KRAS Mutation in Stage III Colon Cancer and Clinical Outcome Following Intergroup Trial CALGB 89803

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Abstract

Purpose: Alterations in the RAS and RAF pathway relate to epigenetic and epigenomic aberrations, and are important in colorectal carcinogenesis. *KRAS* mutation in metastatic colorectal cancer predicts resistance to anti–epidermal growth factor receptor (EGFR)-targeted therapy (cetuximab or panitumumab). It remains uncertain, however, whether *KRAS* mutation predicts prognosis or clinical outcome of colon cancer patients independent of anti-EGFR therapy.

Methods: We conducted a study of 508 cases identified among 1,264 patients with stage III colon cancer who enrolled in a randomized adjuvant chemotherapy trial (5-fluorouracil, leucovorin with or without irinotecan) in 1999-2001 (CALGB 89803). *KRAS* mutations were detected in 178 tumors (35%) by pyrosequencing. Kaplan-Meier and Cox proportional hazard models assessed the prognostic significance of *KRAS* mutation and adjusted for potential confounders including age, sex, tumor location, tumor/node stage, performance status, adjuvant chemotherapy arm, and microsatellite instability status.

Results: Compared with patients with *KRAS*-wild-type tumors, patients with *KRAS*-mutated tumors did not experience any difference in disease-free, recurrence-free, or overall survival. The 5-year disease-free, recurrence-free, and overall survival rates (*KRAS*-mutated versus *KRAS*-wild-type patients) were 62% versus 63% (log-rank $P = 0.89$), 64% versus 66% ($P = 0.84$), and 75% versus 73% ($P = 0.56$), respectively. The effect of *KRAS* mutation on patient survival did not significantly differ according to clinical features, chemotherapy arm, or microsatellite instability status, and the effect of adjuvant chemotherapy assignment on outcome did not differ according to *KRAS* status.

Conclusions: In this large trial of chemotherapy in stage III colon cancer patients, *KRAS* mutational status was not associated with any significant influence on disease-free or overall survival. (Clin Cancer Res 2009;15(23):7322–9)

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Translational Relevance

Activating mutations in the *KRAS* gene are important events during the colorectal carcinogenic process, and predict resistance to anti–epidermal growth factor receptor treatment for metastatic colorectal cancer. However, the literature data on the prognostic significance of *KRAS* mutation in colon cancer have been conflicting. We utilized the database of 508 stage III colon cancers in this adjuvant chemotherapy trial following surgical resection. Because data on pathologic stage, performance status, postoperative treatment, and follow-up were carefully captured in this trial, the simultaneous impact of disease characteristics and the use of adjuvant therapy could be assessed to be controlled for potential confounding. We found that *KRAS* mutation does not have a substantial prognostic or predictive role in stage III colon cancer treated with adjuvant chemotherapy.

KRAS, one of the first genes found to be mutated in human cancer, encodes a G-protein downstream of receptor tyrosine kinases, including epidermal growth factor receptor (EGFR; ref. 1–3). Population-based studies have shown that approximately 30% to 40% of colon cancers harbor mutations in codons 12 and 13 of *KRAS* (4–6). Retrospective observational studies (7–12) as well as randomized controlled trials (13–17) have consistently shown that *KRAS* mutation in stage IV colorectal cancer confers resistance to anti-EGFR targeted treatment (cetuximab or panitumumab). However, whether *KRAS* mutation in colorectal cancer has a prognostic role, independent of anti-EGFR therapy, has been controversial (18–21). Previous data have not been conclusive, even among several large studies (4, 6, 22–26). In addition, whether *KRAS* mutational status modifies the effect of irinotecan-based chemotherapy remains uncertain.

We therefore examined the influence of KRAS on cancer recurrence and survival in a large number (N = 508) of stage III colon cancer patients enrolled in a National Cancer Institute (NCI)-sponsored clinical trial of postoperative adjuvant chemotherapy (27). Within this trial (CALGB 89803), patients were randomized to either fluorouracil and leucovorin or fluorouracil, leucovorin, and irinotecan. Moreover, because data on pathologic stage, performance status, postoperative treatment, and follow-up were carefully captured in this trial, the simultaneous impact of disease characteristics and the use of adjuvant therapy could be assessed to control for potential confounding.

