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KRAS codon 12 and 13 mutations in relation to disease-free survival in *BRAF*-wild type stage III colon cancers from an adjuvant chemotherapy trial (N0147 Alliance)

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Abstract

Purpose—We examined the prognostic impact of specific *KRAS* mutations in stage III colon adenocarcinoma patients receiving adjuvant FOLFOX alone or combined with cetuximab in a phase III trial (N0147). Analysis was restricted to *BRAF*-wild type tumors, since *BRAF* mutation was associated with poor prognosis, and *BRAF* and *KRAS* mutations are mutually exclusive.

Experimental Design—The seven most common *KRAS* mutations in codon 12 and codon 13 were examined in 2,478 *BRAF*-wild type tumors. Because *KRAS* mutations in codon 12 (n=779) or 13 (n=220) were not predictive of adjuvant cetuximab benefit, study arms were pooled for analysis. Disease-free survival (DFS) was evaluated by hazard ratios (HR) using Cox models.

Results—*KRAS* mutations in codon 12 (multivariate HR 1.52; 95% confidence interval [CI] 1.28–1.80; P<.0001) or codon 13 (multivariate HR 1.36; 95% CI 1.04–1.77; P=.0248) were significantly associated with shorter DFS compared to patients with wild type *KRAS*/*BRAF* tumors, independent of covariates. *KRAS* codon 12 mutations were independently associated with proficient mismatch repair (P<.0001), proximal tumor site (P<.0001), low grade, age, and sex, whereas codon 13 mutations were associated with proximal site (P<.0001).

Conclusion—*KRAS* mutations in either codon 12 or 13 are associated with inferior survival in patients with resected stage III colon cancer. These data highlight the importance of accurate molecular characterization and the significant role of *KRAS* mutations in both codons in the progression of this malignancy in the adjuvant setting.

Keywords

KRAS; colon cancer; prognosis; BRAF; survival

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CONFLICT OF INTEREST: none

INTRODUCTION

KRAS is a small G protein that acts as a transducer in the epidermal growth factor receptor (EGFR) signaling pathway (1). Approximately 40% of colorectal cancers (CRCs) harbor activating mutations in *KRAS*, making it the most commonly mutated gene in the RAS/RAF/MAPK pathway. *KRAS* mutations are believed to be an early event in colorectal tumorigenesis and lead to constitutive signaling and downstream activation of MAPK- and PI3K-dependent pathways. Most (90%) *KRAS* mutations occur in codons 12 and 13 in the phosphate-binding loop of KRAS (1), and mutations in either codon possess transforming capacity (2, 3). *In vitro* evidence indicates that *KRAS* codon 12 mutations have greater transforming ability characterized by inhibition of apoptosis, enhanced loss of contact inhibition, and increased predisposition to anchorage-independent growth when compared with codon 13 mutations (2-4). The glycine-to-aspartate transition (p.G13D) is the most frequent codon 13 mutation in CRC. *In vitro* and mouse model data have showed that, although p.G12V-mutated CRC were insensitive to cetuximab, p.G13D-mutated cells were sensitive, as were *KRAS* wild type cells (5).

Whereas the ability of most *KRAS* mutations to predict resistance to anti-EGFR therapy in patients with metastatic colorectal cancer is widely accepted, including recommendations for *KRAS* testing in metastatic disease (6), the prognostic impact of *KRAS* mutations including in stage III disease is uncertain (7-10). Codon 12 mutations have been associated with adverse prognosis in aggregate colorectal cancer populations of diverse disease stages (11, 12). However, recent data suggest that *KRAS* codon 13 mutations may not represent an aggressive phenotype or confer resistance to anti-EGFR therapy compared to wild type. In metastatic CRC, codon 13 (p.G13D) mutation, in contrast to those in codon 12, was associated with sensitivity to anti-EGFR therapy that was similar to wild type (5, 13), though the literature is inconsistent (14). Furthermore, recent population-based data suggest that patients with *KRAS* codon 13 mutations may have similarly favorable prognosis as those with *KRAS* wild type (11). No study to date has demonstrated that *KRAS* codon 13 mutations are significantly associated with worse patient survival in patients with non-metastatic colon cancer (5, 11-19). Data from randomized clinical trials are summarized in Table 1. These findings suggest that *KRAS* codon 13 mutations may not be biologically important in the progression of CRC and question the clinical relevance of analyzing these mutations routinely.

Few studies examining the prognostic impact of specific *KRAS* mutations in CRC have controlled for *BRAF* mutation as a confounder. However, the most rigorous approach to isolate the prognostic impact of *KRAS* is to restrict analysis to *BRAF*-wild type tumors, given that *BRAF* and *KRAS* mutations are mutually exclusive (6) and that *BRAF* mutations are associated with adverse prognosis (7, 18, 20-24). It is also important to account for DNA mismatch repair (MMR) status, since the subset of CRCs with deficient MMR (dMMR) and microsatellite instability (MSI) have a relatively low rate of *KRAS* mutations as compared to proficient MMR (pMMR) and microsatellite stable tumors (25).

In this report, we determined the association of the seven most common *KRAS* mutations in codon 12 and 13 with disease-free survival (DFS) in prospectively collected, stage III colon adenocarcinomas from participants of a phase III trial (N0147). Patients were randomized to adjuvant 5-fluorouracil, oxaliplatin, and leucovorin (mFOLFOX6) alone or combined with cetuximab, and the addition of cetuximab to FOLFOX failed to improve DFS overall or in patients with wild type *KRAS* tumors (26). The current prognostic analysis was restricted to patients whose tumors were wild type for *BRAF*. In this cohort, we previously reported that *KRAS* (all codons combined) or *BRAF* mutations were each associated with shorter DFS (25). In the current study, we examined *KRAS* mutations in codons 12 and 13 separately, with a focus on determining whether codon 13 mutations are prognostic. Our findings indicate that *KRAS* mutations in both codon 12 and 13 confer a worse prognosis in stage III colon cancers.

