Original Study

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Lack of Benefit From Anti-EGFR Treatment in *RAS* and *BRAF* Wild-type Metastatic Colorectal Cancer With Mucinous Histology or Mucinous Component

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Abstract

Adenocarcinoma with mucinous histology or mucinous component are histologic subtypes of metastatic colorectal cancers (mCRCs) with limited benefit from cytotoxic agents. Their sensitivity to anti-epithelial growth factor receptors (EGFRs) is not clear. We retrospectively identified patients with *RAS*/*BRAF* wild-type mCRC treated with anti-EGFRs. Our findings suggest no benefit from anti-EGFRs in mCRC with mucinous histology or mucinous component, irrespective of sidedness.

Background: Adenocarcinoma with mucinous histology or mucinous component are histologic subtypes of metastatic colorectal cancers (mCRCs) with limited benefit from cytotoxic agents. Their sensitivity to anti-epithelial growth factor receptors (EGFRs) is not clear. **Patients and Methods:** The activity and efficacy of anti-EGFRs was retrospectively evaluated among patients with *RAS* and *BRAF* wild-type mCRC with or without mucinous histology or mucinous component. Subgroup analyses according to primary tumor location were conducted. **Results:** Overall, the study population included 22 mucinous or with mucinous component tumors (11 right- and 11 left-sided tumors) and 83 not mucinous tumors. One patient experienced partial response among mucinous tumors, whereas in the not mucinous group, 42 patients experienced partial response, with an overall response rate of 4% and 51%, respectively ($P = .003$). The median progression-free survival was 2.8 versus 6.7 months (hazard ratio, 0.28; 95% confidence interval, 0.13-0.59; $P < .001$), and the median overall survival was 6.5 and 16.7 months (hazard ratio, 0.58; 95% confidence interval, 0.33-1.00; $P = .022$), for the mucinous and not mucinous groups, respectively. Similar results were observed in subgroup analysis according to primary tumor location. **Conclusion:** Anti-EGFRs may not provide clinically meaningful benefit in mCRCs with mucinous histology or mucinous component compared with those without mucinous component, irrespective of sidedness.

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Keywords: Adenocarcinoma with mucinous component, Anti-EGFR monoclonal antibodies, Metastatic colorectal cancer, Mucinous adenocarcinoma, RAS and BRAF wild-type mCRC

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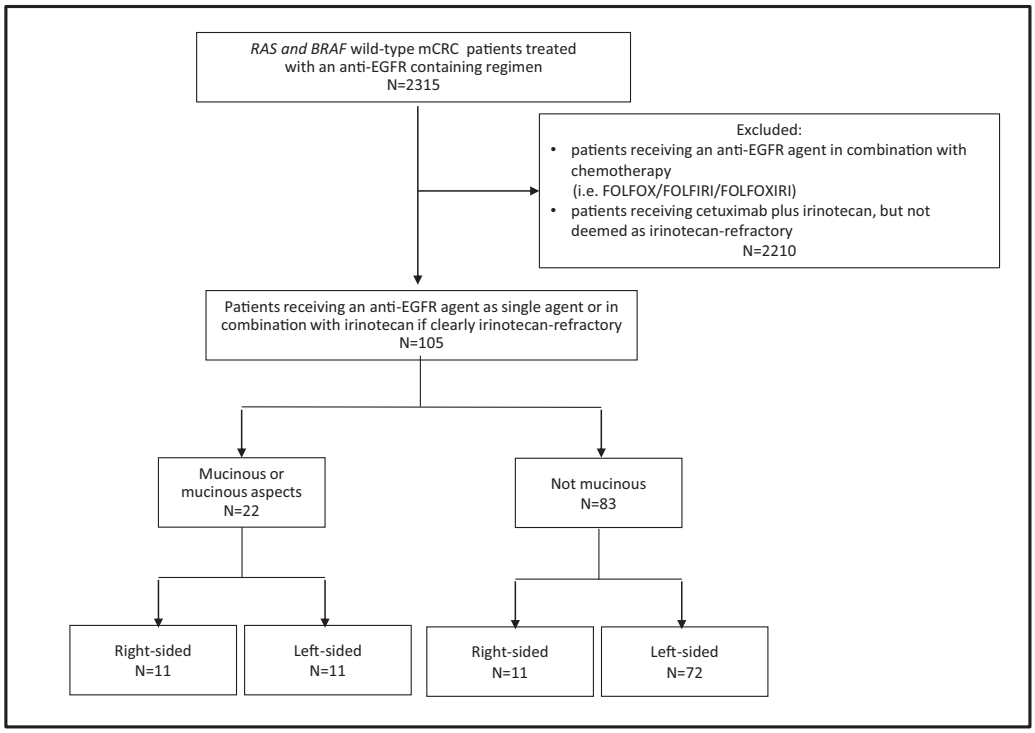
Figure 1 Consolidated Standards of Reporting Trials (CONSORT) Diagram



