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**Original Article**

# Mucinous Histology Is Associated with Resistance to Anti-EGFR Therapy in Patients with Left-Sided *RAS/BRAF* Wild-Type Metastatic Colorectal Cancer

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## Abstract

**Background:** Limited studies have suggested that mucinous histology is associated an attenuated response to anti-epidermal growth factor receptor (EGFR) therapy.

**Methods:** We conducted a single-institution, retrospective study to review the anti-EGFR response and the molecular profile of patients with left-sided microsatellite stable *RAS/BRAF* wild-type mucinous metastatic colorectal cancer.

**Results:** In comparison to nonmucinous population ($n = 98$), mucinous histology ($n = 20$) was associated with a younger age (48 vs 54, $P = .02$), wild-type *APC* (80% vs 15.3%, $P < .0001$), and wild-type *TP53* (40% vs 8.2%, $P = .001$). Guanine nucleotide binding protein, alpha stimulating (*GNAS*) mutations were exclusively found in mucinous tumors (20% vs 0, $P < .0001$). Genomic alterations associated with resistance to anti-EGFR therapy, such as *ERBB2* amplification, *PIK3CA* mutation, *MAP2K1* mutation, and *KRAS* amplification, were identified in patients with left-sided *RAS/BRAF* wild-type mucinous metastatic colorectal cancer. Mucinous histology was not associated with a worse outcome than non-mucinous histology (34.3 vs 42.2 months, $P = .85$). However, patients with left-sided *RAS/BARF* wild-type mucinous colorectal cancer treated with first-line anti-EGFR therapy had significantly worse progression-free survival (4 vs 6.5 months, hazard ratio [HR] = 5.3, 95% confidence interval [CI] 1.3-21.7, $P = .01$) than patients treated with the first-line vascular endothelial growth factor A antibody, bevacizumab. Anti-EGFR therapy was associated with limited responses and a short PFS across all lines of therapy in 12 patients with left-sided *RAS/BRAF* wild-type mucinous colorectal cancer.

**Conclusions:** Mucinous histology is associated with diminished benefits from anti-EGFR therapy in patients with left-sided *RAS/BRAF* wild-type colorectal cancer. These patients should be considered for bevacizumab-based therapy in the first- and second-line settings.

**Key words:** metastatic colorectal cancer; mucinous; anti-EGFR

### Implications for Practice

Incorporating anti-epidermal growth factor receptor therapy offers limited benefits for patients with left-sided *RAS/BARF* wild-type mucinous metastatic colorectal cancer. Physicians should consider bevacizumab as the preferred biological agent in the first-line treatment in this population.

## Introduction

Epidermal growth factor receptor (EGFR) targeting antibodies, such as panitumumab and cetuximab, have been associated with improvements in response rate, progression-free survival (PFS), and overall survival (OS) when combined with systemic chemotherapy in the first-line treatment of left-sided *RAS/BRAF* wild-type metastatic colorectal cancers.[1-6] However, more than 30% patients of patients satisfying these characteristics do not benefit from anti-EGFR therapy, as demonstrated by data from anti-EGFR monotherapy.[1-3,5,6] Identifying additional predictive biomarkers of resistance to anti-EGFR therapy would prevent unnecessary exposure to ineffective therapies and allows for the integration of alternative treatments in these patients.

Mucinous colorectal adenocarcinoma, characterized by more than 50% of extracellular mucin, accounts for 10-20% of patients with colorectal cancer.[7-9] Patients with mucinous metastatic colorectal cancer have inferior response rates and shorter OS to oxaliplatin/irinotecan-based first-line chemotherapy when compared with patients with nonmucinous colorectal cancer.[10] However, limited data exists on the impact of mucinous histology on anti-EGFR response in *RAS/BRAF* wild-type metastatic colorectal cancer.[11] We retrospectively reviewed outcomes of patients with *RAS/BRAF* wild-type left-sided mucinous adenocarcinoma and evaluated their response to anti-EGFR therapy. We focused further on the first-line treatment of this population and compared the impact of first-line bevacizumab-based chemotherapy versus

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anti-EGFR-based chemotherapy. In addition, comprehensive genomic profiling was used to uncover the mechanism of resistance to anti-EGFR therapy.