Materials and Methods

Study population. Patients in this study were participants in the NCI-sponsored Cancer and Leukemia Group B (CALGB) adjuvant therapy trial for stage III colon cancer comparing therapy with the weekly Roswell Park regimen of 5-fluorouracil (FU) and leucovorin (FU/LV) with the weekly bolus regimen of irinotecan, FU, and leucovorin (IFL; CALGB 89803; ref. 27). From April 1999 to May 2001, 1,264 patients were enrolled in the treatment trial. Patients in the treatment trial (and thus this companion study) were eligible if they had undergone a complete surgical resection of the primary tumor within 56 d prior to study entry, and had regional lymph node metastases (stage III colon cancer) but no evidence of distant metastases. Moreover, patients were required to have a baseline Eastern Cooperative Oncology Group performance status of 0 to 2 (ambulatory; ref. 28) and have adequate bone marrow, renal, and hepatic function. The current analysis was limited to 508 patients for whom archived formalin-fixed paraffin-embedded tumor tissue was available and the *KRAS* gene was sequenced. All patients signed informed consent, approved by each site's institutional review board.

We compared the baseline characteristics of the patients who were included in this study (with available *KRAS* data, n = 508) with those who were excluded from this study due to unavailability of tissue data (n = 756). We did not detect any significant or substantial difference between these two groups in terms of age, sex, body mass index, tumor location, T stage, N stage, performance status, bowel perforation, bowel obstruction, or treatment arm. In addition, tumor recurrence or mortality did not substantially differ between these two groups; multivariate hazard ratios (HR; *KRAS* data available versus unavailable) were 1.05 [95% confidence interval (95% CI), 0.87-1.27] for disease-free survival (DFS), 1.05 (95% CI, 0.86-1.28) for recurrence-free survival (RFS), and 1.06 (95% CI, 0.86-1.32) for overall survival (OS).

Definitions of study end points. In this study, the primary end point was DFS, defined as time from the study enrollment to tumor recurrence, occurrence of a new primary colon tumor, or death from any cause. In addition, we defined RFS as the time from the study enrollment to tumor recurrence or occurrence of a new primary colon tumor. For RFS, patients who died without known tumor recurrence were censored at last documented evaluation by treatment provider. Finally, OS was defined as the time from the study enrollment to death from any cause.

DNA extraction from tumor, sequencing of* KRAS*, and microsatellite instability analysis. DNA was extracted from paraffin-embedded tissue of colon cancer as previously described (29). We marked a tumor area on a H&E-stained slide, and dissected the tumor area from another tumor tissue section by a sterile needle for subsequent DNA extraction. PCR and pyrosequencing spanning *KRAS* codons 12 and 13 were done as previously described (29), and validated against Sanger sequencing method (29, 40). In our KRAS pyrosequencing assay, we routinely confirmed the presence of a mutation by two different sequencing primers and by the creation of frameshifted reading of a mutant sequence relative to a wild-type sequence in a pyrogram (29). Microsatellite instability (MSI) was assessed using 10 DNA mononucleotide and dinucleotide microsatellite markers as previously described (30). Tumors showing instability in ≥40% of the loci tested were classified as MSI-high. Tumors showing instability in no or <40% of the loci were classified as microsatellite stable (MSS)/MSI-low.

Statistical analyses. The goal of this correlative study was to determine whether tumoral *KRAS* mutational status influences clinical outcome of patients with stage III colon cancer. Patient registration and clinical data collection were managed by the CALGB Statistical Center, and analyses were conducted collaboratively between the CALGB Statistical Center and Dana-Farber Cancer Institute. All analyses were based on the study database frozen on March 7, 2008, except for the tumoral *KRAS* data. All analyses used SAS version 9.1 (SAS Institute) and all P values were two-sided.

In the treatment trial (comparing two chemotherapy regimens), there was no statistical difference in either DFS or OS between the treatment arms (27). The Kaplan-Meier method was used to describe the distribution of survival time according to *KRAS* status, and the log-rank test was carried out. We used stage-matched (or stratified) Cox proportional hazard models to calculate the HR of events according to tumoral *KRAS* status, adjusted for age at study entry (as a continuous variable), gender, baseline body mass index (≥30 versus <30 kg/m^2), baseline performance status (0 versus 1-2), presence of bowel perforation or obstruction at time of surgery, treatment arm, tumor location (proximal versus distal), and MSI status (high versus low/MSS). Tumor stage (IIIA, IIIB, IIIC, or III unspecified substage) was used as a matching

Table 1. Baseline characteristics according to *KRAS* mutational status in stage III colon cancer