METHODS

Study Population

Subjects with completely resected, stage III colon adenocarcinoma ($T_{any}N_{1\text{-}2}M_0$) participated in a phase III randomized trial (North Central Cancer Treatment Group [NCCTG] N0147; 2004 to 2009) of adjuvant mFOLFOX6 alone or combined with cetuximab, which was previously described (26). Prospective and centralized *KRAS* mutation testing was required, although randomization was done irrespective of *KRAS* status in the original trial design. In August 2008, the trial was amended to restrict randomization to patients with *KRAS*-wild type tumors based upon data demonstrating the predictive utility of *KRAS* for anti-EGFR antibody therapy (26). Post-amendment, eligible patients with *KRAS*-mutated tumors (n=332) were treated at investigator discretion (97% received FOLFOX) and followed for disease recurrence. To avoid selection bias, the current analysis includes all randomized study patients and those with *KRAS*-mutated tumors who enrolled post-amendment (n=3,018 total). Tissues were prospectively collected and required for study participation. Central pathology review was performed. Proximal tumor site included the cecum, ascending and transverse colon; distal site included the splenic flexure, descending and sigmoid colon.

Patients initiated chemotherapy within 10 weeks of surgery. After completing protocol-specified treatment, disease recurrence was assessed every 6 months until 5 years post-randomization with a physical examination, computed tomographic scan, and laboratory assessment. Follow-up colonoscopy was recommended at years 1 and 4 post-resection.

The study was approved by the Mayo Clinic Institutional Review Board (IRB) and the NCCTG (now part of Alliance for Clinical Trials in Oncology). Patients signed an IRB-approved consent.

KRAS and *BRAF* mutation

Assessment of *KRAS* and *BRAF* (NCBI Entrez Gene 673) mutational status was performed centrally at the Mayo Clinic in a Clinical Laboratory Improvement Amendments (CLIA)-compliant laboratory, using appropriate quality control procedures. Both *KRAS* and *BRAF*

mutation status was determined using DNA extracted from macrodissected formalin-fixed, paraffin-embedded tumor tissue.

For *KRAS*, testing was performed with the DxS mutation test kit KR-03/04 (DxS), together with the Light-Cycler 480 (Roche Applied Sciences), which assesses for 7 missense point mutations: six mutations in codons 12 (c.35G>C [p.G12A, GGT>GCT], c.34G>C [p.G12R, GGT>CGT], c.35G>A [p.G12D, GGT>GAT], c.34G>T [p.G12C, GGT>TGT], c.34G>A [p.G12S, GGT>AGT], and c.35G>T [p.G12V, GGT>GTT] and one mutation in codon 13 (c.38G>A [p.G13D, GGC>GAC]). The level of detection was set at 5%. Assessment for the *BRAF* c.1799T>A (p.V600E) mutation was performed using a multiplex allele specific polymerase chain reaction (PCR)–based assay. The polymerase chain reaction primers used for this assay were fluorescently labeled and included the following (wild type forward NEDTGATTTTGGTCATGCTACAGT]; mutant forward [6-Fam-CAGTGATTTTGGTCTAGCTTCAGA]; and reverse [GTTTCTTTCTAGTAACTCAGCAGC]). Following amplification, PCR products were analyzed on an ABI 3130×l instrument (Life Technologies, Applied Biosystems) and scored for the presence or absence of the V600E variant only.

DNA Mismatch Repair Proteins

MMR protein (MLH1, MSH2 and MSH6) expression was analyzed in formalin-fixed, paraffin-embedded tumor sections using an immunoperoxidase method (27). Monoclonal antibodies included mouse anti-human MLH1 (clone G168-15, Biocare Medical, Concord, CA), anti-human MSH2 (clone FE11, Biocare Medical, Concord, CA), and anti-human MSH6 (clone BC/44; Biocare Medical, Concord, CA). MMR protein loss was defined as the absence of nuclear staining in tumor cells in the presence of positive nuclear staining in normal colonic epithelium and lymphocytes. Tumors were classified as MMR-deficient (*vs* MMR-proficient) if loss of one or more MMR proteins was detected.

Statistical Methods

Our primary objective was to compare survival among patients carrying any mutation in codon 12, mutated codon 13, and wild type *KRAS*. The primary clinical endpoint was DFS, and a secondary endpoint was time to recurrence (TTR). DFS was defined as the time from randomization to first documented recurrence or any-cause death, whichever occurred first. TTR was defined as the time from randomization to first documented recurrence. Survival was evaluated by hazard ratios (HR) using Cox models. Kaplan-Meier methods were used to describe the distributions of DFS and TTR, which were censored at 5 years after randomization. Multivariable Cox models were adjusted for age, gender, T stage, N stage, number of examined nodes, histologic grade, performance status, primary tumor site, mismatch repair status, and treatment. Analysis of *KRAS* mutations included analysis of codon 12 mutations grouped together and codon 13, as well as each mutation individually. Interactions between *KRAS* mutation and treatment were assessed. All analyses were based on the study database frozen on Sept. 4, 2012. Two-sided *P* values, with values <.05 considered statistically significant, and 95% confidence intervals (CI) are reported. Analyses were performed using SAS version 9.2 (SAS Institute Inc, Cary NC). Data collection and statistical analyses were conducted by the Alliance Statistics and Data Center.