## Methods

### Patient Population

Patients with metastatic colorectal cancer treated at City of Hope Comprehensive Cancer Center (Duarte, CA) between 2013 and 2020 with available next-generation sequencing by a clinical laboratory improvement amendments (CLIA) certified assay were the subject of this study. Patients were subsequently stratified into mucinous and nonmucinous cohorts based on their official pathology review. We did not include mucinous features (<50% mucinous or minor mucinous component) in the study to maintain a more homogenous population. Signet ring cell cancers were included along with the mucinous adenocarcinomas. Patients with left-sided microsatellite stable *RAS/BRAF* wild-type mucinous adenocarcinoma treated with anti-EGFR therapy were identified and were analyzed for response rate, PFS, and OS across various lines of therapy. To investigate the prognostic versus predictive impact of mucinous cancer on treatment response, we subsequently compared the outcome of left-sided *RAS/BRAF* wild-type metastatic colorectal cancer to first-line anti-EGFR-based chemotherapy vs first-line bevacizumab-based chemotherapy. This study was approved by the Institutional Review Board (IRB 14361). To interrogate potential mechanisms of resistance to anti-EGFR in mucinous left-sided metastatic colorectal cancer, we compared tumor genomic alterations in mucinous versus nonmucinous left-side *RAS/BRAF* wild-type cancer. Genomic alterations previously associated with anti-EGFR resistance were analyzed in both subgroups.

### Statistical Analysis

Patients' characteristics and genomic alterations were analyzed by Wilcoxon rank test (age and tumor mutation burden [TMB]) and Fisher's exact test (categorical variables). Differences in PFS and OS were compared using Kaplan-Meier curves, with *P*-value calculated via log-rank test.

## Results

### Baseline Patients Population Characteristics

Among 430 patients with stage IV colorectal cancer with full genomic profiling, 118 patients were left-sided *RAS/BRAF* wild-type—20 (16.9%) of whom had mucinous tumors. A total of 64 cases of mucinous adenocarcinoma were identified, 67% (43/64) were left-sided, and 41% (26/64) were *RAS/BRAF* wild type. Baseline clinicopathologic and molecular characteristics of patients with left-sided *RAS/BRAF* wild-type metastatic colorectal cancer are shown in Table 1. Genomic alterations that were prevalent in more than 5% of mucinous tumor were included in our data analysis. Mucinous histology was associated with younger age (median, 48 vs 54, $P = .02$) than nonmucinous histology. Mucinous tumors were associated with a high incidence of wild-type *APC* (80% vs 15.3%, $P < .0001$) and wild-type *TP53* (40% vs 8.2%, $P = .001$) compared with nonmucinous tumors. Mutations in *GNAS* (20% vs 0, $P = .0006$) were exclusive to mucinous tumors. *SMAD4* mutation was numerically higher (25% vs 10.2%) in the mucinous versus nonmucinous group. We did not observe a significant difference in *SMAD2* and *PIK3CA* mutations between mucinous and nonmucinous tumors. In addition, no difference in tumor mutation burden was found between mucinous and nonmucinous tumors.