Abbreviations: EGFR = epidermal growth factor receptor; FOLFIRI = folinic acid, fluorouracil, and irinotecan; FOLFOX = folinic acid, fluorouracil, and oxaliplatin; FOLFOXIRI = folinic acid, fluorouracil, irinotecan, and oxaliplatin; mCRC = metastatic colorectal cancer; NOS = not otherwise specified.

Introduction

Mucinous adenocarcinoma is a histologic subtype of colorectal adenocarcinoma characterized by more than 50% of extracellular mucin accounting for 10% to 20% of all colorectal cancers according to the World Health Organization classification.[1] Mucinous histology is often associated with particular clinicopathologic features, such as right sidedness, poor differentiation,[2] synchronous presentation, peritoneal diffusion, microsatellite instability,[3] and CpG island methylator phenotype-high.[4-6] In addition, mucinous tumors frequently harbor mutations in *BRAF*, *KRAS, PIK3CA*, *ERBB2*, *SMAD4*, and *GNAS* genes and are often associated with increased consensus molecular subtype (CMS) 1 and decreased CMS2 prevalence.[7-9] In addition, the higher frequency of advanced tumor stage at diagnosis and poor differentiation often yield worse prognosis for mucinous adenocarcinomas when compared with non-mucinous histologies.[2,4,10-13]

Colorectal adenocarcinomas with mucinous component (less than 50% of extracellular mucin) share comparable clinicopathologic and molecular features with mucinous ones and together differ from not otherwise specified (NOS) adenocarcinomas.[7,14]

Mucinous metastatic colorectal cancer (mCRC) showed decreased benefit from standard chemotherapy combinations with fluoropyrimidines, oxaliplatin, and irinotecan when compared with NOS adenocarcinoma.[15] No predictive role of mucinous histology with regard to benefit from bevacizumab has been reported.[16]

Although translational observations suggest the epidermal growth factor receptor (EGFR)-dependency of mucinous adenocarcinoma, resulting in the overexpression of *MUC2* gene and in mucin hyperproduction,[17,18] no data regarding the predictive impact of mucinous histology and mucinous component towards anti-EGFR monoclonal antibodies (MoAbs) are available.

Drawing from these considerations, we reviewed patients with *RAS* and *BRAF* wild-type mCRC treated with anti-EGFRs to assess the activity of anti-EGFRs in those with mucinous histology or mucinous component compared with those classified as not mucinous. Owing to the well-recognized role of sidedness in affecting sensitivity to anti-EGFRs[19-21] and the higher prevalence of mucinous histology in right-sided tumors, a subgroup analysis according to primary location was planned.