RESULTS

KRAS mutations in colon cancer

The study population comprises patients with completely resected stage III colon cancer (n=3018) who received adjuvant FOLFOX-based chemotherapy in a North American phase III clinical trial (N0147) (Figure 1). *KRAS* and *BRAF* data were available in 93.5% (2822/3018) of patients. Tumors with both *KRAS* and *BRAF* mutations (n=1) or *KRAS* mutation in both codon 12 and 13 (n=1) were excluded.

Figure 2a shows the frequencies and types of *KRAS* mutations, which are consistent with prior reports (28), and the corresponding predicted amino acid sequence alterations. *KRAS* codon 12 or 13 (c.38G>A [p.G13D]) mutations were detected in 35.4% (999/2822) of tumors, with 27.6% in codon 12 and 7.8% in codon 13. Within codon 12, most (82%) mutations occurred in the second base position, and the frequency of transversions (G>C, G>T) and transitions (G>A) were similar (45% and 55%, respectively). *BRAF* mutation occurred in 12.2% (344/2822) (Fig 2a).

KRAS mutations and clinicopathologic characteristics

Table 2 summarizes the baseline clinicopathologic characteristics of study subjects according to *KRAS* and *BRAF* mutation status. Compared to wild type, *KRAS* mutations were significantly associated with older age and female sex, primarily due to mutations in codon 12, and did not differ by T stage or number of positive nodes. Compared to *KRAS* wild type, codon 12 and 13 mutations were each associated with proximal (*vs* distal) tumor site within the colon (*P*<.0001). Codon 12 and 13 mutations were associated with low and high grade histology, respectively, in primary tumors.

A low frequency of *KRAS* mutations was detected in dMMR compared to pMMR tumors (14% [45/318] *vs* 38% [944/2464]; Table 2). Mutations in codon 12 were significantly less frequent in dMMR tumors compared to wild type (3% *vs* 8%; P<.0001; Table 2), and this low frequency was observed across codon 12 mutations (Figure 2b). Deficient MMR showed a strong inverse association with *KRAS* codon 12 mutation (OR 0.28; 95% CI 0.18–0.44; P<.0001), independent of covariates (Table S1). However, the frequency of dMMR was similar in *KRAS* codon 13 mutations and *KRAS/BRAF*-wild type (9% *vs* 8%; P=.7338; Table 2).

Proximal tumor site, older age, female sex, and low grade were each significantly associated with *KRAS* codon 12 mutation independent of covariates (all P values <.030; Table S1). By contrast, only proximal site (P<.0001) showed an independent association with *KRAS* codon 13 mutation compared to *KRAS/BRAF*-wild type (Table S1).

Similar to *KRAS* mutations, *BRAF* mutation was associated with older age, female sex, proximal site, and dMMR; and unlike *KRAS*, *BRAF* mutation was also associated with higher T and N stage, and higher histologic grade (Table 2), as previously reported (25).

KRAS mutation and patient survival in *BRAF*-wild type cases

To remove the confounding effect of *BRAF* mutation on the prognostic impact of *KRAS* mutation, we analyzed *BRAF*-wild type tumors only (n=2478) when examining patient survival and compared *KRAS*-mutated/*BRAF*-wild type cases with *KRAS*-wild type/*BRAF*-wild type cases (Fig. 1). Among the 687 DFS events, there were 353 deaths during a median follow-up of 43.2 (interquartile range, 31.0–55.3) months and 616 TTR events during a median follow-up of 42.4 (interquartile range, 30.4–55.0) months for censored cases.

As shown in Figure 3a and Table 3 (top panel), patient tumors with *KRAS* codon 13 mutations experienced shorter DFS (univariate HR 1.46; 95% CI 1.13–1.89; P=.0035; multivariate HR 1.36; 95% CI 1.04–1.77; P=.0248), compared with those that were wild type for *KRAS* and *BRAF*, independent of clinicopathologic variables and MMR status. *KRAS* codon 12 mutation was also significantly associated with worse DFS (univariate HR 1.50; 95% CI 1.28–1.76; *P*<.0001; multivariate HR 1.52; 95% CI 1.28–1.80; *P*<.0001), compared with patients whose tumors were wild type for *KRAS* and *BRAF*. Results were similar when the full cohort was analyzed adjusting for *BRAF* mutation (codon 13, multivariate HR 1.334 [95% CI 1.003, 1.773], P=.0474; codon 12, multivariate HR 1.584 [95% CI 1.328, 1.890], P<.0001). When TTR was analyzed as the outcome variable in the *BRAF*-wild type subgroup (Figure 3b), results were consistent both for codon 13 (univariate HR 1.46; 95% CI 1.11– 1.92; P=.0064; multivariate HR 1.34; 95% CI 1.01–1.78; P=.0446) and for codon 12 (univariate 1.59; 95% CI 1.34–1.88; P<.0001; multivariate HR 1.60; 95% CI 1.34–1.91; P<.0001).

Individual *KRAS* mutations within codon 12 were also examined in relation to patient survival (Table 3, bottom panel). Each mutation was associated with worse DFS compared to *KRAS/BRAF*-wild type (all HR point estimates >1). Five of the 6 *KRAS* codon 12 mutations (c.34G>A [p.G12D], c.35G>T [p.G12V], c.34G>T [p.G12C], c.35G>C [p.G12A], c.34G>C [p.G12R]) demonstrated a statistically significant association with worse DFS in univariate and multivariate analysis. Results were consistent when TTR was analyzed as the outcome (data not shown).

In an exploratory analysis, we examined the prognostic association of *KRAS* codon 12 or 13 mutations (*vs* wild type) among *BRAF*-wild type tumors within various strata, including tumor site, N stage, and MMR status. No significant modifying effect by these variables was observed (all *P* interaction >.18).