Clinical or molecular feature	No. of cases	*KRAS*	
		Wild-type	Mutant
Total	508	330	178
Sex			
Male	276 (54%)	179 (54%)	97 (54%)
Female	232 (46%)	151 (46%)	81 (46%)
Age (y)			
<50	100 (20%)	62 (19%)	38 (21%)
50-59	130 (26%)	82 (25%)	48 (27%)
60-69	158 (31%)	102 (31%)	56 (31%)
≥70	120 (24%)	84 (25%)	36 (20%)
Mean age ± SD, y	59.8 ± 11.5	60.2 ± 11.6	59.1 ± 11.4
Body mass index (kg/m^2)			
<25	163 (32%)	110 (34%)	53 (30%)
25-29	182 (36%)	114 (35%)	68 (39%)
≥30	157 (31%)	104 (32%)	53 (30%)
Tumor location			
Right (cecum to transverse colon)	291 (58%)	191 (58%)	100 (57%)
Left colon (splenic flexure to sigmoid)	212 (42%)	136 (42%)	76 (43%)
T stage			
T_1-T_2	59 (12%)	43 (13%)	16 (9.1%)
T_3	410 (82%)	260 (80%)	150 (85%)
T_4	33 (6.6%)	23 (7.1%)	10 (5.7%)
N stage			
N_1	321 (64%)	203 (62%)	118 (67%)
N_2	184 (36%)	125 (38%)	59 (33%)
AJCC tumor stage			
IIIA	49 (9.7%)	34 (10%)	15 (8.4%)
IIIB	270 (53%)	167 (51%)	103 (58%)
IIIC	184 (36%)	125 (38%)	59 (33%)
III, unknown substage	5 (1.0%)	4 (1.2%)	1 (0.6%)
Performance status score			
0	384 (76%)	246 (75%)	138 (78%)
1-2	120 (24%)	82 (25%)	38 (22%)
Clinical bowel perforation			
(-)	477 (96%)	310 (96%)	167 (95%)
(+)	22 (4.4%)	14 (4.3%)	8 (4.6%)
Clinical bowel obstruction			
(-)	393 (78%)	252 (77%)	141 (80%)
(+)	112 (22%)	76 (23%)	36 (20%)
MSI status*			
MSS/MSI-low	394 (82%)	247 (78%)	147 (89%)
MSI-high	86 (18%)	68 (22%)	18 (11%)
Treatment arm*			
FU/LV	266 (52%)	157 (48%)	109 (61%)
IFL	242 (48%)	173 (52%)	69 (39%)

NOTE: (%) indicates the proportion of tumors with a specific clinical feature in *KRAS*-wild-type tumors (or *KRAS*-mutated tumors). There were cases with missing value/status for some of the variables.
Abbreviation: AJCC, American Joint Committee on Cancer.
*Distributional differences are significant with $P < 0.01$.

(stratifying) variable (with the "strata" option in the SAS "proc phreg" command) to minimize residual confounding. The proportionality of hazards assumption was satisfied by evaluating time-dependent variables, which were the cross-product of the *KRAS* variable and survival time ($P = 0.10$ for DFS, $P = 0.06$ for RFS, $P = 0.24$ for OS). Covariates with missing variables, including body mass index (1.2% missing), tumor location (1.0% missing), performance status (0.8% missing), perforation status (1.8% missing), and MSI status (5.5% missing), were coded with separate "missing" indicator variables in adjusted models. We assigned three cases (0.6%) with missing information in obstruction status as "no obstruction." We confirmed that excluding cases with missing information in any of the covariates did not substantially alter results (data not shown). An interaction was assessed by including the cross product of the *KRAS* variable and another variable of interest in a multivariate Cox model, and the Wald test was done: *P* values were conservatively interpreted, considering multiple hypothesis testing. To assess an interaction of *KRAS* and stage, we dichotomized American Joint Committee on Cancer stage (IIIA-IIIB, N_1 versus IIIC, N_2) as well as assessed an interaction with T stage ($T_{1\text{-}2}$ versus $T_{3\text{-}4}$). In addition to obtaining a *P* value for interaction, we did stratified analysis to assess potential differential effect of *KRAS* mutation, in which we assessed the effect of *KRAS* mutation simultaneously in two or more strata (of a variable of interest) in a single Cox regression model (31, 32).

As part of the quality assurance program of the CALGB, members of the Audit Committee visit all participating institutions at least once every three years to review source documents. The auditors verify

