > .05$, results can be provided on request).

DISCUSSION

In CALGB/SWOG 80405, the presence or absence of MSI-H, high-TMB, *BRAF*, and extended *RAS* mutations define patient subgroups with different outcomes. To our knowledge, this study provides the first indication that TMB correlates with the prognosis of patients with CRC with MSS tumors. TMB, the cumulative number of somatic DNA point mutations, describes the level of genomic instability in each patient's tumor. Patients with nonhypermutated MSS tumors (93% of patients in our study) had different OS on the basis of whether their TMB was classified as high or low. Patients with a high TMB had better OS than did patients with a low TMB (Fig 4). A high TMB probably reflects the presence of mutation-associated neoantigens, with consequent increased lymphocyte infiltration in the tumor microenvironment. This phenomenon has been observed even in MSS tumors.[5] A subset of MSS tumors are immunogenic, and patients with that subgroup of tumors have a different risk of relapse. In stage I to III CRCs, up to 50% of MSS tumors have a high immunoscore.[6] An elevated neoantigen load and/or high immunoscore was associated with better survival.[5,6] It has also been demonstrated that 16% of nonhypermutated MSS tumors have an immunophenotype similar to that of MSI-H tumors.[7] In our study, detecting a high TMB in patients with stage IV MSS might identify tumors with a favorable immune microenvironment.

In patients with MSS mCRC in this study, the effect of TMB on OS is not as strong as other markers (eg, *BRAF*), and its relevance to direct therapy is still to be determined. Because the relevance of TMB in this setting is more biologic than clinical, additional analyses should evaluate which TMB-high MSS tumors are immunogenic and which mutations give rise to neoantigens that elicit an antitumoral, adaptive response.

With the recent US Food and Drug Administration approval of checkpoint inhibitors for the treatment of patients with MSI-H mCRC refractory to standard chemotherapy, MSI-H testing is now requested routinely. In our study, MSI-H status did not confer a prognostic effect after multivariable adjustment. It should be noted that patients with MSI-H tumors, when not adjusted by all the other covariables in

TABLE 2. Effect of MSI Status, TMB, *BRAF* Mutations, and Extended *RAS* Mutations on OS and PFS

Molecular Marker		PFS				OS			
Patient Subgroup	**No. of Patients**	**Median, months (95% CI)**	**HR (95% CI)**	***P***	**Interaction *P***	**Median, months (95% CI)**	**HR (95% CI)**	***P***	**Interaction *P***
BRAF					.183				.107
BRAF mutant	100	7.6 (6.3 to 8.6)	1.72 (1.30 to 2.29)	< .001		13.5 (11.2 to 18.5)	2.01 (1.49 to 2.71)	< .001	
			1.75 (1.33 to 2.30)*	< .001			2.26 (1.70 to 3.01)*	< .001	
BRAF WT	743	11.4 (10.9 to 12.6)	1 (reference)			30.6 (29.0 to 33.5)	1 (reference)		
BRAF mutant									
Bevacizumab and cetuximab	26	8.6 (7.5 to 10.1)	1.03 (0.57 to 1.86)	.917		16.4 (10.4 to 26.4)	1.38 (0.75 to 2.54)	.307	
Bevacizumab	41	7.6 (5.7 to 9.6)	0.58 (0.33 to 1.02)	.060		15.0 (11.8 to 23.7)	0.67 (0.37 to 1.20)	.176	
Cetuximab	33	6.2 (3.7 to 8.6)	1 (reference)			11.7 (8.6 to 19.7)	1 (reference)		
BRAF WT									
Bevacizumab and cetuximab	181	12.1 (10.2 to 13.2)	0.95 (0.77 to 1.18)	.650		28.3 (24.2 to 34.7)	1.11 (0.88 to 1.39)	.373	
Bevacizumab	274	12.4 (11.1 to 13.4)	0.90 (0.74 to 1.09)	.270		32.0 (29.2 to 35.1)	1.02 (0.83 to 1.25)	.877	
Cetuximab	288	10.9 (9.5 to 12.5)	1 (reference)			30.9 (28.1 to 35.2)	1 (reference)		
Extended *RAS*					.716				.658
RAS mutant	266	9.8 (9.2 to 11.1)	1.36 (1.13 to 1.62)	< .001		25.0 (22.6 to 28.1)	1.52 (1.26 to 1.84)	< .001	
			1.37 (1.15 to 1.63)*	< .001			1.60 (1.33 to 1.92)*	< .001	
RAS WT	572	11.1 (10.2 to 12.4)	1 (reference)			32.1 (28.9 to 35.5)	1 (reference)		
Extended *RAS* mutant									
Bevacizumab and cetuximab	74	9.3 (7.4 to 11.6)	0.93 (0.65 to 1.33)	.689		21.7 (17.7 to 27.2)	1.13 (0.79 to 1.61)	.509	
Bevacizumab	98	12.0 (10.5 to 14.8)	0.77 (0.56 to 1.06)	.110		28.1 (25.0 to 31.2)	1.07 (0.77 to 1.48)	.707	
Cetuximab	94	9.2 (7.8 to 10.9)	1 (reference)			23.6 (20.2 to 30.0)	1 (reference)		
Extended *RAS* WT									
Bevacizumab and cetuximab	131	12.2 (10.2 to 13.7)	0.99 (0.77 to 1.26)	.912		28.4 (24.2 to 36.6)	1.16 (0.89 to 1.51)	.273	
Bevacizumab	217	11.1 (10.0 to 13.1)	0.90 (0.73 to 1.12)	.359		33.3 (29.2 to 36.9)	0.91 (0.72 to 1.16)	.459	
Cetuximab	224	10.9 (9.3 to 12.8)	1 (reference)			31.5 (26.8 to 39.5)	1 (reference)		
MSI					< .001				< .001
MSI-H	52	6.6 (5.7 to 8.6)	1.02 (0.71 to 1.47)	.912		21.5 (14.8 to 26.9)	0.87 (0.60 to 1.28)	.491	
			1.03 (0.72 to 1.47)*	.875			0.93 (0.63 to 1.36)*	.698	
MSS	775	11.0 (10.3 to 11.8)	1 (reference)			29.5 (27.2 to 31.7)	1 (reference)		
MSI-H									
Bevacizumab and cetuximab	15	7.7 (6.6 to 17.6)	0.44 (0.20 to 0.99)	.046		21.5 (16.4 to 41.1)	0.37 (0.16 to 0.85)	.018	
Bevacizumab	21	9.3 (5.4 to 29.0)	0.16 (0.07 to 0.37)	< .001		30.0 (23.6 to NE)	0.13 (0.06 to 0.30)	< .001	
Cetuximab	16	5.4 (4.1 to 8.6)	1 (reference)			11.9 (10.3 to 24.6)	1 (reference)		

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TABLE 2. Effect of MSI Status, TMB, *BRAF* Mutations, and Extended *RAS* Mutations on OS and PFS (continued)

Molecular Marker		PFS				OS			
Patient Subgroup	No. of Patients	Median, months (95% CI)	HR (95% CI)	*P*	Interaction *P*	Median, months (95% CI)	HR (95% CI)	*P*	Interaction *P*
MSS									
Bevacizumab and cetuximab	189	10.9 (9.8 to 12.8)	0.98 (0.80 to 1.21)	.881		26.2 (22.6 to 29.7)	1.18 (0.95 to 1.47)	.134	
Bevacizumab	285	11.2 (10.3 to 12.5)	0.93 (0.77 to 1.12)	.439		30.3 (27.3 to 34.3)	1.06 (0.87 to 1.29)	.539	
Cetuximab	301	10.9 (9.5 to 12.8)	1 (reference)			30.7 (27.6 to 35.0)	1 (reference)		
TMB					.823				.799
> 8	107	12.3 (9.7 to 15.8)	0.91 (0.72 to 1.16)	.457		33.8 (30.1 to 43.1)	0.73 (0.57 to 0.95)	.020	
			0.91 (0.72 to 1.16)*	.461			0.72 (0.56 to 0.94)*	.015	
≤ 8	366	10.9 (10.2 to 12.4)	1 (reference)			28.1 (24.9 to 31.8)	1 (reference)		
TMB high (> 8)									
Bevacizumab and cetuximab	31	12.4 (9.1 to 19.2)	0.93 (0.55 to 1.59)	.799		33.0 (19.8 to 49.5)	1.13 (0.63 to 2.01)	.685	
Bevacizumab	40	12.4 (9.5 to 20.2)	0.84 (0.50 to 1.39)	.490		34.2 (29.7 to 51.5)	0.86 (0.49 to 1.52)	.610	
Cetuximab	36	11.1 (7.8 to 16.4)	1 (reference)			35.8 (23.7 to 70.9)	1 (reference)		
TMB low (≤ 8)									
Bevacizumab and cetuximab	81	10.9 (9.3 to 13.7)	1.07 (0.77 to 1.49)	.688		24.1 (19.7 to 35.6)	1.22 (0.86 to 1.72)	.263	
Bevacizumab	152	11.0 (10.0 to 13.2)	1.00 (0.77 to 1.30)	.993		30.3 (25.3 to 34.3)	1.07 (0.80 to 1.42)	.662	
Cetuximab	133	10.9 (9.2 to 12.8)	1 (reference)			26.5 (23.5 to 35.8)	1 (reference)		

NOTE. The results refer to all patients for whom mutational analysis was available, which includes pre-*KRAS* amendment and post-*KRAS* amendment patients. The interaction *P* reports the statistical significance of interactions between the molecular alteration and the three arms in all patients by Wald test. The biologics are given in combination with chemotherapy.

