

Clinical Features and Outcomes of Patients with Colorectal Cancers Harboring *NRAS* Mutations



Andrea Cercek¹, Maria Iñez Braghiroli¹, Joanne F. Chou², Jaclyn F. Hechtman³, Nancy Kemeny¹, Leonard Saltz¹, Marinela Capanu², and Rona Yaeger¹

Abstract

Purpose: *NRAS* mutations are now routinely included in RAS testing prior to EGFR inhibitor therapy for metastatic colorectal cancer (mCRC). The clinical implications of *NRAS* mutation beyond lack of response to anti-EGFR therapy, however, are not known. We undertook this study to determine the clinical features and treatment outcomes of patients with *NRAS*-mutant mCRC.

Experimental Design: We reviewed clinical characteristics, concurrent mutations, and outcomes for all mCRC cases with *NRAS* mutations undergoing standard genotyping at our institution from 2008 to 2015. Comparison groups consisted of *RAS* wild-type and *KRAS*-mutant mCRC consecutive cases genotyped from 2008 to 2012.

Results: Three percent (87/2764) of mCRC patients had *NRAS*-mutant tumors (45% exon 2 and 55% exon 3), including three cases with concurrent *NRAS* and *KRAS* mutations. Left-sided

primary site and African American self-reported race were associated with *NRAS* mutation ($P < 0.01$). Resection rate at 12 months was lower for *NRAS*-mutant mCRC than for *RAS* wild-type or *KRAS*-mutant mCRC. Median survival from time of first known metastasis was 33 months for *NRAS*-mutant, 47 months for *KRAS*-mutant, and 78 months for *RAS* wild-type cases ($P < 0.001$). Multivariate analysis assigned an HR for overall survival of 2.0 for *NRAS* mutation and 1.5 for *KRAS* mutation ($P < 0.01$).

Conclusions: *NRAS* defines a molecular subset with distinct clinical characteristics from *KRAS*-mutant and wild-type mCRC. *NRAS* mutations are enriched in left-sided primary tumors and among African Americans. Mutations in *NRAS* are associated with poor survival and worse outcomes than either *KRAS*-mutant or wild-type mCRC. *Clin Cancer Res*; 23(16); 4753–60. ©2017 AACR.

Introduction

The *RAS* oncogenes (*KRAS*, *NRAS*, and *HRAS*) encode a family of GTP-regulated switches that are recurrently mutated in human cancer. The four enzymes encoded by the three *RAS* genes are highly homologous to one another. Activating mutations render the *RAS* protein in the GTP-bound activated form by preventing hydrolysis of GTP and so preventing transition to the GDP-bound inactive state. *RAS* mutations occur at conserved hotspots, and oncogenic mutations have been shown to occur at codons 12, 13, 61, 117, and 146. Activation of the *RAS* proteins leads to pleiotropic effects in cells, including cellular proliferation, survival, and differentiation. The differences in signaling and downstream effectors that result from the different activated *RAS* isoforms are not currently clear. In colorectal

cancer, *RAS* mutations predominantly occur in the *KRAS* gene; 45% of metastatic colorectal cancers (mCRC) harbor an activating *KRAS* mutation (1). *NRAS* mutations occur in 2% to 7% of mCRC cases (2–4).

NRAS mutations are now regularly identified in the clinical treatment of mCRC as part of routine *RAS* testing prior to EGFR inhibitor therapy. The clinical implications of *NRAS* mutation beyond lack of response to anti-EGFR therapy (4) and whether these tumors behave similarly to *KRAS*-mutant mCRC are not known. *KRAS* mutations have been associated with right-sided colon tumors, whereas *NRAS* mutations have been associated with left-sided primary tumors and female gender (3), suggesting a distinct biology for *KRAS*- and *NRAS*-mutant molecular subsets of mCRC.

Recent clinical series have examined all *RAS*-mutant colorectal cancer or *NRAS*-mutant colorectal cancer, but have been limited by a small number of *NRAS*-mutant cases. *KRAS* and all *RAS* mutations have been associated with worse survival in mCRC (5, 6). *KRAS* and all *RAS* mutations have been associated with worse outcomes after hepatectomy with increased risk for recurrences in the lungs (7, 8). However, it is unknown if the *NRAS*-mutant cases contributed to these poor outcomes, as they represented the minority of *RAS*-mutant cases studied.

To better characterize the biology of *NRAS*-mutant mCRC, we identified all *NRAS*-mutant mCRCs detected by genotyping of consecutive mCRC cases presenting at Memorial Sloan Kettering Cancer Center (MSKCC) between 2008 and 2015. We now report the analysis of the clinical characteristics, concurrent mutation

¹Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, New York. ²Department of Epidemiology-Biostatistics, Memorial Sloan Kettering Cancer Center, New York, New York. ³Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, New York.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (<http://clincancerres.aacrjournals.org/>).

Corresponding Author: Rona Yaeger, Memorial Sloan Kettering Cancer Center, 300 East 66th Street, 10th Floor, New York, NY 10065. Phone: 646-888-5109; Fax: 646-888-4254; E-mail: yaegerr@mskcc.org

doi: 10.1158/1078-0432.CCR-17-0400

©2017 American Association for Cancer Research.

Translational Relevance

NRAS mutations are being identified in routine clinical testing prior to treatment with EGFR inhibitors, with unknown clinical implications beyond lack of response to anti-EGFR therapy. In this study, we evaluated 87 consecutive cases of *NRAS*-mutant metastatic colorectal cancer (mCRC) to determine the clinical features of *NRAS*-mutant mCRC. We found that *NRAS* mutation, particularly in exon 3, is a strong, independent factor for poor overall survival in mCRC, and *NRAS*-mutant tumors are enriched among African Americans.

spectrum, and outcomes in this large series of 87 *NRAS*-mutant mCRC cases.