**Table 1.** Characteristics of patients with mucinous and nonmucinous left-sided *RAS/BRAF* wild-type metastatic CRC.

| Characteristics | Total ($n = 118$) | Mucinous 16.9% ($n = 20$) | Non-mucinous 83.1% ($n = 9$ 8) | *P*-value |
|---|---|---|---|---|
| Age at diagnosis | | | | |
| Median (range) | 52 (19-88) | 48 (19-88) | 54 (20-84) | .02 |
| Gender | | | | |
| Female | 35.6% (42) | 50% (10) | 32.7% (32) | .2 |
| Male | 64.4% (76) | 50% (10) | 67.3%% (66) | |
| Stage at diagnosis | | | | |
| II/III | 22% (26) | 15% (3) | 23.5% (23) | .6 |
| IV | 78% (92) | 85% (17) | 76.5% (75) | |
| APC | | | | |
| Mutated | 73.7% (87) | 20% (4) | 84.7% (83) | <.0001 |
| Nonmutated | 31.3% (31) | 80% (16) | 15.3% (15) | |
| TP53 | | | | |
| Mutated | 86.4% (102) | 60% (12) | 91.8% (90) | .001 |
| Nonmutated | 13.6% (16) | 40% (8) | 8.2% (8) | |
| GNAS | | | | |
| Mutated | 3.4% (4) | 20% (4) | 0 (0) | .0006 |
| Nonmutated | 96.6% (114) | 80% (16) | 100% (98) | |
| SMAD4 | | | | |
| Mutated | 11.9% (14) | 25% (5) | 10.2% (10) | .13 |
| Nonmutated | 88.1% (104) | 75% (15) | 89.8% (88) | |
| SMAD2 | | | | |
| Mutated | 5.1% (6) | 10% (2) | 4.1% (4) | .27 |
| Nonmutated | 94.9% (112) | 90% (18) | 95.9% (94) | |
| PIK3CA | | | | |
| Mutated | 8.5% (10) | 10% (2) | 8.2% (8) | .68 |
| Nonmutated | 91.5% (108) | 90% (18) | 91.8% (90) | |
| TMB[a] | | | | |
| Median (range) | 5 (0-13) | 5.5 (1-11) | 5 (0-13) | .32 |

[a]Data not available, 4 in mucinous group, 4 in nonmucinous group.

### Response to Anti-EGFR Therapy in Left-Sided *RAS/BRAF* Wild-Type Mucinous Colorectal Cancer

Among the 20 patients with left-sided *RAS/BRAF* wild-type mucinous metastatic colorectal cancer, 12 patients were given panitumumab (7 first-line, 4 second-line, and one fifth-line). Seven patients were given bevacizumab in combination with chemotherapy as first-line treatment, all of whom had stable disease. Among the seven patients who received first-line panitumumab in combination with chemotherapy, one patient had partial response, 2 had stable disease, and 4 had progressive disease as best response (Table 2). No responses were noted in patients receiving panitumumab second-line and beyond treatment (Table 2). The median PFS in patients treated with first-line panitumumab-based therapy was 4 months versus 6.5 months with bevacizumab-based therapy ($P = .01$, HR = 5.3, 95% CI 1.3-21.7, $P = .01$) (Fig. 1). This difference in PFS persisted when we compared left-sided *RAS/BRAF* wild-type mucinous colorectal cancers treated with first-line panitumumab-based therapy to all

**Table 2.** Patients with left-sided *RAS/BRAF* wild-type mucinous metastatic colorectal cancer treated with anti-EGFR.

| Patients | Lines of therapy | Best response | PFS |
|---|---|---|---|
| 01 | First line | PD | 1.4 |
| 02 | First line | PD | 4.0 |
| 03 | First line | SD | 4.6 |
| 04 | First line | PD | 3.8 |
| 05 | First line | PD | 4.0 |
| 06 | First line | SD | 5.1 |
| 07 | First line | PR | 6.1 |
| 08 | Second line | SD | 3.7 |
| 09 | Second line | SD | 3.7 |
| 10 | Second line | SD | 2.8 |
| 11 | Second line | SD | 3.2 |
| 12 | Fifth line | SD | 3.0 |

PD, progressive disease; SD, stable disease; PR, partial response; PFS, progression-free survival.

mucinous metastatic colorectal cancer treated with first-line bevacizumab regardless of sidedness and *RAS/BRAF* status (4 vs 6.5 months, HR = 4.2, 95% CI 1.4-13.2, $P$ = .0077; Supplementary Fig. S1).