BRAF analyses adjusted for age, arm, extended *RAS*, MSI, sex, ethnicity, No. of metastatic sites, synchronous or metachronous metastases, and tumor location.

Extended *RAS* analyses adjusted for age, arm, *BRAF*, MSI, sex, ethnicity, No. of metastatic sites, synchronous or metachronous metastases, and tumor location.

MSI analyses adjusted for age, arm, *BRAF*, *RAS*, sex, ethnicity, No. of metastatic sites, synchronous or metachronous metastases, and tumor location.

TMB analyses adjusted for age, arm, *BRAF*, extended *RAS*, sex, ethnicity, No. of metastatic sites, synchronous or metachronous metastases, and tumor location.

Abbreviations: HR, hazard ratio; MSI, microsatellite instability; MSI-H, microsatellite instability–high; MSS, microsatellite stable; OS, overall survival; PFS, progression-free survival; TMB, tumor mutational burden; WT, wild type.

*Tumor location not included as adjustment factor in this model.

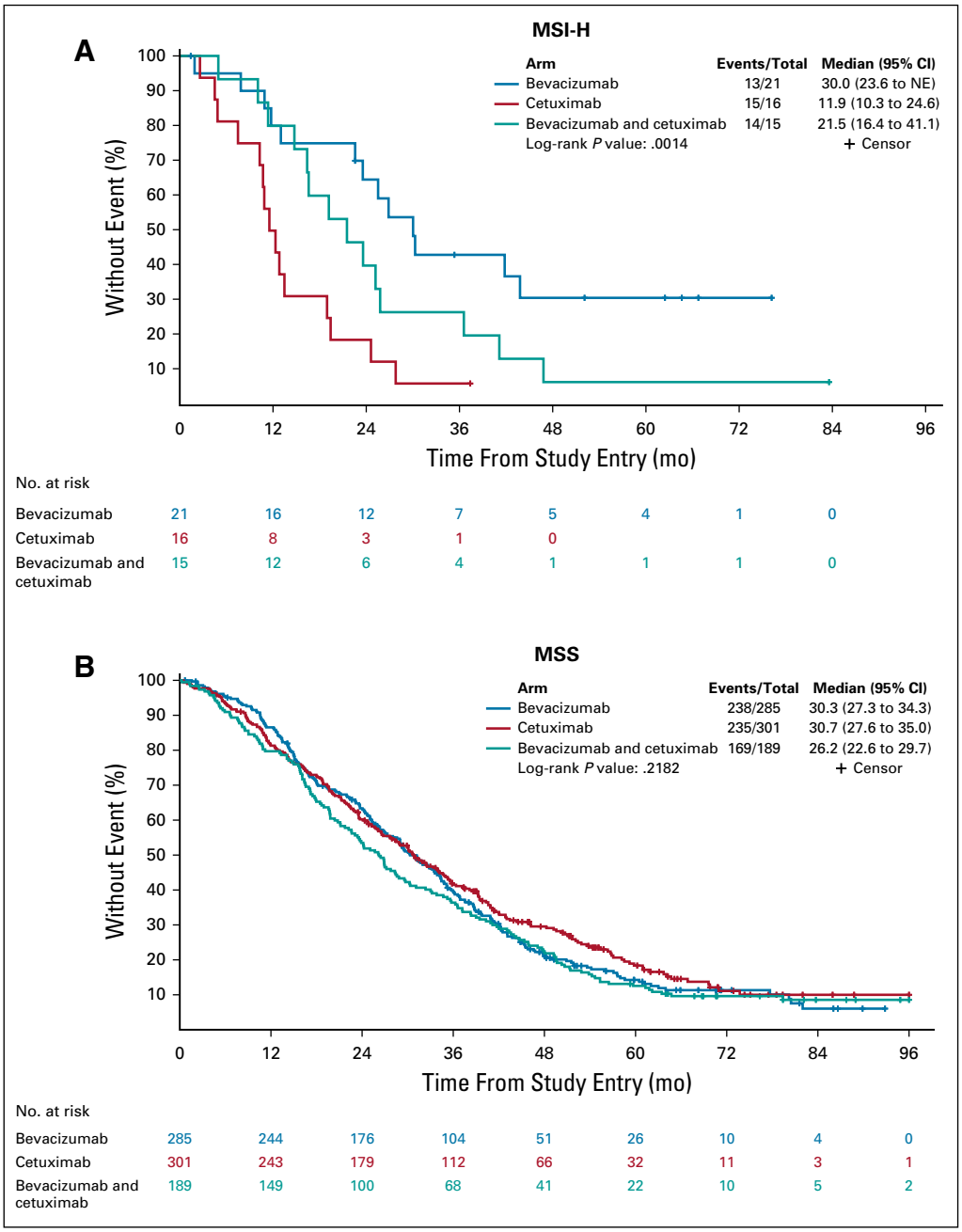

FIG 3. Kaplan-Meier plots of the effect of microsatellite status on overall survival on the basis of treatment arm (microsatellite instability–high [MSI-H], top; microsatellite stable [MSS], bottom). Log-rank *P* values are reported from an unadjusted analysis. The results refer to all patients for whom mutational analysis was available, which includes pre-*KRAS* amendment and post-*KRAS* amendment patients. The proportions of right and transverse and left tumors in MSI-H tumors are 79.2% and 20.8%, respectively.

the model, exhibited a trend to a worse outcome, suggesting that it may be a negative prognostic marker (median OS, 21.5 months [95% CI, 14.8 to 26.9 months] *v* 29.5 months [95% CI, 27.2 to 31.7 months] in patients with MSS tumors; $P = .087$). When patients with MSI-H tumors received chemotherapy and bevacizumab, this treatment conferred a survival advantage over chemotherapy and cetuximab (Fig 3). In the chemotherapy and bevacizumab arm, median OS was 30.0 months versus 11.9 months in the chemotherapy and cetuximab arm, almost a threefold difference. No difference between the two arms has been observed in the MSS group. The effect of MSI-H is observed in a small subset but is corroborated by several lines of evidence. A beneficial effect of FOLFOX and bevacizumab compared with FOLFOX and placebo was reported in patients with MSI-H tumors (and, again, not in patients with MSS tumors) with stage II to III disease.[8] This observation is important because the effect of bevacizumab is against placebo, pointing toward a predictive role of MSI-H. Patients with CMS1 tumors (enriched with MSI-H) show increased OS in the chemotherapy and bevacizumab arm over the chemotherapy and cetuximab arm.[9] The contribution of resistance to EGFR inhibitors in patients with MSI-H tumors cannot be excluded, as suggested by a reduced efficacy of cetuximab in tumors originating in the right or transverse colon (also enriched with MSI-H) compared with