Materials and Methods

Patient population

Cases were derived from patients seen at MSKCC with mCRC who had their tumors submitted for standard genotyping for anti-EGFR treatment selection between 2008 and 2015. We performed a computerized search of electronic medical records to identify all cases that had an *NRAS* mutation in the sequencing report during this period. A comparison group of all *RAS* wild-type or *KRAS*-mutant mCRC came from cases sequenced between 2008 and 2012 (6). During this period, 1,095 unique patients with mCRC had their tumors genotyped, including 786 cases genotyped for extended *RAS* mutations. Cases with a *KRAS* exon 2 mutation or an extended *KRAS* mutation (exon 3 or 4) were analyzed together as a *KRAS*-mutant mCRC comparison group ($n = 423$), and cases with *RAS* wild-type status confirmed on extended testing formed an *RAS* wild-type mCRC comparison group ($n = 475$). Supplementary Fig. S1 presents the cases analyzed and their *RAS* status.

Sequencing

Genomic DNA was extracted from formalin-fixed paraffin-embedded (FFPE) tissue obtained from biopsies or resections. Sequencing was performed on a metastatic specimen in cases where tissue was available from metastasectomy or diagnostic biopsy ($n = 541$; 55%) and on the primary tumor ($n = 444$; 45%) in all other cases. For the *NRAS*-mutant mCRC cases, genotyping was performed by a mass spectrometry-based assay (Sequenom) to detect hotspot mutations in a panel of 8 genes, MiSeq assay of 45 genes, or (starting in 2015) through an exon-capture next-generation sequencing assay (MSK-IMPACT) of >300 cancer-related genes. All known hotspots in *NRAS* were genotyped with these assays. Supplementary Fig. S1 indicates the number of cases analyzed with each sequencing assay. The all *RAS* wild-type mCRC comparison group was genotyped with the Sequenom assay, and the *KRAS*-mutant mCRC comparison group was genotyped with either Sanger sequencing for exon 2 mutations or the Sequenom assay.

Data collection

All cases were reviewed by a medical oncologist (A. Cercek, M.I. Braghiroli, and R. Yaeger) for patient characteristics, treatment history, and survival. Specific clinical characteristics collected included age, gender, race, primary tumor site, stage at diagnosis, sites of metastatic disease, metastasectomy, previous treatment,

and survival. Stage was categorized by timing of metastasis as synchronous (stage IV at diagnosis) or metachronous (stages I–III at diagnosis). Tumors arising from the cecum to distal transverse colon were classified as right-sided, and tumors arising from the splenic flexure to rectosigmoid junction were classified as left-sided. New patient questionnaires included self-reported race with options of white, black, or Asian, designated as Caucasian, African American, and other, respectively, in our analysis. Metastatic sites were identified by review of medical records and/or imaging. Patients' first- and second-line chemotherapy treatments were reviewed in detail including regimen, duration of therapy, and radiographic outcomes. All research was conducted under appropriate Institutional Review Board/Privacy Board protocols and waivers, and the study was conducted in accordance with recognized ethical guidelines.

Statistical analysis

Associations between clinicopathologic characteristics and tumor mutation status were analyzed using the Fisher exact test for categorical variables and the Wilcoxon Rank-Sum test for continuous variables. Overall survival (OS) was examined from date of first known metastatic disease to date of death or last available follow-up. The log-rank test was used to examine whether OS differed by mutation status. Cox proportional hazards model was used to evaluate the independent effect of mutation status on OS after adjusting for the following known confounders: age at diagnosis, gender, race, tumor location, synchronous tumor, and surgery. Surgery and treatment with hepatic arterial infusion were treated as a time-dependent covariate in the multivariate model.

Cumulative incidences of resection of gross metastatic disease were estimated using competing risks methods and compared between mutation status using the Gray test. Recurrence-free survival (RFS) was calculated from the date of complete liver resection to first recurrence or death, whichever occurred first. Recurrence was confirmed by routine CT scan. RFS and OS were estimated using the Kaplan–Meier methods.

All statistical analyses were performed using SAS version 9.3 (SAS Institute, INC.) or R version 3.0.1 (R foundation for Statistical Computing) using the *cmprsk* package. All *P* values were two-sided. *P* values of <0.05 were considered to indicate statistical significance.

All relevant Reporting Recommendations for Tumor Marker Prognostic Studies criteria were followed in this study (9).

Results

RAS genotyping of 2,764 sequential mCRC cases between 2008 and 2015 at our institution identified 87 (3.1%) mCRCs harboring activating *NRAS* mutations. Three cases had concurrent hotspot *KRAS* and *NRAS* mutations and were not included in the analysis of *NRAS*-mutant mCRC. The clinical characteristics of the 84 *NRAS*-mutant mCRC cases and of control groups of *RAS* wild-type mCRC and *KRAS*-mutant mCRC, identified from cases genotyped between 2008 and 2012, are summarized in Table 1.

Median age at diagnosis, gender distribution, and timing of metastasis (synchronous vs. metachronous) did not significantly vary by mutation status. African American self-reported race was enriched among the *NRAS*-mutant mCRC cases ($P < 0.01$), with a greater than 2-fold increase in the frequency of African American reported race in *NRAS*-mutant mCRC compared with *RAS*