### Genomic Alterations Associated with Resistance to Anti-EGFR Therapy Are Common in Left-Sided *RAS/BRAF* Wild-Type Mucinous Metastatic Colorectal Cancer

Underlying primary mechanism of resistance to anti-EGFR therapy was investigated by reviewing the genomic profile of left-sided *RAS/BRAF* wild-type mucinous tumors versus left-sided *RAS/BRAF* wild-type nonmucinous tumors as analyzed by CLIA-certified Next-Generation Sequencing technology. *ERBB2* amplification was identified in 15% of tumors with mucinous histology versus 6% of tumors with nonmucinous histology. *KRAS* amplification was identified in 10% of tumors with mucinous histology versus 5% of tumors with nonmucinous histology. In addition, higher frequencies of *PIK3CA* mutations (10% vs 8.2%), *MAP2K1* mutations (5% vs 0), *FGFR1* amplifications (5% vs 0), and *FGFR2* rearrangement (5% vs 0) were found in tumors with mucinous histology compared with tumors with nonmucinous histology (Fig. 2). In summary, genomic alterations related to resistance to anti-EGFR therapy were enriched in left-sided *RAS/BRAF* wild-type tumors with mucinous histology.

### Association of Mucinous Histology with Clinical Outcomes

To investigate whether mucinous histology is a prognostic marker for worse clinical outcome, we analyzed the OS of patients with mucinous ($n$ = 72) and nonmucinous colorectal cancer ($n$ = 358) regardless of sidedness and molecular profile. The median OS was 34.3 months versus 42.2 months ($P$ = .85, HR = 1.0, 95% CI 0.75-1.42) in patients with mucinous and nonmucinous metastatic colorectal cancer, respectively (Fig. 3).

## Discussion

Mucinous colorectal cancer is often associated with poor differentiation, late stage at diagnosis, and worse prognosis.[12-14]

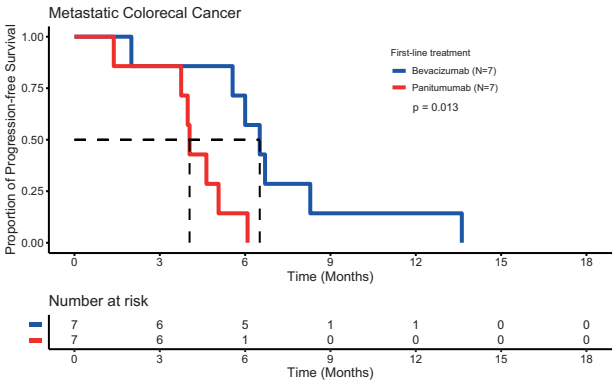


**Figure 1.** Kaplan-Meier curves for PFS of patients with left-sided, *RAS/BRAF* wild-type mucinous metastatic colorectal cancer treated with first-line panitumumab versus first-line bevacizumab.

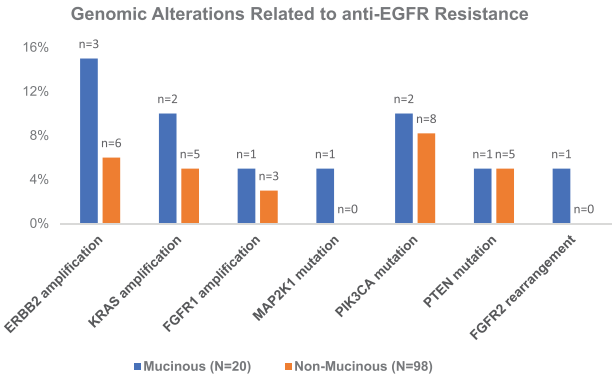


**Figure 2.** Bar chart of genomic alterations associated with resistance to anti-EGFR therapy in patients with mucinous and non-mucinous left-sided *RAS/BRAF* wild-type metastatic colorectal cancer.