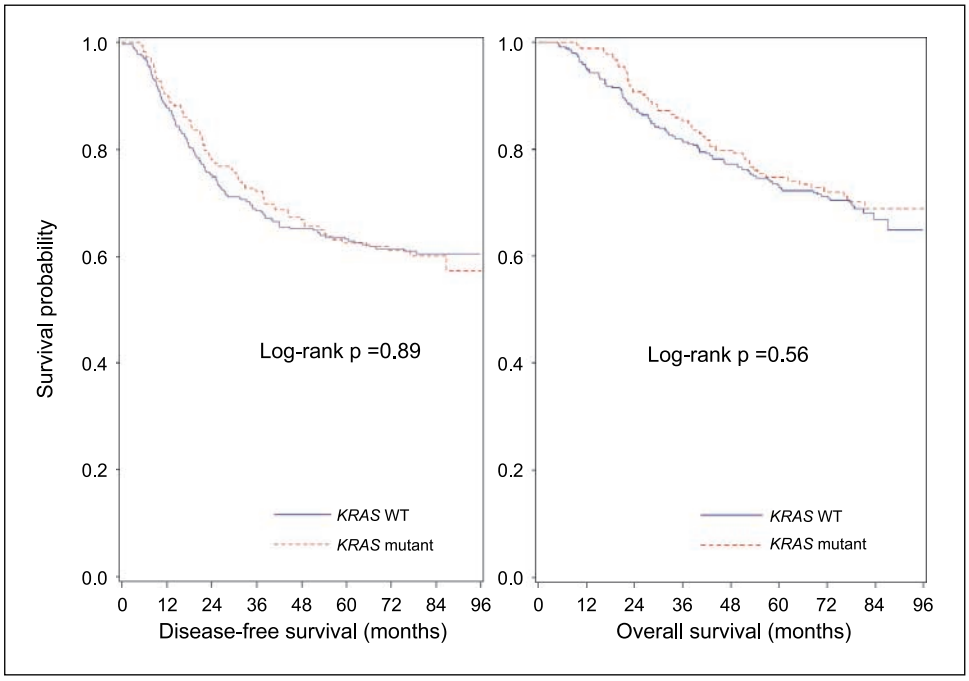

Fig. 1. Kaplan-Meier survival curves for disease-free survival (*left*) and overall survival (*right*) in stage III colon cancer according to *KRAS* mutational status.

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 done for a subgroup of 328 patients (26%) of the 1,264 patients under this study.

Results

KRAS *mutation and clinical outcome in stage III colon cancer*. Study participants were drawn from a multicenter study of postoperative adjuvant chemotherapy in patients with stage III colon cancer who underwent a curative-intent surgical resection. We included 508 cases in this study based on availability of tumor tissue for *KRAS* sequencing, which detected a *KRAS* mutation in 178 (35%) patients. Identified KRAS mutations were as follows: 56 cases with codon 12 GGT > GAT (p.G12D, c.35G > A); 52 with codon 13 GGC > GAC (p.G13D, c.38G > A); 32 with codon 12 GGT > GTT (p.G12V, c.35G > T); 21 with codon 12 GGT > TGT (p.G12C, c.34G > T); 9 with codon 12 GGT > GCT (p.G12A, c.35G > C); and 8 with codon 12 GGT > AGT (p.G12S, c.34G > A). Table 1 summarizes the baseline characteristics of study subjects according to *KRAS* mutational status. Patients with a mutation in *KRAS* were significantly less likely to possess MSI or receive IFL as compared with FU/LV.

We assessed the influence of *KRAS* mutational status on clinical outcome in the 508 patients with stage III colon cancers. With median follow-up of 6.2 years among surviving participants, there were 196 events for DFS analysis, 180 events for RFS analysis, and 149 events for OS analysis. In Kaplan-Meier analysis, there were no significant differences in survival time distributions between patients with *KRAS* mutations and those with wild-type *KRAS* (log-rank $P = 0.89$ for DFS; Fig. 1; log-rank $P = 0.84$ for RFS; log-rank $P = 0.56$ for OS). DFS at 5 years was 62% for *KRAS*-mutated and 63% for *KRAS*-wild-type patients. RFS at 5 years was 64% for *KRAS*-mutated and 66% for *KRAS*-wild-type patients. Finally, OS at 5 years was 75% for *KRAS*-mutated and 73% for *KRAS*-wild-type patients.

In a univariate Cox regression analysis, when compared with *KRAS*-wild-type patients, *KRAS*-mutated patients did not experience a significant difference in DFS (HR, 0.98; 95% CI, 0.73-1.31), RFS (HR, 0.97; 95% CI, 0.71-1.32), or OS (HR,

Table 2. *KRAS* mutational status and clinical outcome in stage III colon cancer

KRAS	Total, *n*	Disease-free survival			Recurrence-free survival			Overall survival		
		No. of events	Univariate HR (95% CI)	Multivariate HR (95% CI)	No. of events	Univariate HR (95% CI)	Multivariate HR (95% CI)	No. of events	Univariate HR (95% CI)	Multivariate HR (95% CI)
Wild-type	330 (65%)	127	1 (referent)	1 (referent)	117	1 (referent)	1 (referent)	100	1 (referent)	1 (referent)
Mutant	178 (35%)	69	0.98 (0.73-1.31)	0.95 (0.70-1.28)	63	0.97 (0.71-1.32)	0.93 (0.68-1.28)	49	0.90 (0.64-1.27)	0.86 (0.60-1.23)

NOTE: The multivariate Cox regression model included age, sex, body mass index, tumor location, T and N stage, the presence or absence of perforation and/or obstruction at diagnosis, performance status, MSI status, and treatment arm.

