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ARTICLE

Predictive and Prognostic Analysis of *PIK3CA* Mutation in Stage III Colon Cancer Intergroup Trial

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Background Somatic mutations in *PIK3CA* (phosphatidylinositol-4,5-bisphosphonate 3-kinase [PI3K], catalytic subunit alpha gene) activate the PI3K-AKT signaling pathway and contribute to pathogenesis of various malignancies, including colorectal cancer.

Methods We examined associations of *PIK3CA* oncogene mutation with relapse, survival, and treatment efficacy in 627 stage III colon carcinoma case subjects within a randomized adjuvant chemotherapy trial (5-fluorouracil and leucovorin [FU/LV] vs irinotecan [CPT11], fluorouracil and leucovorin [IFL]; Cancer and Leukemia Group B 89803 [Alliance]). We detected *PIK3CA* mutation in exons 9 and 20 by polymerase chain reaction and pyrosequencing. Cox proportional hazards model was used to assess prognostic and predictive role of *PIK3CA* mutation, adjusting for clinical features and status of routine standard molecular pathology features, including *KRAS* and *BRAF* mutations and microsatellite instability (mismatch repair deficiency). All statistical tests were two-sided.

Results Compared with *PIK3CA* wild-type cases, overall status of *PIK3CA* mutation positivity or the presence of *PIK3CA* mutation in either exon 9 or 20 alone was not statistically significantly associated with recurrence-free, disease-free, or overall survival (log-rank $P > .70$; $P > .40$ in multivariable regression models). There was no statistically significant interaction between *PIK3CA* and *KRAS* (or *BRAF*) mutation status in survival analysis ($P_{interaction} > .18$). *PIK3CA* mutation status did not appear to predict better or worse response to IFL therapy compared with FU/LV therapy ($P_{interaction} > .16$).

Conclusions Overall tumor *PIK3CA* mutation status is not associated with stage III colon cancer prognosis. *PIK3CA* mutation does not appear to serve as a predictive tumor molecular biomarker for response to irinotecan-based adjuvant chemotherapy.

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Phosphatidylinositol-4,5-bisphosphonate 3-kinase (PI3K) can activate the AKT signaling pathway and facilitate cellular growth, proliferation, and survival (1). Activating mutations in *PIK3CA* (the phosphatidylinositol-4,5-bisphosphonate 3-kinase, catalytic subunit alpha gene; HGNC ID; HGNC:8975) have been found in various human malignancies, including colon cancers (2). A subset (10%–30%) of colorectal cancers harbor *PIK3CA* mutations, which have been associated with various clinical and molecular features, including proximal tumor location and *KRAS* mutation (3–16).

Despite many previous studies (10–24), a prognostic role of overall *PIK3CA* mutation status in colorectal cancer remains uncertain, although coexistence of *PIK3CA* mutations in both exons 9 and 20 may be associated with shorter survival (13). Most of these previous studies were underpowered for robust statistical analysis (mostly with total sample size of less than 500 and 10%–20% frequency of *PIK3CA* mutation). Therefore, additional studies with a large sample size are needed.

A recent study suggests that *PIK3CA* mutation in colorectal cancer may serve as a molecular biomarker to predict response to aspirin therapy (25). Nonetheless, clinical utility of *PIK3CA* mutation test on colorectal cancer as a predictive tumor biomarker remains to be fully characterized (22,26–29).

We therefore conducted this study to examine prognostic and predictive roles of *PIK3CA* mutation in stage III colon cancer patients who enrolled in the National Cancer Institute–sponsored randomized clinical trial comparing postoperative adjuvant 5-fluorouracil (FU)/leucovorin (LV) with irinotecan/FU/LV (IFL) (CALGB 89803 [Alliance]) (30). Because data on postoperative treatment, performance status, and disease stage were carefully recorded in the trial, we could assess prognostic and predictive roles of *PIK3CA* mutations in colon cancer while controlling for potential confounding by

those covariables and key molecular characteristics, including *KRAS*, *BRAF*, and microsatellite instability (MSI) status. It is important to consider *KRAS*, *BRAF*, and MSI status because these are now routinely assessed in colorectal cancer for patient management.

Methods

Study Population

Patients in this study were participants in the National Cancer Institute–sponsored Cancer and Leukemia Group B (CALGB) phase III adjuvant therapy trial for stage III colon cancer comparing therapy with the weekly Roswell Park regimen of FU/LV with weekly bolus regimen of IFL (CALGB 89803; ClinicalTrials.gov Identifier: NCT00003835) (30). CALGB is now part of the Alliance for Clinical Trials in Oncology. Between April 1999 and May 2001, 1264 patients were enrolled. Patients in the treatment trial (and thus this companion study) were eligible if they underwent a complete surgical resection of the primary tumor within 56 days before study entry and had regional lymph node metastases but no evidence of distant metastases (ie, stage III). Considering the colorectal continuum model, we included cases from cecal cancers to sigmoid cancers (31). Moreover, patients were required to have a baseline Eastern Cooperative Oncology Group performance status of 0 to 2 (ambulatory) and have adequate bone marrow, renal, and hepatic function. Cancer staging was based on Tumor Node Metastatis (TNM) classification (http://www.cancer.org). This analysis represents correlative research based on a subset of patients within the trial and was limited to 627 patients for whom archived formalin-fixed paraffin-embedded tumor tissue and *PIK3CA* sequencing data were available. All patients signed informed consent, approved by each site's institutional review board.

We compared baseline characteristics of the patients who were included in this study (with available *PIK3CA* data: n = 627) with those who were excluded from this study because of unavailability of tissue data (n = 637). We did not detect any statistically significant or substantial difference between these two groups in terms of age, sex, body mass index, family history, tumor location, extent of invasion through bowel wall (pT stage), lymph node involvement (pN stage), performance status, clinical bowel perforation, clinical bowel obstruction, or treatment arm (all $P > .05$). In addition, recurrence-free (RFS), disease-free (DFS), or overall survival (OS) did not statistically significantly differ in subjects with available *PIK3CA* data as compared with those without *PIK3CA* data (multivariable hazard ratio [HR] = 1.05, 95% confidence interval [CI] = 0.87 to 1.27; multivariable HR = 1.10, 95% CI = 0.93 to 1.31; multivariable HR = 1.12, 95% CI = 0.93 to 1.36, respectively).

As part of the quality assurance program of the Alliance, members of the Audit Committee visit all participating institutions at least once every 3 years to review source documents. The auditors verify compliance with federal regulations and protocol requirements, including those pertaining to eligibility, treatment, adverse events, tumor response, and outcome in a sample of protocols at each institution. Such on-site review of medical records was performed for a subgroup of 328 (26%) of the 1264 patients included in the treatment trial. Data quality was also ensured by review of data by the Alliance Statistics and Data Center and by the study chairperson following Alliance policies.