Patients and Methods

Patient Population

From a dataset of 2315 consecutive patients with *RAS* and *BRAF* wild-type mCRC referred to 3 Italian institutions (Azienda Ospedaliero-Universitaria Pisana, Pisa; Istituto Nazionale dei Tumori, Milan; and Azienda Sanitaria Universitaria Integrata di Udine, Udine) from August 2010 to May 2017 and treated with anti-EGFR MoAb-containing regimens, we retrospectively identified patients with known histology regarding mucinous component who were treated with panitumumab, cetuximab, or cetuximab plus

Table 1 Patients' Characteristics

Characteristics	Mucinous or Mucinous Component N = 22 N (%)	Not Mucinous N = 83 N (%)	*P* Value
Age, y			
Median	64	67	.24
Range	37-82	36-88	
Gender			
Male	12 (55)	50 (60)	.81
Female	10 (45)	33 (40)	
ECOG PS at the beginning of anti—EGFR-containing treatment			
0	7 (32)	41 (49)	.27
1-2	13 (59)	38 (46)	
NA	2 (9)	4 (5)	
Time to metastases			
Synchronous	18 (82)	47 (57)	.028
Metachronous	4 (18)	36 (43)	
Primary tumor resected			
Yes	19 (86)	83 (100)	.008
No	3 (14)	0 (0)	
Primary sidedness			
Right	11 (50)	11 (13)	<.001
Left or rectum	11 (50)	72 (87)	
pT			
1-2	2 (9)	9 (11)	1.0
3-4	17 (77)	74 (89)	
X	3 (14)	0 (0)	
pN			
0	3 (14)	15 (18)	1.0
1-2	16 (73)	68 (82)	
X	3 (14)	0 (0)	
Grading[a]			
1-2	5 (23)	59 (71)	<.001
3-4	14 (63)	24 (29)	
X	3 (14)	0 (0)	
Microsatellite status			
MSS/MSI-L	14 (63)	66 (80)	.036
MSI-H	2 (9)	0 (0)	
NA	6 (28)	17 (20)	
Previous adjuvant treatment			
No	18 (82)	67 (81)	.27
Fluoropyrimidine	0 (0)	7 (8)	
Fluoropyrimidine plus oxaliplatin	4 (18)	9 (11)	
Previous lines of treatment for metastatic disease			
0	4 (18)	21 (25)	.71
1	4 (18)	17 (21)	
2	14 (64)	45 (54)	
Anti—EGFR-containing regimen			
Cetuximab or panitumumab	12 (55)	63 (76)	.088
Cetuximab plus irinotecan	10 (45)	20 (24)	

Table 1 Continued

Characteristics	Mucinous or Mucinous Component N = 22 N (%)	Not Mucinous N = 83 N (%)	*P* Value
No. of metastatic sites at the beginning of anti–EGFR-containing treatment			
1	6 (27)	24 (29)	.81
>1	14 (64)	55 (66)	
NA	2 (9)	4 (5)	

P Value from the Mann-Whitney, χ^2, or Fisher exact test when appropriate.
Abbreviations: anti-EGFR: anti-epidermal growth factor receptor monoclonal antibody; ECOG PS = Eastern Cooperative Oncology Group performance status; MSI-H = microsatellite instability-high; MSI-L = microsatellite instability-low; MSS = microsatellite stable; NA = not available; pN = pathologic N-stage; pT = pathologic T-stage.
[a]According to American Society of Clinical Oncology/College of American Pathologists guidelines.

irinotecan (only if irinotecan-refractoriness was documented by the evidence of disease progression during or within 3 months from the last irinotecan-containing therapy). Mucinous histology was assessed as per standardized protocols according to international guidelines[22] and retrieved through the revision of pathologic reports. No central re-assessment was planned. Patients with undefined histology, receiving an anti-EGFR agent in combination with chemotherapy (ie, FOLFOX [folinic acid, fluorouracil, and oxaliplatin]/FOLFIRI [folinic acid, fluorouracil, and irinotecan]/FOLFOXIRI [folinic acid, fluorouracil, irinotecan, and oxaliplatin]), or receiving cetuximab plus irinotecan but with no demonstrated refractoriness to a previous irinotecan-containing regimen were excluded from the analysis (Figure 1). Only patients who had not been previously treated with anti-EGFR MoAbs, with measurable disease according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 and radiologic reassessments performed every 2 or 3 months as per clinical practice were eligible.