Table 3. *KRAS* mutation in stage III colon cancer and clinical outcome according to treatment arm

	Total, *n*	Disease-free survival			Recurrence-free survival			Overall survival		
		No. of events	Univariate HR (95% CI)	Multivariate HR (95% CI)	No. of events	Univariate HR (95% CI)	Multivariate HR (95% CI)	No. of events	Univariate HR (95% CI)	Multivariate HR (95% CI)
FU/LV										
KRAS(-)	157	59	1 (referent)	1 (referent)	52	1 (referent)	1 (referent)	50	1 (referent)	1 (referent)
KRAS(+)	109	44	1.06 (0.72-1.57)	1.05 (0.70-1.60)	39	1.07 (0.70-1.62)	1.02 (0.66-1.59)	30	0.85 (0.54-1.33)	0.82 (0.50-1.32)
IFL										
KRAS(-)	173	68	1 (referent)	1 (referent)	65	1 (referent)	1 (referent)	50	1 (referent)	1 (referent)
KRAS(+)	69	25	0.88 (0.56-1.39)	0.85 (0.53-1.36)	24	0.88 (0.55-1.41)	0.86 (0.53-1.38)	19	0.97 (0.57-1.65)	0.95 (0.55-1.64)
P for interaction (*KRAS* and treatment arm)			0.55	0.64		0.56	0.67		0.69	0.60

NOTE: The multivariate Cox regression model included the *KRAS* variable stratified by the treatment arm variable, age, sex, tumor location, T and N stage, the presence or absence of perforation and/or obstruction at diagnosis, performance status, and MSI status.

0.90; 95% CI, 0.64-1.27; Table 2). These findings persisted in multivariate analysis that adjusted for clinical, pathologic, or molecular predictors of patient outcome, and no substantial confounding was identified.

KRAS *mutation and clinical outcome in strata of treatment arm.* We assessed whether the effect of *KRAS* mutational status on patient outcome was modified by adjuvant chemotherapy (Table 3). In both treatment arms (FU/LV and IFL), the presence of a mutation in *KRAS* was not associated with any significant difference in patient survival. Moreover, statistical tests for interaction failed to show any significant interaction between chemotherapy assignment and *KRAS* mutational status (*P* for interaction = 0.64, 0.67, and 0.60 for DFS, RFS, and OS, respectively).

Effect of irinotecan on clinical outcome in strata of* KRAS *status. We also assessed whether the effect of adjuvant chemotherapy arm on patient survival was modified by *KRAS* mutational status (Table 4). In both *KRAS*-wild-type and *KRAS*-mutated cases, there were no significant differences in DFS, RFS, or OS between the two treatment arms.

No significant modifying effect on the relation between* KRAS *and clinical outcome by any of the other covariates. Finally, we examined whether there was significant modifying effect on the relation between *KRAS* mutation and clinical outcome by any of the other covariates (age, gender, body mass index, baseline performance status, tumor location, T stage, N stage, stage III substage, status of bowel perforation or obstruction, and MSI status). There was no evidence of significant effect modification by any of the variables examined (all $P_{interaction} > 0.23$).

Discussion

In this study of 508 patients with stage III colon cancer treated with surgery and adjuvant chemotherapy, *KRAS* mutational

Table 4. Treatment arm and clinical outcome of patients with stage III colon cancer in strata of *KRAS* status

	Total, *n*	Disease-free survival			Recurrence-free survival			Overall survival		
		No. of events	Univariate HR (95% CI)	Multivariate HR (95% CI)	No. of events	Univariate HR (95% CI)	Multivariate HR (95% CI)	No. of events	Univariate HR (95% CI)	Multivariate HR (95% CI)
KRAS(-)										
FU/LV	157	59	1 (referent)	1 (referent)	52	1 (referent)	1 (referent)	50	1 (referent)	1 (referent)
IFL	173	68	1.06 (0.75-1.50)	1.07 (0.75-1.55)	65	1.15 (0.80-1.65)	1.16 (0.79-1.69)	50	0.90 (0.61-1.33)	0.94 (0.62-1.41)
KRAS(+)										
FU/LV	109	44	1 (referent)	1 (referent)	39	1 (referent)	1 (referent)	30	1 (referent)	1 (referent)
IFL	69	25	0.89 (0.54-1.45)	0.88 (0.53-1.47)	24	0.96 (0.58-1.60)	0.97 (0.57-1.65)	19	1.04 (0.58-1.84)	1.06 (0.58-1.93)
P for interaction (*KRAS* and treatment arm)			0.55	0.64		0.56	0.67		0.69	0.60

NOTE: The multivariate Cox regression model included the treatment arm variable stratified by the *KRAS* variable, age, sex, tumor location, T and N stage, the presence or absence of perforation and/or obstruction at diagnosis, performance status, and MSI status.