The predictive value of *KRAS* status for cetuximab benefit was determined among patients that enrolled prior to August 2008, when both *KRAS*-mutated and -wild type patients were randomized to chemotherapy with or without cetuximab (see *Methods*). *KRAS* codon 12 or 13 mutations were not associated with differential DFS among treatment arms (any *KRAS* mutation *vs* wild type, $P_{interaction}$ =.988; codon 12 *vs* codon 13 *KRAS* mutations *vs* wild type, $P_{interaction}$ =.628; Figure S1). Individual mutations within codon 12 were also not predictive of cetuximab benefit (Figure S1).

DISCUSSION

We analyzed the frequency of *KRAS* codon 12 and 13 mutations in prospectively collected stage III colon cancers from a clinical trial of adjuvant chemotherapy. *KRAS* mutations were detected in 35.4% (999/2822) of tumors, with 27.6% detected in codon 12 and 7.8% in codon 13 (c.38G>A [p.G13D]). The specific *KRAS* mutations identified and their relative frequencies are consistent with other studies across tumor stages (28). We also determined the association of *KRAS* codon 12 and 13 mutations with clinicopathologic variables and survival. The study arms were combined for analysis since the addition of cetuximab to FOLFOX trial did not improve outcome in the parent trial, and no interaction between treatment and *KRAS* mutation status was observed. We restricted prognostic analysis to *BRAF*-wild type tumors so as to control for the confounding effect of *BRAF* c.1799T>A mutations. We found that *KRAS* mutations in codons 12 or 13 (c.38G>A) were each significantly associated with worse DFS compared with *KRAS*-wild type/*BRAF*-wild type cases. Specifically, patients whose tumors carried *KRAS* codon 12 or 13 mutations experienced a 52% or 36% higher relative risk, respectively, of colon cancer recurrence or any-cause death that was independent of clinicopathological variables or MMR status. Results were similar when TTR was used as the outcome variable. We emphasize that only the c.38G>A mutation was analyzed in codon 13, whereas multiple mutations within codon 12 were found that showed a consistent association with adverse outcome.

To our knowledge, our data are the first to demonstrate that *KRAS* codon 13 (c.38G>A) mutations adversely impact survival in non-metastatic colon cancer. In both a population-based cohort and a meta-analysis using individual patient data of stage I to IV CRCs, codon 13 mutations were not prognostic, in contrast to codon 12 mutations (11, 12). In smaller studies examining CRCs of metastatic or mixed stage, non-significant trends were reported between codon 13 mutations and worse prognosis (13, 15, 17, 29). Furthermore, a study of 160 CRCs of varying tumor stages and treatments found that *KRAS* codon 13, but not codon 12, mutations were associated with higher S-phase fractions, increased nodal metastases, and adverse outcome compared to wild type (16). A Swedish population-based study of 525 CRCs reported that individuals with *KRAS* codon 13 (but not codon 12) mutations experienced shorter cancer-specific survival in unadjusted, but not adjusted, analysis (30). Limitations of prior studies include the inconsistent incorporation of patients with *BRAF* mutations (in the comparison group) and variable patient therapies, which can confound the interpretation of the *KRAS* prognostic data (31-33). Most prior studies included stage IV patients and had fewer codon 13 mutation patients. Of note, the adverse impact of *KRAS* codon 13 mutations on survival in our study appeared to be attenuated compared to codon 12 mutations (36% *vs* 52%, respectively, higher risk of DFS). Consistent with this finding are laboratory data showing that *KRAS* codon 12 mutations display greater transforming ability, enhanced anchorage-independent growth, and an increased ability to suppress apoptosis when compared with codon 13 mutants (2-4). Computational analysis of the structural implications of *KRAS* mutations suggests that codon 12 mutation (c.35G>A, p.G12D) may impair hydrolysis of GTP, leaving KRAS in an active GTP-bound state, to a greater degree than codon 13 mutation (c.38G>A, p.G13D) or wild type *KRAS* (34). In metastatic CRCs codon 13 mutations (p.G13D), but not codon 12 mutations, were associated

with sensitivity to anti-EGFR therapy that was similar to wild type tumors (5, 13), However, cetuximab was ineffective in our study and, therefore, *KRAS* mutations including those in codon 13 did not predict outcomes from adjuvant cetuximab treatment.

Within *KRAS* codon 12, each of the six individual mutations showed an association with shorter DFS compared to wild type *KRAS/BRAF*. Although c.35G>A (p.G12D) was most common, four other mutations (c.35G>T [p.G12V], c.34G>C [p.G12R], c.34G>T [p.G12C], c.35G>C [p.G12A]) also demonstrated a significant association with adverse outcome that was independent of covariates and sometimes appeared to be stronger. The c.34G>A [p.G12S] mutation showed the weakest association. Codon 12 *RAS* mutations encoding valine (p.G12V) or arginine (p.G12R) have been reported to demonstrate stronger transforming ability and a more aggressive tumorigenic phenotype than other codon 12 mutations (35-37) and to be associated with shorter patient survival compared to wild type (11, 12). Interestingly, c.34G>C [p.G12R] demonstrated the strongest association with poor survival in both our study (HR >5 for DFS) and in a population-based cohort (HR > 3 for cancer-specific death), suggesting that this codon 12 mutation is particularly aggressive despite being rare (<1%). Our findings confirm the adverse prognostic impact of c.35G>T (p.G12V) and, consistent with prior studies, suggest that c.34G>C (p.G12R) mutations are also adverse. In addition, our findings suggest the adverse impact of lower frequency mutations within codon 12 (c.34G>T [p.G12C], c.35G>C [p.G12A]) and c.35G>A (p.G12D) that has not been previously reported in non-metastatic colon cancers.