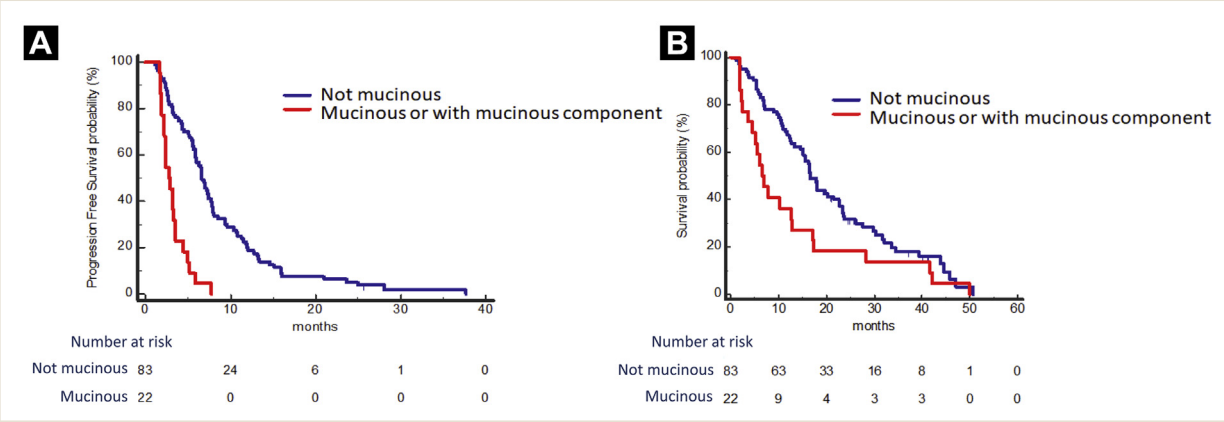
According to previous subgroup analyses of other randomized studies,[19,21,23-26] tumors located in the cecum and the ascending and transverse colon were defined as right-sided, whereas those

Table 2 Response and Survival Parameters

	Mucinous or Mucinous Component N = 22	Not Mucinous N = 83	Overall Population N = 105
Evaluable for response, n (%)			
CR	0 (0)	0 (0)	0 (0)
PR	1 (4)	42 (51)	43 (41)
SD	3 (14)	20 (24)	23 (22)
PD	18 (82)	21 (25)	39 (37)
ORR, %	4%	51%	41%
OR (95% CI)	21.51 (2.76-167.39)		
P	.003		
DCR, %	18	75	63
OR (95% CI)	13.29 (4.04-43.72)		
P	<.001		
PFS			
Events, n (%)	22 (100)	81 (98)	103 (98)
Median PFS, mos	2.8	6.7	5.9
HR (95% CI)	0.28 (0.13-0.59)		
P	<.001		
OS			
Events, n (%)	22 (100)	69 (83)	91 (87)
Median OS, mos	6.5	16.7	16.2
HR (95% CI)	0.58 (0.33-1.00)		
P	.022		

Abbreviations: CI = confidence interval; CR = complete response; DCR = disease control rate; HR = hazard ratio; OR = odds ratio; ORR = overall response rate; OS = overall survival; PD = progression disease; PFS = progression-free survival; PPS = post-progression survival; PR = partial response; SD = stable disease.

Figure 2 Kaplan-Meier Analyses of Progression-free Survival and Overall Survival in Mucinous Tumors (A) and Not Mucinous Tumors (B)



located within the splenic flexure, descending colon, sigma, and rectum were defined as left-sided.