Table 1. Patient characteristics

	Total (n = 982)	RAS WT (n = 475)	KRAS MUT (n = 423)	NRAS MUT (n = 84)	P ^a overall	P ^b KRAS vs. NRAS
Age at diagnosis					0.99	0.92
Mean (years)	58.3	58.2	58.4	58.3		
Gender					0.23	0.28
Male	527 (54%)	265 (56%)	214 (51%)	48 (57%)		
Female	455 (46%)	210 (44%)	209 (49%)	36 (43%)		
Self-reported race ^c					<0.01	0.23
Caucasian	839 (85%)	419 (88%)	356 (84%)	64 (76%)		
African American	68 (7%)	19 (4%)	37 (9%)	12 (14%)		
Other	56 (6%)	32 (7%)	21 (5%)	3 (4%)		
Timing of metastasis					0.98	0.90
Synchronous (stage IV at diagnosis)	553 (56%)	267 (56%)	238 (56%)	48 (57%)		
Site of primary tumor ^d					<0.01	<0.01
Right colon	280 (29%)	100 (21%)	161 (38%)	19 (23%)		
Left colon	149 (15%)	71 (15%)	46 (11%)	32 (38%)		
Rectosigmoid/rectum	552 (56%)	304 (64%)	216 (51%)	32 (38%)		
MMR status					0.96	1.00
Proficient (pMMR)	547 (56%)	259 (55%)	260 (61%)	48 (57%)		
Deficient (dMMR)	17 (2%)	8 (2%)	8 (2%)	1 (1%)		
Unknown/missing	417(42%)	208 (44%)	155 (37%)	35 (42%)		
First site of metastasis					0.04	0.81
Liver	580 (59%)	300 (63%)	233 (55%)	47 (56%)		
Lung	86 (9%)	31 (7%)	47 (11%)	8 (10%)		
Liver + lung	48 (5%)	16 (3%)	25 (6%)	7 (8%)		
Other	268 (27%)	128 (27%)	118 (28%)	22 (26%)		

Abbreviations: MUT, mutant; WT, wild-type; MMR, mismatch repair.

^aP value comparing the three groups, and unknown/missing categories were excluded in the P value calculations.

^bP value comparing KRAS-MUT and NRAS-MUT patients.

^cRace was unknown for 19 patients (5 with RAS wild-type, 9 with KRAS-mutant, and 5 with NRAS-mutant tumors), and these cases were excluded in the P value calculations.

^dSite of primary tumor was unknown for 1 patient with NRAS-mutant colorectal cancer.

wild-type cases (14% vs. 4%). The race distribution of patients did not significantly vary between NRAS- and KRAS-mutant mCRC ($P = 0.23$; African Americans, 14% vs. 9%). Among African Americans whose tumors were genotyped between 2008 and 2012 when all consecutive cases were reviewed, the distribution of tumor molecular subtypes was 32% RAS wild-type, 61% KRAS mutant, and 7% NRAS mutant. Seventeen patients were found to be mismatch repair (MMR) protein deficient by immunohistochemistry. Of these, 5 were germline mutations and 11 were somatic. One patient initiated germline testing but died shortly after, and the results were not obtained.

The primary tumor site varied significantly by molecular status with the highest frequency of right-sided tumors among KRAS-mutant cases (Table 1). Compared with all other cases and with the KRAS-mutant mCRC, NRAS-mutant cases had a significantly higher frequency of left-sided primary tumors.

The sites of metastasis involved at the time of diagnosis of metastasis also varied by molecular alteration (Table 1), but did not significantly differ between KRAS- and NRAS-mutant cases. Like KRAS-mutant tumors, the NRAS-mutant tumors had a lower frequency of liver-limited metastases at diagnosis and a higher frequency of pulmonary metastases. At first diagnosis of metastatic disease, metastases were limited to the liver in 63% of RAS wild-type, 55% of KRAS-mutant, and 56% of NRAS-mutant cases ($P = 0.04$). Metastases were limited to the lung in 7% of RAS wild-type, 11% of KRAS-mutant, and 10% of NRAS-mutant cases.

Mutation genotype and concurrent alterations

The distribution of mutations in the RAS genes and concurrent alterations in the PI3K pathway varied significantly between the

NRAS- and KRAS-mutant mCRCs. Mutations in NRAS occurred in exon 2 in 45% of cases and in exon 3 in 55% of cases (Fig. 1A). In contrast, in the KRAS-mutant mCRC cohort, 93% of mutations were in exon 2, 2% in exon 3, and 4% in exon 4 (Fig. 1B). Of the three cases with concurrent KRAS and NRAS mutations, NRAS mutations occurred in exon 2 (G12V, G13V) in two cases and in exon 3 (Q61K) in one case; concurrent KRAS mutations all occurred at codon 12. Overall, the frequency of concurrent NRAS and KRAS mutations was low: 5% of NRAS exon 2-mutant cases and 2% of NRAS exon 3-mutant cases. Concurrent PIK3CA mutations were identified in four NRAS-mutant cases (5%; Supplementary Fig. S2), a significantly lower frequency than in KRAS-mutant mCRC (18%; $P < 0.01$). In addition, two NRAS-mutant mCRC cases harbored concurrent AKT1 E17K-activating mutations. The PI3K pathway alterations consisted of less common alterations, including three mutations in C2 (N345K twice, R357Q) and one mutation in the helical domain of PIK3CA (Fig. 1C).

Outcomes by tumor mutational status

With a median follow-up among survivors of 35.2 months (range, 0.4–286), we observed a total of 483 deaths. OS varied by mutational status (Fig. 2A): median OS from diagnosis of first metastasis was 33 months [95% confidence interval (CI), 23–59] for NRAS-mutant mCRC, 47 months (95% CI, 40–53) for KRAS-mutant mCRC, and 78 months (95% CI, 67–98) for RAS wild-type mCRC ($P < 0.01$). There was a trend toward worse OS between the NRAS-mutant and KRAS-mutant mCRC patients (log-rank $P = 0.05$). A Cox proportional model that adjusted for age at metastasis, gender, self-reported race, primary tumor site,

