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KRAS Amplification in Metastatic Colon Cancer is Associated with a History of Inflammatory Bowel Disease and May Confer Resistance to anti-EGFR Therapy

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Abstract

Mutations in *RAS* occur in 30–50% of metastatic colorectal carcinomas (mCRCs) and correlate with resistance to anti-EGFR therapy. Consequently, mCRC biomarker guidelines state *RAS* mutational testing should be performed when considering EGFR inhibitor treatment. However, a small subset of mCRCs are reported to harbor *RAS* amplification. In order to elucidate the clinicopathologic features and anti-EGFR treatment response associated with *RAS* amplification, we retrospectively reviewed a large cohort of mCRC patients that underwent targeted next-

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generation sequencing and copy number analysis for *KRAS*, *NRAS*, *HRAS*, *BRAF* and *PIK3CA*. Molecular testing was performed on 1,286 consecutive mCRC from 1,271 patients as part of routine clinical care, and results were correlated with clinicopathologic findings, mismatch repair (MMR) status and follow-up. *RAS* amplification was detected in 22 (2%) mCRCs and included: *KRAS*, *NRAS* and *HRAS* for 15, 5 and 2 cases, respectively (6 to 21 gene copies). Patients with a *KRAS*-amplified mCRC were more likely to report a history of inflammatory bowel disease ($p < 0.001$). In contrast, mutations in *KRAS* were associated with older patient age, right-sided colonic origin, low-grade differentiation, mucinous histology and MMR proficiency ($p \leq 0.017$). Four patients with a *KRAS*-amplified mCRC and no concomitant *RAS*/*BRAF*/*PIK3CA* mutations received EGFR inhibitor-based therapy, and none demonstrated a clinicoradiographic response. The therapeutic impact of *RAS* amplification was further evaluated using a separate, multi-institutional cohort of 23 patients. Eight of 23 patients with *KRAS*-amplified mCRC received anti-EGFR therapy and all 8 patients exhibited disease progression on treatment. Although the number of *KRAS*-amplified mCRCs is limited, our data suggests the clinicopathologic features associated with mCRC harboring a *KRAS* amplification are distinct from those associated with a *KRAS* mutation. However, both alterations seem to confer EGFR inhibitor resistance and, therefore, *RAS* testing to include copy number analyses may be of consideration in the treatment of mCRC.

Keywords

locally advanced; metastasis; recurrent; colorectal adenocarcinoma; colon cancer; treatment; cetuximab; panitumumab

Introduction

Colorectal cancer (CRC) is the third most common malignancy in the United States and prognosis is highly dependent on the occurrence of distant metastases.(1) Approximately 30% of newly diagnosed CRC patients present with metastatic disease and up to 50% of all CRC patients will develop metastases as the disease progresses.(2) Unfortunately, metastatic CRC (mCRC) is often considered incurable. Historically, mCRC was associated with an overall survival of 6 months. However, with recent progress in therapy, the median overall survival for mCRC patients has improved to 30 months or longer.(3)

Combination systemic chemotherapy is the mainstay of mCRC treatment. The National Comprehensive Care Network (NCCN) currently recommends patients receive a first-line regimen to include 5-fluorouracil, oxaliplatin or irinotecan, and a targeted agent to vascular endothelial growth factor or epidermal growth factor receptor (EGFR).(4) Cetixumab and panitumumab are monoclonal antibodies that target the EGFR extracellular domain and, consequently, inhibit the mitogen-activated protein kinase signaling pathway. Their efficacy has been demonstrated in several phase III clinical trials but restricted to patients whose mCRC is wild-type for mutations in *KRAS* and *NRAS*.(5–10) To aid in the identification of patients eligible for treatment with EGFR inhibitors, biomarker testing guidelines from the American Society for Clinical Pathology (ASCP), Association for Molecular Pathology (AMP), College of American Pathologists (CAP) and American Society of Clinical

Oncology (ASCO) recommend mutational analysis for exons 2, 3 and 4 for *KRAS* and *NRAS*.(11)

While mutations in *KRAS* and *NRAS* occur in 30 to 50% of mCRCs and are mutually exclusive, a small subset of mCRCs are reported to harbor *KRAS* amplification.(12–15) However, little is known regarding patients harboring *KRAS*-amplified mCRC and treatment response data to anti-EGFR therapy is lacking. Herein, we retrospectively reviewed our clinical and pathologic experience with targeted next-generation sequencing (NGS) for *KRAS*, *NRAS*, *HRAS*, *BRAF* and *PIK3CA* on a consecutive series of 1,271 patients with mCRC. The aims of this study were to: (1) identify the prevalence of *RAS*-amplified mCRC, (2) evaluate the clinicopathologic features of *RAS* amplification in comparison to *RAS* mutations and (3) correlate *RAS* amplification with treatment response to EGFR inhibitors.

Materials and Methods

Study population and design

Study approval was obtained from the University of Pittsburgh Institutional Review Board (IRB# STUDY19110319). Between February 2016 and December 2019, 1,286 consecutive metastatic CRC (mCRC) specimens from 1,271 patients were prospectively submitted to the Molecular and Genomic Pathology (MGP) Laboratory at the University of Pittsburgh Medical Center (UPMC) for targeted NGS. Metastatic CRC was defined as pathologically confirmed distant organ metastases and/or locoregional recurrence. Patients with metastasis to regional lymph nodes without pathologically confirmed distant organ metastases/ locoregional recurrence were excluded from this study. The MGP Laboratory is a Clinical Laboratory Improvement Amendments (CLIA)-certified and CAP-accredited laboratory. Colorectal carcinoma specimens were received from 34 hospitals within the UPMC system and other medical institutions within and outside of Pennsylvania, USA. In all cases, molecular testing was performed to at least determine *RAS* mutational status for oncologic treatment with EGFR inhibitors (e.g., cetuximab and panitumumab). Medical records and pathology slides/reports were reviewed to document patient demographics, clinical presentation and history, primary tumor location, primary tumor histologic subtype and grade (according to the 2019 WHO Classification of Tumours of the Digestive System(16)), mismatch repair (MMR) status and follow-up to include treatment history and response data. Among 1,271 patients, available medical records for 70 patients were insufficient to determine a history of Lynch syndrome and inflammatory bowel disease.

