HHS Public Access

Author manuscript

Clin Cancer Res. Author manuscript; available in PMC 2018 August 15.

Published in final edited form as:

Clin Cancer Res. 2017 August 15; 23(16): 4753–4760. doi:10.1158/1078-0432.CCR-17-0400.

Clinical features and outcomes of patients with colorectal cancers harboring NRAS mutations

Andrea Cercek[1], **Maria Ignez Braghiroli**[1], **Joanne F. Chou**[2], **Jaclyn F. Hechtman**[3], **Nancy Kemeny**[1], **Leonard Saltz**[1], **Marinela Capanu**[2], and **Rona Yaeger**[1]

[1]Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY 10065

[2]Department of Epidemiology-Biostatistics, Memorial Sloan Kettering Cancer Center, New York, NY 10065

[3]Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY 10065

Abstract

Purpose—*NRAS* mutations are now routinely included in RAS testing prior to EGFR (epidermal growth factor receptor) 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 2008–2015. Comparison groups consisted of *RAS* wild-type and *KRAS* mutant mCRC consecutive cases genotyped from 2008–2012.

Results—Three percent (87/2764) of mCRC patients had *NRAS* mutant tumors (45% exon 2, 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 a hazard ratio 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.

Keywords

NRAS; colorectal cancer; survival; race

Address correspondence to Rona Yaeger, MD, Memorial Sloan Kettering Cancer Center, 300 E 66th Street, 10th Floor, New York, NY 10065. yaegerr@mskcc.org.

Disclosures: There are no conflicts.

Introduction

The *RAS* oncogenes (*KRAS, NRAS, 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 (CRC), *RAS* mutations predominantly occur in the *KRAS* gene; 45% of metastatic CRC (mCRC) harbor an activating *KRAS* mutation(1). *NRAS* mutations occur in 2–7% 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 (epidermal growth factor receptor) 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, while *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 CRC or *NRAS* mutant CRC, 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 mCRC 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 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 1095 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 (exons 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 a *RAS* wild-type mCRC comparison group (n=475). Supplementary Figure 1 diagrams 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 Figure 1 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 by reviewed a medical oncologist (A.C., M.I.B., R.Y.) 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 (stage 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's 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. 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 Gray's 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., Cary, NC, USA) or R version 3.0.1 (R foundation for Statistical Computing, Vienna, Austria) 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 (REMARK) criteria were followed in this study(9).

Results

RAS genotyping of 2764 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 versus 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 two-fold increase in the frequency of African-American reported race in *NRAS* mutant mCRC compared to *RAS* wild-type cases (14% versus 4%). The race distribution of patients did not significantly vary between *NRAS* and *KRAS* mutant mCRC ($p=0.23$; African Americans 14% versus 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 protein deficient (dMRR) by immunohistochemistry. Of these five 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 to all other cases and to 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 phosphatidylinositol 3-kinase (PI3K) pathway varied significantly between the *NRAS* and *KRAS* mutant mCRC. 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 2 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). Additionally, 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% 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 towards worse OS between the *NRAS* mutant and *KRAS* mutant mCRC patients (logrank p=0.05). A Cox proportional model that adjusted for age at metastasis, gender, self-reported race, primary tumor site, 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 to *RAS* wild-type mCRC, *NRAS* mutant and *KRAS* mutant mCRC had a hazard ratio (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 to *RAS* wild-type mCRC patients (HR 2.85 [95% CI: 1.87–4.36], p<0.01)

and to *NRAS* exon 2 mutant mCRC patients (HR: 2.0 [95% CI: [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 to *RAS* wild-type mCRC patients, *NRAS* mutation was associated with a HR of 1.7 (95% CI: 1.1–1.6, p=0.02) and *KRAS* mutation was associated with a 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 to *RAS* wild-type or *KRAS* mutant mCRC cases (Supplementary Table 1). Thirty-four percent of *NRAS* mutant mCRC patients underwent metastasectomy by 12 months compared to 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. Recurrence-free survival at 24 months from resection of colorectal liver metastases was 62% (95% CI: 46–82%) (Fig. 3). Forty-four patients received first line 5-fluourouracil-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 placement of hepatic arterial infusion pump (n=7), and treatment break (n=3). Two patients remain 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, three patients received EGFR inhibitors in the first line setting in combination with the FOLFOX or FOLFIRI regimens, two patients received EGFR