status was not associated with any significant influence on cancer recurrence or death. These results were not materially altered in multivariate analyses that adjusted for other predictors for patient outcome. Moreover, the effect of *KRAS* mutation on patient survival did not significantly differ according to clinical features, chemotherapy arm, or MSI status, and the effect of adjuvant chemotherapy arm did not differ according to *KRAS* status. In separate independent cohort studies (6, 33), we previously showed that *KRAS* mutation was not significantly associated with survival of colon cancer patients in univariate analysis as well as multivariate analysis that adjusted for tumor stage, MSI, *BRAF* mutation, and other related molecular features. Thus, together with our previous data, our current data do not support a substantial prognostic role of *KRAS* mutation in colon cancer.

Although *KRAS* mutation does not seem to be a significant prognostic marker in colon cancer, its importance in colorectal carcinogenesis has been well documented. *KRAS* is one of the most commonly mutated oncogenes in human cancer. *KRAS* mutation activates the RAS-RAF pathway as well as the phosphoinositide 3-kinase-AKT pathway, leading to cellular growth and proliferation (2). Indeed, *KRAS* and *PIK3CA* mutations are associated with each other in colorectal cancer (34, 35), and *KRAS* and *PIK3CA* mutations seem to interact in survival analysis (33). Recently, a link between *KRAS* mutation and epigenomic aberrations in colorectal cancer has been suggested (31, 36–38). Specifically, *KRAS* mutation has been associated with low-level CpG island methylator phenotype (31, 36, 38, 39), and this relation has been shown in another independent dataset (22). In contrast to somatic mutations including those in *KRAS*, epigenomic aberrations are potentially reversible. Although a mechanistic link between epigenomics and *KRAS* mutation remains uncertain, analysis of *KRAS* mutation in colon cancer may shed light on epigenomic aberrations in cancer and provide targeted therapeutic opportunities.

Studying patient outcome has been an important area in cancer research. Accumulating evidence suggests *KRAS* mutational status is a critical biomarker to predict response or resistance to anti-EGFR targeted therapy in patients with metastatic colorectal cancer. Retrospective observational studies (7–12) as well as randomized controlled trials (13–17) have consistently shown that *KRAS* mutation in stage IV colorectal cancer confers resistance to cetuximab or panitumumab treatment. Thus, *KRAS* mutation testing is rapidly emerging as a routine clinical test for patients with metastatic colorectal cancers who are potential candidates for treatment with either cetuximab or panitumumab (1, 2, 40).

In contrast to anti-EGFR targeted therapy, the role of *KRAS* mutation in predicting response to other therapies remains unclear. For example, a couple of previous studies have examined the relationship between *KRAS* mutation and response to bevacizumab, and have shown that *KRAS* mutation does not predict response or resistance to bevacizumab in colon cancer (25, 41).

Although the "predictive" role for *KRAS* mutational testing in defining sensitivity to anti-EGFR targeted therapy in stage IV colorectal cancer is now widely accepted, the "prognostic" role for *KRAS* as an independent predictor of survival in patients with colorectal cancer remains less conclusive (18–20). Previous meta-analyses (RASCAL and RASCAL II; refs. 42, 43) showed that *KRAS* mutation was associated with worse outcome in colorectal cancer. However, these meta-analyses substantially suffered from publication bias; especially as most studies used were relatively small (N <150 in most studies; N <290 in all included studies). Compared with small studies with significant results, small studies with null results were more likely unpublished, and thus more likely excluded from these meta-analyses. Larger studies (e.g., N >290) have tended to show no independent prognostic significance of *KRAS* mutation in colorectal cancer. A large population-based study of 569 colorectal cancer patients reported that *KRAS* mutation was independently associated with worse survival (22), whereas most other large studies found no independent prognostic role of *KRAS* mutation (4, 6, 23–25, 44), including a recent study on 1,379 stage II-III colon cancers (26). Our current findings were limited to only stage III colon cancers. Nonetheless, our results are consistent with most previous large studies on colon cancers including stage III and other stages (4, 6, 23–26, 44). Moreover, although one small study of 35 patients suggested that *KRAS* mutational status influenced irinotecan sensitivity (45), *KRAS* mutational status did not seem to modify the influence of irinotecan-based adjuvant therapy in our trial.

There are several advantages in examining associations of molecular markers with outcome of patients in a NCI-sponsored clinical trial of adjuvant chemotherapy. All patients had stage III colon cancer, thus reducing the impact of heterogeneity by disease stage. Moreover, treatment and follow-up care were all standardized within the clinical trial, and the date and nature of recurrence were prospectively recorded. In addition, detailed information on other prognostic variables was routinely collected at study entry.