In order to further evaluate the therapeutic effect of *RAS* amplification in mCRC, a separate multi-institutional cohort was collected from Henry Ford Health System, University of Texas MD Anderson Cancer Center and UPMC. All three medical institutions utilize the Oncomine™ Comprehensive Assay v3 for selected stage IV neoplasms according to the manufacturer's instructions (Thermo Fisher Scientific, Waltham, MA). Molecular testing archives were retrospectively searched for *RAS*-amplified mCRC at all three institutions. Twenty-three patients were identified, and individual electronic medical records and pathology slides/reports were reviewed similarly to the aforementioned UPMC consecutive cohort of mCRC patients.

Targeted NGS to include copy number analysis

Tumor DNA was isolated from formalin-fixed paraffin-embedded (FFPE) sections of surgical resection, biopsy and cytopathology fine needle aspirate cell block material. The DNeasey Blood and Tissue kit on the QIAcube instrument (QIAGEN, Germantown, MD) was used for tumor DNA isolation. Extracted DNA was quantitated on the GloMax Discover Plate Reader using the QuantiFluor ONE dsDNA System (Promega, Madison, WI). Amplification-based targeted NGS was performed using custom AmpliSeq primers for hotspot mutations in *KRAS*, *NRAS*, *HRAS*, *BRAF* and *PIK3CA* with primer sequences and performance characteristics as previously described.(17, 18) Of note, coverage for *KRAS*, *NRAS* and *HRAS* included exons 2, 3 and 4. Amplicons were barcoded, ligated with specific adapters and purified. DNA library quantity and quality checks were performed using the 4200 TapeStation (Agilent Technologies, Santa Clara, CA). The Ion Chef was used to prepare and enrich templates and enable testing via Ion Sphere Particles on a semiconductor chip. Massive parallel sequencing was carried out on an Ion S5 XL System according to the manufacturer's instructions (Thermo Fisher Scientific, Waltham, MA) and data was analyzed with the Torrent Suite Software v5.8 (Thermo Fisher Scientific, Waltham, MA) and in-house Variant Explorer (UPMC, Pittsburgh, PA) for point mutations, small insertions/deletions and copy number alterations. Each variant was prioritized according to the 2017 AMP/ASCO/CAP joint consensus guidelines.(19) Tier I, Tier II and Tier III variants were reported; however, only Tier I and Tier II variants were used clinically. The limit of detection was at 3% allele frequency (AF). The minimum depth of coverage for testing was 300x; however, the mean coverage attained was 3,225x. For each mutation, an AF was calculated based on the number of reads of the mutant allele versus the wild-type allele and reported as a percentage.

Copy number analysis was performed as previously described and validated.(20, 21) The total depth of sequencing coverage for each sequenced region was normalized and calculated per sequenced case. A decrease in sequencing coverage below established cut-offs was considered a copy number loss. In contrast, an increase in sequencing coverage above established cut-offs was interpreted as a copy number gain. A gene amplification was defined by the presence of ≥6 copies of a variant as previously described and previously validated using fluorescence *in situ* hybridization analysis.(20) Of note, the mechanism of gene amplification, such as chromosomal aneuploidy, cannot be determined using this assay. In addition, results of copy number analysis were not reported clinically in accordance with ASCP/AMP/CAP/ASCO biomarker testing guidelines for mCRC.(11)

Statistical analysis

Chi-squared analysis or Fisher exact tests were used to compare categorical data, and Mann-Whitney *U* test was used to compare continuous variables. All statistical analyses were performed using the SPSS Statistical software, version 24 (IBM, Armonk, NY) and statistical significance was defined as a p-value of <0.05.

Results

Metastatic colorectal carcinoma study cohort

The clinicopathologic features of the study cohort are summarized in Table 1. Briefly, 1,286 consecutive CRC specimens from 1,271 patients with distant metastatic/recurrent CRC were prospectively submitted for targeted NGS. At the time of initial cancer diagnosis, the patient age range was 18 to 99 years (median, 64.0 years; mean, 63.2 years) and there was a female-to-male ratio of 1:1.2. A history of Lynch syndrome and inflammatory bowel disease (IBD) was documented in 10 of 1,201 (1%) patients and 23 of 1,201 (2%) patients, respectively. Among the 23 patients with an IBD history, 8 patients had Crohn's disease and 15 patients had ulcerative colitis.

The specimens used for sequencing consisted of 373 (29%) primary CRCs and 913 (71%) distant metastases/recurrences (Supplementary Table 1). Primary CRCs were used for targeted NGS if sufficient tumor from the corresponding metastasis was unavailable at the time of metastatic presentation. Of note, the NCCN guidelines state *RAS* testing can be performed on either primary or metastatic/recurrent CRCs as both specimen types are reported to have a similar prevalence in *RAS* mutational status.(4, 22) The site of colonic origin for the tumors was predominantly left-sided (n = 801, 63%) (Supplementary Table 2). The histologic subtypes of the primary tumors included 1,060 (83%) conventional adenocarcinomas, 150 (12%) mucinous adenocarcinomas, 37 (3%) signet-ring cell carcinomas, 11 (1%) neuroendocrine carcinomas, 6 micropapillary carcinomas, 3 medullary, 2 adenosquamous/squamous carcinomas and 2 undifferentiated carcinomas. In addition, most of the primary tumors were histologically graded as well-to-moderately differentiated (n = 1,042, 82%). Results of targeted NGS among 1,286 specimens revealed mutations in *KRAS*, *PIK3CA*, *BRAF* and *NRAS* for 563 (44%), 170 (13%), 154 (12%), 29 (2%) mCRCs, respectively. Further, one mCRC harbored an *HRAS* mutation. Except for one mCRC with *KRAS* and *NRAS* mutations, mutations in the *RAS* genes were mutually exclusive with one another. Mismatch repair (MMR) was also evaluated in 1,258 (98%) cases with the identification of 100 (8%) MMR deficient-mCRCs.