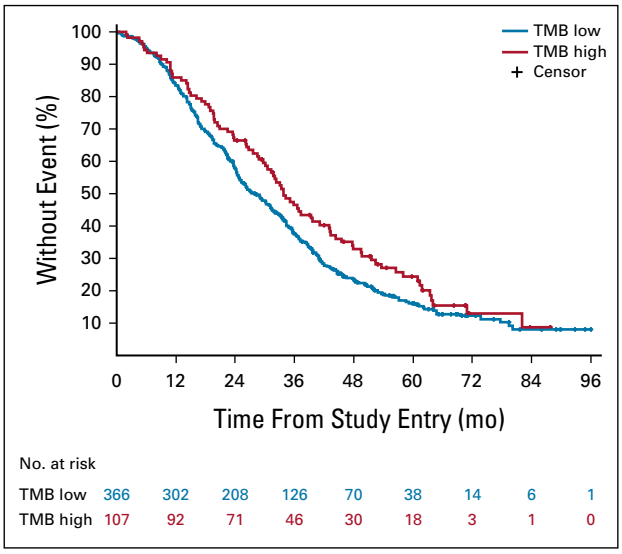

FIG 4. Kaplan-Meier plots of the effect of tumor mutational burden (TMB) status on overall survival. Log-rank *P* values are reported from an unadjusted analysis. The results refer to all patients for whom mutational analysis was available, which includes pre-*KRAS* amendment and post-*KRAS* amendment patients. In TMB-high tumors, the proportions of right and transverse and left tumors were 45.1% and 54.9%, respectively. In TMB-low tumors, the proportions of right and transverse and left tumors were 40.3% and 59.7%, respectively.

those tumors originating in the left colon.[10,11] It is also interesting to observe that in CALGB/SWOG 80405, the effect of the chemotherapy and bevacizumab and cetuximab arm on OS is intermediate between the arms with the single biologics (Fig 3).

What is the biologic underpinning of the effect of MSI-H on the activity of biologics? We hypothesize a concurrent effect on both cetuximab (negative) and bevacizumab (positive). For the effect on cetuximab, it is well established that hypermethylation typical of MSI-H tumors results in lower expression of EGFR ligands,[12] reducing the efficacy of EGFR inhibitors.[13,14] For the effect of bevacizumab, vessel normalization induced by bevacizumab correlated with immunostimulatory pathways, especially Th1 lymphocyte infiltration and activity,[15] possibly potentiating the antitumor effects of an already T-cell–infiltrated and activated microenvironment, such as that of MSI-H tumors.

This study represents one of the largest series on the negative prognostic effect of *BRAF* V600E in a single clinical trial in the first-line setting of mCRC. Although *BRAF* V600E is more prevalent in right and transverse (81%) versus left (19%) tumors, removal of tumor location as an adjusting covariable does not modify the effect of *BRAF* V600E HR by a great extent (HR from 2.01 to 2.26, Table 2), suggesting that *BRAF* V600E has an effect on survival that is independent of tumor location. By looking at the effect of treatment, despite a weak signal of improved OS in the chemotherapy and bevacizumab arm compared with the chemotherapy and cetuximab arm in patients with *BRAF* V600E tumors (HR, 0.67 [95% CI, 0.37 to 1.20]; $P = .176$; Appendix Fig A4; Table 2), median OS remains poor in both arms (15.0 and 11.7 months, respectively), as also reported in FIRE-3.[16]

The analysis of extended *RAS* clearly supports a negative prognostic effect. Patients with *RAS* mutant tumors have worse OS than do patients with *RAS* WT tumors, irrespective of treatment (Fig 2 [bottom]; Table 2). A detrimental effect of *RAS* mutations in patients enrolled in the chemotherapy and cetuximab arm compared with the chemotherapy and bevacizumab arm is more evident in the primary cohort for PFS (median, 9.2 *v* 11.4 months; $P = .006$) than for OS (median, 22.9 *v* 26.2 months; $P = .855$), probably because of the compensatory effects of postprogression therapies.[2]

One potential limitation of this article is that the mutational analysis was not available from all patients enrolled in the trial, particularly for TMB. This is a limitation for studies such as this one that use material from primary tumors retrospectively, because specimen collection might introduce bias.

In conclusion, molecular DNA analysis of tumor alterations in patients with mCRC can provide new tools to predict patient outcome and improve therapeutic decision making. Molecularly driven, novel immunotherapy-based combinations are urgently needed in patients with first-line MSS mCRC. In patients with MSI-H tumors, if the benefit of bevacizumab combined with chemotherapy is further confirmed, this regimen could be a preferable therapeutic option in patients with MSI-H, either as front-line therapy or in patients who are not responsive and/or suffer from clinical and financial toxicity of checkpoint inhibitors. Additional evaluation of the neoantigen-specific T-cell responses associated with TMB is needed to identify new targets and pathways and to guide the testing of targeted interventions. TMB can be obtained readily using a reliable and simple US Food and Drug Administration–approved diagnostic test that is often used in the clinic.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST AND DATA AVAILABILITY STATEMENT

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Mutational Analysis of Patients With Colorectal Cancer in CALGB/SWOG 80405 Identifies New Roles of Microsatellite Instability and Tumor Mutational Burden for Patient Outcome

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APPENDIX

The proportional hazard assumption was examined by the Kolmogorov-type supremum test and graphical method using weighted residuals (Grambsch PM, Therneau TM: Biometrika 81:515-526, 1994). On the basis of the Kolmogorov-type supremum test, microsatellite instability (MSI) status and *BRAF* status violated the proportional hazard assumption. After closer examination of the weighted residual figures (Fig A5), it seems that the nonproportionality for MSI status is probably inconsequential because the confidence band for the time-varying coefficient covers 0 at all time and we reported a nonsignificant prognostic effect (Fig A5A). For *BRAF*, it does seem that there is a slightly diminished effect over time (Fig A5A and B). The negative prognostic effect is strong for the first 2 years after registration but then diminishes toward the null after 2 years. Given that the hazard ratio (HR) is still the most widely accepted method of reporting time-to-event end points and the fact that the effect never crosses 0 (ie, maintaining a negative prognostic effect), we reported the HR for *BRAF* in this article. When nonproportionality exists, the HR is simply an average of the time-varying effect over time; therefore, the HR may be pulled toward the null. Even so, because *BRAF* is a known negative prognostic factor, the HR has still a big effect size. Therefore, we decided to report the HR because it is easier to understand than a time-varying coefficient.

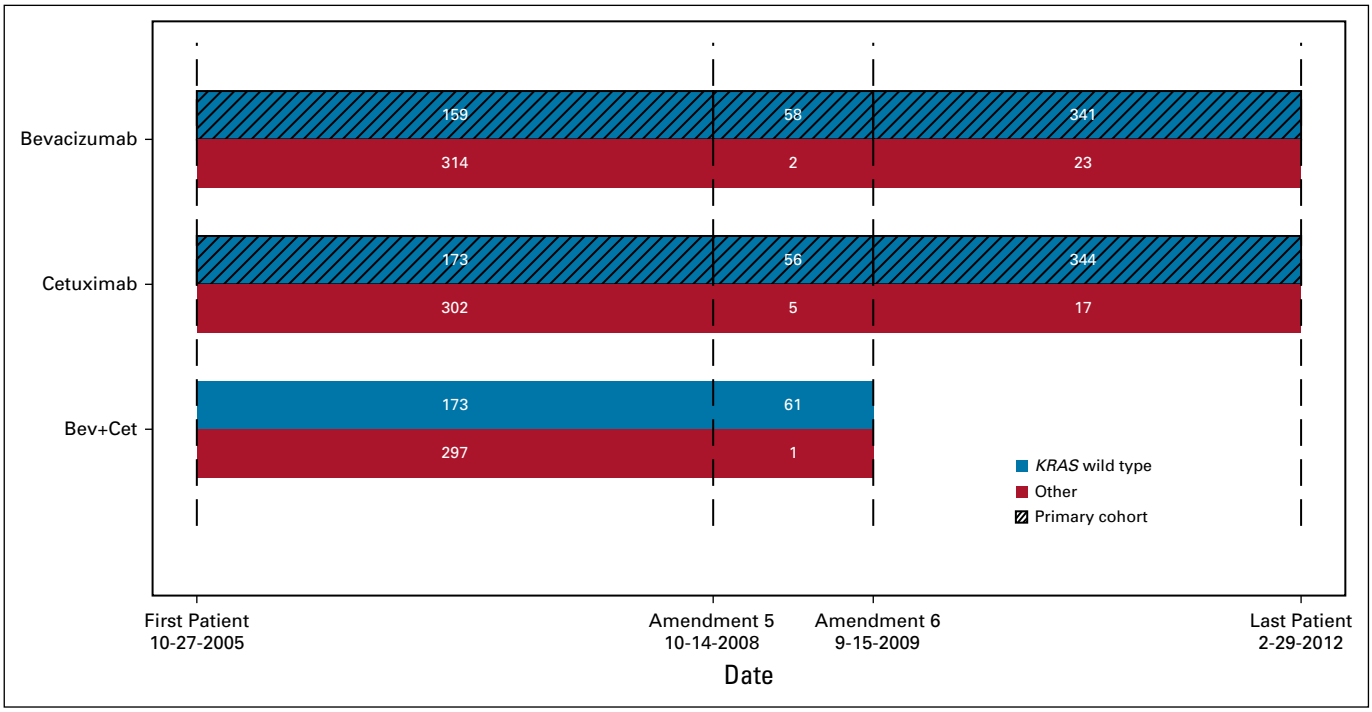

FIG A1. Flow chart of patients by arm, *KRAS* status, and amendments (amendment 5: to enroll only *KRAS* WT for codon 12/13, amendment 6: to stop the bevacizumab-cetuximab [Bev+Cet] combination arm).