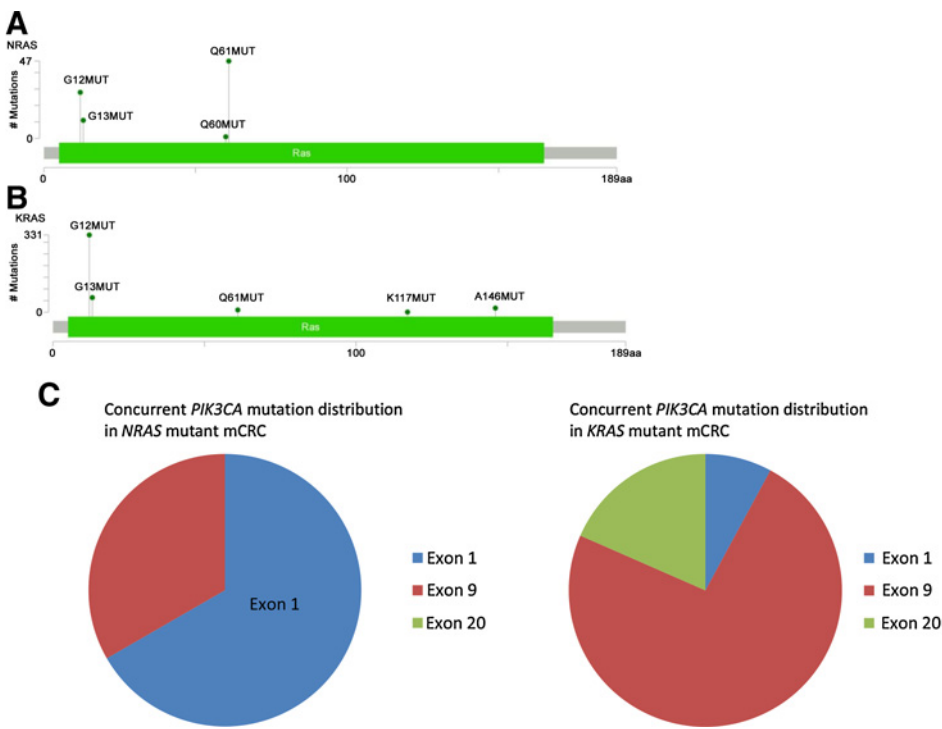


Figure 1. **A** and **B**, Mutation map showing the location of mutations in *NRAS* (**A**) and *KRAS* (**B**). The height of the lollipops in the plot corresponds to the number of cases with each *RAS* variant, as indicated on the y-axis. **C**, Distribution of concurrent mutations in *PIK3CA*, which encodes the catalytic subunit of PI3K, in *NRAS*-mutant colorectal cancer (left) and *KRAS*-mutant colorectal cancer (right).

synchronous disease, liver-limited metastases, and metastasectomy or hepatic arterial infusion treatment as time-dependent covariates identified mutation status, the presence of extrahepatic disease, and liver resection as significant predictors of OS (Table 2). Compared with *RAS* wild-type mCRC, *NRAS*-mutant and *KRAS*-mutant mCRC had an HR of 2.0 (95% CI, 1.3–2.8, $P < 0.01$) and 1.5 (95% CI, 1.2–1.8, $P < 0.01$) for OS, respectively.

We also evaluated OS by mutation genotype (Fig. 2B), comparing survival among three groups—*NRAS* exon 2-mutant mCRC ($n = 39$), *NRAS* exon 3-mutant mCRC ($n = 48$), and *RAS* wild-type mCRC ($n = 475$). Among these three groups, patients whose mCRC harbored exon 3 *NRAS*-mutant mCRC exhibited the shortest OS. OS did not vary significantly between *NRAS* exon 2-mutant mCRC patients and *RAS* wild-type mCRC patients (HR, 1.33; 95% CI, 0.77–2.34; $P = 0.32$). OS was significantly shorter for *NRAS* exon 3-mutant mCRC patients compared with *RAS* wild-type mCRC patients (HR, 2.85; 95% CI, 1.87–4.36; $P < 0.01$) and with *NRAS* exon 2-mutant mCRC patients (HR, 2.0; 95% CI, 1.04–4.0; $P = 0.039$).

To address a potential referral bias to MSKCC for metastasectomy, we also evaluated OS among patients with mCRC who did not undergo complete resection of metastatic disease ($n = 397$). In this group, median OS was 30 months (95% CI, 25.5–33.3) with a breakdown by mutational status of median OS 35 months (95% CI, 28–44) for *RAS* wild-type mCRC patients ($n = 169$), 20 months (95% CI, 16–26) for *NRAS*-mutant mCRC patients ($n = 52$), and 28 months (95% CI, 25–33) for *KRAS*-mutant mCRC patients ($n = 176$). Compared with *RAS* wild-type mCRC patients, *NRAS* mutation was associated with an HR of 1.7 (95% CI, 1.1–1.6; $P = 0.02$), and *KRAS* mutation was associated with an HR of 1.5 (95% CI, 1.1–1.8; $P < 0.01$).

To understand the decreased survival among patients with *NRAS*-mutant mCRC, several subset analyses were conducted.

Recent data suggest that in *RAS* wild-type tumors, left-sided primary site is associated with significantly better prognosis than right-sided primary site (10, 11). In our study, we saw a higher frequency of left-sided primary tumor sites in the *NRAS*-mutant cases. However, among the *NRAS*-mutant mCRC, there was no difference in OS for left-sided versus right-sided colon primary tumor site ($P = 0.31$). We also evaluated survival among *NRAS*-mutant mCRC patients based on outcomes after surgery or first-line chemotherapy. Significantly fewer patients with *NRAS*-mutant mCRC underwent resection of all gross metastatic disease by 12 months from time of diagnosis of metastasis compared with *RAS* wild-type or *KRAS*-mutant mCRC cases (Supplementary Table S1). Thirty-four percent of *NRAS*-mutant mCRC patients underwent metastasectomy by 12 months compared with 44% of *KRAS*-mutant mCRC patients and 49% of *RAS* wild-type mCRC patients ($P < 0.01$). Forty-seven patients with *NRAS*-mutant mCRC had metastatic disease limited to the liver at the time of diagnosis of metastases. Thirty-two of these patients underwent hepatectomy. RFS at 24 months from resection of colorectal liver metastases was 62% (95% CI, 46%–82%; Fig. 3). Forty-four patients received first-line 5-fluorouracil-based combination chemotherapy (with either the FOLFOX or FOLFIRI regimen) for unresectable metastatic disease; the other patients received other regimens ($n = 12$), no chemotherapy for metastatic disease ($n = 10$), preoperative chemotherapy before resection of metastasis ($n = 9$), preoperative chemotherapy before radiation treatment for isolated metastasis ($n = 1$), or were lost to follow-up ($n = 8$). The median duration of this first-line treatment was 4.7 months (range, 0.5–17.9 months) in this population, a substantially shorter period than the 8-month duration for these first-line regimens in clinical trials (12). Reasons for stopping first-line treatment were progression of disease ($n = 24$), toxicity ($n = 7$), palliative surgery for symptomatic metastasis ($n = 1$), surgery for