Statistical Analysis

The primary objective of the study was to assess the activity of anti-EGFRs in *RAS* and *BRAF* wild-type mCRC with mucinous histology or with mucinous component compared with not mucinous tumors. Taking into account the role of sidedness in predicting resistance to anti-EGFR MoAbs, subgroup analyses based on primary tumor location were planned.

The χ^2 test, Fisher exact test, and Mann-Whitney test were used, when appropriate, to compare clinical and biological features, overall response rate (ORR), and disease control rate between mucinous (or with mucinous component) and not mucinous tumors. Progression-free survival (PFS), defined as the time elapsed from the first administration of anti-EGFR-containing treatment to the first occurrence of radiologic disease progression according to RECIST v.1.1 or death from any cause, and overall survival (OS), defined as the time between the first administration of anti-EGFR-containing treatment and death for any cause, were determined according to the Kaplan-Meier estimates method. Survival curves were compared using the log-rank test. Statistical significance was set at $P = .05$ for a bilateral test. All analyses were carried out with MedCalc Statistical Software (https://www.medcalc.org).

Results

Overall, among 2315 patients screened, 105 patients with mCRC who fulfilled the eligibility criteria were identified. The study population included 11 mucinous tumors and 11 tumors with mucinous component, that were grouped together, and 83 not mucinous tumors. Patients' characteristics are summarized in Table 1. Patients in the 2 groups shared similar characteristics in terms of performance status at the beginning of the anti-EGFR treatment, and number of previous lines of therapy. As expected, higher rates of poorly differentiated, synchronous, right-sided, unresected, and microsatellite-instable tumors were reported among patients affected by mucinous or with mucinous component tumors.

Response and survival parameters are listed in Table 2. In the overall population, no complete responses were observed. Among patients with mucinous or with mucinous component tumors, 1 patient experienced partial response, whereas in the not mucinous

Table 3 Response and Survival Parameters

	Mucinous	Mucinous Component
	N = 11	N = 11
Evaluable for response, n (%)		
CR	0 (0)	0 (0)
PR	0 (0)	1 (9)
SD	1 (9)	2 (18)
PD	11 (91)	8 (73)
ORR, %	0	9
OR (95% CI)	3.29 (0.12-89.82)	
P	.48	
DCR, %	9	27
OR (95% CI)	3.75 (0.32-43.32)	
P	.29	
PFS		
Events, n (%)	11 (100)	11 (100)
Median PFS, mos	3.2	2.3
HR (95% CI)	0.89 (0.39-2.06)	
P	.78	
OS		
Events, n (%)	11 (100)	11 (100)
Median OS, mos	7.9	6.0
HR (95% CI)	1.16 (0.50-2.69)	
P	.71	

Abbreviations: CI = confidence interval; CR = complete response; DCR = disease control rate; HR = hazard ratio; OR = odds ratio; ORR = overall response rate; OS = overall survival; PD = progression disease; PFS = progression-free survival; PPS = post-progression survival; PR = partial response; SD = stable disease.

Table 4 Response and Survival Parameters Based on Primary Tumor Location

	Right-sided		Left-sided	
	Mucinous or Mucinous Component	Not Mucinous	Mucinous or Mucinous Aspects	Not Mucinous
	N = 11	N = 11	N = 11	N = 72
Evaluable for response, n (%)				
CR	0 (0)	0 (0)	0 (0)	0 (0)
PR	0 (0)	5 (45.5)	1 (9)	37 (52)
SD	0 (0)	1 (9)	3 (27)	19 (26)
PD	11 (100)	5 (45.5)	7 (64)	16 (22)
ORR, %	0	45	9	52
OR (95% CI)	19.46 (0.92-411.22)		10.57 (1.29-86.93)	
P	.056		.028	
DCR, %	0	54	36	88
OR (95% CI)	27.18 (1.29-574.35)		6.12 (1.59-23.59)	
P	.034		.008	
PFS				
Events, n (%)	11 (100)	11 (100)	11 (100)	70 (97)
Median PFS, mos	2.3	3.9	3.5	6.9
HR (95% CI)	0.40 (0.16-1.0)		0.29 (0.10-0.85)	
P	.013		<.001	
OS				
Events, n (%)	11 (100)	10 (91)	11 (100)	59 (82)
Median OS, mos	6.0	8.9	6.9	17.9
HR (95% CI)	1.21 (0.51-2.87)		0.31 (0.11-0.87)	
P	.64		<.001	