Metastatic colorectal carcinomas with RAS amplification

RAS amplification was detected for 22 (2%) mCRCs and involved *KRAS, NRAS* and *HRAS* for 15, 5 and 2 cases, respectively (Table 2 and Figure 1). Copy number gains in *BRAF* and *PIK3CA* were not identified. Of note, in accordance with mCRC biomarker testing guidelines, *RAS* amplification results were not reported.(11) Gene amplification ranged from 6 to 21 copies and, among the individual *RAS* genes, was mutually exclusive with one another. However, this mutually exclusive relationship did not extend between *RAS* amplification and *RAS* mutations ($p = 0.396$). Eight of 22 (36%) *RAS*-amplified mCRCs harbored mutations in *KRAS* (n = 7) or *NRAS* (n = 1). Further, *BRAF* mutations were found in 3 *RAS*-amplified mCRCs. Interestingly, amplification of a *RAS* gene correlated with a younger median patient age at initial diagnosis (58.0 years vs. 64.0 years, $p = 0.042$) and a history of IBD (41% [9 of 22] vs. 1% [14 of 1,249], $p < 0.001$). However, upon excluding IBD patients from statistical analysis, the association between *RAS* amplification and young median age was no longer significant ($p = 0.786$). Further, there were no statistically

significant differences among other clinicopathologic features including site of distant metastasis and/or locoregional recurrence.

A subanalysis of *KRAS* status also revealed that patients with a *KRAS*-amplified mCRC were younger (54.0 years vs. 64.0 years, p = 0.016) and had a history of IBD (60% [9 of 15] vs 1% [14 of 1,256], p < 0.001) as compared to patients without a *KRAS*-amplified mCRC (Table 3). But, after excluding a history of IBD, the association between *KRAS* amplification and young median age was not statistically significant (p = 0.776). In contrast, *KRAS*-mutated mCRCs frequently occurred in older patients (65.0 years vs. 63.0 years, p = 0.017), right-sided in origin (42% vs. 33%, p = 0.003), well-to-moderate differentiation (89% vs. 77%, p < 0.001), mucinous histology (17% vs. 7%, p < 0.001) and MMR proficient (96% vs. 89%, p < 0.001) in comparison to *KRAS* wild-type mCRC. Further, *KRAS* mutations were essentially mutually exclusive of mutations in *NRAS*, *HRAS* and *BRAF* (p < 0.001); but, among 170 *PIK3CA*-mutant mCRCs, 107 (63%) cases harbored a *KRAS* mutation. Due to the identification of only 5 *NRAS*-amplified mCRCs, no statistically significant associations were found (Supplementary Table 3). Mutations in *NRAS* also did not correlate with any of the evaluated clinicopathologic features.

Follow-up for patients with a RAS-amplified metastatic colorectal carcinoma

Follow-up was available for 20 of 22 *RAS*-amplified mCRC patients and ranged from 2–156 months (median, 16.5 months; mean, 25.2 months). Fourteen of 20 (70%) patients died from disease, and 5 (25%) patients were documented to have received the EGFR inhibitor, panitumumab. Among the five patients treated with panitumumab, other than amplification of *KRAS* (n = 4) or *NRAS* (n = 1), no additional genomic alterations were identified. After one cycle of panitumumab-based therapy, the mCRC patient with an *NRAS* amplification developed a severe grade III skin toxicity necessitating discontinuation of the EGFR inhibitor. The remaining four patients with *KRAS*-amplified mCRC received a therapeutic regimen that included panitumumab as either first-line or second-line therapy. All four patients demonstrated clinical and radiographic disease progression while on treatment (Figure 2).

Treatment data from a multi-institutional cohort of metastatic colorectal carcinomas with RAS amplification

In order to further evaluate the therapeutic impact of *RAS* amplification in the setting of mCRC, a cohort of *RAS*-amplified mCRCs without concomitant mutations in *KRAS*, *NRAS*, *HRAS*, *BRAF* and *PIK3CA* were collected from three institutions and identified using the Oncomine™ Comprehensive Assay v3 testing platform (Table 4). This cohort consisted of 23 patients ranging in age of 33 to 81 years (median, 55.0 years; mean, 53.6 years), a female-to-male ratio of 1:1.6 and a history of ulcerative colitis for 5 (22%) patients. Amplification of *RAS* included *KRAS* (n = 21), *NRAS* (n = 1) and both genes (n = 1) with copy number alterations ranging between 6 and 42 gene copies. Eight mCRC patients with *KRAS* amplification received cetuximab (n = 6) or panitumumab (n = 2), and all 8 patients exhibited clinical and radiographic disease progress while on EGFR inhibitor-based treatment.

Discussion

Effective chemotherapy for mCRC is a key determinant for patient overall survival and is influenced by multiple factors that include clinicoradiographic findings, pathologic features and molecular biomarkers, such as mutations in the *RAS* genes when considering anti-EGFR therapy. Consistent with previous studies, the prevalence of a *RAS* mutation within our mCRC patient cohort was 48% and, except for one case, mutations in the *RAS* genes were mutually exclusive with one another. In addition, we found 2% of mCRCs harbored a gene amplification in either *KRAS*, *NRAS* or *HRAS*. None of the *RAS*-amplified mCRCs had amplification of more than one *RAS* gene. However, a mutually exclusive relationship between *RAS* mutations and *RAS* amplification was not identified.

Likely due to the combination of biomarker guideline recommendations in *RAS* testing, consequent limitations in testing algorithms and techniques, and its low prevalence, the description of *RAS*-amplified mCRCs has been restricted to a few publications and is primarily focused on *KRAS* amplification. Within a study of 1,039 cases, Valtorta el al found 0.7% of mCRCs were *KRAS* amplified, which was slightly lower than 1% in our patient cohort.(12) While the authors did not assess the status of *NRAS* or *HRAS*, they employed a two-tiered immunohistochemical and fluorescence *in situ* hybridization (FISH) screen for KRAS overexpression and *KRAS* amplification, respectively. This screening protocol is similar to what is currently advocated for HER2/neu overexpression and *ERBB2* amplification in gastric and gastroesophageal adenocarcinomas.(23) It is, however, important to note that the false negative rate for KRAS immunohistochemistry has not been documented and current mCRC biomarker testing guidelines have not approved its clinical use. To date, the largest published cohort of *RAS*-amplified mCRCs is by Serebriiskii et al. (13) The authors retrospectively reviewed Foundation Medicine's NGS database of 13,336 mCRCs and, consistent with our findings, determined the prevalence of *RAS* amplification was 2%. However, this database contains minimal clinicopathologic data. Within our cohort, patients with mCRC that harbored a *KRAS* amplification frequently had a history of IBD. In comparison, mutations in *KRAS* were associated with older patient age, right-sided colonic origin, low-grade differentiation, mucinous histology and MMR proficiency. Moreover, considering the lack of mutual exclusivity between *KRAS* mutation and *KRAS* amplification, our findings suggest the clinicopathologic features of *KRAS*-amplified mCRC are different from those of *KRAS*-mutated mCRC.