inhibitors in combination with irinotecan on which they did not previously progress, and two patients received EGFR inhibitors together with a MEK inhibitor. The remaining nine patients received EGFR inhibitors alone or in combination with irinotecan-containing regimens after progression on irinotecan, allowing assessment of potential benefit of treatment; eight of these patients (89%) had progression of disease on first imaging and one 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 CRC; 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 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 5–10% 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 which 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 regards to possible differences in development of CRC in African-American patients. We saw an increase in the proportion of *RAS* mutant tumors, both *KRAS* and *NRAS,* among African-American patients compared to Caucasians, that was more pronounced for *NRAS.* Consistent with the analysis by Yoon *et al* 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 biologic 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 *et al* reported that in a retrospective analysis of stage I–IV CRCs the presence of an *NRAS* mutation was associated with significantly shorter survival(21), and in the COIN trial of advanced CRC, irrespective of treatment, patients with *KRAS* or *NRAS* mutant tumors had shorter OS compared to 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 to 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 *et al*(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 (hazard ratio of 2.85 versus *RAS* mutant tumors and of 2.0 versus *NRAS* exon 2 mutant tumors) and find no significant effect for exon 2 *NRAS* mutants compared to *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 to patients with *RAS* wild-type or *KRAS* mutant mCRC. Additionally, 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 co-mutation pattern. Further understanding of the biology of this aggressive subset of mCRC is needed to better target these tumors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Supported by a Career Development Award from the Conquer Cancer Foundation of the American Society of Clinical Oncology (R. Yaeger) and the National Institutes of Health Memorial Sloan Kettering Cancer Center Core Grant (P30 CA 008748 to Craig Thompson).

References

1. 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. [PubMed: 20570890]
2. De Roock W, Claes B, Bernasconi D, De Schutter J, Biesmans B, Fountzilas 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. The lancet oncology. 2010; 11:753–62. [PubMed: 20619739]
3. Irahara N, Baba Y, Nosho K, Shima K, Yan L, Dias-Santagata D, et al. NRAS mutations are rare in colorectal cancer. Diagnostic molecular pathology: the American journal of surgical pathology, part B. 2010; 19:157–63.
4. 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. [PubMed: 24024839]
5. 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. [PubMed: 9586664]
6. 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. 2014
7. 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
8. Vauthey JN, Zimmitti 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. Annals of surgery. 2013; 258:619–26. discussion 26–7. [PubMed: 24018645]

9. 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. [PubMed: 16172462]
10. 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 oncology. 2016
11. Venook AP, Niedzwiecki D, Innocenti F, Fruth B, Greeene C, O'Neill BH, et al. Impact of pirmary 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). Journal of Clinical Oncology. 2016; 34 abstract 3504.
12. 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. [PubMed: 14657227]
13. Benson AB 3rd, Venook AP, Bekaii-Saab T, Chan E, Chen YJ, Cooper HS, et al. Colon cancer, version 3.2014. Journal of the National Comprehensive Cancer Network: JNCCN. 2014; 12:1028–59. [PubMed: 24994923]
14. 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. The lancet oncology. 2014; 15:1065–75. [PubMed: 25088940]
15. Venook AP, Niedzwiecki D, Lenz H, Innocenti F, Mahoney MR, 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. 2014; 32(suppl) abstr LBA3.
16. 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. [PubMed: 22184373]
17. 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. [PubMed: 27120810]
18. Williams R, White P, Nieto J, Vieira D, Francois F, Hamilton F. Colorectal Cancer in African Americans: An Update. Clinical and translational gastroenterology. 2016; 7:e185. [PubMed: 27467183]
19. 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.
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. [PubMed: 15741570]
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 discovery. 2013; 3:294–307. [PubMed: 23274911]
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. [PubMed: 21641636]
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. [PubMed: 24806288]
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. [PubMed: 27358379]

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

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. [PubMed: 24737664]

STATEMENT OF TRANSLATIONAL RELEVANCE

NRAS mutations are being identified in routine clinical testing prior to treatment with epidermal growth factor receptor (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.