Abbreviations: CI = confidence interval; CR = complete response; DCR = disease control rate; HR = hazard ratio; OR = odds ratio; ORR = overall response rate; OS = overall survival; PD = progression disease; PFS = progression-free survival; PPS = post-progression survival; PR = partial response; SD = stable disease.

tumor group, 42 patients experienced partial response with an ORR of 4% and 51%, respectively (odds ratio [OR], 21.51; 95% confidence interval [CI], 2.76-167.39; $P = .003$).

At a median follow up of 16.2 months, disease progression during anti-EGFR treatment (irrespective of the anti-EGFR treatment line) and death occurred in 103 patients (98%) and 91 patients (87%), respectively. Both PFS and OS were higher in the not mucinous tumor group. The median PFS was 2.8 months in the mucinous tumor group and 6.7 months in the not mucinous tumor group (hazard ratio [HR], 0.28; 95% CI, 0.13-0.59; $P < .001$) (Figure 1, panel A). The median OS was 6.5 and 16.7 months (HR, 0.58; 95% CI, 0.33-1.00; $P = .022$), for the mucinous and not mucinous groups, respectively (Figure 2, panel B).

Moreover, when comparing the outcome of patients with pure mucinous tumors with that of patients whose tumors show mucinous component, no differences in terms of activity and efficacy were observed (Table 3). When looking at primary tumor location (Table 4), among right-sided tumors, no objective responses were observed in the mucinous group, whereas 5 patients harboring not mucinous tumors experienced partial response with a ORR of 0% versus 45.5% (OR, 19.46; 95% CI, 0.92-411.22; $P = .056$). In addition, a better PFS was reported in favor of not mucinous tumors (2.3 vs. 3.9 months; HR, 0.40; 95% CI, 0.16-1.00; $P = .013$) (Figure 3, panel A), whereas no differences in terms of OS were shown (6.0 vs. 8.9 months; HR, 1.21; 95% CI, 0.51-2.87; $P = .64$) (Figure 3, panel B).