In our study, tumors with *KRAS* codon 12 mutations had a lower frequency of deficient MMR compared to tumors with codon 13 mutation or wild type, consistent with findings from a smaller report (38). Admittedly, this difference may be related to smaller size of the codon 13 subgroup, yet the frequency of deficient MMR was consistently low across all *KRAS* codon 12 mutations. In addition, codon 12 mutations were associated with low-grade histology whereas cancers with codon 13 mutations were more likely to show high-grade histology. These findings are consistent with evidence indicating that *KRAS* mutations may arise in unique molecular and clinical contexts, as the mutational spectrum can depend on the nature of the underlying genetic instability (38, 39). Epidemiologically, colorectal cancers with codon 12 and 13 mutations have been associated with different dietary intake patterns (40, 41). Furthermore, laboratory studies have shown that codon 12 mutations demonstrate increased PI3K pathway activation (2) and a distinct metabolic phenotype that promotes resistance to apoptosis (42) compared to codon 13 mutations. We found that *KRAS* mutations showed a higher frequency in proximal (*vs* distal) colon tumors, independent of other variables (43, 44). The distribution of *KRAS* codon 12 *vs* 13 mutations did not differ considerably by tumor subsite (data not shown). Proximal colon tumors are more likely than distal tumors to be *KRAS*-, *BRAF*-, and hypermutated, hypermethylated, and MMR-deficient (45). The explanation for why *KRAS* mutations show a predilection for the proximal tumor is unknown except to invoke molecular differences based on midgut and hindgut embryology. As expected, *BRAF* c.1799T>A mutations were enriched in tumors with dMMR and showed clinicopathologic features in common that included proximal tumor predominance, high-grade histology, older age, and female sex (46). In the N0147 study

cohort and other reports, *BRAF* mutations are associated with shorter patient survival rates (9, 18, 21, 25).

This study is the largest to evaluate the prognostic impact of specific *KRAS* codons 12 and 13 in stage III colon cancer. Other strengths of this study include prospective collection of tissue specimens from a large clinical trial with meticulous collection of survival data. Systemic treatment consisted of a modern chemotherapy regimen (FOLFOX) generalizable to most stage III patients in the world. *KRAS* and *BRAF* mutation status was determined in a CLIA-certified laboratory. Limitations of the study include the fact that overall survival data have not yet matured; however, the reliability of DFS as a surrogate for OS in a stage III colon cancer population has been demonstrated by our group and others (47). We await biomarker results from PETACC-8, a phase 3 trial of colon cancer patients in which the addition of cetuximab to FOLFOX did not improve DFS or OS (48). We did not examine other less common mutations in *KRAS*, *NRAS*, or *HRAS;* recent data suggest that 17%-18% of patients with metastatic CRC that are wild type for *KRAS* codon 12 or 13 harbor additional *RAS* activating mutations that predict a lack of response to panitumumab (49, 50).

In conclusion, we found that *KRAS* mutations in codon 12 and 13 were each significantly associated with shorter DFS, compared to tumors with wild type *KRAS*/*BRAF*. In contrast to prior reports, our data establish codon 13 mutations as being adversely associated with outcome in stage III colon cancers. *KRAS* mutations were significantly more frequent in proximal tumors, and codon 12 mutations were less frequent in tumors with deficient *vs* proficient MMR. Our findings support testing for *KRAS* mutations in codons 12 and 13 in stage III colon cancers as these results provide important prognostic information.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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STATEMENT OF TRANSLATIONAL RELEVANCE

The most common mutations in the EGFR pathway in colorectal cancers occur in *KRAS* codons 12 and 13. However, recent data suggests that codon 13 mutations may not represent an aggressive phenotype. We examined the prognostic impact of the seven most common *KRAS* mutations in codon 12 and 13 in stage III colon adenocarcinomas from a phase III adjuvant trial of FOLFOX with or without cetuximab. To minimize confounding, analysis was restricted to 2,478 *BRAF*-wild type tumors. *KRAS* mutations, including those in codon 13 only, were prognostic, showing a significant association with shorter disease-free survival compared to wild type *KRAS*/*BRAF*. These data demonstrate for the first time that *KRAS* codon 13 mutations are associated with inferior survival in patients with non-metastatic colon cancer, and highlight the important role of both codon 12 and 13 mutations in the progression of this malignancy in the adjuvant setting.