Indeed, the relationship between IBD and *KRAS*-amplified mCRC is intriguing. IBD-associated CRCs are characterized by several clinical, pathologic and molecular features that contrast those associated with sporadic CRC. For instance, IBD-associated CRCs often occur in young patients.(24) Further, rather than developing from a polypoid adenoma, IBD-associated CRCs frequently arise from flat dysplasia with indistinct margins and a field of inflammation and scarring.(25) Recent genomic studies have also found IBD-associated CRCs are molecularly distinct from their sporadic counterparts. IBD-associated CRCs tend to have a high burden of recurrent chromosomal gains and loss.(26, 27) Interestingly, copy number alterations begin to accrue prior to cancer formation with high-grade dysplastic lesions demonstrating a similar frequency of specific chromosomal gains and losses as matched IBD-associated CRCs, and are distinctly different from sporadic CRCs.(26)

Although a defining set of copy number alterations in IBD-associated tumorigenesis has not been elucidated, we found 60% of patients with *KRAS*-amplified mCRC had a history of IBD. Therefore, our data implicates *KRAS* amplification in the molecular pathogenesis of IBD-associated CRC.

Besides understanding the clinicopathologic features associated with *RAS*-amplified mCRC, determining whether amplification of *RAS* confers resistance to anti-EGFR monoclonal antibodies is critical for patient management. Utilizing preclinical models, Valtorta el al assessed whether *KRAS* amplification could affect response to EGFR inhibitors.(12) The authors found the occurrence of *KRAS* amplification in otherwise anti-EGFR sensitive CRC cell lines dramatically impairs their response to cetuximab. Moreover, silencing of *KRAS* was able to restore cetuximab sensitivity in the *KRAS*-amplified NIH-H630 CRC cell line. Prior to our study, the only documented patient with a *KRAS*-amplified CRC and received anti-EGFR therapy was reported by Mekenkamp et al.(14) Within a retrospective analysis of the multicenter phase III CAIRO2 trial, a subset of mCRC patients that underwent three cycles of cetuximab-based treatment and either exhibited long or short progression-free survival were evaluated for both *KRAS* and *BRAF* status. Among 17 mCRC patients with poor progression-free survival after cetuximab therapy, *KRAS* amplification was detected in one case. However, no additional patient data was provided regarding clinicopathologic findings or a description of clinical course while on cetuximab. Furthermore, mutational analysis for *KRAS* was limited to exon 2, and did not include extended *RAS* testing as recommended by current mCRC biomarker testing guidelines.(11) Considering *RAS* amplification and *RAS* mutations are not mutually exclusive, it is uncertain from the authors' study whether cetuximab resistance was due to the presence of a *KRAS* amplification, mutations in other *KRAS* exons, or *NRAS* and *HRAS* mutations, which were not evaluated. In contrast, we identified twelve *KRAS*-amplified mCRCs without additional alterations in *KRAS*, *NRAS*, *HRAS*, *BRAF* or *PIK3CA*, and all twelve exhibited clinicoradiographic resistance to either a cetuximab- or panitumumab-based regimen. While we acknowledge that it's difficult to draw conclusions based on twelve mCRC cases alone, analogous results have been published for other tumor types.(28–33) In fact, *KRAS*-amplified non-small cell lung cancers are well-known to be refractory to anti-EGFR targeted therapies.(34) Altogether, preclinical and clinical evidence points to *RAS* amplification in mCRC as a potential resistance mechanism for EGFR inhibitors. Thus, it seems prudent to at least consider *RAS* copy number analysis when determining whether a mCRC patient is a candidate for anti-EGFR therapy.

It is also worth noting that there are a few limitations to our study. It is retrospective by design and although it represents one of the largest series of consecutive mCRCs to be molecularly analyzed for routine patient care, the number of *RAS*-amplified cases is relatively small. This is, however, to be expected based on the low prevalence of *RAS* amplification in mCRC. In addition, amplification of the *RAS* genes was assessed by NGS rather than conventional methods, such as FISH. Classically, FISH has been recognized as the "gold standard" for gene amplification, but NGS is proven to show comparable performance to FISH.(20) NGS testing also permits simultaneous testing of single nucleotide variants, small insertions/deletions and copy number analysis for *KRAS*, *NRAS* and *HRAS* at a fraction of the cost to perform FISH for each *RAS* gene. However, the

targeted NGS platform used within this study to evaluate consecutive mCRCs for *KRAS*, *NRAS*, *HRAS*, *BRAF* and *PIK3CA* cannot determine the mechanism of a gene amplification, such as chromosomal aneuploidy. It is also interesting to note that the only *NRAS*-amplified mCRC patient to receive panitumumab had a severe grade III skin toxicity. An EGFR inhibitor-induced rash has been reported to correlate with improved survival in patients treated with anti-EGFR antibodies.(35, 36) In other words, the presence of these eruptions may predict tumor response. Hence, an *NRAS* amplification in mCRC may not confer EGFR inhibitor resistance as compared to *KRAS*-amplified mCRCs. Finally, in the absence of a clinical trial, the true effect of *RAS* amplification on anti-EGFR therapy remains unknown. The low prevalence of *RAS*-amplified mCRC will pose a formidable challenge for clinical trial accrual, but the lack of clinical trial data in light of additional studies in other *RAS*-amplified tumors types should not be a contraindication to modify treatment.