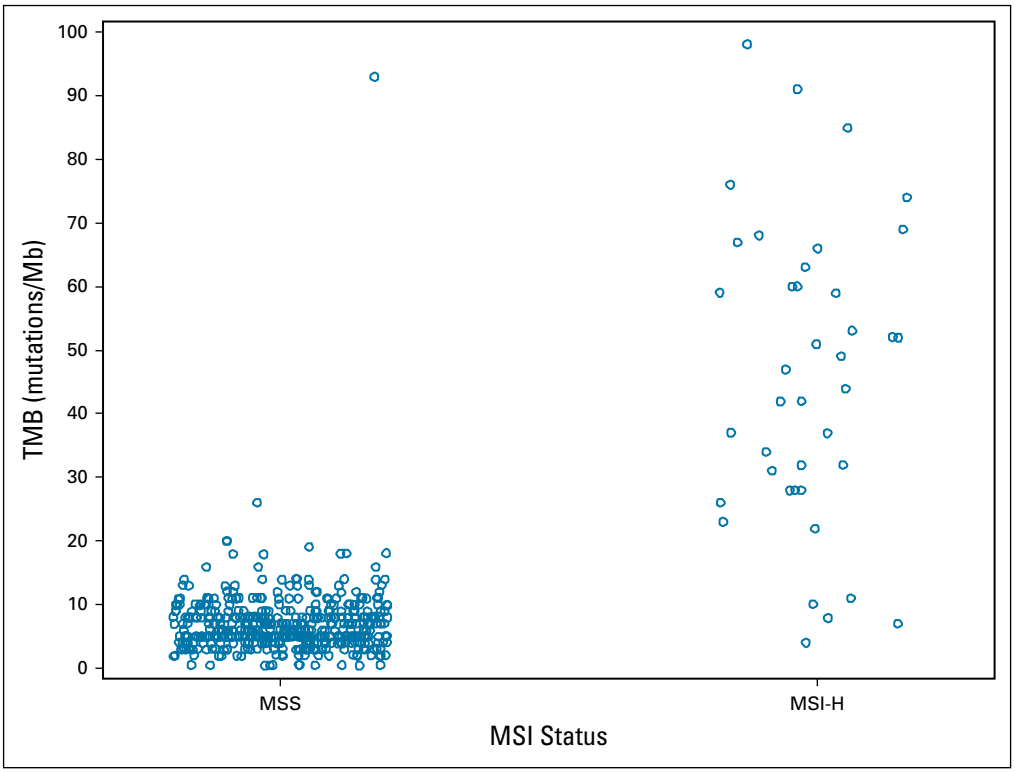

FIG A2. Tumor mutational burden (TMB) values by microsatellite instability (MSI) status. One hypermuytated outlier in the microsatellite stable (MSS) group (TMB=361) and one hypermutated outlier in the microsatellite instability–high (MSI-H) group (TMB=208) are not shown in the figure.

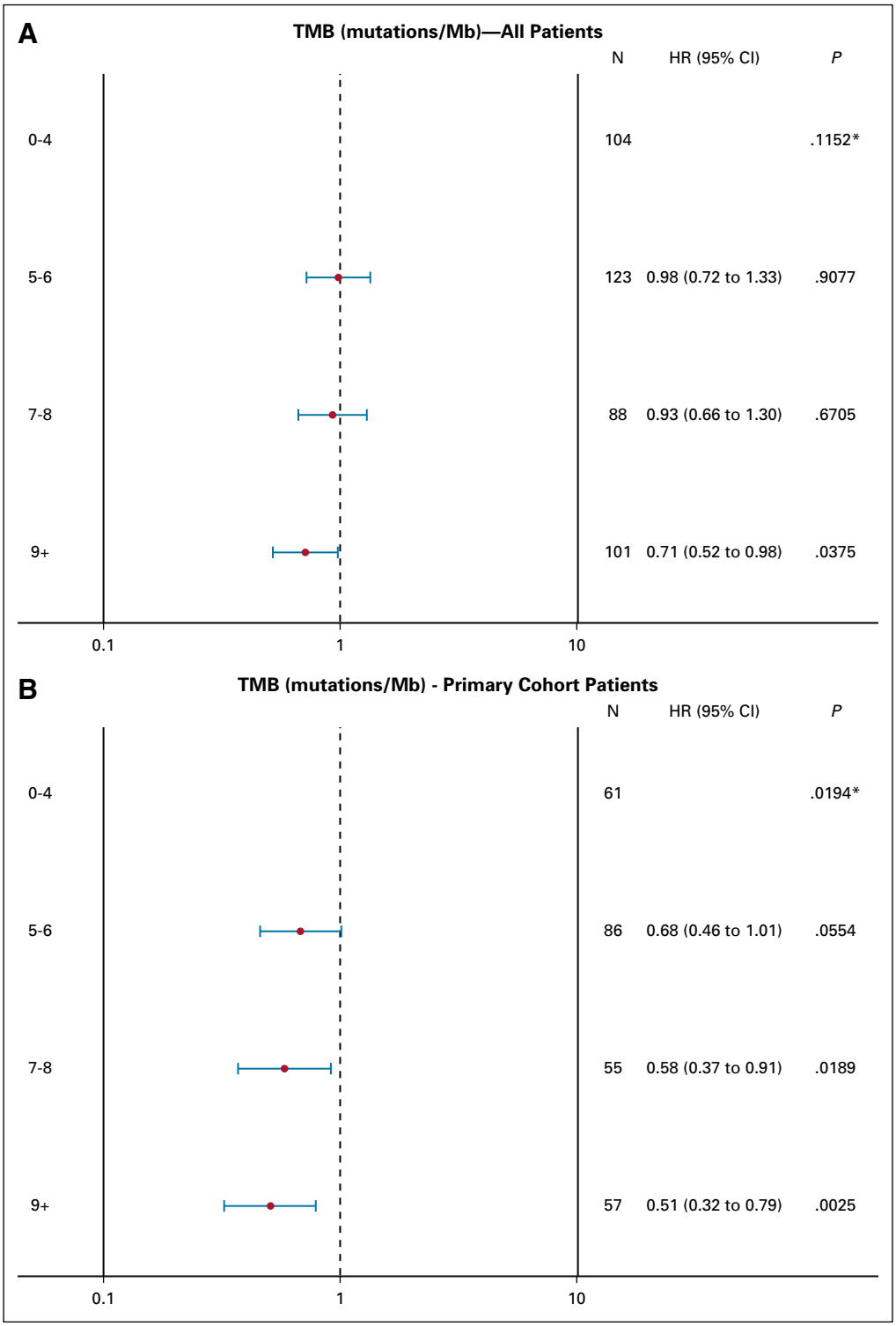

FIG A3. Inverse correlation between tumor mutational burden (TMB) and hazard ratio (HR) of overall survival (A) in all patients and (B) in the primary cohort patients. (*) A 3-degree-of-freedom test for detecting whether there is any difference in outcome across different TMB levels.