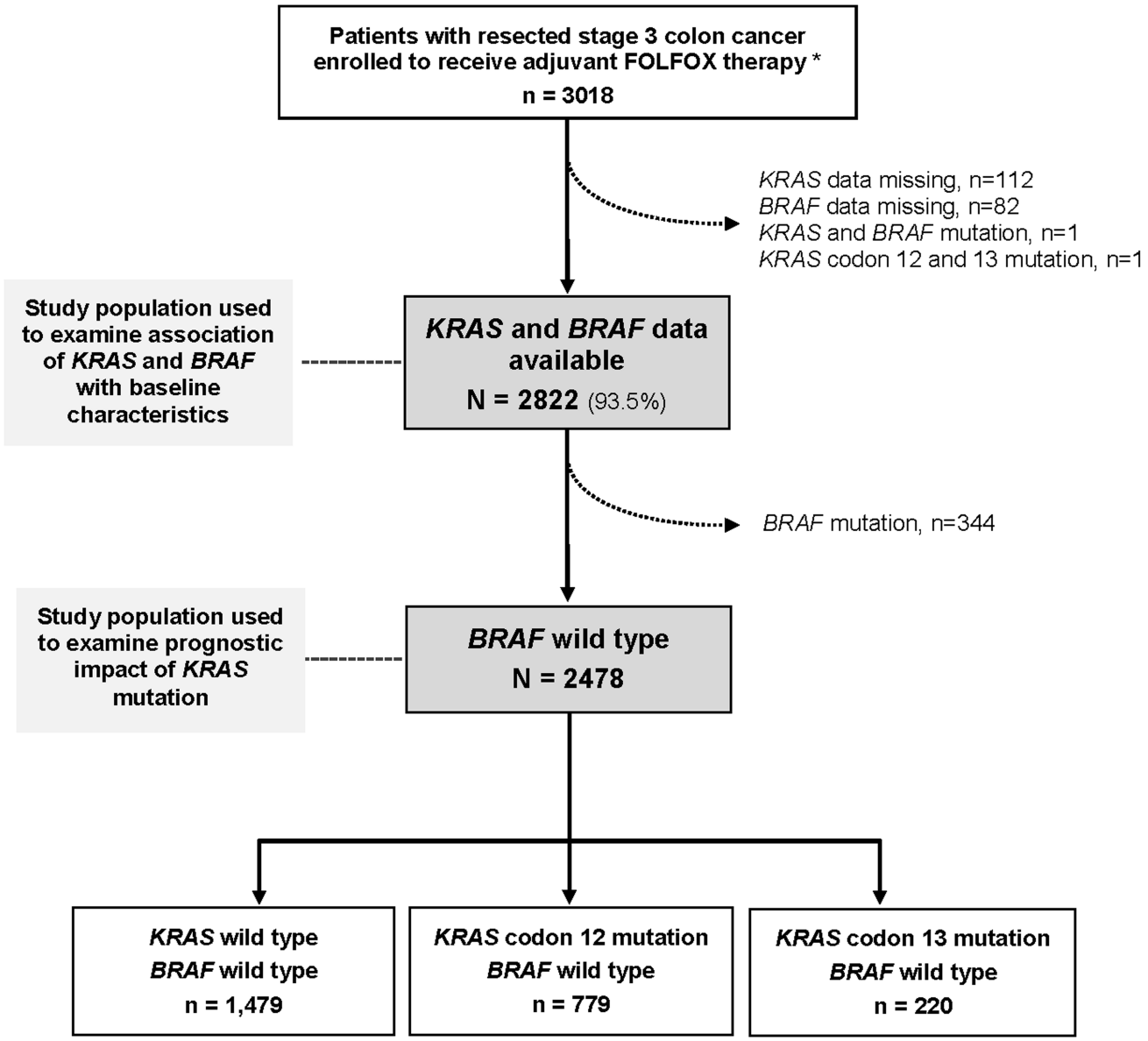

Figure 1. Study profile

BRAF-mutated cases were excluded to assess the prognostic role of *KRAS* mutation in *BRAF*-wild type tumors. * Includes patients with *KRAS*-mutated tumors (n=332) enrolled post-study modification (see *Methods*), of whom 97% received FOLFOX.