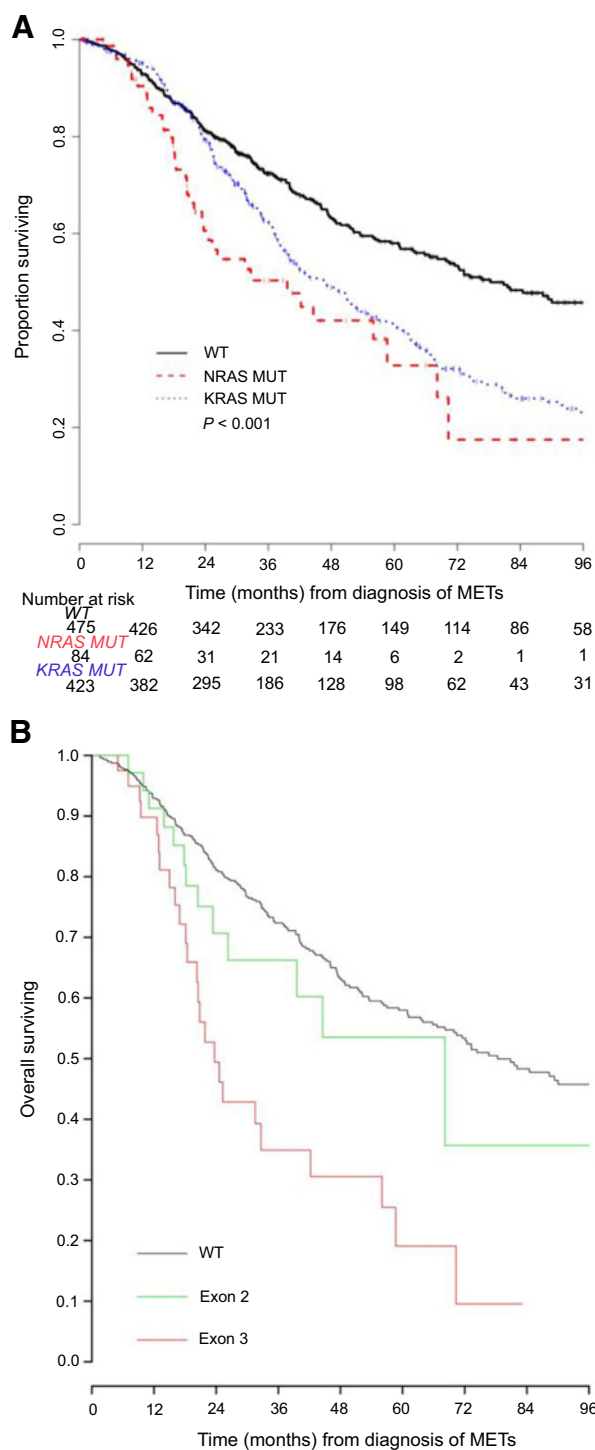


Figure 2. Kaplan-Meier estimates of OS from diagnosis of metastatic disease (MET). **A**, OS by RAS mutation status: RAS wild-type (WT) cases are marked in black, KRAS-mutant cases (KRAS MUT) are marked in blue, and NRAS-mutant cases (NRAS MUT) are marked in red. **B**, OS by NRAS mutation genotype: NRAS WT cases are marked in black, NRAS exon 2-mutant cases (codon 12 or 13) in green, and NRAS exon 3-mutant cases (codon 60 or 61) in red.

Table 2. Multivariate OS model

Characteristics	HR (95% CI)	P
Gene group		<0.01
RAS WT	Reference	
NRAS MUT	2.0 (1.3-2.8)	
KRAS MUT	1.5 (1.2-1.8)	
Age at diagnosis ^a	1.1 (0.9-1.2)	0.12
Gender		0.06
Male	1.2 (0.9-1.4)	
Female	Reference	
Race/ethnicity		0.08
African American	1.5 (1.0-2.1)	0.03
Others	1.1 (0.7-1.6)	0.70
Caucasian	Reference	
Primary tumor site		0.19
Right colon	1.2 (0.9-1.5)	
Left colon	1.1 (0.8-1.5)	
Rectosigmoid/rectum	Reference	
Synchronous metastatic disease		0.30
Yes	1.1 (0.9-1.3)	
No	Reference	
Extrahepatic disease		<0.01
Lung	1.1 (0.7-1.6)	
Liver and lung	1.8 (1.2-2.7)	
Other location	1.7 (1.4-2.2)	
Liver only	Reference	
HAI ^b	0.8 (0.6-1.1)	0.10
Liver resection ^b	0.5 (0.4-0.6)	<0.01

Abbreviations: MUT, mutant; WT, wild-type.

^aHR reflects 10 years of increase in age.

^bHAI and liver resection were coded as time-dependent covariates in the multivariable model.

placement of hepatic arterial infusion pump ($n = 7$), and treatment break ($n = 3$). Two patients remained on first-line treatment at this time.