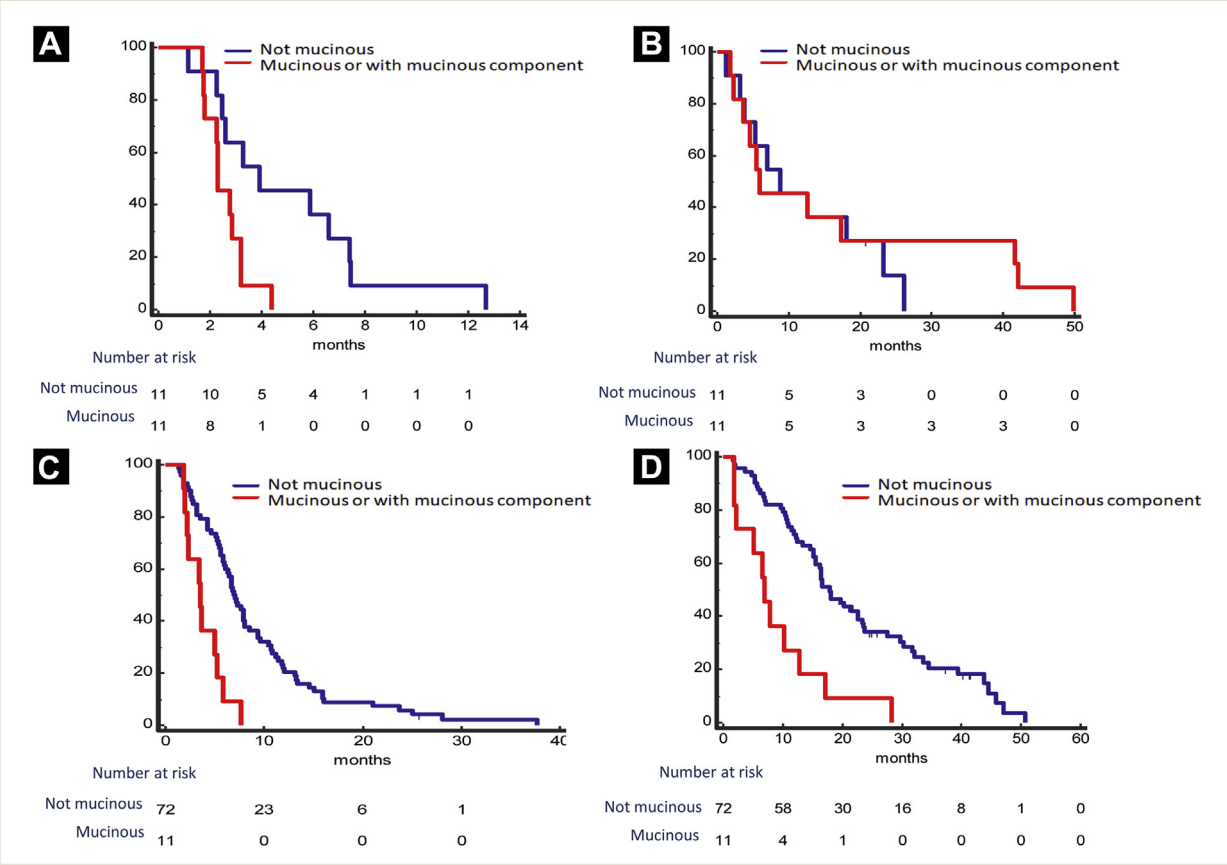
Among left-sided tumors, the response rate and survival parameters were higher in those with not mucinous histology, with an ORR of 9% versus 52% (OR, 10.57; 95% CI, 1.29-86.93; $P = .028$), a median PFS of 3.5 versus 6.9 months (HR, 0.29; 95% CI, 0.10-0.85; $P < .001$) (Figure 3, panel C), and a median OS of 6.9 versus 17.9 months (HR, 0.31; 95% CI, 0.11-0.87; $P < .001$) for mucinous and not mucinous tumors, respectively (Figure 3, panel D).

Discussion

To our knowledge, this is the first attempt to address the issue of the efficacy of anti-EGFRs in *RAS* and *BRAF* wild-type mCRC with mucinous histology or with mucinous component. Although acknowledging the limitations of our study, including the small sample size and, in particular, the small number of mucinous tumors compared with not mucinous ones, the retrospective nature of data collection, and the lack of a central reassessment of histology, our results suggest that mucinous subtypes seem not to derive substantial benefit from anti-EGFR MoAbs.

Although the molecular and biological basis of this intrinsic resistance is not known, an EGFR-independent constitutive activation of EGFR-pathway[27] may be hypothesized. In fact,

Figure 3 Kaplan-Meier Analyses of Progression-free Survival and Overall Survival in Right-sided (A, B) and Left-sided (C, D) metastatic colorectal cancer