Definitions of Study Endpoints

The study endpoints were 1) RFS, defined as the time from the study enrollment to tumor recurrence or occurrence of a new primary colon cancer; 2) DFS, defined as time from the study enrollment to cancer recurrence, occurrence of a new primary colon cancer, or death from any cause; and 3) OS, defined as the time from the study enrollment to death from any cause. For RFS, patients who died without known cancer recurrence were censored at last documented evaluation by a treating provider.

DNA Extraction From Tumor, *PIK3CA*, *BRAF*, and *KRAS* sequencing and MSI Analysis

Tumor molecular analyses were performed blinded to patient and outcome data. DNA was extracted from paraffin-embedded colon cancer tissue (32). We marked tumor areas on hematoxylin and eosin–stained slides and dissected tumor tissue by a sterile needle. Polymerase chain reaction (PCR) and pyrosequencing targeted for mutation hotspots in *PIK3CA* exons 9 and 20 (13), *BRAF* codon 600 (33), and *KRAS* codons 12 and 13 were performed, as previously described (32), in the laboratory at the Dana-Farber Cancer Institute. Pyrosequencing assay has been found to be more sensitive than Sanger sequencing and can detect approximately 5% to 10% of mutant alleles among a mixture of mutant and normal alleles (32). MSI was assessed by PCR for 10 microsatellite markers (BAT25, BAT26, D17S250, D5S346, ACTC, D18S55, BAT40, D10S197, BAT34c4, and MycL) (34). Tumors with instability in 50% or more of the loci were classified as MSI-high, and those with instability in 0% to 49% of the loci were classified as microsatellite stable; and the concordance between MSI testing and immunohistochemistry for MLH1 or MSH2 loss was 97% (34). For 28 cases without PCR MSI results, those with loss of MLH1 or MSH2 were classified as MSI-high, and those with intact expression of MLH1 and MSH2 were classified as microsatellite stable.

Statistical Analyses

Detailed statistical methods are described in the Supplementary Methods (available online). All analyses were based on the clinical study database frozen on November 9, 2009. SAS version 9.2 (SAS Institute, Cary, NC) was used for all statistical analyses, and all *P* values were two-sided. Statistical significance was set at *P* equal to .05 for main hypothesis testing on *PIK3CA* status and outcome. For exploratory analyses of interactions (between *PIK3CA* mutation and each of the variables, including age, sex, etc.) and clinical, pathological, and molecular associations, we adjusted statistical significance level by Bonferroni correction to *P* equal to .004 (.05 divided by 13) to account for multiple hypothesis testing. Because a vast majority of participants were non-Hispanic whites, we did not perform analyses stratified by ethnic group.

The Kaplan–Meier method was used to estimate the distribution of survival time according to *PIK3CA* status, and the log-rank test was used to compare survival between subgroups. We used the multivariable Cox proportional hazards model to estimate survival hazard ratio according to tumor *PIK3CA* status. We conducted power calculations for survival analyses (Supplementary Table 1, available online). The proportionality of hazards assumption was assessed using standard survival plots and by evaluating a time-dependent variable, which was the cross-product of *PIK3CA* and

Table 1. Baseline characteristics according to *PIK3CA* mutation status in stage III colon cancer*

Clinical or molecular feature	Total No.	Tumor *PIK3CA* status				*P* (wild-type vs exon 9 mutant vs exon 20 mutant)	*P* (wild-type vs overall mutant)
		Wild-type	Mutation present in only exon 9†	Mutation present in only exon 20†	Overall mutation positive (exon 9 or 20)‡		
Total No.	627	553	48	25	74		
Sex, No. (%)						.001	.15
Male	337 (54)	303 (55)	15 (31)	18 (72)	34 (46)		
Female	290 (46)	250 (45)	33 (69)	7 (28)	40 (54)		
Age, y, mean ± SD	60.2 ± 11.4	60.2 ± 11.4	59.0 ± 10.7	61.9 ± 12.6	60.0 ± 11.4	.59	.91
Body mass index, kg/m^2, No. (%)						.24	.74
<25	162 (30)	140 (29)	19 (42)	3 (15)	22 (33)		
25–29	183 (34)	163 (34)	12 (27)	8 (40)	20 (30)		
≥30	197 (36)	173 (36)	14 (31)	9 (45)	24 (36)		
Family history of colorectal cancer in any first-degree relative, No. (%)						.23	.97
No	454 (83)	399 (83)	35 (78)	19 (95)	55 (83)		
Yes	92 (17)	81 (17)	10 (22)	1 (5.0)	11 (17)		
Tumor location, No. (%)						.78	.90
Proximal, cecum to transverse colon	359 (58)	317 (58)	26 (55)	16 (64)	42 (58)		
Distal, splenic flexure to sigmoid	258 (42)	227 (42)	21 (44)	9 (36)	31 (42)		
pT stage, No. (%)						.65	.31
pT1–pT2	75 (12)	66 (12)	5 (11)	3 (12)	9 (13)		
pT3	492 (80)	438 (81)	35 (76)	19 (76)	54 (75)		
pT4	49 (8.0)	40 (7.4)	6 (13)	3 (12)	9 (13)		
pN stage, No. (%)						.94	.80
pN1	381 (62)	337 (62)	28 (60)	15 (60)	44 (60)		
pN2	237 (38)	208 (38)	19 (40)	10 (40)	29 (40)		
Performance status score						.20	.07
0	472 (76)	410 (75)	40 (85)	21 (84)	62 (85)		
1–2	146 (24)	135 (25)	7 (15)	4 (16)	11 (15)		
Clinical bowel perforation						.54	.49
No	578 (95)	511 (95)	42 (91)	24 (96)	67 (93)		
Yes	32 (5.3)	27 (5.0)	4 (8.7)	1 (4.0)	5 (6.9)		
Clinical bowel obstruction						.85	.63
No	480 (78)	425 (78)	35 (74)	19 (76)	55 (75)		
Yes	139 (22)	121 (22)	12 (26)	6 (24)	18 (25)		
Microsatellite instability (MSI) status§, No. (%)						.19	.70
Microsatellite stable	470 (81)	420 (81)	35 (85)	14 (67)	50 (79)		
MSI-high	109 (19)	96 (19)	6 (15)	7 (33)	13 (21)		
BRAF mutation status, No. (%)						.07	.048
Wild-type	486 (84)	419 (83)	45 (96)	21 (84)	67 (92)		
Mutant (c.1799T>A, p.V600E)	94 (16)	88 (17)	2 (4.3)	4 (16)	6 (8.2)		
KRAS mutation status, No. (%)						.07	.09
Wild-type	376 (65)	335 (66)	24 (50)	17 (71)	41 (56)		
Mutant	203 (35)	171 (34)	24 (50)	7 (29)	32 (44)		

(Table continues)

Table 1 (Continued).