We recognize that patients who enroll in randomized trials may differ from the population-at-large. To participate, patients must meet eligibility criteria, be selected as appropriate candidates, and be motivated to participate. In addition, patients were particularly selected for this study on the basis of availability of colon cancer tissue specimens. Nonetheless, the demographic data of the patients in this study did not suggest significant selection bias. Moreover, because the study included patients from both community and academic centers across North America, our findings should reflect the general population of stage III patients in North America. In addition, although data on *KRAS* mutational status were available on a subset of patients enrolled in the trial, baseline characteristics and patient survival did not differ for patients with and without available archived tumor tissue in this trial.

In conclusion, we found that KRAS mutational status did not significantly predict clinical outcome in this study of stage III colon cancer patients. Although *KRAS* mutational testing should be routinely utilized to assess for appropriate use of anti-EGFR therapy in advanced colorectal cancer, *KRAS* status is unlikely to meaningfully predict patient prognosis.

Disclosure of Potential Conflicts of Interest

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References

1. Jimeno A, Messersmith WA, Hirsch FR, Franklin WA, Eckhardt SG. KRAS mutations and sensitivity to epidermal growth factor receptor inhibitors in colorectal cancer: practical application of patient selection. J Clin Oncol 2009;27:1130–6.
2. Riely GJ, Ladanyi M. KRAS mutations: an old oncogene becomes a new predictive biomarker. J Mol Diagn 2008;10:493–5.
3. Hamilton SR. Targeted therapy of cancer: new roles for pathologists in colorectal cancer. Mod Pathol 2008;21 Suppl 2:S23–30.
4. Samowitz WS, Curtin K, Schaffer D, Robertson M, Leppert M, Slattery ML. Relationship of Ki-ras mutations in colon cancers to tumor location, stage, and survival: a population-based study. Cancer Epidemiol Biomarkers Prev 2000;9:1193–7.
5. Brink M, de Goeij AF, Weijenberg MP, et al. K-ras oncogene mutations in sporadic colorectal cancer in The Netherlands Cohort Study. Carcinogenesis 2003;24:703–10.
6. Ogino S, Nosho K, Kirkner GJ, et al. CpG island methylator phenotype, microsatellite instability, BRAF mutation and clinical outcome in colon cancer. Gut 2009;58:90–6.
7. Lievre A, Bachet JB, Boige V, et al. KRAS mutations as an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab. J Clin Oncol 2008;26:374–9.
8. Lievre A, Bachet J-B, Le Corre D, et al. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Res 2006;66:3992–5.
9. De Roock W, Piessevaux H, De Schutter J, et al. KRAS wild-type state predicts survival and is associated to early radiological response in metastatic colorectal cancer treated with cetuximab. Ann Oncol 2008;19:508–15.
10. Di Fiore F, Blanchard F, Charbonnier F, et al. Clinical relevance of KRAS mutation detection in metastatic colorectal cancer treated by Cetuximab plus chemotherapy. Br J Cancer 2007;96:1166–9.
11. Frattini M, Saletti P, Romagnani E, et al. PTEN loss of expression predicts cetuximab efficacy in metastatic colorectal cancer patients. Br J Cancer 2007;97:1139–45.
12. Freeman DJ, Juan T, Reiner M, et al. Association of K-ras mutational status and clinical outcomes in patients with metastatic colorectal cancer receiving panitumumab alone. Clin Colorectal Cancer 2008;7:184–90.
13. Khambata-Ford S, Garrett CR, Meropol NJ, et al. Expression of epiregulin and amphiregulin and K-ras mutation status predict disease control in metastatic colorectal cancer patients treated with cetuximab. J Clin Oncol 2007;25:3230–7.
14. Karapetis CS, Khambata-Ford S, Jonker DJ, et al. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. N Engl J Med 2008;359:1757–65.
15. Amado RG, Wolf M, Peeters M, et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. J Clin Oncol 2008;26:1626–34.
16. Van Cutsem E, Kohne CH, Hitre E, et al.

Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. N Engl J Med 2009;360:1408–17.

17. Bokemeyer C, Bondarenko I, Makhson A, et al. Fluorouracil, leucovorin, and oxaliplatin with and without cetuximab in the first-line treatment of metastatic colorectal cancer. J Clin Oncol 2009;27:663–71.

18. Klump B, Nehls O, Okech T, et al. Molecular lesions in colorectal cancer: impact on prognosis? Original data and review of the literature. Int J Colorectal Dis 2004;19:23–42.

19. Anwar S, Frayling IM, Scott NA, Carlson GL. Systematic review of genetic influences on the prognosis of colorectal cancer. Br J Surg 2004; 91:1275–91.

20. Westra JL, Plukker JT, Buys CH, Hofstra RM. Genetic alterations in locally advanced stage II/III colon cancer: a search for prognostic markers. Clin Colorectal Cancer 2004;4: 252–9.