While studies have shown that patients with mucinous colorectal cancer may have a poorer response to chemotherapy, it remains unclear whether left-sided *RAS/BRAF* wild-type mucinous colorectal cancer derive benefit from anti-EGFR therapy.[10,11] In 2019, Moretto et al reported that mucinous histology was associated with diminished benefit to anti-EGFR therapy.[11] While these data point to the poor response to anti-EGFR therapy, the lack of comparative data to a non-anti-EGFR therapy cohort limits the interpretation of this data. In addition, the lack of extensive assessment of tumor genomic profiling did not shed a good understanding on the potential mechanisms of resistance.

In our study, patients treated with first-line panitumumab-based chemotherapy had a significantly shorter PFS and OS than patients treated with bevacizumab. In addition, patients treated with anti-EGFR therapies in subsequent lines had similarly poor responses. We also evaluated whether mucinous histology could serve as a prognostic biomarker for colorectal cancer. Unlike some prior reports, we did not observe a significant difference in OS between the mucinous and the nonmucinous group. Our study suggests that mucinous histology is a predictive biomarker for resistance to anti-EGFR therapy in left-sided *RAS/BRAF* wild-type colorectal cancer. This is at least partly explained by enrichment with genomic alterations associated with resistance to anti-EGFR therapy.

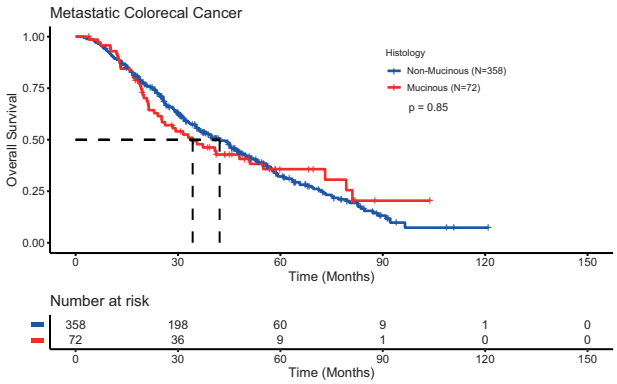


**Figure 3.** Kaplan-Meier curves for overall survival of patients with mucinous and nonmucinous metastatic colorectal cancer.

We identified significant differences in the genomic characteristics between mucinous and nonmucinous *RAS/BRAF* wild-type left-sided colorectal cancer. Like prior studies, mucinous histology was associated with increased wild-type *APC* and *TP53*, which suggest a distinct oncogenic pathway from that of nonmucinous colorectal cancer.[13,15] Our study found significantly more frequent mutations of *GNAS* among the mucinous group, which is also consistent with prior reports.[13,16] Of note, *GNAS* and *SMAD4* alterations have been associated with mucinous neoplasms of the pancreas and appendix, indicating their unique roles in the pathogenesis of mucinous neoplasms.[17-19] In addition, prior studies have shown strong correlation between *GNAS* mutation and peritoneal metastasis of mucinous appendix and colorectal adenocarcinoma.[19,20] In this study, we found 4 patients with *GNAS* alterations, 3 of them had peritoneal carcinomatosis. The exact role of mutant *GNAS* in the pathogenesis of mucinous colorectal cancer and the development of peritoneal metastasis remains to be demonstrated. *GNAS* encodes for the Gs-α subunit of G-proteins. *GNAS* mutation or amplification are found in about 10% of colorectal cancer.[21] Active alteration of *GNAS* results in increased activation of Wnt/β-catenin and ERK/MAPK signaling, which may limit the activity of anti-EGFR therapy.[22] In addition, a recent report associated *GNAS* amplification with resistance to cetuximab in patients with *KRAS* wild-type colorectal cancer.[23] These results indicate that *GNAS* alterations may represent a novel mechanism of resistance to anti-EGFR therapy in *RAS/BRAF* wild-type colorectal cancer.