Clinical or molecular feature	Total No.	Tumor *PIK3CA* status				*P* (wild-type vs exon 9 mutant vs exon 20 mutant)	*P* (wild-type vs overall mutant)
		Wild-type	Mutation present in only exon 9†	Mutation present in only exon 20†	Overall mutation positive (exon 9 or 20)‡		
Treatment arm, No. (%)						.71	.87
FU/LV	319 (51)	282 (51)	26 (54)	11 (44)	37 (50)		
IFL	308 (49)	271 (49)	22 (46)	14 (56)	37 (50)		

* The percentages indicate the proportion of tumors with a specific clinical or molecular feature among tumors with specific *PIK3CA* status. There were cases with missing value/status for some of the variables. A χ^2 test was used to assess associations, and all *P* values were two-sided. FU/LV = 5-fluorouracil and leucovorin; IFL = irinotecan, 5-fluorouracil and leucovorin; SD = standard deviation.

† One case subject with *PIK3CA* mutations in both exons 9 and 20 was excluded.

‡ This category includes one case subject with mutations in both exons 9 and 20.

§ For 29 cases without MSI results by polymerase chain reaction, those with loss of MLH1 or MSH2 were classified as MSI-high, and those with intact expression of MLH1 and MSH2 as microsatellite stable because concordance between MSI polymerase chain reaction and immunohistochemistry for MLH1 and MSH2 was very high (97%) among case subjects with both results available (34).

survival time (*P* = .46 for RFS; *P* = .76 for DFS; *P* = .35 for OS). To assess the potential differential effect of treatment arm according to *PIK3CA* status, we performed a single multivariable Cox regression analysis, in which we could estimate the effect of treatment arm simultaneously in two strata of *PIK3CA* status, using a reparameterization of the interaction term(s) (35). Interaction was also assessed by including the cross-product of *PIK3CA* and another variable of interest (without data-missing cases) in a multivariable model using the Wald test.

Results

PIK3CA Mutation and Patient Survival in Stage III Colon Cancer

Study participants were drawn from a multicenter study of postoperative adjuvant chemotherapy in stage III colon cancer patients who underwent a curative-intent surgical resection (CALGB 89803) (30). We included 627 case subjects in this study based on availability of tumor tissue for sequencing of *PIK3CA* exons 9 and 20, which detected mutation in 74 (12%) patients. One case subject showed mutations in both exons 9 and 20. Table 1 summarizes baseline characteristics according to *PIK3CA* mutation status. Overall prevalence of postdiagnosis regular aspirin use (defined as two or more tablets per week) was 7.2% (n = 45 of 627 patients), and there were only four patients who had *PIK3CA*-mutated tumor and regularly used aspirin after colon cancer diagnosis.

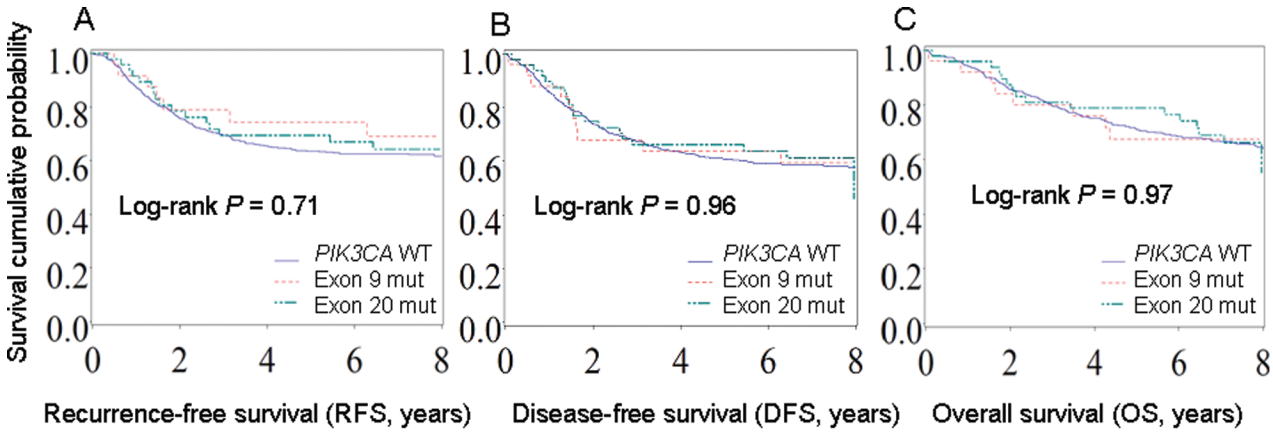
With median follow-up of 7.6 (interquartile range = 7.1–8.1) years among those who were censored for overall survival outcome, there were 225 events for RFS analysis, 258 events for DFS analysis, and 210 events for OS analysis.

In a Kaplan–Meier analysis (Figure 1), compared with *PIK3CA* wild-type patients, *PIK3CA* mutation in either exon 9 or 20 was not statistically significantly associated with RFS, DFS, or OS outcome (log-rank *P* > .70).

In multivariable Cox regression analysis, we examined the prognostic association of *PIK3CA* mutation adjusting for other predictors of patient survival (Table 2; Supplementary Table 2, available online, shows data on all variables in the final multivariable models). Compared with *PIK3CA* wild-type cases, *PIK3CA* mutation in only exon 9, or only exon 20, or overall *PIK3CA* mutation status was not statistically significantly associated with RFS, DFS, or OS outcome in univariate or multivariable analysis (*P* > .40 in multivariable regression models). Multivariable hazard ratios for RFS, DFS, and OS in overall *PIK3CA* mutated tumors compared with *PIK3CA* wild-type tumors were 0.84 (95% CI = 0.54 to 1.29), 0.92 (95% CI = 0.62 to 1.35), and 0.95 (95% CI = 0.63 to 1.45), respectively.