In summary, we report *RAS* amplification occurs in 2% of mCRC patients and is not mutually exclusive from *RAS* mutations. Among the *RAS* genes, *KRAS* amplification was the most prevalent and frequently found among mCRC patients with a history of inflammatory bowel disease. Further, the clinicopathologic features of mCRC associated with *KRAS* amplification contrasted those associated with *KRAS* mutations. However, both alterations in mCRC seem to confer resistance to anti-EGFR therapy and, therefore, *RAS* testing to include copy number analyses may be of consideration in the treatment of mCRC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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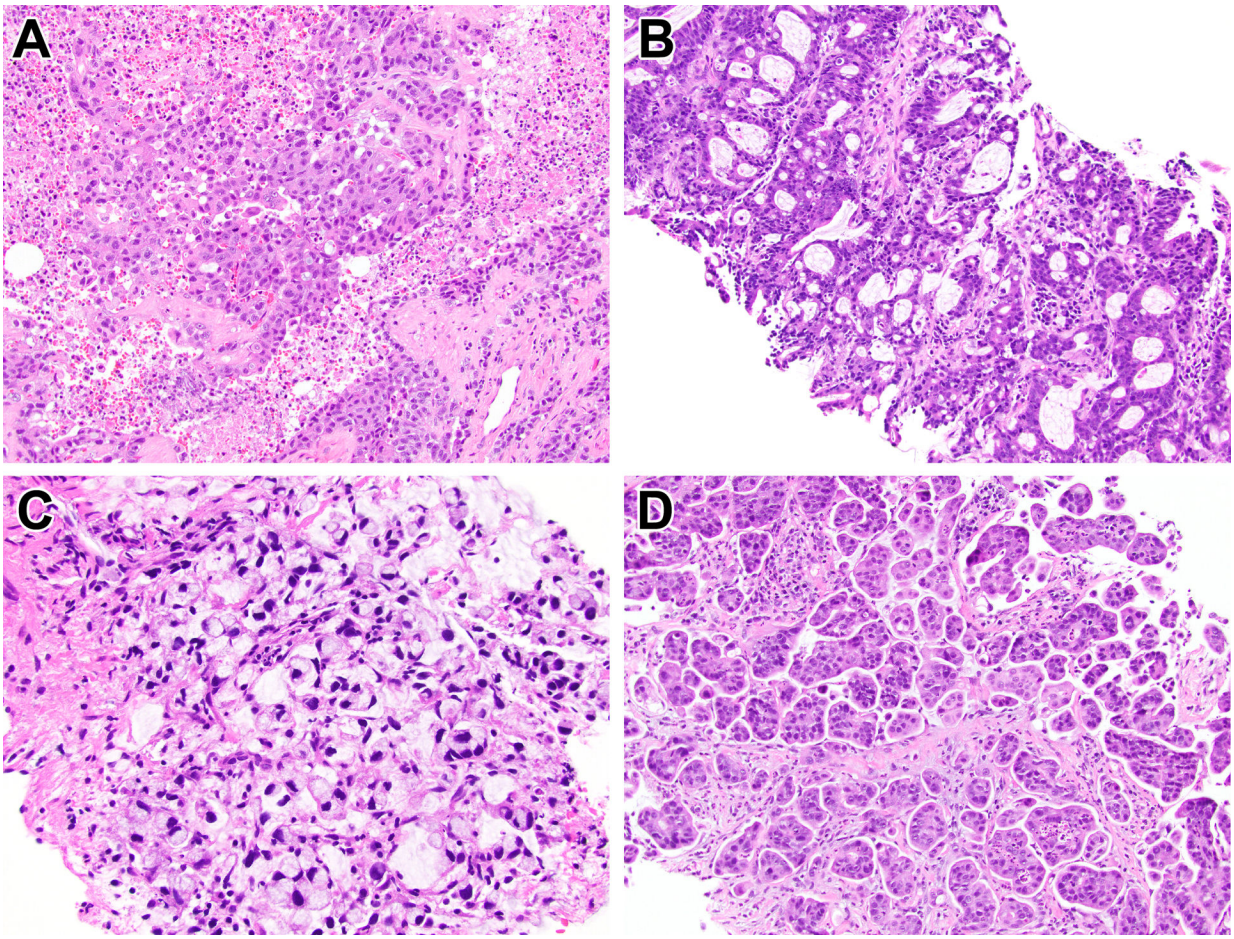

Figure 1.

Metastatic colorectal carcinomas (mCRC) with *RAS* amplifications exhibited diverse histopathologic findings. Most *RAS*-amplified mCRC were characterized by histopathologic features of conventional adenocarcinoma (A, Case 1, Table 2), but other histologic subtypes were also identified and included mucinous adenocarcinoma (B, Case 19, Table 2), signet ring cell carcinoma (C, Case 5, Table 2) and micropapillary carcinoma (D, Case 7, Table 2).