mucinous histology is often associated with alterations in MAPK signaling pathways as well as *RAS* and *BRAF* mutations[28] that could lead to an EGFR-independent constitutive activation of the EGFR pathway. Moreover, the CpG island methylator phenotype is an epigenetic mechanism of gene silencing more frequently observed in mucinous tumors,[29] so that the methylation of the *EGFR* promoter may be responsible for the loss of EGFR expression[30] and the inefficacy of anti-EGFR MoAbs. Moreover, the "canonical" CMS2 subtype, characterized by epithelial activation,[9] and therefore, potentially enhanced sensitivity to EGFR inhibition, is poorly represented among mucinous tumors,[4] whereas the highest frequency of mucinous colorectal tumors is comprised of the CMS1 subtype,[9] in which more benefit from bevacizumab rather than cetuximab was reported in terms of PFS and OS.[31] Finally, as confirmed in the present study, mucinous tumors are associated with microsatellite instability, which could represent another mechanism of anti-EGFRs resistance. Indeed, the retrospective analysis of the CALGB/SWOG (Cancer and Leukemia Group B/South West Oncology Group) 80405 trial[32] seems to suggest a potential worse outcome of microsatellite instable tumors when receiving a cetuximab-based rather than a bevacizumab-based therapy, and impaired sensitivity to anti-EGFRs was reported also in the case-control PRESSING (PRimary rESiStance IN RAS and BRAF wild-type metastatic colorectal cancer patients treated with anti-eGfr monoclonal antibodies) study.[26]

A clear limitation of our study is the lack of a control arm including untreated patients. This prevents us from drawing definitive conclusions about the predictive role of the mucinous histology. Nevertheless, the evidence of a significant difference in terms of relative risk and PFS, although limited by the small sample size, might suggest an additional predictive impact of the histology on anti-EGFR treatment-related outcomes.

The choice to restrict the current analysis to patients receiving anti-EGFR MoAbs as single agents or in combination with irinotecan only in the case of strictly defined irinotecan-refractoriness was driven by the objective to avoid the confounding effect of the associated chemotherapy backbone in interpreting treatment outcome. In other words, although chemotherapy plus anti-EGFR is today the most common use of anti-EGFRs, this combination does not allow catching the relative contribution of the targeted drug to treatment outcome, which is jeopardized by the contemporary administration of chemotherapy. To this end, and in order to confirm these findings in an earlier setting, the analysis of randomized studies evaluating the addition of the anti-EGFR to

first- or second-line chemotherapy or comparing chemotherapy plus anti-EGFR and chemotherapy plus bevacizumab would be of paramount importance and should be encouraged.

Of note, the potential role of mucinous histology as a negative predictor of benefit from anti-EGFRs is reported also in the subgroup of patients with left-sided tumors, where only 1 objective response was observed. Based on these findings, the selection of patients to be treated with anti-EGFR MoAbs could be further refined by identifying *RAS* and *BRAF* wild-type patients with not mucinous left-sided tumors as the best candidates to receive these targeted agents.

Conclusion

Our results suggest the potential contribution of a traditional histopathologic feature (ie, the evaluation of mucinous histology) in tailoring the best treatment for every patient with mCRC, and even more in left-sided tumors, where anti-EGFR-based therapies are nowadays considered the best upfront option in *RAS* and *BRAF* wild-type tumors.[33] To this purpose, confirmation in subgroup analyses of clinical trials randomizing patients to receive or not anti-EGFR MoAbs might improve the level of evidence of our findings and highlight the importance of collecting this information in ongoing and future trials.

Clinical Practice Points

- Adenocarcinoma with mucinous histology or mucinous component are histologic subtypes of mCRC often associated with particular clinicopathologic features, with worse prognosis and with limited benefit from cytotoxic agents.
- Although no data about the predictive impact of mucinous histology and mucinous component with regard to anti-EGFRs MoAbs are available, translational observations suggest the EGFR-dependency of mucinous adenocarcinoma.
- We reviewed patients with *RAS* and *BRAF* wild-type mCRC treated with anti-EGFR monotherapy or cetuximab plus irinotecan (only if irinotecan-refractory) to assess the activity of these agents in those with mucinous histology or mucinous component compared with those classified as not mucinous. A subgroup analysis according to primary tumor location was conducted.
- Our results suggest that mucinous histology could predict the lack of benefit from anti-EGFR MoAbs independently of primary tumor location.

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Disclosure

The authors have stated that they have no conflicts of interest.

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