PIK3CA Mutation and Prognosis in Strata of Combined *KRAS* and *BRAF* Status

We assessed a prognostic role of *PIK3CA* mutation in strata of combined *KRAS* and *BRAF* status to examine possible effect modification by *KRAS* or *BRAF* status on *PIK3CA* mutation (Table 3). We did not observe statistically significant effect modification by *KRAS* status. The number (n = 6) of cases with both *PIK3CA* and *BRAF* mutations precluded robust assessment of effect modification by *BRAF* status. There was no statistically significant interaction



Number of patients at risk

RFS (years)	0	2	4	6	8
PIK3CA WT	553	404	331	289	94
Exon 9 mut	48	35	30	27	3
Exon 20 mut	25	17	15	14	8

Number of patients at risk

DFS (years)	0	2	4	6	8
PIK3CA WT	553	404	331	289	94
Exon 9 mut	48	35	30	27	3
Exon 20 mut	25	17	15	14	8

Number of patients at risk

OS (years)	0	2	4	6	8
PIK3CA WT	553	404	331	289	94
Exon 9 mut	48	35	30	27	3
Exon 20 mut	25	17	15	14	8

Figure 1. Kaplan–Meier curves according to *PIK3CA* mutation in stage III colon cancers for recurrence-free survival (RFS) (**A**), disease-free survival (DFS) (**B**), and overall survival (OS) (**C**). Tables of the numbers of patients at risk are below the graphs. Exon 9 mut = mutation in only exon 9; Exon 20 mut = mutation in only exon 20; WT = wild-type.

between *PIK3CA* and *KRAS* (or *BRAF*) status ($P_{interaction}$ > .18). Multivariable hazard ratios for DFS in *PIK3CA* mutant tumors compared with *PIK3CA* wild-type tumors were 1.21 (95% CI = 0.71 to 2.06) among the *KRAS* wild-type *BRAF* wild-type subtype and 0.76 (95% CI = 0.40 to 1.43) among the *KRAS* mutant *BRAF* wild-type subtype. Five-year survival probabilities for DFS among *BRAF* wild-type tumors were 0.63, 0.62, 0.60, and 0.68 in *KRAS* wild-type *PIK3CA* wild-type; *KRAS* wild-type *PIK3CA* mutant; *KRAS* mutant *PIK3CA* wild-type; and *KRAS* mutant *PIK3CA* mutant subtypes, respectively.

Predictive Role of *PIK3CA* Mutation for IFL-Based Therapy

We assessed the prognostic role of *PIK3CA* mutation within each treatment arm and the effect of treatment according to *PIK3CA* status (Table 4). In either treatment arm, *PIK3CA* mutation was not statistically significantly associated with RFS, DFS, or OS outcome. Multivariable hazard ratios for DFS in *PIK3CA* mutant tumors compared with *PIK3CA* wild-type tumors were 0.75 (95% CI = 0.43 to 1.31) among the FU/LV arm and 1.13 (95% CI = 0.65 to 1.96) among the IFL arm.

In either stratum of patients with *PIK3CA* mutated or wild-type tumors, IFL treatment was not statistically significantly associated with RFS, DFS, or OS outcome compared with FU/LV treatment (Table 4), and there was no statistically significant interaction between treatment arm and *PIK3CA* status ($P_{interaction}$ > .16). Multivariable hazard ratios for DFS in the IFL treatment group compared with the FU/LV treatment group were 0.88 (95% CI = 0.68 to 1.15) among the *PIK3CA* wild-type subtype and 1.33 (95% CI = 0.64 to 2.78) among the *PIK3CA* mutant subtype.

Interaction Analysis Between *PIK3CA* and Other Variables

In exploratory analyses, we further examined whether prognostic association of *PIK3CA* mutation was modified by any other variables, including clinical features and MSI status. We did not observe statistically significant or appreciable effect modification by any of the variables examined for RFS, DFS, or OS outcomes (all $P_{interaction}$ > .04; given multiple hypothesis testing, a *P* value for statistical significance was adjusted to *P* = .004).

Discussion

In this study, we found that tumor *PIK3CA* mutation was not statistically significantly associated with recurrence or survival among more than 600 stage III colon cancer patients, who participated in the randomized trial comparing postoperative IFL with FU/LV (CALGB 89803). We found no evidence for a predictive role of *PIK3CA* mutation status in IFL-based treatment.

It is a challenge to optimize treatment decision-making for patients with colon cancer because of heterogeneity of colon cancer with regard to both biology and clinical response (36). Heterogeneity exists in tumors with different mutations, even in one oncogene such as *KRAS* (37, 38), and treatment and other host factors can influence (or can be influenced by) tumor characteristics through tumor microenvironment (and vice versa) (39–43).

Table 2. *PIK3CA* mutation status and clinical outcome in stage III colon cancer*

		Recurrence-free survival			Disease-free survival			Overall survival		
***PIK3CA* status**	**No.**	**Five-year survival probability**	**Univariate HR (95% CI)**	**Multivariable HR (95% CI)**	**Five-year survival probability**	**Univariate HR (95% CI)**	**Multivariable HR (95% CI)**	**Five-year survival probability**	**Univariate HR (95% CI)**	**Multivariable HR (95% CI)**
Wild-type	553	0.64	1 (referent)	1 (referent)	0.61	1 (referent)	1 (referent)	0.71	1 (referent)	1 (referent)
Overall mutation positive, exon 9 or 20 †	74	0.71	0.84 (0.54 to 1.29)	0.84 (0.54 to 1.29)	0.66	0.93 (0.63 to 1.37)	0.92 (0.62 to 1.35)	0.75	0.99 (0.65 to 1.50)	0.95 (0.63 to 1.45)
Mutation in only exon 9‡	48	0.69	0.89 (0.54 to 1.49)	0.88 (0.53 to 1.46)	0.66	0.94 (0.59 to 1.50)	0.91 (0.57 to 1.46)	0.79	1.03 (0.63 to 1.70)	0.98 (0.59 to 1.62)
Mutation in only exon 20‡	25	0.74	0.76 (0.36 to 1.62)	0.76 (0.36 to 1.63)	0.64	0.95 (0.51 to 1.79)	0.94 (0.50 to 1.78)	0.67	0.93 (0.46 to 1.88)	0.90 (0.44 to 1.84)

* The multivariable Cox proportional hazards regression model was used. CI = confidence interval; HR = hazard ratio.

† This category includes one case with mutations in both exons 9 and 20.

‡ One case with *PIK3CA* mutations in both exons 9 and 20 was excluded.