Response to targeted therapies

Sixteen patients (19%) received EGFR inhibitors at some point during their treatment course. Among them, 3 patients received EGFR inhibitors in the first-line setting in combination with the FOLFOX or FOLFIRI regimens, 2 patients received EGFR inhibitors in combination with irinotecan on which they did not previously progress, and 2 patients received EGFR inhibitors together with an MEK inhibitor. The remaining 9 patients received EGFR inhibitors alone or in combination with irinotecan-containing regimens after progression on irinotecan, allowing assessment of potential benefit of treatment; 8 of these patients (89%) had progression of disease on first imaging, and 1 patient had disease stability for about 8 months.

Discussion

In a large series of 84 patients with NRAS-mutant mCRC, we have analyzed clinical characteristics, outcomes, and response to therapy. We find that NRAS-mutant mCRC is an aggressive subset of colorectal cancer; OS for patients with NRAS-mutant mCRC, particularly with exon 3 NRAS mutations, is worse than that for patients with RAS wild-type tumors or KRAS-mutant mCRC. NRAS mutation defines a clinically distinct subgroup of mCRC, with increased left-sided colon primary tumors and more common in the African American race. NRAS-mutant mCRC may have a distinct molecular pathogenesis: concurrent alterations in the PI3K pathway were relatively uncommon and

Downloaded from http://aacrjournals.org/clinccancerres/article-pdf/23/16/4753/203937/4753.pdf by guest on 11 November 2024

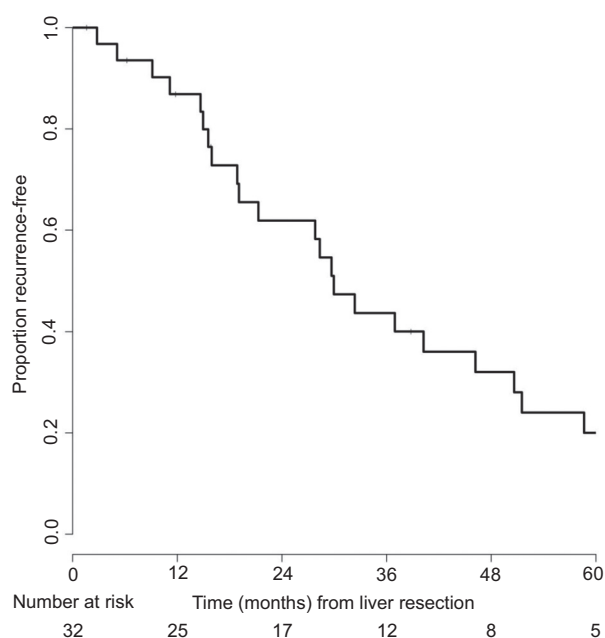


Figure 3. Kaplan-Meier estimate of RFS from time of hepatectomy in patients with liver-limited *NRAS*-mutant metastatic colorectal cancer.

often involved less common pathway alterations (*PIK3CA* C2 domain mutations, *AKT1* mutations).

As a retrospective study of patients undergoing molecular analyses and care at a single center, our study has some inherent limitations. It is possible that the prevalence of *NRAS* mutations in this population may not be reflective of the general population, but the incidence of *NRAS* mutations we found was in concordance with previously published data (2, 3). Several sequencing assays were used as technology improved during the period of the study. The sensitivity of the assay to detect hotspot mutations in *RAS* improved from about 5% to 10% to about 2% for our current multi-gene next-generation sequencing panel MSK-IMPACT. Several cases were sequenced both by the sequenom assay and MSK-IMPACT, and we found no discordant cases. Genotyping for *NRAS* mutations began at our institution before the presence of these mutations was shown to cause resistance to EGFR inhibitors (4), and about a fifth of the patients with *NRAS*-mutant tumors received an EGFR inhibitor at some point in their treatment. The management of these patients may be variable in other institutions which would affect the overall outcomes, although first- and second-line therapies would be expected to be similar in clinical practice (13). The median OS in our cohort was better than would be expected based on published data (14, 15). This may be in part due to a referral bias for patients to undergo resection of colorectal liver metastases or other metastases and a focus on liver directed therapy, including hepatic arterial infusion, at our institution. To address this potential bias, we analyzed OS in patients with unresectable metastatic disease and found median OS of 30 months, in line with recent OS estimates from large clinical trials (15). In this group, *NRAS* mutation remained a significant poor prognostic factor for OS.

Our study identifies a molecular marker that is enriched in African Americans and associated with a worse outcome. Several studies have reported on disparaging outcomes in patients with mCRC between Caucasians and African Americans (16). To date, the reports have focused on socioeconomic standing including access to care and therefore a diagnosis in a more advanced stage of the disease (17, 18). Analyses of epigenetic and genetic differences in tumorigenesis in African-American patients describe discordant findings for the incidence of microsatellite instability with regard to possible differences in development of colorectal cancer in African American patients. We saw an increase in the proportion of *RAS*-mutant tumors, both *KRAS* and *NRAS*, among African American patients compared with Caucasians, that was more pronounced for *NRAS*. Consistent with the analysis by Yoon and colleagues of North-American patients participating in a large adjuvant colon cancer trial (Alliance No147), we found that *KRAS*-mutant tumors are the most common molecular subtype among African American patients (19), and our data further suggest that about 68% of mCRCs in African Americans have a *RAS* mutation, possibly contributing to the poor outcomes seen among African Americans.