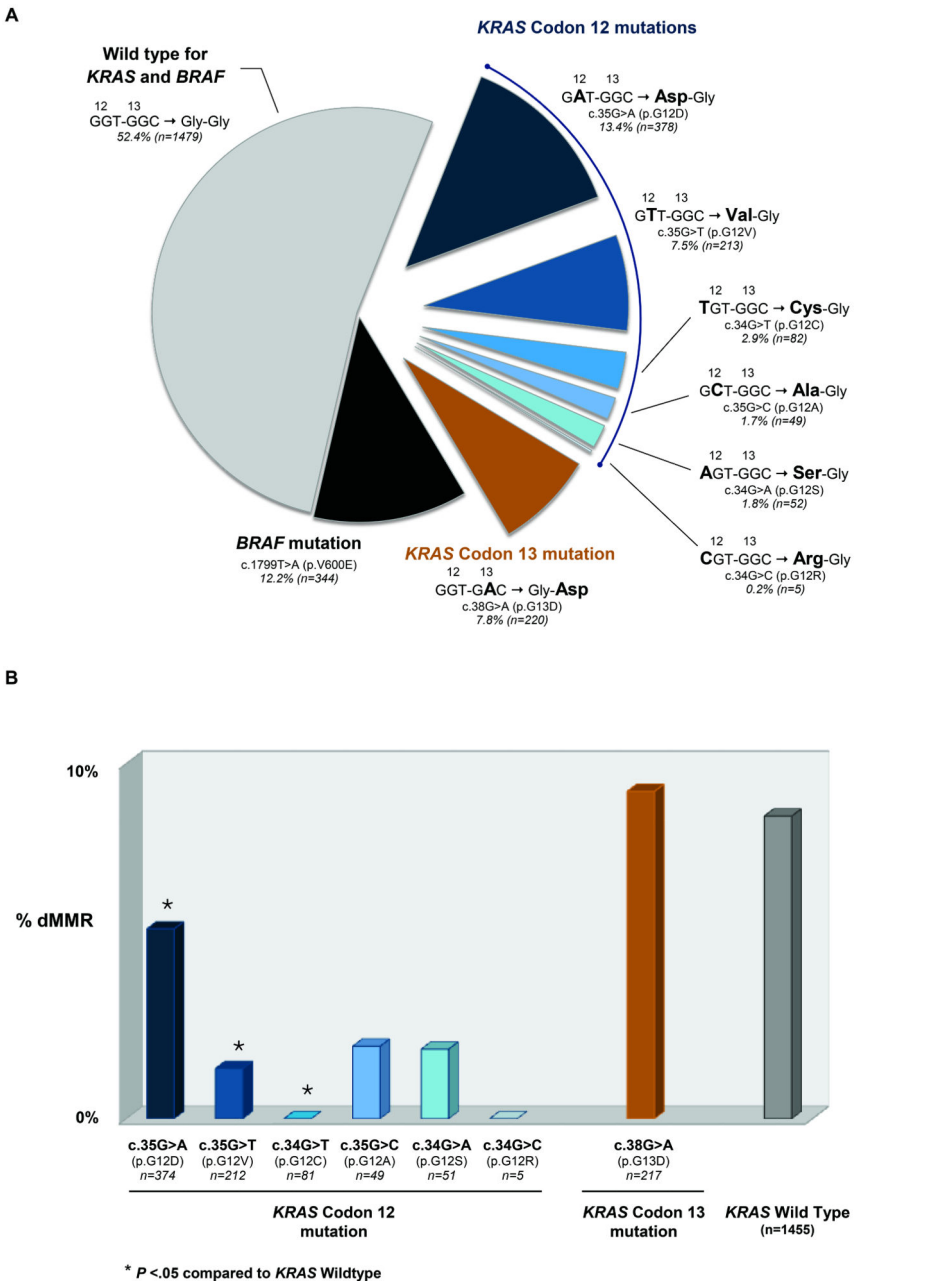

Figure 2. *KRAS* (codon 12 and 13) and *BRAF* mutation frequencies in 2,904 stage III colon adenocarcinomas

(a) Frequencies of *KRAS* mutations and corresponding amino acid sequence alterations are shown. (b) Frequency of deficient mismatch repair (MMR) among *KRAS*-mutated and *BRAF*-wild type tumors are shown (numbers differ slightly from [a] due to missing MMR data). Asterisks (*) denote statistically significant differences compared to *KRAS/BRAF* wild type (P <.05).

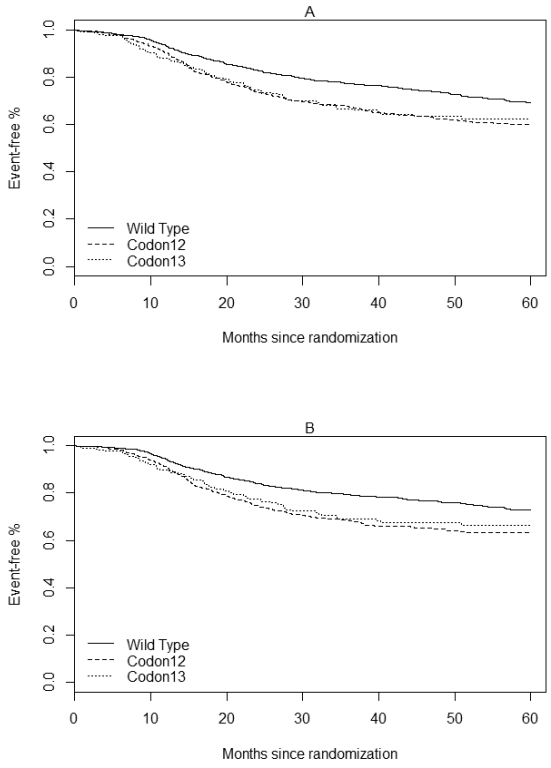

Figure 3. Prognostic impact of specific *KRAS* mutations in 2,478 patients with *BRAF*-wild type resected stage III colon cancer

KRAS mutations in codon 12 and 13, compared to wild type *BRAF* and *KRAS*, are shown in relation to *(a)* disease free-survival and *(b)* time to recurrence.

Table 1

Randomized clinical trials examining the prognostic impact of *KRAS* codon 12 and 13 mutations in colorectal cancer

Cohort	No. of Tumors Total (Codon 12 / 13)	% of Total Cohort	Tumor, Stage	Treatment	Findings		
					Multivariate HRs for *KRAS* mutations		Reference Group [a]
					Codon 12	Codon 13	
Co.17, BOND, MABEL, EMR202600, EVEREST, BABEL, SALVAGE (5)	579 (~260 / 45)		CRC IV	BSC +/− cetuximab; Cetuximab +/− chemotherapy		c.38G>A HR 1.82 (p =.053) for overall survival [b]	*BRAF/KRAS* wild type or *BRAF* mutated
OPUS, CRYSTAL (13)	1378 (125 / 83)	90%	CRC IV	FOLFIRI or FOLFOX +/− cetuximab	c.35G>T, HR 1.11 (p =.53) for overall survival [c]	c.38G>A, HR 1.39 (p=.079) for overall survival [c]	*BRAF/KRAS* wild type or *BRAF* mutated
NSABP C07, C08 (9)	2299 (- / -)	48%	Colon II—III	5FU +/− oxaliplatin, FOLFOX +/− bevacizumab	c.35G>T, HR 1.22 (p=.16) for time to recurrence [d]		*BRAF/KRAS* wild type or *BRAF* mutated
PETACC-3 (18)	1321 (368 / 102)	40%	Colon II—III	5FU +/− irinotecan	c.35G>A, HR 0.98 (p =.91) c.35G>C, HR 0.97 (p =.92) c.35G>T, HR 1.09 (p =.64) c.34G>T, HR 1.40 (p =.15) c.34G>A, HR 0.99 (p =.97) for relapse-free survival [d]	c.38G>A, HR 0.99 (p =.97) for relapse-free survival [d]	*BRAF/KRAS* wild type or *BRAF* mutated
CALGB 89803 (21)	506 (123 / 53)	40%	Colon III	5FU +/− irinotecan	Any Codon 12, HR 1.09 (NS) for disease-free survival [d]	c.38G>A, HR 0.82 (NS) for disease-free survival [d]	*BRAF/KRAS* wild type or *BRAF* mutated
NCCTG N0147 (Alliance); Current Study	2478 (779 / 220) *BRAF wild type only*	**82%**	Colon III	FOLFOX +/− cetuximab	**Any Codon 12, HR 1.52 (p <.0001)** for disease-free survival [d]	**c.38G>A, HR 1.36 (p =.025)** for disease-free survival [d]	***BRAF/KRAS* wild type only**

BSC, best supportive care; CRC, colorectal; HR, hazard ratio; 5FU, fluorouracil; NS, not statistically significant

[a] Refers to the patient reference group used for prognostic analysis.