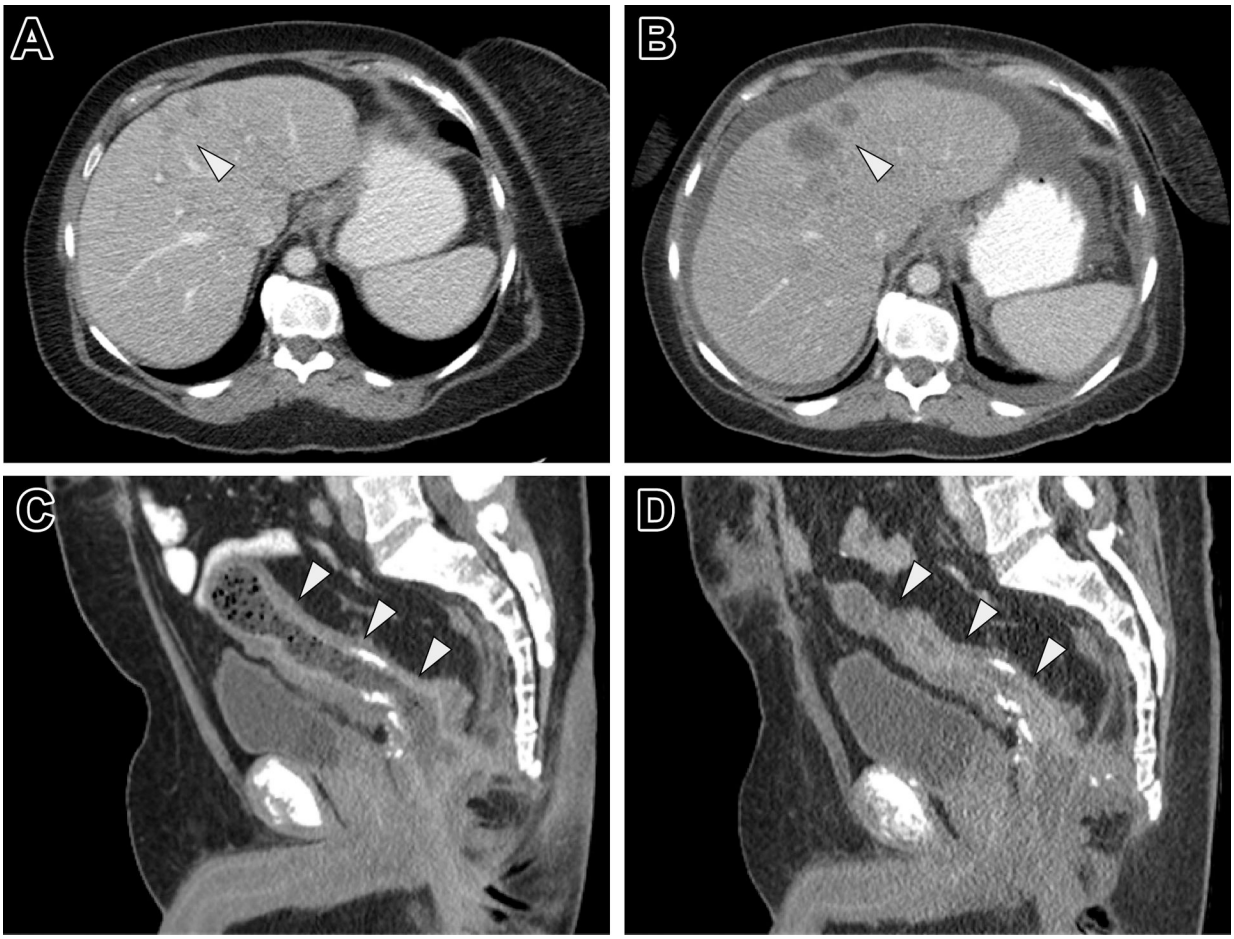

Figure 2.

An EGFR inhibitor-based treatment regimen was characterized by disease progression in patients with *KRAS*-amplified mCRC. Case 9 (Table 2) was a 60-year-old female with mCRC to the liver (white arrowhead) that failed first line FOLFOX and bevacizumab (A) and continued to progress after 3 months on FOLFIRI and panitumumab (B). Similarly, Case 1 (Table 2) was a 49-year-old male with ulcerative colitis, status post subtotal proctocolectomy, and developed an adenocarcinoma of his rectal cuff (C, white arrowhead). Surgical resection was aborted upon identification of peritoneal carcinomatosis and, thus, the patient received 6 cycles of FOLFOX and panitumumab. However, the patient's disease continued to progress within 4 months and resulted in excessive distal colonic stricturing (D).

Table 1.

The clinicopathologic features of *RAS*-amplified and *RAS* non-amplified metastatic colorectal carcinomas.

Clinical and pathologic features	Total	*RAS*-amplified, *n = 22 (2%)*	*RAS* non-amplified, *n = 1,264 (98%)*	*p*
Gender	*n = 1,271*			
Female	571 (45%)	6 (27%)	565 (45%)	0.129
Male	700 (55%)	16 (73%)	684 (55%)	
Median age (range), years	64.0 (18 – 99)	58.0 (26 – 79)	64.0 (18 – 99)	0.042 **
History of Lynch syndrome (*n = 1,201*)	10 (1%)	0 (0%)	10 (1%)	1.000
History of IBD (*n = 1,201*)	23 (2%)	9 (41%)	14 (1%)	< 0.001
Specimen sequenced	*n = 1,286*			
Primary	373 (29%)	6 (27%)	367 (29%)	1.000
Distant metastasis/recurrence	913 (71%)	16 (73%)	897 (71%)	
Primary tumor site	*n = 1,271*			
Right colon	470 (37%)	8 (36%)	462 (37%)	1.000
Left colon	801 (63%)	14 (64%)	787 (63%)	
Primary tumor grade				
Well–to–moderate	1,042 (82%)	16 (73%)	1026 (82%)	0.262
Poor–to–undifferentiated	229 (18%)	6 (27%)	223 (18%)	
Primary tumor histology				
Conventional	1,060 (83%)	14 (65%)	1046 (84%)	0.055
Mucinous/mucinous features	150 (12%)	6 (27%)	144 (11%)	
Signet ring cell	37 (3%)	1 (4%)	36 (3%)	
Micropapillary	6 (<1%)	1 (4%)	5 (<1%)	
Medullary	3 (<1%)	0 (0%)	3 (<1%)	
Adenosquamous/squamous	2 (<1%)	0 (0%)	2 (<1%)	
Undifferentiated	2 (<1%)	0 (0%)	2 (<1%)	
Neuroendocrine	11 (1%)	0 (0%)	11 (1%)	
Mismatch repair deficient (*n = 1,258*)	100 (8%)	2 (9%)	98 (8%)	0.692
Mutations (excluding gene amplification) *	*n = 1,286*			
Negative	509 (40%)	11 (50%)	498 (39%)	0.380
KRAS	563 (44%)	7 (32%)	556 (44%)	0.286
NRAS	29 (2%)	1 (5%)	28 (2%)	0.397
HRAS	1 (<1%)	0 (0%)	1 (<1%)	1.000
RAS (*KRAS*, *NRAS* and *HRAS*)	592 (46%)	8 (36%)	584 (46%)	0.396
BRAF	154 (12%)	3 (14%)	151 (12%)	0.740
PIK3CA	170 (13%)	0 (0%)	170 (13%)	0.102
RAS/*BRAF*/*PIK3CA*	777 (60%)	11 (50%)	766 (61%)	0.380

Abbreviations: IBD, inflammatory bowel disease

* The following genes were evaluated for mutations: *KRAS*, *NRAS*, *HRAS*, *BRAF* and *PIK3CA*.

** This association was not significant upon exclusion of patients with a history of IBD.

Table 2.

The clinicopathologic features, molecular findings and treatment/follow-up data for 22 *RAS*-amplified metastatic colorectal carcinomas.