Table 3. *PIK3CA* mutation status in strata of combined *KRAS* and *BRAF* mutation status and clinical outcome in stage III colon cancer*

		Recurrence-free survival		Disease-free survival		Overall survival	
Combined *KRAS* / *BRAF* status, and *PIK3CA* status	**No.**	**Five-year survival probability**	**Multivariable HR (95% CI)**	**Five-year survival probability**	**Multivariable HR (95% CI)**	**Five-year survival probability**	**Multivariable HR (95% CI)**
KRAS wild-type *BRAF* wild-type							
PIK3CA wild-type	245	0.65	1 (referent)	0.63	1 (referent)	0.74	1 (referent)
PIK3CA mutant	34	0.64	1.18 (0.65 to 2.09)	0.62	1.21 (0.71 to 2.06)	0.73	1.30 (0.73 to 2.31)
KRAS mutant *BRAF* wild-type							
PIK3CA wild-type	169	0.65	1 (referent)	0.60	1 (referent)	0.69	1 (referent)
PIK3CA mutant	32	0.78	0.60 (0.28 to 1.32)	0.68	0.76 (0.40 to 1.43)	0.77	0.76 (0.38 to 1.53)
KRAS wild-type *BRAF* mutant							
PIK3CA wild-type	87	0.54	1 (referent)	0.50	1 (referent)	0.62	1 (referent)
PIK3CA mutant	6	0.83	0.27 (0.04 to 2.01)	0.83	0.25 (0.04 to 1.85)	0.83	0.29 (0.04 to 2.12)

* The multivariable Cox proportional hazards regression model included the same set of covariables that were selected in the final models of the analyses in Table 2, except for stratifying variables *KRAS* and *BRAF* mutation status. Cases with mutations in both *KRAS* and *BRAF* were excluded. CI = confidence interval; HR = hazard ratio.

Table 4. Stage III colon cancer and clinical outcome according to treatment arm and *PIK3CA* mutation status*

Treatment arm and tumor *PIK3CA* status	No.	Recurrence-free survival		Disease-free survival		Overall survival	
		Five-year survival probability	Multivariable HR (95% CI)	Five-year survival probability	Multivariable HR (95% CI)	Five-year survival probability	Multivariable HR (95% CI)
FU/LV							
PIK3CA wild-type	282	0.64	1 (referent)	0.62	1 (referent)	0.72	1 (referent)
PIK3CA mutant	37	0.71	0.70 (0.37 to 1.31)	0.64	0.75 (0.43 to 1.31)	0.77	0.72 (0.39 to 1.32)
IFL							
PIK3CA wild-type	271	0.63	1 (referent)	0.60	1 (referent)	0.71	1 (referent)
PIK3CA mutant	37	0.72	1.00 (0.55 to 1.84)	0.67	1.13 (0.65 to 1.96)	0.73	1.30 (0.72 to 2.34)
$P_{interaction}$ (*PIK3CA* and treatment arm)†		—	.36	—	.27	—	.17
PIK3CA wild-type							
FU/LV	282	0.64	1 (referent)	0.62	1 (referent)	0.72	1 (referent)
IFL	271	0.63	0.90 (0.68 to 1.19)	0.60	0.88 (0.68 to 1.15)	0.71	0.84 (0.62 to 1.12)
PIK3CA mutant							
FU/LV	37	0.71	1 (referent)	0.64	1 (referent)	0.77	1 (referent)
IFL	37	0.72	1.29 (0.56 to 2.95)	0.67	1.33 (0.64 to 2.78)	0.73	1.51 (0.68 to 3.34)

* The multivariable Cox proportional hazards regression model included the same set of covariables that were selected in the final models of the analyses in Table 2. CI = confidence interval; FU/LV = 5-fluorouracil and leucovorin; HR = hazard ratio; IFL = irinotecan, 5-fluorouracil and leucovorin.

† Interaction was assessed by including the cross-product of *PIK3CA* and treatment arm variables in a multivariable model, and the Wald test was used. All *P* values are two-sided.

Therefore, studies examining tumor biomarkers and clinical outcome are crucial in colon cancer research (44–49).

Although previous studies (34,50–55) have assessed potential predictive roles of germline genetic or tumor tissue markers for IFL-based chemotherapy, no biomarker has been proven to be useful in predicting response or resistance to IFL-based therapy (54). A previous analysis of patients in the clinical trial studied in this report suggested that MSI-high might predict an improved patient outcome for treatment with IFL relative to FU/LV (34); however, an independent trial of more than 1200 case subjects comparing IFL vs FU/LV failed to prove a predictive value of MSI status (53). Additional studies are necessary to identify and validate predictors for IFL-based chemotherapy against colorectal cancer.

Several other predictive roles for *PIK3CA* mutation in colorectal cancer have been examined (22,26–28,56). A recent study (25) suggests that *PIK3CA* mutation in colorectal cancer may predict response to aspirin treatment, and this finding appears to be replicated (56). Challenges include relatively low prevalence (10%–20%) of *PIK3CA* mutations, possible differential effects of exon 9 and 20 mutations, and potential confounding effect by associated *KRAS* mutations. A meta-analysis suggested that *PIK3CA* exon 20 mutation in metastatic colorectal cancer may predict response to anti-EGFR therapy (57). Finally, a recent preclinical study suggested that *PIK3CA* mutant colorectal cancer may respond to the SRC inhibitor saracatinib (58). Thus, it is possible that *PIK3CA* mutation status may serve as a predictive biomarker for a number of different therapeutic agents and their combinations, which include aspirin and other drugs. Additional large-scale trials are needed to define predictive roles of *PIK3CA* mutations in colorectal cancer.

Studies have examined the prognostic significance of *PIK3CA* mutations in colorectal cancer (10–24). Although two studies linked tumor *PIK3CA* mutation to poor prognosis (10,17), statistical power in these reports was limited (each total sample size was less than 160). In one study (n = 586) (6), the presence of a mutation in any one of *KRAS*, *BRAF*, and *PIK3CA* oncogenes was associated with inferior prognosis; however, this could be purely because of the effects of *BRAF* mutations (59–62). Two other studies showed that *PIK3CA* exon 20 mutations were associated with poor prognosis, whereas *PIK3CA* exon 9 mutations were not associated with prognosis (20,23). Other reports showed that *PIK3CA* mutations were associated with neither distant metastasis to liver (63) nor patient survival (11,12,21). The two largest prognostic studies [n = 2091 (14), and n = 1170 (13)] did not support a prognostic role of overall *PIK3CA* mutation status. In our analysis, neither *PIK3CA* overall mutation status nor *PIK3CA* mutation in exon 9 or 20 alone was statistically significantly associated with tumor recurrence or overall survival. Our study further underscores the importance of large, multicenter, collaborative studies; it should be noted that small, underpowered studies with null findings experience higher likelihood of being unwritten and unpublished when compared with small studies with "statistically significant" results. Well-designed, large-scale studies with appropriate statistical power can be published irrespective of positive or null findings, hence being less susceptible to "publication bias." Thus, more weight should be placed on the data from large-scale studies upon evaluation of published data on the prognostic significance of tumor biomarkers (13).

Interestingly, Liao et al. (13) demonstrated that the coexistence of *PIK3CA* mutations in both exons 9 and 20 was associated with shorter survival. Experimental evidence suggests that *PIK3CA* helical and kinase domain mutations differentially activate protein function and that the presence of *PIK3CA* mutations in both exon 9 and exon 20 results in a synergistic gain of enzymatic function (64). However, in our analysis, there was only one case subject with *PIK3CA* mutations in both exons 9 and 20, which precluded robust outcome assessment.