In other tumor types, differences in tumor molecular profile by patient race have been reported. Most notably, mutations in *EGFR* in non-small cell lung adenocarcinoma are more common among patients of East Asian ethnicity (20). Somatic *NRAS* mutations have not been previously associated with African American race. Melanoma with *NRAS* mutation is rare among African Americans as *NRAS* mutations are associated with melanoma in sun-exposed skin. Review of the molecular profiles of melanoma cases seen at MSKCC suggests that 1% of *NRAS*-mutant melanoma occur in African Americans. Similarly, *NRAS*-mutant non-small cell lung cancer does not appear more common among African Americans; 5% of *NRAS*-mutant non-small cell lung cancers genotyped at MSKCC occurred in African American patients, a similar number to the relative proportion of African Americans in the patient population at MSKCC. The etiology for the increased proportion of *RAS*-mutant mCRC among African Americans is unclear, and future studies are needed to evaluate the biological basis of this observation.

We found that *NRAS* mutation is associated with more aggressive disease and worse outcomes, a striking finding in the setting of a higher frequency of left-sided primaries with *NRAS* mutation. In our series, primary tumor site was not associated with outcome suggesting a complicated relationship between primary tumor site, tumor molecular profile, and outcomes. Several previous studies have looked at the prognostic effect of *NRAS* mutations. Wang and colleagues reported that in a retrospective analysis of stage I-IV colorectal cancers, the presence of an *NRAS* mutation was associated with significantly shorter survival (21), and in the COIN trial of advanced colorectal cancer, irrespective of treatment, patients with *KRAS*- or *NRAS*-mutant tumors had shorter OS compared with those with wild-type tumors (22). A case series from Italy of mCRC patients that included 47 cases with *NRAS*-mutant disease found a significant association between *NRAS* mutation and shorter OS compared with wild-type cases on univariate and multivariate analyses (23). A recent pooled analysis of five randomized trials in mCRC that included 39 patients with *NRAS*-mutant tumors, however, did not find a significant effect of *NRAS* mutation status on survival (24). An analysis of

NRAS mutation locus by Summers and colleagues (25) suggests that prognostic effects of *NRAS* mutation in mCRC vary by mutation locus with no clear effect for exon 2 mutants, but shorter survival for codon 61 mutants. Consistent with this study, we find that exon 3 *NRAS* mutants are associated with a strong effect on survival (HR of 2.85 vs. *RAS*-mutant tumors and of 2.0 vs. *NRAS* exon 2-mutant tumors) and find no significant effect for exon 2 *NRAS* mutants compared with *RAS* wild-type tumors. These data suggest exon 3-mutant *NRAS* tumors are an aggressive subset of mCRC and may be driving the poor outcomes we see among patients with *NRAS*-mutant tumors. We found in our series, that despite the poor prognosis of *NRAS*-mutant mCRC, a substantial portion of patients with liver-limited disease who were able to get to resection achieved long periods of disease control, in contrast to *BRAF*-mutant mCRC (26). However, a lower proportion of *NRAS*-mutant mCRC patients underwent resection of all metastases compared with patients with *RAS* wild-type or *KRAS*-mutant mCRC. In addition, patients with *NRAS*-mutant mCRC often progressed quickly through first-line 5-fluorouracil-based combination chemotherapy, suggesting that new approaches are needed to better treat *NRAS*-mutant mCRC.

In summary, mutations in *NRAS* occur in about 3% of mCRC cases and are being identified in routine clinical practice. The presence of an *NRAS* mutation serves as a marker of more aggressive mCRC and is enriched among African Americans. These tumors have a distinct biology, both in sites of development and comutation pattern. Further understanding of the biology of this aggressive subset of mCRC is needed to better target these tumors.