It is well-established that *RAS/BRAF* mutations are predictive biomarkers for intrinsic and acquired resistance to anti-EGFR therapy. There is increasing evidence that other genomic alterations might confer resistance to anti-EGFR therapy as well. *ERBB2* amplification was identified in 15% of our patients with mucinous histology, which is proportionally higher than in unselected patients with metastatic colorectal cancer.[24] Aberrant *ERBB2* activation leads to bypass of the *RAS/MEK/ERK* signalizing, thereby blunting the efforts of EGFR inhibition.[25] Preclinical and clinical studies have demonstrated that *ERBB2* amplification is a predictive biomarker for resistance to anti-EGFR therapy.[26,27] *KRAS* amplification was observed in 10% of tumors with mucinous histology which is also considerably higher than unselected patients with metastatic colorectal tumors.[28] We have previously reported, among others, that high levels of *RAS* amplification confer resistance to anti-EGFR therapy.[28,29]

*MAP2K1* alterations were also encountered in our mucinous left-sided *RAS/BRAF* wild-type cohort. *MAP2K1* gene codes for protein MEK1 which located at the downstream of BRAF. The noted *MAP2K1*(E102_I103del) results in constitutive phosphorylation of MEK1 and thus activation of MEK/ERK signaling.[30] Studies from our group and others have shown that activating *MAP2K1* mutations is associated with resistance to anti-EGFR therapy[31-33].

Other alterations enriched in our population of interest that may have conferred resistance to anti-EGFR include *PIK3CA*, *FGFR*, and *SMAD4*. *PIK3CA-PTEN-AKT* signaling is a parallel pathway to *RAS-RAF-MAPK* under EGFR. Activating mutations in the *PIK3CA/PTEN/AKT* pathway have been associated with resistance to anti-EGFR therapy.[34] *FGFR* fusions are oncogenic drivers and can substitute for EGFR signaling and have been linked to primary and acquired mechanisms of resistance to anti-EGFR therapy in prior clinical studies in colorectal cancer.[35-37] Similarly, FGFR1 amplification is another oncogenic driver that has been correlated with resistance to anti-EGFR therapy.[36] Additionally, studies have shown that *SMAD4* mutation may lead to resistance to anti-EGFR therapy. A clinical study analyzed the genomic alterations in 65 colorectal tumors treated with cetuximab or panitumumab found that, in addition to mutations in *RAS/BRAF/PIC3CA/PTEN*, *SMAD4* and *FBXW7* mutations were significantly more prevalent in anti-EGFR resistant tumors.[38] *SMAD4* is a key mediator of TGF-β signaling. Loss of *SMAD4* leads to abnormal activation of TGF-β pathway, which may confer resistance to anti-EGFR therapy[39].

While our study shows limited benefits to anti-EGFR therapy in mucinous left-sided RAS/BRAF wild-type tumors, we note the limitations of a small sample size and the potential limitations of a retrospective analysis. The enrichment of genomic alterations associated with absolute or relative resistance to anti-EGFR therapy in left-sided mucinous *RAS/BRAF* wild-type colorectal cancer may partially explain the lack of anti-EGFR benefits within this group. However, additional mechanisms of resistance on the genomic expression level may also co-exist in this population and are yet to be elucidated. Our findings should at least generate caution regarding the integration of anti-EGFR therapy in the front-line treatment of this population and trigger additional studies to interrogate this issue more conclusively.

## Funding

None declared.

## Conflict of Interest

**Marwan Fakih:** Amgen (H), AstraZeneca, Amgen, Novartis (RF), Amgen, Array, Bayer, Pfizer (SAB), Amgen, Guardant Health (Other- speaker bureau). The other authors indicated no financial relationships.

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## Author Contributions

Conception/design: C.W., M.F. Provision of study material or patients: M.F. Collection and/or assembly of data:

C.W., J.S., M.F. Data analysis and interpretation: C.W., J.S., M.F. Manuscript writing: C.W., M.F. Final approval of manuscript: C.W., J.S., M.F.

## Data Availability

The data underlying this article are available in the article and in its online Supplementary Material.

## Supplementary Material

Supplementary material is available at *The Oncologist* online.

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