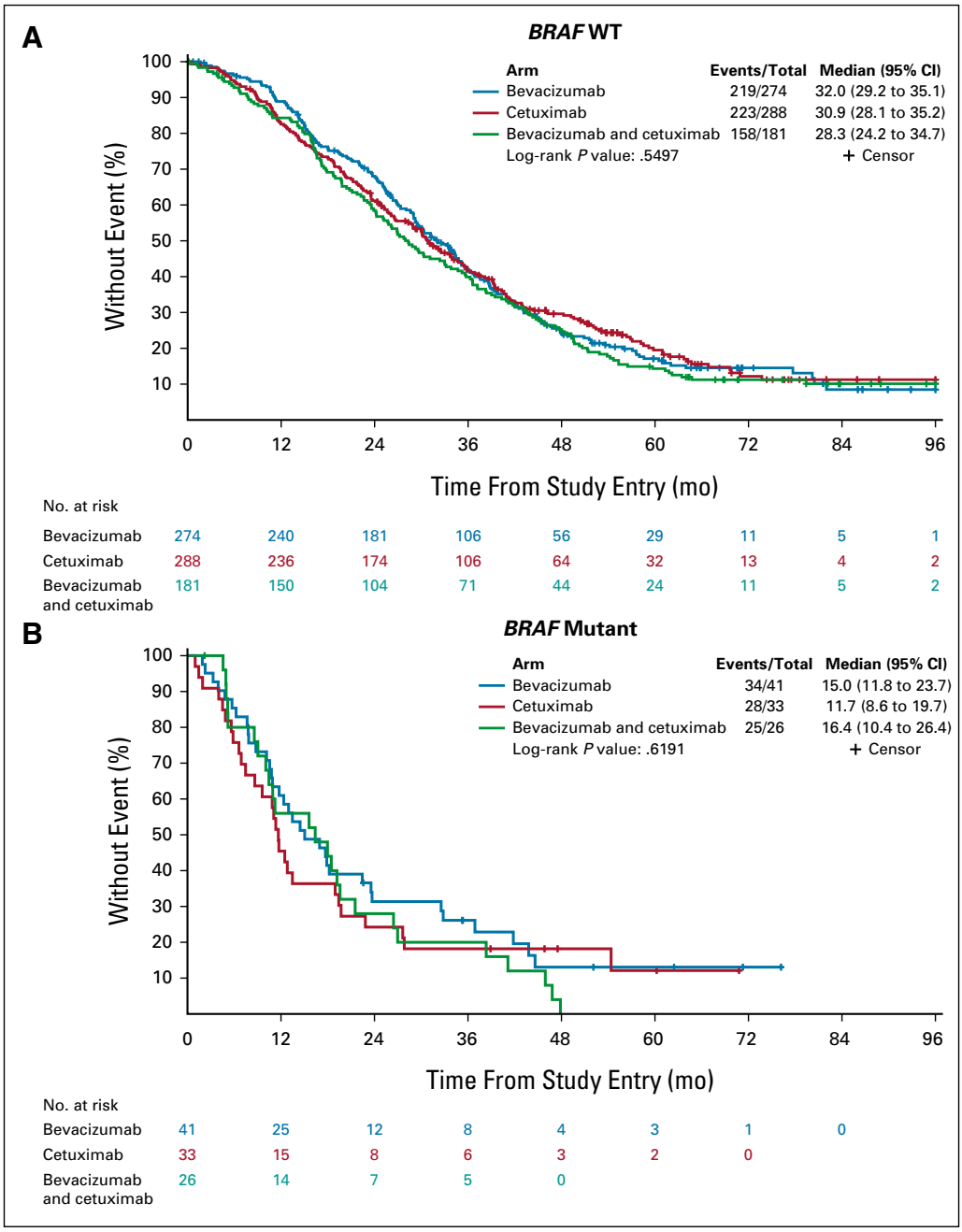

FIG A4. Kaplan-Meier plots of the effect of *BRAF* mutations on overall survival based upon treatment arm: (A) *BRAF* wild type (WT) or (B) *BRAF* mutant. Almost all *BRAF* mutations are V600E except for two patients. Log-rank *P* values are reported from an unadjusted analysis. The results refer to all patients from whom mutational analysis was available, which includes pre-*KRAS* amendment and post-*KRAS* amendment patients.

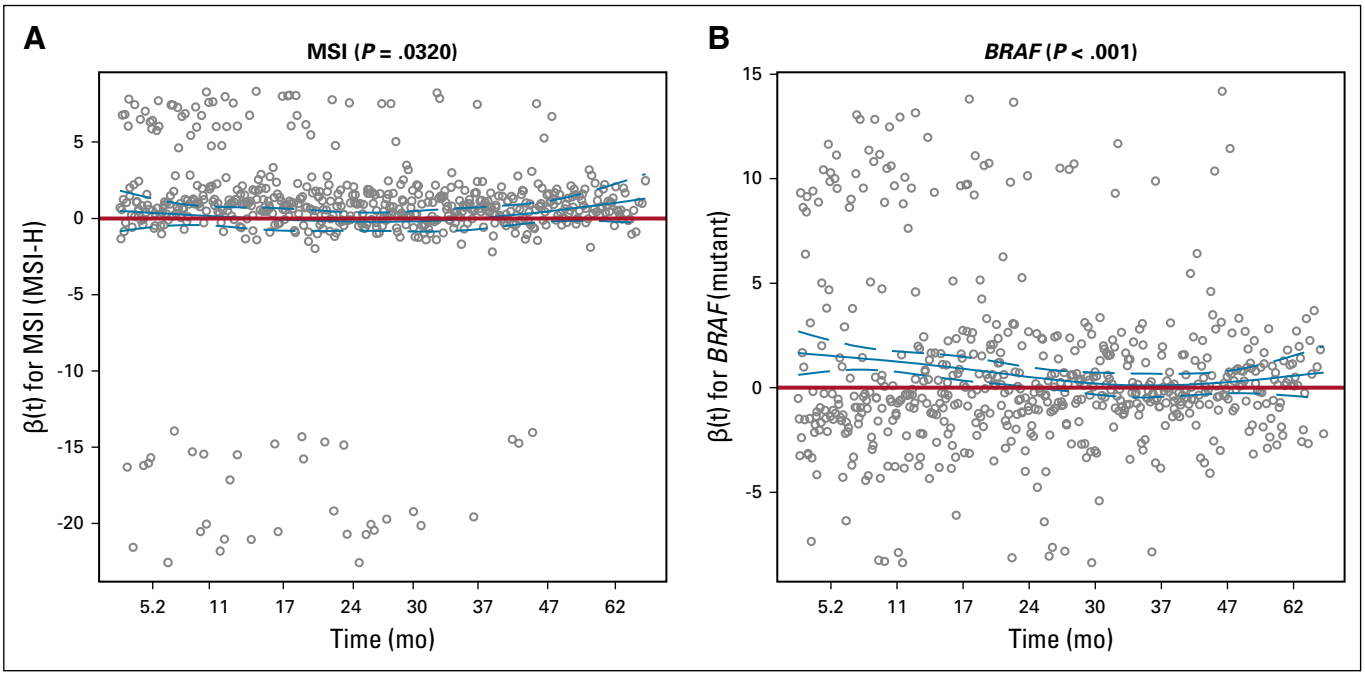

FIG A5. Smoothed scaled Schoenfeld residual plots. MSI, microsatellite instability; MSI-H, microsatellite instability–high.

TABLE A1. Somatic Mutations: Type and Frequency

Mutation	No. (%)
AKT	
Mutation status	
Missing	1 (0.1)
WT	842 (99.9)
Point mutation	
E17K	WT
Missing	1 (0.1)
APC	
Mutation status	
Mutant	139 (16.5)
WT	704 (83.5)
Point mutation	
Q1367X	7 (0.8)
Q1367X/R1450X	1 (0.1)
R1450X	30 (3.6)
R213X	32 (3.8)
R213X/R1450X	2 (0.2)
R302X	12 (1.4)
R564X	13 (1.5)
R564X/Q1367X	1 (0.1)
R876X	39 (4.6)
R876X/R1450X	2 (0.2)
BRAF	
Mutation status	
Mutant	100 (11.9)
WT	743 (88.1)
Point mutation	
K601E	1 (0.1)
V600E	98 (11.6)
V600M	1 (0.1)
K205Q	WT
F247L	WT
A305V	WT
V600K	WT
V600L	WT
V600Q	WT
K601E	WT
A718V	WT
CTNNB1	
Mutation status	
Missing	5 (0.6)
Mutant	7 (0.8)
WT	831 (98.6)

(continued in next column)

TABLE A1. Somatic Mutations: Type and Frequency (continued)