References

- Janakiraman M, Vakiani E, Zeng Z, Pratilas CA, Taylor BS, Chitale D, et al. Genomic and biological characterization of exon 4 *KRAS* mutations in human cancer. *Cancer Res* 2010;70:5901–11.
- De Roock W, Claes B, Bernasconi D, De Schutter J, Biesmans B, Fountzilias G, et al. Effects of *KRAS*, *BRAF*, *NRAS*, and *PIK3CA* mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: A retrospective consortium analysis. *Lancet Oncol* 2010;11:753–62.
- Irahara N, Baba Y, Noshio K, Shima K, Yan L, Dias-Santagata D, et al. *NRAS* mutations are rare in colorectal cancer. *Diagn Mol Pathol* 2010;19:157–63.
- Douillard JY, Oliner KS, Siena S, Tabernero J, Burkes R, Barugel M, et al. Panitumumab-FOLFOX4 treatment and *RAS* mutations in colorectal cancer. *N Engl J Med* 2013;369:1023–34.
- 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.
- Yaeger R, Cowell E, Chou JF, Gewirtz AN, Borsu L, Vakiani E, et al. *RAS* mutations affect pattern of metastatic spread and increase propensity for brain metastasis in colorectal cancer. *Cancer* 2015;121:1195–203.
- Kemeny NE, Chou JF, Capanu M, Gewirtz AN, Cercek A, Kingham TP, et al. *KRAS* mutation influences recurrence patterns in patients undergoing hepatic resection of colorectal metastases. *Cancer* 2014;120:3965–71.
- Vauthey JN, Zimmiti G, Kopetz SE, Shindoh J, Chen SS, Andreou A, et al. *RAS* mutation status predicts survival and patterns of recurrence in patients undergoing hepatectomy for colorectal liver metastases. *Ann Surg* 2013;258:619–26; discussion 26–7.
- McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM, et al. Reporting recommendations for tumor marker prognostic studies. *J Clin Oncol* 2005;23:9067–72.
- Tejpar S, Stintzing S, Ciardiello F, Tabernero J, Van Cutsem E, Beier F, et al. Prognostic and predictive relevance of primary tumor location in patients with *RAS* wild-type metastatic colorectal cancer: Retrospective analyses of the CRYSTAL and FIRE-3 trials. *JAMA Oncol* 2016 Oct 10. [Epub ahead of print].
- Venook AP, Niedzwiecki D, Innocenti F, Fruth B, Greeene C, O'Neill BH, et al. Impact of primary tumor location on overall survival (OS) and progression-free survival (PFS) in patients (pts) with metastatic colorectal cancer (mCRC): Analysis of CALGB/SWOG 80405 (Alliance). *J Clin Oncol* 34, 2016 (suppl; abstr 3504).
- Tournigand C, Andre T, Achille E, Lledo G, Flesh M, Mery-Mignard D, et al. FOLFIRI followed by FOLFOX6 or the reverse sequence in advanced colorectal cancer: A randomized GERCOR study. *J Clin Oncol* 2004;22:229–37.
- Benson AB III, Venook AP, Bekaii-Saab T, Chan E, Chen YJ, Cooper HS, et al. Colon cancer, version 3.2014. *J Natl Compr Cancer Network* 2014;12:1028–59.
- Heinemann V, von Weikersthal LF, Decker T, Kiani A, Vehling-Kaiser U, Al-Batran SE, et al. FOLFIRI plus cetuximab versus FOLFIRI plus bevacizumab as first-line treatment for patients with metastatic colorectal cancer (FIRE-3): A randomised, open-label, phase 3 trial. *Lancet Oncol* 2014;15:1065–75.
- Venook AP, Niedzwiecki D, Lenz H, Innocenti F, Mahoney MR, O'Neil BH, et al. CALGB/SWOG 80405: Phase III trial of irinotecan/5-FU/leucovorin (FOLFIRI) or oxaliplatin/5-FU/leucovorin (mFOLFOX6) with bevacizumab (BV) or cetuximab (CET) for patients (pts) with *KRAS* wild-type (wt) untreated metastatic adenocarcinoma of the colon or rectum (mCRC). *J Clin Oncol* 32, 2014; (suppl; abstr LBA3).
- Robbins AS, Siegel RL, Jemal A. Racial disparities in stage-specific colorectal cancer mortality rates from 1985 to 2008. *J Clin Oncol* 2012;30:401–5.
- Ashktorab H, Ahuja S, Kannan L, Llor X, Ellis NA, Xicola RM, et al. A meta-analysis of MSI frequency and race in colorectal cancer. *Oncotarget* 2016;7:34546–57.
- Williams R, White P, Nieto J, Vieira D, Francois F, Hamilton F. Colorectal cancer in African Americans: an update. *Clin Translat Gastroenterol* 2016;7:e185.
- Yoon HH, Shi Q, Alberts SR, Goldberg RM, Thibodeau SN, Sargent DJ, et al. Racial differences in *BRAF*/*KRAS* mutation rates and survival in stage III colon cancer patients. *J Natl Cancer Inst* 2015;107:djv186.

Disclosure of Potential Conflicts of Interest

N. Kemeny reports receiving commercial research grants from Amgen Inc. L. Saltz reports receiving commercial research grants from Taiho Pharmaceuticals. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: A. Cercek, J.F. Chou, L. Saltz, R. Yaeger
Development of methodology: A. Cercek, M.I. Braghiroli, J.F. Hechtman, R. Yaeger
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A. Cercek, M.I. Braghiroli, L. Saltz, R. Yaeger
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A. Cercek, J.F. Chou, J.F. Hechtman, N. Kemeny, L. Saltz, M. Capanu, R. Yaeger
Writing, review, and/or revision of the manuscript: A. Cercek, M.I. Braghiroli, J.F. Chou, J.F. Hechtman, N. Kemeny, L. Saltz, M. Capanu, R. Yaeger
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A. Cercek, N. Kemeny, R. Yaeger
Study supervision: A. Cercek, R. Yaeger

Grant Support

This study was supported by a Career Development Award from the Conquer Cancer Foundation of the American Society of Clinical Oncology (to R. Yaeger) and the NIH Memorial Sloan Kettering Cancer Center Core Grant (P30 CA 008748).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received February 10, 2017; revised March 22, 2017; accepted April 18, 2017; published OnlineFirst April 26, 2017.

20. Shigematsu H, Lin L, Takahashi T, Nomura M, Suzuki M, Wistuba II, et al. Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. *J Natl Cancer Inst* 2005;97:339–46.
21. Wang Y, Velho S, Vakiani E, Peng S, Bass AJ, Chu GC, et al. Mutant N-RAS protects colorectal cancer cells from stress-induced apoptosis and contributes to cancer development and progression. *Cancer Discov* 2013;3:294–307.
22. Maughan TS, Adams RA, Smith CG, Meade AM, Seymour MT, Wilson RH, et al. Addition of cetuximab to oxaliplatin-based first-line combination chemotherapy for treatment of advanced colorectal cancer: Results of the randomised phase 3 MRC COIN trial. *Lancet* 2011;377:2103–14.
23. Schirripa M, Cremolini C, Loupakis F, Morvillo M, Bergamo F, Zoratto F, et al. Role of NRAS mutations as prognostic and predictive markers in metastatic colorectal cancer. *Int J Cancer* 2015;136:83–90.
24. Modest DP, Ricard I, Heinemann V, Hegewisch-Becker S, Schmiegel W, Porschen R, et al. Outcome according to KRAS-, NRAS- and BRAF-mutation as well as KRAS mutation variants: Pooled analysis of five randomized trials in metastatic colorectal cancer by the AIO colorectal cancer study group. *Ann Oncol* 2016;27:1746–53.
25. Summers M, Smith CG, Maughan TS, Kaplan R, Escott-Price V, Cheadle JP. BRAF and NRAS locus specific variants have different outcomes on survival to colorectal cancer. *Clin Cancer Res* 2016 Nov 4. [Epub ahead of print].
26. Yaeger R, Cercek A, Chou JF, Sylvester BE, Kemeny NE, Hechtman JF, et al. BRAF mutation predicts for poor outcomes after metastasectomy in patients with metastatic colorectal cancer. *Cancer* 2014;120:2316–24.