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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-EGFR targeted therapy (cetuximab or panitumumab). However, it remains uncertain 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 1264 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

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(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 (MSI) status.

Results—Compared to patients with *KRAS*-wild-type tumors, patients with *KRAS*-mutated tumors did not experience any difference in disease-free (DFS), recurrence-free (RFS), or overall survival (OS). Five-year DFS, RFS and OS (*KRAS*-mutated vs. *KRAS*-wild-type patients) were: 62% vs. 63% (log-rank p=0.89); 64% vs. 66% (p=0.84); and 75% vs. 73% (p=0.56), respectively. 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 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.

Keywords

colorectal cancer; K-RAS; predictive; prognostic; response

INTRODUCTION

KRAS, one of the first genes found to be mutated in human cancer, encodes a G-protein downstream of receptor tyrosine kinases, including EGFR (1–3). Population-based studies have shown that approximately 30–40% 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, since 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.

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 and leucovorin (FU/LV) to weekly bolus regimen of irinotecan, 5-FU, and leucovorin (IFL) (CALGB 89803) (27). Between April 1999 and May 2001, 1,264 patients were enrolled on the treatment trial. Patients in the treatment trial (and thus this companion study) were eligible if they underwent a complete surgical resection of the primary tumor within 56 days prior to study entry, and had regional lymph node metastases (stage III colon cancer) but no evidence of distant metastases. Moreover, patients were required to have a baseline Eastern Cooperative Oncology Group (ECOG) performance status of 0–2

(ambulatory) (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 were available and the *KRAS* gene was sequenced. 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 *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 (BMI), 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 (*KRAS* data available vs. unavailable) were 1.05 (95% CI, 0.87–1.27) for disease-free survival; 1.05 (95% CI, 0.86–1.28) for recurrence-free survival; and 1.06 (95% CI, 0.86–1.32) for overall survival.

Definitions of study endpoints

In this study, the primary endpoint was disease-free survival (DFS), defined as time from the study enrollment to tumor recurrence, occurrence of a new primary colon tumor, or death from any cause. In addition, we defined recurrence-free survival (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 treating provider. Finally, overall survival (OS) was defined as the time from the study enrollment to death from any cause.

DNA extraction from tumor, sequencing of *KRAS*, and MSI analysis

DNA was extracted from paraffin-embedded tissue of colon cancer as previously described (29). We marked a tumor area on a hematoxylin and eosin (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 performed as previously described (29), and validated against Sanger sequencing method (29). 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 at least 40% of the loci tested were classified as MSI-high. Tumors showing instability in no or less than 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 influence 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, Cary, NC) and all p values were two-sided.

In the treatment trial (comparing two chemotherapy regimens), there was no statistical difference in either disease-free or overall survival 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 performed. We used stage-matched (or stratified) Cox proportional hazard models to calculate hazard ratios (HRs) of events according to tumoral

KRAS status, adjusted for age at study entry (as a continuous variable), gender, baseline body mass index (BMI; ≥30 vs. <30 kg/m^2), baseline performance status (0 vs. 1–2), presence of bowel perforation or obstruction at time of surgery, treatment arm, tumor location (proximal vs. distal) and MSI status (high vs. low/MSS). Tumor stage (IIIA, IIIB, IIIC or III unspecified substage) was used as a matching (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 disease-free survival; p=0.06 for recurrence-free survival; p=0.24 for overall survival). Covariates with missing variables [including BMI (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 3 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 performed: p values were conservatively interpreted, considering multiple hypothesis testing. To assess an interaction of *KRAS* and stage, we dichotomized AJCC stage (IIIA-IIIB, N1 vs. IIIC, N2) as well as assessed an interaction with T stage (T1–2 vs. T3–4). In addition to obtaining a P value for interaction, we performed 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 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 patients (26%) of the 1264 patients under this study.