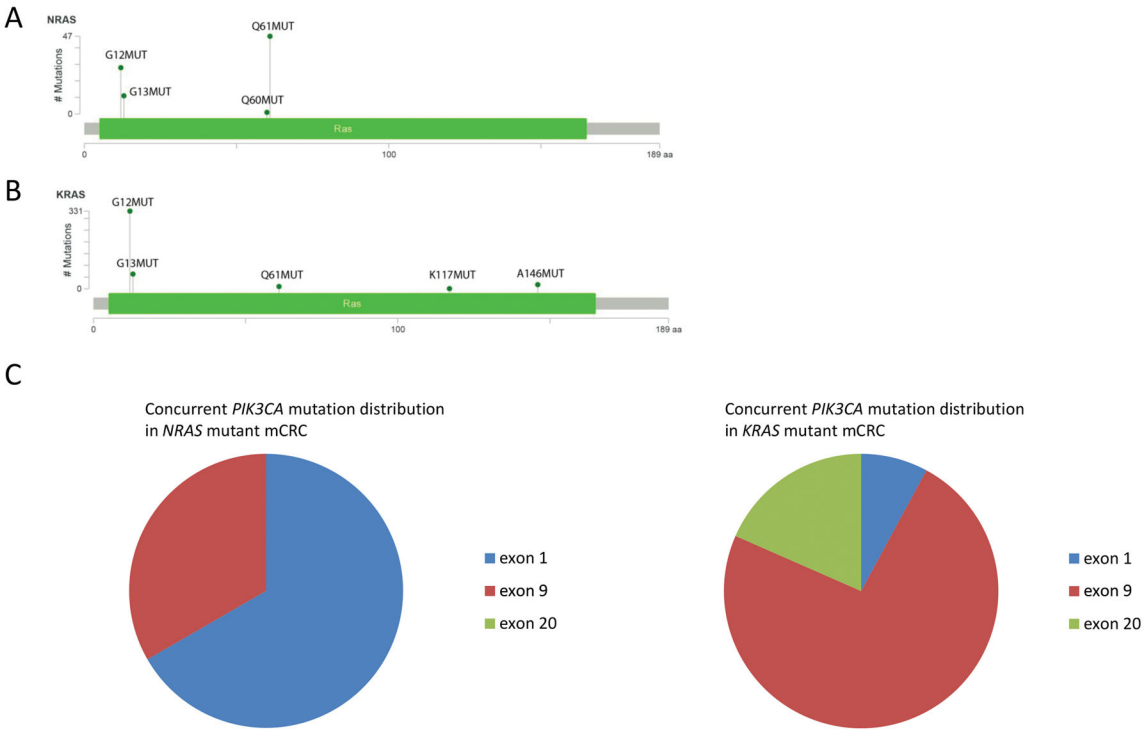

Figure 1.

(A, B) Mutation map showing the location of mutations in (A) *NRAS* and (B) *KRAS*. 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 panel) and *KRAS* mutant colorectal cancer (right panel).