A ligand–receptor interaction of EGFR (HGNC ID: HGNC:3236) leads to activation of two main signal transduction pathways, RAS-RAF-MAPK and PI3K-AKT. Activating mutations in *KRAS*, *BRAF*, and/or *PIK3CA* are well-known carcinogenic mechanisms, and there may be interactive effects of *KRAS* and *PIK3CA* mutations (65). In our study on colon cancer, we did not observe statistically significant interactive effects of *PIK3CA* and *KRAS* (or *BRAF*) mutations.

This study used the multi-institutional clinical trial of adjuvant chemotherapy and had several strengths. All study subjects were stage III cancer patients, which decreased potential residual confounding by disease stage. Methods of follow-up and treatment were standardized, and the date and nature of recurrence were recorded. Furthermore, integrative database of treatment, behavioral and lifestyle factors, tumor molecular characteristics (including *PIK3CA*, *KRAS*, *BRAF*, and MSI status), and clinical outcomes enable molecular pathological epidemiology research (66,67) and controlling for confounding by lifestyle factors. The paradigm of molecular pathological epidemiology has been widely used (68–76). In colorectal cancer, *KRAS*, *BRAF*, and MSI tests are a part of routine clinical practice (77), and *PIK3CA* test is an emerging clinical test (25,29,56).

We recognize limitations of our study. Patients who enrolled in the clinical trials constituted a selected group of individuals and might differ from the general population. Patients needed to meet enrollment criteria and be motivated to participate and were further selected based on availability of tissue specimens. Nonetheless, demographic, clinical, or prognostic data of the patients selected in this study did not substantially differ from those without available tumor tissue. Because this trial included patients from both academic and community hospitals across the United States and Canada, our results might reflect stage III colon cancers in the general North American population. Finally, because *PIK3CA* status was not available on all patients, statistical power was attenuated, especially for predictive assessment for response to IFL use.

In conclusion, our current study of stage III colon cancer patients has shown that tumor *PIK3CA* mutation is not statistically significantly associated with recurrence or survival and that *PIK3CA* mutation status is not a predictive marker for response to IFL-based chemotherapy. Additional large-scale studies are needed to define predictive roles of *PIK3CA* mutations in colon cancer.

References

1. Samuels Y, Diaz LA Jr, Schmidt-Kittler O, et al. Mutant PIK3CA promotes cell growth and invasion of human cancer cells. *Cancer Cell*. 2005;7(6):561–573.
2. Samuels Y, Wang Z, Bardelli A, et al. High frequency of mutations of the *PIK3CA* gene in human cancers. *Science*. 2004;304(5670):554.
3. Velho S, Oliveira C, Ferreira A, et al. The prevalence of *PIK3CA* mutations in gastric and colon cancer. *Eur J Cancer*. 2005;41(11):1649–1654.
4. Baldus SE, Schaefer KL, Engers R, Hartleb D, Stoecklein NH, Gabbert HE. Prevalence and heterogeneity of *KRAS*, *BRAF*, and *PIK3CA* mutations in primary colorectal adenocarcinomas and their corresponding metastases. *Clin Cancer Res*. 2010;16(3):790–799.
5. Benvenuti S, Frattini M, Arena S, et al. *PIK3CA* cancer mutations display gender and tissue specificity patterns. *Hum Mutat*. 2008;29(2):284–288.
6. Barault L, Veyries N, Jooste V, et al. Mutations in the RAS-MAPK, PI(3)K (phosphatidylinositol-3-OH kinase) signaling network correlate with poor survival in a population-based series of colon cancers. *Int J Cancer*. 2008;122(10):2255–2259.
7. Yamauchi M, Morikawa T, Kuchiba A, et al. Assessment of colorectal cancer molecular features along bowel subsites challenges the conception of distinct dichotomy of proximal versus distal colorectum. *Gut*. 2012;61(6):847–854.
8. Whitehall VL, Rickman C, Bond CE, et al. Oncogenic PIK3CA mutations in colorectal cancers and polyps. *Int J Cancer*. 2012;131(4):813–820.
9. Voutsina A, Tzardi M, Kalikaki A, et al. Combined analysis of *KRAS* and *PIK3CA* mutations, *MET* and *PTEN* expression in primary tumors and corresponding metastases in colorectal cancer. *Mod Pathol*. 2013;26(2):302–313.
10. Kato S, Iida S, Higuchi T, et al. *PIK3CA* mutation is predictive of poor survival in patients with colorectal cancer. *Int J Cancer*. 2007;121(8):1771–1778.
11. Abubaker J, Bavi P, Al-Harbi S, et al. Clinicopathological analysis of colorectal cancers with *PIK3CA* mutations in Middle Eastern population. *Oncogene*. 2008;27(25):3539–3545.
12. Souglakos J, Philips J, Wang R, et al. Prognostic and predictive value of common mutations for treatment response and survival in patients with metastatic colorectal cancer. *Br J Cancer*. 2009;101(3):465–472.
13. Liao X, Morikawa T, Lochhead P, et al. Prognostic role of *PIK3CA* mutation in colorectal cancer: cohort study and literature review. *Clin Cancer Res*. 2012;18(8):2257–2268.
14. Gavin P, Colangelo LH, Fumagalli D, et al. Mutation profiling and microsatellite instability in stage II and III colon cancer: an assessment of their prognostic and oxaliplatin predictive value. *Clin Cancer Res*. 2012;18(23):6531–6541.
15. Rosty C, Young JP, Walsh MD, et al. *PIK3CA* activating mutation in colorectal carcinoma: associations with molecular features and survival. *PLoS One*. 2013;8(6):e65479.
16. Day FL, Jorissen RN, Lipton L, et al. *PIK3CA* and *PTEN* gene and exon mutation-specific clinicopathological and molecular associations in colorectal cancer. *Clin Cancer Res*. 2013;19(12):3285–3296.
17. Sartore-Bianchi A, Martini M, Molinari F, et al. *PIK3CA* mutations in colorectal cancer are associated with clinical resistance to EGFR-targeted monoclonal antibodies. *Cancer Res*. 2009;69(5):1851–1857.
18. Ogino S, Nosho K, Kirkner GJ, et al. *PIK3CA* mutation is associated with poor prognosis among patients with curatively resected colon cancer. *J Clin Oncol*. 2009;27(9):1477–1484.
19. He Y, Van't Veer LJ, Mikolajewska-Hanclich I, et al. *PIK3CA* mutations predict local recurrences in rectal cancer patients. *Clin Cancer Res*. 2009;15(22):6956–6962.
20. De Roock W, Claes B, Bernasconi D, et al. Effects of *KRAS*, *BRAF*, *NRAS*, and *PIK3CA* mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. *Lancet Oncol*. 2010;11(8):753–762.
21. Tol J, Dijkstra JR, Klomp M, et al. Markers for EGFR pathway activation as predictor of outcome in metastatic colorectal cancer patients treated with or without cetuximab. *Eur J Cancer*. 2010;46(11):1997–2009.
22. Prenen H, De Schutter J, Jacobs B, et al. *PIK3CA* mutations are not a major determinant of resistance to the epidermal growth factor receptor inhibitor cetuximab in metastatic colorectal cancer. *Clin Cancer Res*. 2009;15(9):3184–3188.
23. Farina Sarasqueta A, Zeestraten EC, van Wezel T, et al. *PIK3CA* kinase domain mutation identifies a subgroup of stage III colon cancer patients with poor prognosis. *Cell Oncol (Dordr)*. 2011;34(6):523–531.
24. Phipps AI, Makar KW, Newcomb PA. Descriptive profile of *PIK3CA*-mutated colorectal cancer in postmenopausal women [published online