RESULTS

KRAS mutation and clinical outcome in stage III colon cancer

Study participants were drawn from a multi-center study of post-operative 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); 8 with codon 12 GGT>AGT (p.G12S, c.34G>A). Table 1 summarizes baseline characteristics of study subjects according to *KRAS* mutational status. Patients with a mutation in *KRAS* were significantly less likely to possess microsatellite instability (MSI) or receive irinotecan, 5-fluorouracil, and leucovorin (IFL) as compared to 5-fluorouracil and leucovorin (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 disease-free survival analysis, 180 events for recurrence-free survival analysis, and 149 events for overall survival 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 disease-free survival (DFS, Figure 1); log-rank p=0.84 for recurrence-free survival (RFS); log-rank p=0.56 for overall survival (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 to *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 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 demonstrate 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 for 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 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, microsatellite instability (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 appear 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 colon cancer. *KRAS* mutation activates the RAS-RAF pathway as well as the PI3K-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 appear 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 (CIMP-low) (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 lights 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 previous studies have examined relationship between *KRAS* mutation and response to bevacizumab, and shown that *KRAS* mutation does not predict response or resistance to bevacizumab in colon cancer (25,41).

While 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) (42,43) showed that *KRAS* mutation was associated with worse outcome in colorectal cancer. However, these meta-analyses substantially suffered from publication bias; especially most studies used were relatively small (N<150 in most studies; N<290 in all included studies). Compared to 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), while most other large studies found no independent prognostic role of *KRAS* mutation (4,6,23–25,44), including a recent study on 1379 stage II-III colon cancers (26). Our current findings were limited to only stage III colon cancers. Nonetheless, our results were 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 appear 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, 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 an appropriate

candidate, and be motivated to participate. In addition, patients were particularly selected for this study on the basis of availability of colon cancer tissue specimens. Nonetheless, demographic data of the patients in this study did not suggest 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 was 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.

Statement of Translational Relevance

Activating mutations in the *KRAS* gene are important events during colorectal carcinogenic process, and predict resistance to anti-EGFR treatment for metastatic colorectal cancer. However, the literature data on prognostic significance of *KRAS* mutation in colon cancer have been conflicting. We have utilized the database of 508 stage III colon cancers in this adjuvant chemotherapy trial following surgical resection. Since data on pathologic stage, performance status, post-operative 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 have found that *KRAS* mutation does not have a substantial prognostic or predictive role in stage III colon cancer treated with adjuvant chemotherapy.

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Abbreviations

AJCC, American Joint Committee on Cancer
CALGB, Cancer and Leukemia Group B
CI, confidence interval
DFS, disease-free survival
FU/LV, 5-fluorouracil and leucovorin
HR, hazard ratio
IFL, irinotecan, 5-fluorouracil and leucovorin
MSI, microsatellite instability
MSS, microsatellite stable
NCI, National Cancer Institute
OS, overall survival
RFS, recurrence-free survival