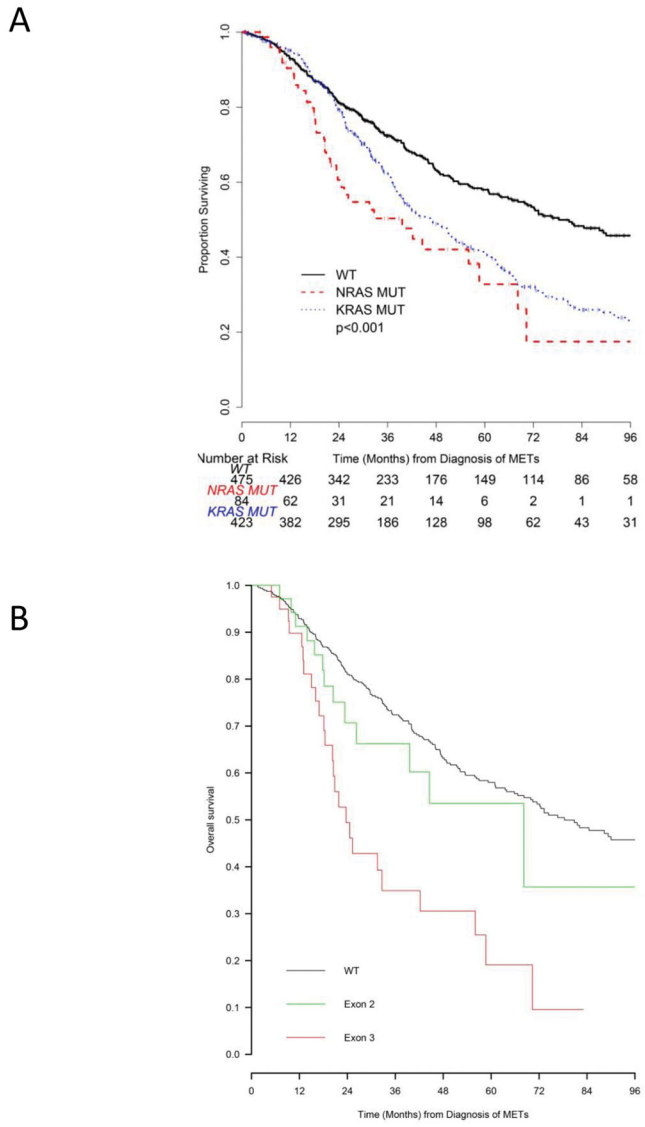

Figure 2.

Kaplan-Meier estimates of overall survival from diagnosis of metastatic disease (METs). (A) Overall survival 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) Overall survival 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.

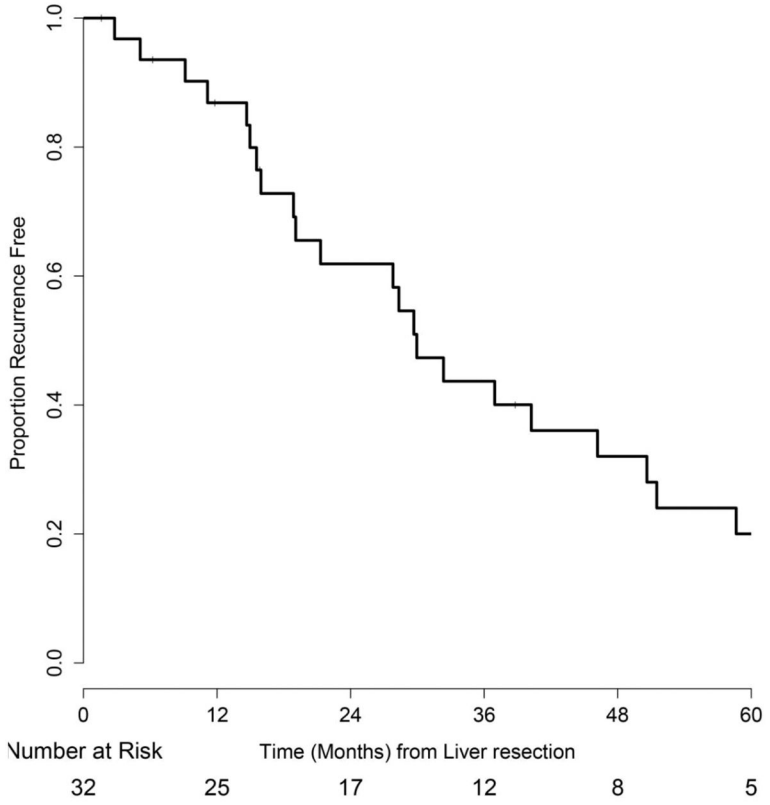

Figure 3.

Kaplan-Meier estimate of recurrence free survival from time of hepatectomy in patients with liver limited *NRAS* mutant metastatic colorectal cancer.

Clin Cancer Res. Author manuscript; available in PMC 2018 August 15.

Table 1

Patient characteristics.

	Total (n=982)	RAS WT (n=475)	KRAS MUT (n=423)	NRAS MUT (n=84)	*p*-value[a] overall	*p*-value[b] KRAS *v* 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 *					<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 **					<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%)		

* Race 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.

** Site of primary tumor was unknown for one patient with *NRAS* mutant colorectal cancer.

[a] *p*-value comparing the 3 groups, Unknown/missing categories were excluded in the *p*-value calculations;

[b] *p*-value comparing KRAS MUT and NRAS MUT patients.

WT – wild-type; MUT - mutant

Table 2

Multivariate overall survival model.

Characteristics	HR (95%CI)	*p*-value
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 *	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 & Lung	1.8 (1.2,2.7)	
Other location	1.7 (1.4,2.2)	
Liver only	Reference	
HAI **	0.8 (0.6,1.1)	0.10
Liver resection **	0.5 (0.4,0.6)	<0.01

* Hazard ratio reflects 10 years increase in age

** HAI and liver resection were coded as time-dependent covariates in the multivariable model

WT – wild-type; MUT - mutant