Case	Age (years)	Sex	History of IBD	Primary Colonic Origin	Histologic Subtype	Histologic Grade	*RAS* Gene Amplification (CN)	Other Genomic Alterations*	MMR Protein Status	Anti-EGFR Treatment	Follow-up (months)
1	49	M	Yes (UC)	Rectum	Conventional	Moderate	*KRAS* (19)		Preserved	Yes (panitumumab) **	AWD (18)
2	45	M	Yes (UC)	Rectum	Conventional	Moderate	*KRAS* (17)		Preserved	No	AWD (27)
3	73	M	Yes (UC)	Sigmoid Colon	Conventional	Moderate	*KRAS* (10)		Preserved	Yes (panitumumab) **	AWD (57)
4	63	M	Yes (UC)	Sigmoid Colon	Conventional	Moderate	*KRAS* (6)		Preserved	Yes (panitumumab) **	AWD (30)
5	38	F	Yes (Crohn's)	Cecum	Signet Ring Cell	Poor	*KRAS* (13)		Preserved	No	DOD (5)
6	54	F	Yes (UC)	Sigmoid Colon	Conventional	Moderate	*KRAS* (7)		Preserved	N/A	N/A
7	59	M	No	Cecum	Micropapillary	Moderate	*KRAS* (14)		Preserved	No	DOD (6)
8	61	M	No	Cecum	Mucinous	Moderate	*KRAS* (8)	*BRAF* p.V600E	Preserved	No	DOD (31)
9	60	F	No	Ascending Colon	Mucinous	Moderate	*KRAS* (8)		Preserved	Yes (panitumumab) **	DOD (23)
10	26	M	No	Sigmoid	Conventional	Poor	*KRAS* (7)		Preserved	No	AWD (15)
11	78	M	No	Rectum	Conventional	Moderate	*KRAS* (6)	*KRAS* p.G12V	Preserved	No	DOD (7)
12	74	M	No	Rectum	Conventional	Moderate	*KRAS* (7)	*KRAS* p.G12R	Preserved	No	DOD (21)
13	41	M	Yes (Crohn's)	Rectum	Conventional	Poor	*KRAS* (12)	*KRAS* p.G12D	Preserved	No	DOD (2)
14	57	M	Yes (Crohn's)	Rectum	Conventional	Moderate	*KRAS* (6)	*KRAS* p.A146T	Preserved	No	DOD (8)
15	53	M	Yes (Crohn's)	Rectum	Conventional	Moderate	*KRAS* (6)	*KRAS* p.G12D	Preserved	No	DOD (15)
16	57	M	No	Rectum	Conventional	Moderate	*NRAS* (16)		Preserved	Yes (panitumumab) ***	AWD (10)
17	52	M	No	Rectum	Conventional	Moderate	*NRAS* (14)		Preserved	N/A	N/A
18	51	F	No	Rectum	Conventional	Poor	*NRAS* (8)	*NRAS* p.G12D	Preserved	No	DOD (156)
19	72	M	No	Cecum	Mucinous	Moderate	*NRAS* (7)	*KRAS* p.G12R	Preserved	No	DOD (11)
20	66	M	No	Cecum	Mucinous	Moderate	*NRAS* (6)	*KRAS* p.G12S	Preserved	No	DOD (11)
21	67	F	No	Cecum	Mucinous	Poor	*HRAS* (21)	*BRAF* p.V600E	MLH1/ PMS2-deficient	No	DOD (31)

Case	Age (years)	Sex	History of IBD	Primary Colonic Origin	Histologic Subtype	Histologic Grade	*RAS* Gene Amplification (CN)	Other Genomic Alterations*	MMR Protein Status	Anti-EGFR Treatment	Follow-up (months)
22	79	F	No	Cecum	Mucinous	Poor	*HRAS* (19)	*BRAF* p.V600E	MLH1/ PMS2-deficient	No	DOD (20)

Abbreviations: AWD, alive with disease; CN, copy number; DOD, died of disease; F, female; IBD, inflammatory bowel disease; M, male; MMR, mismatch repair; N/A, not available; UC, Ulcerative colitis

*The following genes were evaluated for genomic alterations: *KRAS*, *NRAS*, *HRAS*, *BRAF* and *PIK3CA*.

**Clinical and radiographic disease progression on panitumumab-based treatment.

***Discontinuation of panitumumab after one cycle due to severe grade III skin toxicity.

Table 3.

The clinicopathologic features of *KRAS*-amplified and *KRAS* non-amplified metastatic colorectal carcinomas.