[b] BSC-alone arm

[c] Chemotherapy-alone arms across both trials

[d] Data pooled across both arms

Table 2

KRAS codon 12 and 13 mutations in relation to clinicopathologic and molecular characteristics in stage III colon cancers (N = 2,822)

Variable	Wild type for *KRAS* and *BRAF* (n=1479)	Any *KRAS* mutation in Codon 12 or 13 (n=999)		Specific *KRAS* Mutation				*BRAF* Mutation (n=344)	
				Codon 12 only (n=779)		Codon 13 only (n=220)			
	N (%)	N (%)	p [a]	N (%)	p [a]	N (%)	p [a]	N (%)	p [a]
Age, *years*									
Median (range)	56 (19-84)	58 (22-85)	**0.0008**	58 (22-85)	**.0002**	57 (22-82)	.6052	65 (31-86)	**<.0001**
Gender									
Female (n=1336)	630 (43)	484 (48)	**0.0041**	387 (50)	**.0013**	97 (44)	.6759	222 (65)	**<.0001**
Male (n=1486)	849 (57)	515 (52)		392 (50)		123 (56)		122 (35)	
T stage									
T1-2 (n=423)	238 (16)	149 (15)	0.4346	111 (14)	.2545	38 (17)	.6578	36 (11)	**.0085**
T3-4 (n=2398)	1241 (84)	849 (85)		667 (86)		182 (83)		308 (89)	
missing	0	1		1		0		0	
Grade									
Low (n=2116)	1145 (77)	792 (79)	0.2710	639 (82)	**.0105**	153 (70)	**.0103**	179 (52)	**<.0001**
High (n=706)	334 (23)	207 (21)		140 (18)		67 (30)		165 (48)	
No. positive nodes									
1-3 (n=1650)	871 (59)	610 (61)	0.2799	487 (63)	.0944	123 (56)	.4023	169 (49)	**.0010**
4 or more (n=1172)	608 (41)	389 (39)		292 (37)		97 (44)		175 (51)	
Tumor Site									
Proximal (n=1407)	545 (37)	577 (59)	**<.0001**	443 (58)	**<.0001**	134 (62)	**<.0001**	285 (84)	**<.0001**
Distal (n=1370)	914 (63)	402 (41)		321 (42)		81 (38)		54 (16)	
Missing	20	20		15		5		5	
Mismatch Repair									
Deficient (n=318)	124 (8)	45 (5)	**0.0001**	25 (3)	**<.0001**	20 (9)	**.7338**	149 (44)	**<.0001**
Proficient (n=2464)	1331 (92)	944 (95)		747 (97)		197 (91)		189 (56)	
Missing	24	10		7		3		6	

[a] Comparison with *KRAS/BRAF* wild type.

Table 3

Cox proportional hazards models examining association of *KRAS* mutation status with disease-free survival in 2,478 *BRAF*-wild type colon cancer patients

***KRAS* status**	**N (Events)**	**3-year disease-free survival rate (95% CI)**	**Univariate**		**Multivariate [a]**	
			HR (95% CI)	**P**	**HR (95% CI)**	**P**
Model 1						
Any codon 12 mutation	779 (256)	68% (64%-71%)	1.50 (1.28, 1.76)	**<.0001**	1.52 (1.28, 1.80)	**<.0001**
Codon 13 mutation	220 (71)	67% (60%-73%)	1.46 (1.13, 1.89)	**0.0035**	1.36 (1.04, 1.77)	**0.0248**
Wild type [b]	1479 (360)	77% (75%-80%)	reference		Reference	
Model 2						
Individual codon 12 mutations						
c.35G>A (p.G12D)	378 (122)	68% (63%-73%)	1.51 (1.23, 1.85)	**<.0001**	1.53 (1.23, 1.89)	**0.0001**
c.35G>T (p.G12V)	213 (68)	70% (63%-76%)	1.38 (1.07, 1.79)	**0.0145**	1.40 (1.07, 1.82)	**0.0139**
c.34G>T (p.G12C)	82 (30)	61% (50%-73%)	1.66 (1.14, 2.41)	**0.0078**	1.63 (1.11, 2.41)	**0.0128**
c.35G>C (p.G12A)	49 (19)	63% (49%-77%)	1.78 (1.12, 2.82)	**0.0148**	1.75 (1.10, 2.79)	**0.0178**
c.34G>A (p.G12S)	52 (14)	72% (59%-85%)	1.28 (0.75, 2.19)	**0.3624**	1.37 (0.80, 2.35)	**0.2485**
c.34G>C (p.G12R)	5 (3)	50% (1%-99%)	3.81 (1.23, 11.87)	**0.0209**	5.30 (1.69, 16.64)	**0.0043**
Codon 13 mutation						
c.38G>A (p.G13D)	220 (71)	67% (60%-73%)	1.46 (1.13, 1.89)	**0.0035**	1.36 (1.04, 1.77)	**0.0246**
Wild type [b]	1479 (360)	77% (75%-80%)	reference		reference	

CI, confidence interval; HR, hazard ratio.

[a] Adjusted for age, gender, T stage, N stage, no. examined nodes, grade, performance status, tumor site, mismatch repair status, treatment.

[b] *KRAS* and *BRAF* wild type.