Mutation	No. (%)
Point mutation	
S45F	5 (0.6)
S45P	WT
T41A	2 (0.2)
S33Y	WT
Missing	5 (0.6)
EGFR	
Mutation status	
Missing	61 (7.2)
Mutant	2 (0.2)
WT	780 (92.5)
Point mutation	
R671C	1 (0.1)
S768T	1 (0.1)
E114K	WT
R165Q	WT
S492R	WT
G719X	WT
G724S	WT
Del(30)	WT
Ins(5)	WT
S768G	WT
S768I	WT
T790M	WT
L858R	WT
L861Q	WT
Missing	61 (7.2)
FBXW7	
Mutation status	
Missing	11 (1.3)
Mutant	31 (3.7)
WT	801 (95.0)
Point mutation	
R367X	1 (0.1)
R465C	2 (0.2)
R465H	7 (0.8)
R479Q	8 (0.9)
R505C	8 (0.9)
S582L	5 (0.6)
R367X	WT
Missing	11 (1.3)

(continued on following page)

TABLE A1. Somatic Mutations: Type and Frequency (continued)

Mutation	No. (%)
HRAS	
Mutation status	
Missing	10 (1.2)
WT	833 (98.8)
Point mutation	
G12S	WT
G13S	WT
Missing	10 (1.2)
KRAS	
Mutation status	
Missing	5 (0.6)
Mutant	238 (28.2)
WT	600 (71.2)
Point mutation	
A146T	19 (2.3)
A146V	7 (0.8)
G12A	14 (1.7)
G12C	15 (1.8)
G12C/Q61R	10 (1.2)
G12D	62 (7.4)
G12F	1 (0.1)
G12R	6 (0.7)
G12S	9 (1.1)
G12S/Q61K	3 (0.4)
G12V	38 (4.5)
G13A	WT
G13C	7 (0.8)
G13D	35 (4.2)
G13R	2 (0.2)
G13S	2 (0.2)
G13S/Q61HC	2 (0.2)
K117N	2 (0.2)
K146T	WT
K146V	WT
L19F	1 (0.1)
Q22K	2 (0.2)
Q22R	WT
Q61HC	1 (0.1)
Q61L	WT
Q61Ht	WT
Q61K	WT
Q61R	WT
R68S	WT
Missing	5 (0.6)

(continued in next column)

TABLE A1. Somatic Mutations: Type and Frequency (continued)

Mutation	No. (%)
MET	
Mutation status	
Missing	3 (0.4)
Mutant	55 (6.5)
WT	785 (93.1)
Point mutation	
N375S	31 (3.7)
T1010I	24 (2.8)
Y1248C	WT
Y1253D	WT
Missing	3 (0.4)
NRAS	
Mutation status	
Mutant	29 (3.4)
WT	814 (96.6)
Point mutation	
G12X	2 (0.2)
G13X	5 (0.6)
Q61K	14 (1.7)
Q61R	8 (0.9)
G12A	WT
G12C	WT
G12R	WT
G12S	WT
G12V	WT
G12D	WT
G13A	WT
G13C	WT
G13D	WT
G13R	WT
G13V	WT
Q61L	WT
Q61P	WT
Q61Hc	WT
Q61Ht	WT
PIK3CA	
Mutation status	
Missing	3 (0.4)
Mutant	93 (11.0)
WT	747 (88.6)
Point mutation	
C420R	2 (0.2)
E542K	17 (2.0)

(continued on following page)

TABLE A1. Somatic Mutations: Type and Frequency (continued)

Mutation	No. (%)
E542K/H1047X	1 (0.1)
E545A	WT
E545X	25 (3.0)
E545D	WT
E545G	WT
E545K	WT
E982G	WT
F909L	WT
G1049R	3 (0.4)
H1047L	WT
H1047R	WT
H1047X	24 (2.8)
H1047Y	WT
K111E	1 (0.1)
K111N/C420R	1 (0.1)
M1043I	1 (0.1)
M1043V	WT
N345K	2 (0.2)
Q546K	WT
Q546R	WT
Q546E	WT
Q546L	WT
Q546X	3 (0.4)
R108H/C420R	1 (0.1)
R38C	3 (0.4)
R88Q	6 (0.7)
T1025A	WT
Y1021C	3 (0.4)
R108H	WT
Missing	3 (0.4)
TP53	
Mutation status	
Mutant	257 (30.5)
WT	586 (69.5)
Point mutation	
C176F	3 (0.4)
G245S	21 (2.5)
R175H	57 (6.8)
R175H/R248W	1 (0.1)
R196X	14 (1.7)
R196X/R282W	1 (0.1)
R213X	15 (1.8)
R213X/R282W	1 (0.1)
R248Q	34 (4.0)
R248Q/R273C	1 (0.1)
R248Q/R273H	1 (0.1)
R248W	18 (2.1)
R248W/R282W	1 (0.1)
R273C	26 (3.1)
R273H	33 (3.9)
R282W	29 (3.4)

Abbreviation: WT, wild type.

TABLE A2. Gene List of the FoundationOne Platform

Entire Coding Sequence	Selected Rearrangements
ABL1	ALK
ABL2	BCL2
ACVR1B	BCR
AKT1	BRAF
AKT2	BRCA1
AKT3	BRCA2
ALK	BRD4
ALOX12B	EGFR
AMER1 (FAM123B)	ETV1
APC	ETV4
APCDD1	ETV5
AR	ETV6
ARAF	EWSR1
ARFRP1	FGFR1
ARID1A	FGFR2
ARID1B	FGFR3
ARID2	KIT
ASXL1	KMT2A (MLL)
ATM	MSH2
ATR	MYB
ATRX	MYC
AURKA	NOTCH2
AURKB	NTRK1
AXIN1	NTRK2
AXL	PDGFRA
BACH1	RAF1
BAP1	RARA
BARD1	RET
BCL2	ROS1
BCL2A1	RSPO2
BCL2L1	TMPRSS2
BCL2L2	
BCL6	
BCOR	
BCORL1	
BLM	
BMPR1A	
BRAF	
BRCA1	
BRCA2	
BRD4	
BRIP1	
BTG1	

(continued in next column)

TABLE A2. Gene List of the FoundationOne Platform (continued)

Entire Coding Sequence	Selected Rearrangements
BTK	
C11orf30 (EMSY)	
CARD11	
CASP8	
CBFB	
CBL	
CCND1	
CCND2	
CCND3	
CCNE1	
CD274	
CD79A	
CD79B	
CDC73	
CDH1	
CDH2	
CDH20	
CDH5	
CDK12	
CDK4	
CDK6	
CDK8	
CDKN1A	
CDKN1B	
CDKN2A	
CDKN2B	
CDKN2C	
CEBPA	
CHD2	
CHD4	
CHEK1	
CHEK2	
CHUK	
CIC	
CRBN	
CREBBP	
CRKL	
CRLF2	
CSF1R	
CTCF	
CTNNA1	
CTNNB1	
CUL3	

(continued on following page)

TABLE A2. Gene List of the FoundationOne Platform (continued)

Entire Coding Sequence	Selected Rearrangements
CUL4A	
CUL4B	
CYLD	
CYP17A1	
DAXX	
DDR1	
DDR2	
DICER1	
DIS3	
DNMT3A	
DOT1L	
EGFR	
EP300	
EPHA3	
EPHA5	
EPHA6	
EPHA7	
EPHB1	
EPHB4	
EPHB6	
ERBB2	
ERBB3	
ERBB4	
ERCC4	
ERG	
ERRFI1	
ESR1	
EZH2	
FAM175A	
FAM46C	
FANCA	
FANCC	
FANCD2	
FANCE	
FANCF	
FANCG	
FANCI	
FANCL	
FANCM	
FAS	
FAT1	
FAT3	
FBXW7	

(continued in next column)

TABLE A2. Gene List of the FoundationOne Platform (continued)

Entire Coding Sequence	Selected Rearrangements
FGF10	
FGF12	
FGF14	
FGF19	
FGF23	
FGF3	
FGF4	
FGF6	
FGF7	
FGFR1	
FGFR2	
FGFR3	
FGFR4	
FH	
FLCN	
FLT1	
FLT3	
FLT4	
FOXL2	
FOXP1	
FRS2	
FUBP1	
GABRA6	
GALNT12	
GATA1	
GATA2	
GATA3	
GATA4	
GATA6	
GEN1	
GID4 (C17orf39)	
GLI1	
GNA11	
GNA13	
GNAQ	
GNAS	
GPR124	
GREM1	
GRIN2A	
GRM3	
GSK3B	
H3F3A	
HGF	