21. van Krieken JH, Jung A, Kirchner T, et al. KRAS mutation testing for predicting response to anti-EGFR therapy for colorectal carcinoma: proposal for an European quality assurance program. Virchows Arch 2008;453:417–31.

22. Barault L, Charon-Barra C, Jooste V, et al. Hypermethylator phenotype in sporadic colon cancer: study on a population-based series of 582 cases. Cancer Res 2008;68:8541–6.

23. Wang C, van Rijnsoever M, Grieu F, et al. Prognostic significance of microsatellite instability and Ki-ras mutation type in stage II colorectal cancer. Oncology 2003;64:259–65.

24. Gnanasampanthan G, Elsaleh H, McCaul K, Iacopetta B. Ki-ras mutation type and the survival benefit from adjuvant chemotherapy in Dukes' C colorectal cancer. J Pathol 2001;195:543–8.

25. Ince WL, Jubb AM, Holden SN, et al. Association of k-ras, b-raf, and p53 status with the treatment effect of bevacizumab. J Natl Cancer Inst 2005;97:981–9.

26. Roth A, Tejpar S, Yan P, et al. Correlation of molecular markers in colon cancer with stage-specific prognosis: Results of the translational study on the PETACC 3 - EORTC 40993-SAKK 60–00 trial [abstract No. 288]. J Clin Oncol 2009.

27. 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;25:3456–61.

28. Zubrod C, Scheiderman M, Frei E, et al. Appraisal of methods for the study of chemotherapy in man: comparative therapeutic trial of nitrogen mustard and triethylene thiophosphoramide. J Chron Dis 1960;11:7–33.

29. Ogino S, Kawasaki T, Brahmandam M, et al. Sensitive sequencing method for KRAS mutation detection by Pyrosequencing. J Mol Diagn 2005;7:413–21.

30. 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;27:1814–21.

31. 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:e3698.

32. Ogino S, Nosho K, Kirkner GJ, et al. A cohort study of tumoral LINE-1 hypomethylation and prognosis in colon cancer. J Natl Cancer Inst 2008;100:1734–8.

33. 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;27:1477–84.

34. Velho S, Oliveira C, Ferreira A, et al. The prevalence of PIK3CA mutations in gastric and colon cancer. Eur J Cancer 2005;41:1649–54.

35. Nosho K, Kawasaki T, Ohnishi M, et al. PIK3CA mutation in colorectal cancer: relationship with genetic and epigenetic alterations. Neoplasia 2008;10:534–41.

36. 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;8:582–8.

37. Shen L, Toyota M, Kondo Y, et al. Integrated genetic and epigenetic analysis identifies three different subclasses of colon cancer. Proc Natl Acad Sci U S A 2007;104:18654–9.

38. Ogino S, kawasaki T, Kirkner GJ, Suemoto Y, Meyerhardt JA, Fuchs CS. Molecular correlates with MGMT promoter methylation and silencing support CpG island methylator phenotype-low (CIMP-low) in colorectal cancer. Gut 2007;56: 1409–16.

39. Ogino S, Goel A. Molecular classification and correlates in colorectal cancer. J Mol Diagn 2008;10:13–27.

40. Ogino S, Meyerhardt JA, Cantor M, et al. Molecular alterations in tumors and response to combination chemotherapy with gefitinib for advanced colorectal cancer. Clin Cancer Res 2005;11:6650–6.

41. Hurwitz HI, Yi J, Ince W, Novotny WF, Rosen O. The clinical benefit of bevacizumab in metastatic colorectal cancer is independent of K-ras mutation status: analysis of a phase III study of bevacizumab with chemotherapy in previously untreated metastatic colorectal cancer. Oncologist 2009;14:22–8.

42. Andreyev HJ, Norman AR, Cunningham D, et al. Kirsten ras mutations in patients with colorectal cancer: the 'RASCAL II' study. Br J Cancer 2001;85:692–6.

43. Andreyev HJ, Norman AR, Cunningham D, Oates JR, Clarke PA. Kirsten ras mutations in patients with colorectal cancer: the multicenter "RASCAL" study. J Natl Cancer Inst 1998;90: 675–84.

44. Finkelstein SD, Sayegh R, Bakker A, Swalsky P. Determination of tumor aggressiveness in colorectal cancer by K-ras-2 analysis. Arch Surg 1993;128:526–31; discussion 31–2.

45. Nemunaitis J, Cox J, Meyer W, Courtney A, Mues G. Irinotecan hydrochloride (CPT-11) resistance identified by K-ras mutation in patients with progressive colon cancer after treatment with 5-fluorouracil (5-FU). Am J Clin Oncol 1997;20:527–9.