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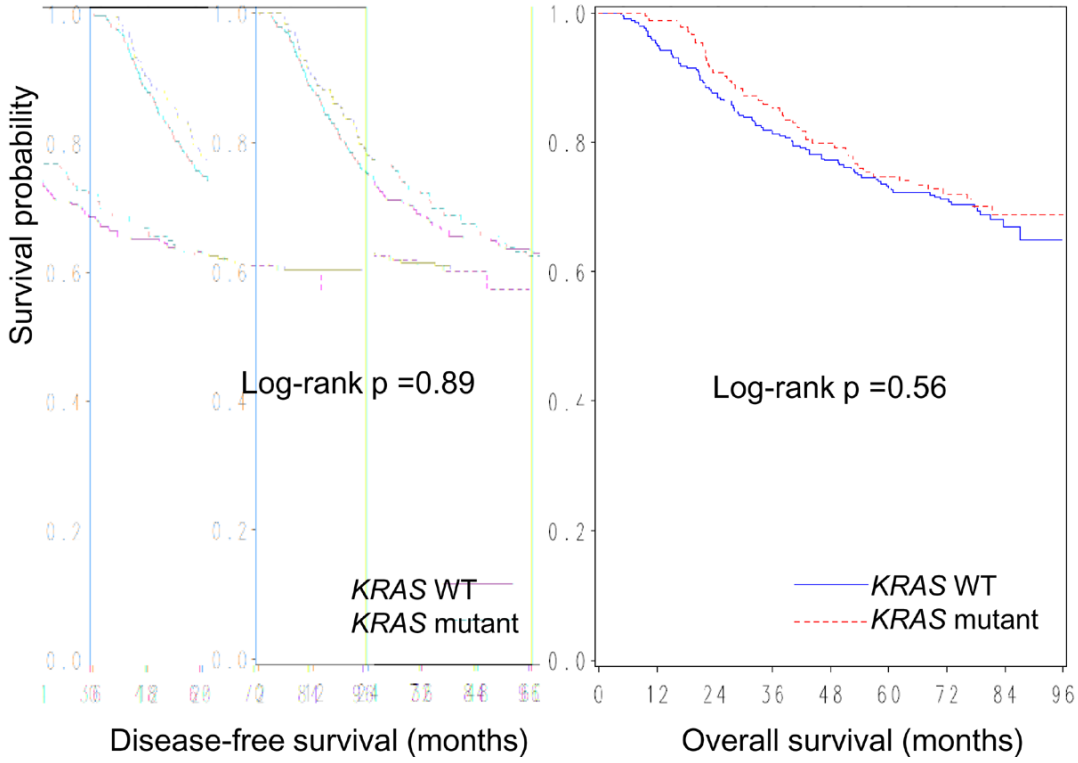

Figure 1.
Kaplan-Meier survival curves for disease-free survival (left panel) and overall survival (right panel) in stage III colon cancer according to *KRAS* mutational status.

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 N	508	330	178
Sex			
Male	276 (54%)	179 (54%)	97 (54%)
Female	232 (46%)	151 (46%)	81 (46%)
Age (years)			
<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	59.8 ± 11.5	60.2 ± 11.6	59.1 ± 11.4
Body mass index (BMI; 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			
T1–T2	59 (12%)	43 (13%)	16 (9.1%)
T3	410 (82%)	260 (80%)	150 (85%)
T4	33 (6.6%)	23 (7.1%)	10 (5.7%)
N stage			
N1	321 (64%)	203 (62%)	118 (67%)
N2	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%)

(%) 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.

FU/LV, 5-fluorouracil and leucovorin; IFL, irinotecan, 5-fluorouracil and leucovorin; MSI, microsatellite instability; MSS, microsatellite stable; SD, standard deviation.

* Distributional differences are significant with $p<0.01$.

Table 2

KRAS mutational status and clinical outcome in stage III colon cancer

KRAS	Total N	Disease-free survival No. of events	Univariate HR (95% CI)	Multivariate HR (95% CI)	Recurrence-free survival No. of events	Univariate HR (95% CI)	Multivariate HR (95% CI)	Overall survival 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)

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, microsatellite instability (MSI) status, and treatment arm.

CI, confidence interval; HR, hazard ratio.

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

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 microsatellite instability (MSI) status.

CI, confidence interval; FU/LV, 5-fluorouracil and leucovorin; HR, hazard ratio; IFL, irinotecan, 5-fluorouracil and leucovorin.

Table 4

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

	Total N	Disease-free survival No. of events	Univariate HR (95% CI)	Multivariate HR (95% CI)	Recurrence-free survival No. of events	Univariate HR (95% CI)	Multivariate HR (95% CI)	Overall survival 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

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 microsatellite instability (MSI) status.

CI, confidence interval; FU/LV, 5-fluorouracil and leucovorin; HR, hazard ratio; IFL, irinotecan, 5-fluorouracil and leucovorin.