ahead of print June 1, 2013]. *Int J Colorectal Dis*. 2013: doi:10.1007/s00384-00013-01715-00388.
25. Liao X, Lochhead P, Nishihara R, et al. Aspirin use, tumor *PIK3CA* mutation status, and colorectal cancer survival. *N Engl J Med*. 2012;367(17):1596–1606.
26. De Roock W, Vriendt VD, Normanno N, Ciardiello F, Tejpar S. *KRAS*, *BRAF*, *PIK3CA*, and *PTEN* mutations: implications for targeted therapies in metastatic colorectal cancer. *Lancet Oncol*. 2011;12(6):594–603.
27. Lievre A, Blons H, Laurent-Puig P. Oncogenic mutations as predictive factors in colorectal cancer. *Oncogene*. 2010;29(21):3033–3043.
28. Febbo PG, Ladanyi M, Aldape KD, et al. NCCN Task Force report: evaluating the clinical utility of tumor markers in oncology. *J Natl Compr Canc Netw*. 2011;9(Suppl 5):S1–32; quiz S33.
29. Ogino S, Lochhead P, Giovannucci E, Meyerhardt JA, Fuchs CS, Chan AT. Discovery of colorectal cancer PIK3CA mutation as potential predictive biomarker: power and promise of molecular pathological epidemiology [published online ahead of print June 24, 2013]. *Oncogene*. 2013; doi:10.1038/onc.2013.244.
30. Saltz LB, Niedzwiecki D, Hollis D, et al. Irinotecan fluorouracil plus leucovorin is not superior to fluorouracil plus leucovorin alone as adjuvant treatment for stage III colon cancer: results of CALGB 89803. *J Clin Oncol*. 2007;25(23):3456–3461.
31. Yamauchi M, Lochhead P, Morikawa T, et al. Colorectal cancer: a tale of two sides or a continuum? *Gut*. 2012;61(6):794–797.
32. Ogino S, Kawasaki T, Brahmandam M, et al. Sensitive sequencing method for *KRAS* mutation detection by pyrosequencing. *J Mol Diagn*. 2005;7(3):413–421.
33. Ogino S, Kawasaki T, Kirkner GJ, Loda M, Fuchs CS. CpG island methylator phenotype-low (CIMP-low) in colorectal cancer: possible associations with male sex and *KRAS* mutations. *J Mol Diagn*. 2006;8(5):582–588.
34. Bertagnolli MM, Niedzwiecki D, Compton CC, et al. Microsatellite instability predicts improves response to adjuvant therapy with irinotecan, fluorouracil, and leucovorin in stage III colon cancer: Cancer and Leukemia Group B protocol 89803. *J Clin Oncol*. 2009;27(11):1814–1821.
35. Nosho K, Irahara N, Shima K, et al. Comprehensive biostatistical analysis of CpG island methylator phenotype in colorectal cancer using a large population-based sample. *PLoS ONE*. 2008;3(11):e3698.
36. Ogino S, Fuchs CS, Giovannucci E. How many molecular subtypes? Implications of the unique tumor principle in personalized medicine. *Expert Rev Mol Diagn*. 2012;12(6):621–628.
37. Imamura Y, Morikawa T, Liao X, et al. Specific mutations in KRAS codons 12 and 13, and patient prognosis in 1075 *BRAF*-wild-type colorectal cancers. *Clin Cancer Res*. 2012;18(17):4753–4763.
38. Chen CC, Er TK, Liu YY, et al. Computational analysis of *KRAS* mutations: implications for different effects on the KRAS p.G12D and p.G13D mutations. *PLoS One*. 2013;8(2):e55793.
39. Straussman R, Morikawa T, Shee K, et al. Tumor microenvironment contributes to innate RAF-inhibitor resistance through HGF secretion. *Nature*. 2012;487(7408):500–504.
40. Ogino S, Galon J, Fuchs CS, Dranoff G. Cancer immunology—analysis of host and tumor factors for personalized medicine. *Nat Rev Clin Oncol*. 2011;8(12):711–719.
41. Dahlin AM, Henriksson ML, Van Guelpen B, et al. Colorectal cancer prognosis depends on T-cell infiltration and molecular characteristics of the tumor. *Mod Pathol*. 2011;24(5):671–682.
42. Nosho K, Baba Y, Tanaka N, et al. Tumour-infiltrating T-cell subsets, molecular changes in colorectal cancer and prognosis: cohort study and literature review. *J Pathol*. 2010;222(4):350–366.
43. Galon J, Franck P, Marincola FM, et al. Cancer classification using the Immunoscore: a worldwide task force. *J Transl Med*. 2012;10(1):205.
44. Lao VV, Grady WM. Epigenetics and colorectal cancer. *Nat Rev Gastroenterol Hepatol*. 2011;8(12):686–700.
45. Goel A, Boland CR. Epigenetics of colorectal cancer. *Gastroenterology*. 2012;143(6):1442–1460.
46. Colussi D, Brandi G, Bazzoli F, Ricciardiello L. Molecular pathways involved in colorectal cancer: implications for disease behavior and prevention. *Int J Mol Sci*. 2013;14:16365–16385.
47. Bardhan K, Liu K. Epigenetics and colorectal cancer pathogenesis. *Cancers*. 2013;5:676–713.
48. Kunzmann AT, Murray LL, Cardwell CR, McShane CM, McMenamin UC, Cantwell MM. PTGS2 (Cyclooxygenase-2) expression and survival amongst colorectal cancer patients: a systematic review. *Cancer Epidemiol Biomarkers Prev*. 2013;22(9):1490–1497.
49. Ogino S, Nosho K, Kirkner GJ, et al. CpG island methylator phenotype, microsatellite instability, *BRAF* mutation and clinical outcome in colon cancer. *Gut*. 2009;58(1):90–96.
50. Dopeso H, Mateo-Lozano S, Elez E, et al. Aprataxin tumor levels predict response of colorectal cancer patients to irinotecan-based treatment. *Clin Cancer Res*. 2010;16(8):2375–2382.
51. Glimelius B, Garmo H, Berglund A, et al. Prediction of irinotecan and 5-fluorouracil toxicity and response in patients with advanced colorectal cancer. *Pharmacogenomics J*. 2011;11(1):61–71.
52. Vallbohmer D, Iqbal S, Yang DY, et al. Molecular determinants of irinotecan efficacy. *Int J Cancer*. 2006;119(10):2435–2442.
53. Tejpar S, Bosman F, Delorenzi M, et al. Microsatellite instability (MSI) in stage II and III colon cancer treated with 5FU-LV or 5FU-LV and irinotecan (PETACC 3-EORTC 40993-SAKK 60/00 trial). *J Clin Oncol*. 2009;27(15s):abstract 4001.
54. Ogino S, Shima K, Meyerhardt J, et al. Predictive and prognostic roles of *BRAF* mutation in stage III colon cancer: results from intergroup trial CALGB 89803. *Clin Cancer Res*. 2012;18(3):890–900.
55. Ogino S, Meyerhardt JA, Irahara N, et al. *KRAS* mutation in stage III colon cancer and clinical outcome following intergroup trial CALGB 89803. *Clin Cancer Res*. 2009;15(23):7322–7329.
56. Domingo E, Church DN, Sieber O, et al. Evaluation of *PIK3CA* mutation as a predictor of benefit from NSAID therapy in colorectal cancer. *J Clin Oncol*. 2013.
57. Mao C, Yang ZY, Hu XF, Chen Q, Tang JL. *PIK3CA* exon 20 mutations as a potential biomarker for resistance to anti-EGFR monoclonal antibodies in *KRAS* wild-type metastatic colorectal cancer: a systematic review and meta-analysis. *Ann Oncol*. 2012;23(6):1518–1525.
58. Arcaroli JJ, Quackenbush KS, Powell RW, et al. Common *PIK3CA* mutants and a novel 3' UTR mutation are associated with increased sensitivity to saracatinib. *Clin Cancer Res*. 2012;18(9):2704–2714.
59. Phipps AI, Buchanan DD, Makar KW, et al. *BRAF* mutation status and survival after colorectal cancer diagnosis according to patient and tumor characteristics. *Cancer Epidemiol Biomarkers Prev*. 2012;21(10):1792–1798.
60. Zlobec I, Bihl MP, Schwarb H, Terracciano L, Lugli A. Clinicopathological and protein characterization of BRAF- and K-RAS-mutated colorectal cancer and implications for prognosis. *Int J Cancer*. 2010;127(2):367–380.
61. Popovici V, Budinska E, Tejpar S, et al. Identification of a poor-prognosis *BRAF*-mutant-like population of patients with colon cancer. *J Clin Oncol*. 2012;30(12):1288–1295.
62. Lochhead P, Kuchiba A, Imamura Y, et al. Microsatellite instability and *BRAF* mutation testing in colorectal cancer prognostication. *J Natl Cancer Inst*. 2013;105(15):1151–1156.
63. Bruin SC, He Y, Mikolajewska-Hanclich I, et al. Molecular alterations associated with liver metastases development in colorectal cancer patients. *Br J Cancer*. 2011;105(2):281–287.
64. Zhao L, Vogt PK. Helical domain and kinase domain mutations in p110alpha of phosphatidylinositol 3-kinase induce gain of function by different mechanisms. *Proc Natl Acad Sci U S A*. 2008;105(7):2652–2657.
65. Kim A, Lee JE, Lee SS, et al. Coexistent mutations of *KRAS* and *PIK3CA* affect the efficacy of NVP-BEZ235, a dual PI3K/MTOR inhibitor, in regulating the PI3K/MTOR pathway in colorectal cancer. *Int J Cancer*. 2013;133(4):984–996.
66. Ogino S, Stampfer M. Lifestyle factors and microsatellite instability in colorectal cancer: the evolving field of molecular pathological epidemiology. *J Natl Cancer Inst*. 2010;102(6):365–367.
67. Ogino S, Chan AT, Fuchs CS, Giovannucci E. Molecular pathological epidemiology of colorectal neoplasia: an emerging transdisciplinary and interdisciplinary field. *Gut*. 2011;60(3):397–411.
68. Curtin K, Slattery ML, Samowitz WS. CpG island methylation in colorectal cancer: past, present and future. *Pathol Res Int*. 2011;2011:902674.