(continued on following page)

TABLE A2. Gene List of the FoundationOne Platform (continued)

Entire Coding Sequence	Selected Rearrangements
HLA-A	
HLA-B	
HLA-C	
HNF1A	
HOXB13	
HRAS	
HSD3B1	
HSP90AA1	
IDH1	
IDH2	
IGF1	
IGF1R	
IGF2	
IGF2R	
IKBKE	
IKZF1	
IL7R	
INHBA	
INPP4B	
INSR	
IRF2	
IRF4	
IRS2	
JAK1	
JAK2	
JAK3	
JUN	
KAT6A (MYST3)	
KDM5A	
KDM5C	
KDM6A	
KDR	
KEAP1	
KEL	
KIT	
KLHL6	
KMT2A (MLL)	
KMT2C (MLL3)	
KMT2D (MLL2)	
KRAS	
LMO1	
LRP1B	
LRP6	

(continued in next column)

TABLE A2. Gene List of the FoundationOne Platform (continued)

Entire Coding Sequence	Selected Rearrangements
LTK	
LYN	
LZTR1	
MAGI2	
MAP2K1	
MAP2K2	
MAP2K4	
MAP3K1	
MAP3K13	
MCL1	
MDM2	
MDM4	
MED12	
MEF2B	
MEN1	
MERTK	
MET	
MITF	
MKNK1	
MKNK2	
MLH1	
MPL	
MRE11A	
MSH2	
MSH6	
MST1R	
MTOR	
MUTYH	
MYC	
MYCL (MYCL1)	
MYCN	
MYD88	
NBN	
NCOR1	
NF1	
NF2	
NFE2L2	
NFKBIA	
NKX2-1	
NOTCH1	
NOTCH2	
NOTCH3	
NOTCH4	

(continued on following page)

TABLE A2. Gene List of the FoundationOne Platform (continued)

Entire Coding Sequence	Selected Rearrangements
NPM1	
NRAS	
NSD1	
NTRK1	
NTRK2	
NTRK3	
NUDT1	
NUP93	
PAK3	
PAK7	
PALB2	
PARK2	
PARP1	
PARP2	
PARP3	
PARP4	
PAX5	
PBRM1	
PDCD1LG2	
PDGFRA	
PDGFRB	
PDK1	
PHLPP2	
PIK3C2B	
PIK3C2G	
PIK3C3	
PIK3CA	
PIK3CB	
PIK3CG	
PIK3R1	
PIK3R2	
PLCG2	
PMS2	
PNRC1	
POLD1	
POLE	
PPARG	
PPP2R1A	
PRDM1	
PREX2	
PRKAR1A	
PRKCI	
PRKDC	

(continued in next column)

TABLE A2. Gene List of the FoundationOne Platform (continued)

Entire Coding Sequence	Selected Rearrangements
PRSS1	
PRSS8	
PTCH1	
PTCH2	
PTEN	
PTPN11	
PTPRD	
QKI	
RAC1	
RAD50	
RAD51	
RAD51B (RAD51L1)	
RAD51C	
RAD51D (RAD51L3)	
RAD52	
RAD54L	
RAF1	
RANBP2	
RARA	
RB1	
RBM10	
REL	
RET	
RICTOR	
RNF43	
ROS1	
RPA1	
RPTOR	
RUNX1	
RUNX1T1	
SDHA	
SDHB	
SDHC	
SDHD	
SETD2	
SF3B1	
SH2B3	
SLIT2	
SMAD2	
SMAD3	
SMAD4	
SMARCA4	
SMARCB1	

(continued on following page)

TABLE A2. Gene List of the FoundationOne Platform (continued)

Entire Coding Sequence	Selected Rearrangements
SMARCD1	
SMO	
SNCAIP	
SOCS1	
SOX10	
SOX2	
SOX9	
SPEN	
SPOP	
SPTA1	
SRC	
STAG2	
STAT3	
STAT4	
STK11	
SUFU	
SYK	
TAF1	
TBX3	
TEK	
TERC	
TERT (promoter only)	
TET2	
TGFBR2	
TIPARP	
TNF	
TNFAIP3	
TNFRSF14	
TNKS	
TNKS2	
TOP1	
TOP2A	
TP53	
TP53BP1	
TRRAP	
TSC1	
TSC2	
TSHR	
TYRO3	
U2AF1	
VEGFA	
VHL	
WISP3	

(continued in next column)

TABLE A2. Gene List of the FoundationOne Platform (continued)

Entire Coding Sequence	Selected Rearrangements
WT1	
XPO1	
XRCC2	
XRCC3	
ZBTB2	
ZNF217	
ZNF703	
ZNRF3	

NOTE. This platform interrogates the entire coding sequence of 395 cancer-related genes and of 31 genes often rearranged or altered in cancer.

TABLE A3. Effect of MSI Status, TMB, *BRAF* Mutations, and Extended *RAS* Mutations on OS and PFS in Patients in the Primary Cohort Only (*KRAS* WT for Codons 12 and 13, Two Arms)

Molecular Marker		PFS				OS			
Patient Subgroup	**No. of Patients**	**Median, months (95% CI)**	**HR (95% CI)**	***P***	**Interaction *P***	**Median, months (95% CI)**	**HR (95% CI)**	***P***	**Interaction *P***
BRAF					.210				.293
BRAF mutant	72	7.1 (5.4 to 8.6)	1.61 (1.13 to 2.30)	.008		12.9 (11.1 to 19.0)	1.72 (1.18 to 2.51)	.005	
			1.64 (1.17 to 2.31)*	.005			1.94 (1.36 to 2.77)*	< .001	
BRAF WT	432	12.0 (11.1 to 13.1)	1 (reference)			34.2 (31.0 to 36.4)	1 (reference)		
BRAF mutant									
Bevacizumab	41	7.6 (5.7 to 9.6)	0.60 (0.33 to 1.09)	.093		15.0 (11.8 to 23.7)	0.68 (0.37 to 1.24)	.207	
Cetuximab	31	6.2 (3.5 to 8.6)	1 (reference)			11.7 (8.6 to 19.7)	1 (reference)		
BRAF WT									
Bevacizumab	207	12.6 (11.2 to 14.3)	0.90 (0.72 to 1.13)	.373		34.4 (30.3 to 37.6)	0.96 (0.76 to 1.22)	.755	
Cetuximab	225	11.4 (10.0 to 13.1)	1 (reference)			33.4 (29.1 to 39.2)	1 (reference)		
Extended *RAS*					.168				.602
Extended *RAS*	72	10.0 (8.9 to 13.0)	1.30 (1.00 to 1.86)	.049		25.0 (20.2 to 29.1)	1.53 (1.11 to 2.10)	.010	
			1.38 (1.01 to 1.87)*	.041			1.59 (1.16 to 2.18)*	.004	
Extended *RAS* WT	429	11.1 (10.1 to 12.6)	1 (reference)			33.5 (30.1 to 36.8)	1 (reference)		
Extended *RAS* mutant									
Bevacizumab	35	11.4 (9.6 to 15.9)	0.60 (0.34 to 1.04)	.067		26.2 (24.4 to 34.2)	1.05 (0.60 to 1.85)	.855	
Cetuximab	37	9.2 (7.4 to 12.9)	1 (reference)			22.9 (18.4 to 32.4)	1 (reference)		
Extended *RAS* WT									
Bevacizumab	213	11.2 (10.2 to 13.1)	0.91 (0.73 to 1.14)	.422		33.6 (29.4 to 37.5)	0.90 (0.70 to 1.15)	.378	
Cetuximab	216	10.9 (9.4 to 12.8)	1 (reference)			31.8 (26.8 to 39.5)	1 (reference)		
MSI					< .001				< .001
MSI-H	35	6.0 (5.1 to 9.6)	0.90 (0.56 to 1.45)	.665		21.0 (12.8 to 30.3)	0.80 (0.49 to 1.31)	.380	
			0.91 (0.56 to 1.46)*	.685			0.83 (0.50 to 1.35)*	.448	
MSS	457	11.2 (10.6 to 12.6)	1 (reference)			32.8 (30.1 to 35.7)	1 (reference)		
MSI-H									
Bevacizumab	20	7.5 (5.4 to NE)	0.16 (0.07 to 0.38)	< .001		30.3 (23.6 to NE)	0.14 (0.06 to 0.34)	< .001	
Cetuximab	15	5.7 (4.1 to 8.6)	1 (reference)			12.3 (10.7 to 24.6)	1 (reference)		
MSS									
Bevacizumab	223	11.2 (10.3 to 13.1)	0.94 (0.76 to 1.16)	.540		32.8 (29.0 to 35.7)	1.02 (0.81 to 1.29)	.852	
Cetuximab	234	11.3 (9.8 to 12.9)	1 (reference)			33.4 (29.1 to 39.3)	1 (reference)		