Clinical and pathologic features	***KRAS*-amplified, *n* = *15* (1%)**	***KRAS* non-amplified, *n* = *1,271 (99%)***	***p***	***KRAS*-mutant, *n* = *563 (44%)***	***KRAS* non-mutant, *n* = *723 (56%)***	***p***
Gender	*n = 15*	*n = 1,256*		*n = 553*	*n = 718*	
Female	3 (20%)	568 (45%)	0.066	258 (47%)	313 (44%)	0.280
Male	12 (80%)	688 (55%)		295 (53%)	405 (56%)	
Median age (range), years	54.0 (26 – 78)	64.0 (18 – 99)	0.016	65.0 (18 – 91)	63.0 (24 – 99)	0.017**
History of Lynch syndrome (*n = 1,201*)	0 (0%)	10 (1%)	1.000	5 (1%)	5 (1%)	0.754
History of IBD (*n = 1,201*)	9 (60%)	14 (1%)	< 0.001	6 (1%)	17 (3%)	0.135
Specimen sequenced	*n = 15*	*n = 1,271*		*n = 563*	*n = 723*	
Primary	2 (13%)	371 (29%)	0.255	128 (23%)	245 (34%)	< 0.001
Distant metastasis/recurrence	13 (87%)	900 (71%)		435 (77%)	478 (66%)	
Primary tumor site	*n = 15*	*n = 1,256*		*n = 553*	*n = 718*	
Right colon	4 (27%)	466 (37%)	0.592	230 (42%)	240 (33%)	0.003
Left colon	11 (73%)	790 (63%)		323 (58%)	478 (67%)	
Primary tumor grade						
Well–to–moderate	12 (80%)	1,030 (82%)	0.742	490 (89%)	552 (77%)	< 0.001
Poor–to–undifferentiated	3 (20%)	226 (18%)		63 (11%)	166 (23%)	
Primary tumor histology						
Conventional	11 (73%)	1,049 (84%)	0.136	445 (80%)	615 (86%)	< 0.001
Mucinous/mucinous features	2 (13%)	148 (12%)		95 (17%)	54 (7%)	
Signet–ring cell	1 (7%)	36 (3%)		5 (1%)	32 (4%)	
Micropapillary	1 (7%)	5 (<1%)		2 (<1%)	5 (1%)	
Adenosquamous/Squamous	0 (0%)	2 (<1%)		0 (0%)	2 (<1%)	
Undifferentiated	0 (0%)	2 (<1%)		1 (<1%)	1 (<1%)	
Neuroendocrine	0 (0%)	11 (1%)		5 (1%)	6 (1%)	
Medullary	0 (0%)	3 (<1%)		0 (0%)	3 (<1%)	
Mismatch repair deficient (*n = 1,258*)	0 (0%)	100 (8%)	0.624	21 (4%)	79 (11%)	< 0.001
Mutations (excluding gene amplification)*	*n = 15*	*n = 1,271*		*n = 563*	*n = 723*	
Negative	9 (60%)	500 (39%)	0.117			
KRAS	5 (33%)	558 (44%)	0.448			
NRAS	0 (0%)	29 (2%)	1.000	1 (<1%)	28 (4%)	< 0.001
HRAS	0 (0%)	1 (<1%)	1.000	0 (0%)	1 (<1%)	1.000
RAS (*KRAS*, *NRAS* and *HRAS*)	5 (33%)	587 (46%)	0.437			
BRAF	1 (7%)	153 (12%)	1.000	4 (1%)	150 (21%)	< 0.001
PIK3CA	0 (0%)	170 (13%)	0.243	107 (19%)	63 (9%)	< 0.001
RAS/*BRAF*/*PIK3CA*	6 (40%)	771 (61%)	0.117			

Abbreviations: IBD, inflammatory bowel disease

*The following genes were evaluated for mutations: *KRAS*, *NRAS*, *HRAS*, *BRAF* and *PIK3CA*.

**This association was not significant upon exclusion of patients with a history of IBD.

Table 4.

The clinicopathologic features, molecular findings and treatment/follow-up data for a multi-institutional cohort of 23 *RAS*-amplified mCRC.

Case	Age (years)	Sex	History of IBD	Primary Colonic Origin	Histologic Subtype	Histologic Grade	*RAS* Gene Amplification (CN)*	MMR Protein Status	Anti-EGFR Treatment	Follow-up (months)
1	73	F	No	Cecum	Conventional	Poor	*KRAS (14)*	Preserved	Yes (panitumumab) **	DOD (12)
2	38	F	Yes (UC)	Sigmoid	Conventional	Poor	*KRAS* (6)	Preserved	No	AWD (27)
3	34	F	No	Rectum	Conventional	Poor	*KRAS* (16)	Preserved	Yes (cetuximab) **	AWD (46)
4	33	M	No	Rectum	Conventional	Moderate	*KRAS* (7)	Preserved	No	DOD (26)
5	57	M	No	Rectosigmoid	Conventional	Moderate	*KRAS* (7)	Preserved	Yes (cetuximab) **	DOD (3)
6	67	M	No	Splenic flexure	Conventional	Moderate	*KRAS* (42)	Preserved	Yes (cetuximab) **	AWD (52)
7	49	M	No	Rectum	Conventional	Moderate	*KRAS* (7)	Preserved	No	AWD (47)
8	38	M	Yes (UC)	Rectum	Mucinous and Signet Ring Cell	Poor	*KRAS* (7)	Preserved	Yes (panitumumab) **	DOD (14)
9	55	M	No	Sigmoid	Conventional	Poor	*KRAS* (7)	Preserved	No	DOD (14)
10	63	F	No	Sigmoid	Signet Ring Cell	Poor	*KRAS* (7)	Preserved	No	DOD (12)
11	56	M	No	Rectum	Conventional	Poor	*KRAS* (7)	Preserved	Yes (cetuximab) **	AWD (12)
12	48	F	No	Rectum	Conventional	Moderate	*KRAS* (7)	Preserved	No	AWD (21)
13	42	M	Yes (UC)	Hepatic flexure	Mucinous	Moderate	*KRAS* (7)	Preserved	No	NED (16)
14	55	M	No	Rectum	Conventional	Moderate	*KRAS* (7)	Preserved	No	AWD (11)
15	48	M	No	Rectum	Conventional	Moderate	*KRAS* (7)	Preserved	No	AWD (103)
16	64	F	Yes (UC)	Sigmoid	Signet Ring Cell	Poor	*KRAS* (7)	Preserved	No	AWD (11)
17	54	M	No	Sigmoid	Conventional	Moderate	*KRAS* (7)	Preserved	Yes (cetuximab) **	AWD (31)
18	61	F	No	Rectosigmoid	Conventional	Moderate	*KRAS* (7)	Preserved	No	AWD (76)
19	46	M	No	Cecum	Conventional	Poor	*KRAS* (7)	Preserved	No	AWD (6)
20	56	F	No	Sigmoid	Conventional	Moderate	*KRAS* (7); *NRAS* (7)	Preserved	Yes (cetuximab) **	AWD (6)
21	66	M	No	Sigmoid	Conventional	Moderate	*NRAS* (7)	Preserved	No	DOD (18)
22	81	M	No	Rectosigmoid	Conventional	Moderate	*KRAS* (8)	Preserved	No	AWD (8)
23	49	F	Yes (UC)	Rectum	Mucinous	Moderate	*KRAS* (10)	Preserved	No	DOD (20)

Abbreviations: AWD, alive with disease; CN, copy number; DOD, died of disease; F, female; IBD, inflammatory bowel disease; M, male; mCRC, metastatic colorectal carcinoma; MMR, mismatch repair; N/A, not available; NED, no evidence of disease; UC, Ulcerative colitis

* The following genes were evaluated for genomic alterations: *KRAS*, *NRAS*, *HRAS*, *BRAF* and *PIK3CA*.

** Clinical and radiographic disease progression on anti-EGFR therapy.