69. Hughes LA, Khalid-de Bakker CA, Smits KM, et al. The CpG island methylator phenotype in colorectal cancer: progress and problems. *Biochim Biophys Acta*. 2012;1825(1):77–85.
70. Ku CS, Cooper DN, Wu M, et al. Gene discovery in familial cancer syndromes by exome sequencing: prospects for the elucidation of familial colorectal cancer type X. *Mod Pathol*. 2012;25(8):1055–1068.
71. Gay LJ, Mitrou PN, Keen J, et al. Dietary, lifestyle and clinico-pathological factors associated with *APC* mutations and promoter methylation in colorectal cancers from the EPIC-Norfolk Study. *J Pathol*. 2012;228(3):405–415.
72. Chia WK, Ali R, Toh HC. Aspirin as adjuvant therapy for colorectal cancer-reinterpreting paradigms. *Nat Rev Clin Oncol*. 2012;9(10):561–570.
73. Spitz MR, Caporaso NE, Sellers TA. Integrative cancer epidemiology—the next generation. *Cancer Disc*. 2012;2(12):1087–1090.
74. Rosty C, Young JP, Walsh MD, et al. Colorectal carcinomas with *KRAS* mutation are associated with distinctive morphological and molecular features. *Mod Pathol*. 2013;26(6):825–834.
75. Buchanan DD, Win AK, Walsh MD, et al. Family history of colorectal cancer in *BRAF* p.V600E mutated colorectal cancer cases. *Cancer Epidemiol Biomarkers Prev*. 2013;22(5):917–926.
76. Burnett-Hartman AN, Newcomb PA, Potter JD, et al. Genomic aberrations occuring in subsets of serrated colorectal lesions but not conventional adenomas. *Cancer Res*. 2013;73(9):2863–2872.
77. Funkhouser WK, Lubin IM, Monzon FA, et al. Relevance, pathogenesis, and testing algorithm for mismatch repair-defective colorectal carcinomas: a report of the Association for Molecular Pathology. *J Mol Diagn*. 2012;14(2):91–103.

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