(continued on following page)

TABLE A3. Effect of MSI Status, TMB, *BRAF* Mutations, and Extended *RAS* Mutations on OS and PFS in Patients in the Primary Cohort Only (*KRAS* WT for Codons 12 and 13, Two Arms) (continued)

Molecular Marker		PFS				OS			
Patient Subgroup	**No. of Patients**	**Median, months (95% CI)**	**HR (95% CI)**	***P***	**Interaction *P***	**Median, months (95% CI)**	**HR (95% CI)**	***P***	**Interaction *P***
TMB					.999				.848
> 8	60	12.3 (9.5 to 20.1)	0.87 (0.62 to 1.21)	.399		36.9 (31.2 to 54.6)	0.70 (0.48 to 1.01)	.053	
			0.86 (0.62 to 1.20)*	.371			0.70 (0.48 to 1.01)*	.056	
≤ 8	232	11.0 (10.1 to 12.9)	1 (reference)			30.1 (25.8 to 34.3)	1 (reference)		
TMB high (> 8)									
Bevacizumab	34	11.4 (9.1 to 20.9)	0.94 (0.52 to 1.68)	.823		35.1 (31.2 to 58.0)	0.91 (0.46 to 1.81)	.793	
Cetuximab	26	12.6 (9.3 to 25.0)	1 (reference)			39.3 (30.1 to 63.7)	1 (reference)		
TMB low (≤ 8)									
Bevacizumab	124	11.0 (10.0 to 13.5)	0.94 (0.69 to 1.26)	.663		30.3 (25.3 to 35.2)	0.98 (0.71 to 1.36)	.919	
Cetuximab	108	11.3 (9.2 to 13.5)	1 (reference)			29.1 (24.3 to 38.5)	1 (reference)		

NOTE. Interaction *P* reports the statistical significance of interactions between the molecular alteration and the two treatment arms in the primary cohort patients by the Wald test. The biologics are given in combination with chemotherapy.

BRAF analyses adjusted for age, arm, extended *RAS*, MSI, sex, ethnicity, No. of metastatic sites, synchronous or metachronous metastases, and tumor location.

Extended *RAS* analyses adjusted for age, arm, *BRAF*, MSI, sex, ethnicity, No. of metastatic sites, synchronous or metachronous metastases, and tumor location.

MSI analyses adjusted for age, arm, *BRAF*, RAS, sex, ethnicity, No. of metastatic sites, synchronous or metachronous metastases, and tumor location.

TMB analyses adjusted for age, arm, *BRAF*, extended *RAS*, sex, ethnicity, No. of metastatic sites, synchronous or metachronous metastases, and tumor location.

Abbreviations: HR, hazard ratio; MSI, microsatellite instability; MSI-H, microsatellite instability–high; MSS, microsatellite stable; OS, overall survival; PFS, progression-free survival; TMB, tumor mutational burden; WT, wild type.

*Tumor location not included as adjustment factor in this model.

TABLE A4. TMB and Patient Characteristics (clinical and molecular)

Clinical and Molecular Characteristics	TMB		Total (N = 473)	*P*
	Low (≤ 8; n = 366)	High (> 8; n = 107)		
Arm				.3404*
Bevacizumab	152 (41.5)	40 (37.4)	192 (40.6)	
Cetuximab	133 (36.3)	36 (33.6)	169 (35.7)	
Bevacizumab and cetuximab	81 (22.1)	31 (29.0)	112 (23.7)	
Chemotherapy				.2943*
FOLFOX	280 (76.5)	87 (81.3)	367 (77.6)	
FOLFIRI	86 (23.5)	20 (18.7)	106 (22.4)	
Prior adjuvant chemotherapy				.9715*
No	322 (88.0)	94 (87.9)	416 (87.9)	
Yes	44 (12.0)	13 (12.1)	57 (12.1)	
Prior pelvic radiation				.3180*
No	343 (93.7)	103 (96.3)	446 (94.3)	
Yes	23 (6.3)	4 (3.7)	27 (5.7)	
Age, years				.0368†
Mean ± SD	58.3 ± 12.0	61.0 ± 11.5	58.9 ± 11.9	
Median	58.9	60.8	59.1	
Q1, Q3	50.1, 66.9	53.3, 70.9	50.9, 67.6	
Range	(20.8-84.3)	(31.4-84.5)	(20.8-84.5)	
Sex				.3676*
Male	223 (60.9)	60 (56.1)	283 (59.8)	
Female	143 (39.1)	47 (43.9)	190 (40.2)	
Ethnicity				.5954*
Missing	3	0	3	
Other	55 (15.2)	14 (13.1)	69 (14.7)	
White	308 (84.8)	93 (86.9)	401 (85.3)	
No. of metastatic sites				.6126*
Missing	4	0	4	
0	3 (0.8)	0 (0.0)	3 (0.6)	
1	194 (53.6)	55 (51.4)	249 (53.1)	
2	114 (31.5)	39 (36.4)	153 (32.6)	
≥ 3	51 (14.1)	13 (12.1)	64 (13.6)	
Synchronous or metachronous metastases				.6039*
Missing	13	2	15	
Synchronous	281 (79.6)	86 (81.9)	367 (80.1)	
Metachronous	72 (20.4)	19 (18.1)	91 (19.9)	
Tumor location				.3902*
Missing	36	5	41	
Left	197 (59.7)	56 (54.9)	253 (58.6)	
Right and transverse	133 (40.3)	46 (45.1)	179 (41.4)	
Liver metastases only				.7012*
Missing	4	0	4	
No	234 (64.6)	67 (62.6)	301 (64.2)	
Yes	128 (35.4)	40 (37.4)	168 (35.8)	

(continued on following page)

TABLE A4. TMB and Patient Characteristics (clinical and molecular) (continued)

Clinical and Molecular Characteristics	TMB		Total (N = 473)	*P*
	Low (≤ 8; n = 366)	High (> 8; n = 107)		
MSI status				.3475*
MSS	363 (99.2)	107 (100.0)	470 (99.4)	
MSI-H	3 (0.8)	0 (0.0)	3 (0.6)	
BRAF				.1739*
Missing	9	1	10	
WT	323 (90.5)	91 (85.8)	414 (89.4)	
Mutant	34 (9.5)	15 (14.2)	49 (10.6)	
KRAS				.4463*
Missing	9	1	10	
WT	249 (69.7)	78 (73.6)	327 (70.6)	
Mutant	108 (30.3)	28 (26.4)	136 (29.4)	
NRAS				.3903*
Missing	9	1	10	
WT	340 (95.2)	103 (97.2)	443 (95.7)	
Mutant	17 (4.8)	3 (2.8)	20 (4.3)	
Extended *RAS*				.2930*
Missing	9	1	10	
WT	233 (65.3)	75 (70.8)	308 (66.5)	
Mutant	124 (34.7)	31 (29.2)	155 (33.5)	
Triple negative (*NRAS*/*KRAS*/*BRAF*)				.8753*
Missing	9	1	10	
All WT	199 (55.7)	60 (56.6)	259 (55.9)	
Any mutation	158 (44.3)	46 (43.4)	204 (44.1)	
Quadruple negative (*NRAS*/*KRAS*/*BRAF*/*PIK3CA*)				
Missing	9	1	10	
All WT	186 (52.1)	53 (50.0)	239 (51.6)	
Any mutation	171 (47.9)	53 (50.0)	224 (48.4)	

NOTE. Data are presented as No. (%) unless indicated otherwise.

Abbreviations: FOLFIRI, fluorouracil, leucovorin, and irinotecan; FOLFOX, infusional fluorouracil, leucovorin, and oxaliplatin; MSI, microsatellite instability; MSI-H, microsatellite instability–high; MSS, microsatellite stable; TMB, tumor mutational burden; WT, wild type.

*χ^2 test.

†Kruskal-Wallis test.