KRAS Status as a Predictor of Chemotherapy Activity in Patients With Metastatic Colorectal Cancer

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Abstract

This is a retrospective analysis of the effect of *KRAS* status on chemotherapy efficacy. Among those treated with capecitabine-based regimens, survival was longer for patients with KRAS wild-type status (hazard ratio, 0.47; 95% confidence interval, 0.23-0.95; *P* < .0001), when compared with those with mutant status. The median overall survival was 46.7 versus 32.6 months for patients with *KRAS* wild-type versus mutant status, respectively.

Background: *KRAS* mutations occur in 40% of colorectal cancers (CRCs), affecting the efficacy of agents targeting the epidermal growth factor receptor. However, the effect of *KRAS* mutation status on the activity of non-epidermal growth factor receptor-targeting chemotherapy has not been fully elucidated. The aim of the present study is to evaluate the effect of *KRAS* status on the activity of different chemotherapeutic regimens. **Patients and Methods**: A retrospective chart review of chemotherapy-treated patients with metastatic CRC with known *KRAS* status was undertaken. Chemotherapy effects were measured by progression-free survival, time to chemotherapy resistance, and overall survival. Analysis was performed for the different chemotherapy regimens, and according to the *KRAS* mutation status while adjusting for potential confounders. **Results**: *KRAS* mutations were detected in 43% of 223 patients with metastatic CRC who were treated at the Ottawa Hospital. The baseline distribution of *KRAS* wild-type (WT) and mutant status was similar. The median follow-up was 27.2 months. Regimens received included single agents or combinations of 2 or 3 chemotherapies. Among those treated with capecitabine-based regimens, survival was longer for patients with *KRAS* WT status (hazard ratio, 0.47; 95% confidence interval, 0.23-0.95; $P < .0001$) when compared with those with mutant status. The median overall survival was 46.7 versus 32.6 months for patients with *KRAS* WT versus mutant status, respectively. The time to chemotherapy resistance was also significantly longer for patients with WT status (hazard ratio, 0.49; 95% confidence interval, 0.25-0.97; $P = .0398$). A trend for progression-free survival did not reach statistical significance. **Conclusion**: Patients with *KRAS* WT tumors may benefit more from capecitabine-based treatments than patients with mutant status. Further research is needed to explain this data.

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Introduction

KRAS is a key component of the epidermal growth factor receptor (EGFR) signal transduction pathway, affecting proliferation and angiogenesis. *KRAS* mutations are present in approximately 40% of colorectal cancers (CRC). A mutated *KRAS* is constitutively activated, enabling the downstream effects of EGFR/renin–angiotensin system (RAS)/rapidly accelerated fibrosarcom (RAF)/mitogen-activated protein kinase (MAP) kinase signaling pathway independent of EGFR activation by the ligand, thus leading to uninterrupted proliferation.[1] This mutation has been shown to occur early in the course of oncogenesis, with no difference in the rate of mutation in early versus later stage disease.[2] The clinical benefit of EGFR-targeted agents has been shown to be limited to *KRAS* wild-type (WT) tumors. However, there is no agreement in the literature as to whether *KRAS* mutation status could serve as a prognostic factor in metastatic CRC (mCRC). The RASCAL study,[2] which presented overall

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survival (OS) and disease-free survival of 4268 patients with CRC according to *KRAS* status, showed that patients with mutated *KRAS* had a worse prognosis. However, *KRAS* cannot be considered as a prognostic marker from this large study, as there were no data on the treatment the patients did or did not receive. In order to conclude that *KRAS* is a prognostic marker, the data have to be driven from patients who did not receive any treatment, or else the conclusion is on the effect of the marker on treatment effectiveness. The mutational analysis from the NCIC CO-17 study reported 398 patients with *KRAS* status, half of whom were in the best supportive care arm. In this arm, *KRAS* status did not affect progression-free survival (PFS) and OS.[3] Thus, the conclusion that *KRAS* status affects prognosis of disease still cannot be drawn from the available evidence.

The addition of an anti-EGFR monoclonal antibody (eg, cetuximab or panitumumab) to chemotherapy has been shown to increase response rate, PFS, and OS in patients with WT *KRAS* mCRC, although results are inconsistent across studies.[4-7] Lièvre et al[8] first reported an association between *KRAS* gene mutation status and response to treatment with anti-EGFR agents in patients with mCRC. In the NORDIC VII trial, the addition of cetuximab to the Nordic FLOX (fluorouracil, leucovorin, oxaliplatin) regimen did not improve outcomes in first-line treatment of mCRC patients with WT *KRAS*.[9] In contrast, results from the PRIME study demonstrated that the addition of panitumumab to FOLFOX (folinic acid, fluorouracil, oxaliplatin) resulted in an improvement in overall response rate (ORR), PFS, and OS in patients without *RAS* mutations.[3,10] A meta-analysis of trials, assessing the effect of chemotherapy with versus without cetuximab, suggested that the difference in cetuximab benefit between trials might have been influenced by the choice of fluoropyrimidine backbone. Specifically, the improvement in ORR was greater when cetuximab was added to an infusional 5-fluorouracil (5-FU)-based chemotherapy regimen (eg, leucovorin, 5-fluorouracil, irinotecan, oxaliplatin [FOLFIRI], FOLFOX) versus the addition of cetuximab to a noninfusional regimen.[7] In the NORDIC and COIN trials, bolus 5-FU was the backbone, and no difference was observed in ORR with the addition of cetuximab, as opposed to the OPUS and CRYSTAL trials, where the 5-FU was administered as a continuous infusion and where there was a significant improvement in OS and response rate with the addition of cetuximab.

The goal of this study was to examine whether *KRAS* mutation status is associated with chemotherapy benefit in mCRC. We hypothesized that *KRAS* mutation status might correlate with reduced chemotherapy response and shorter disease control in the absence of anti-EGFR treatment effects. In the period studied, all patients had disease refractory to fluoropyrimidines, irinotecan, and oxaliplatin with or without bevacizumab before receiving EGFR inhibitors. The effectiveness of chemotherapy was evaluated before EGFR antibody treatment.

Patients and Methods

After receipt of ethics approval, data of patients with metastatic CRC with known *KRAS* mutation status treated at a single institution were retrospectively reviewed for response to chemotherapy before initiation of EGFR monoclonal antibody therapy.

Definition of Exposure

The primary independent variable was *KRAS* mutation status, obtained by either the DxS Kit method (DxS Diagnostic Innovations) or by the Qiagen QIAamp DNA Mini Kit (Qiagen Sample and Assay Technologies). Tumors in this patient series were only assessed for *KRAS* codon 12 and 13 mutation status, not for other *RAS* mutations, because testing was done at a time when the value of assessing other *RAS* mutations was unknown.

Fifty-nine patients were screened for *KRAS* status as part of a clinical trial. For these patients, genomic DNA was extracted from formalin-fixed, paraffin-embedded tissue slides or sections with the use of the QIAamp DNA Mini Kit (Qiagen), and PCR with the specific primers was performed.[11] The remaining patients had *KRAS* analyzed using the DxS *KRAS* mutation test kit (DxS Limited) to detect the 7 most common mutations in codons 12 and 13 of the *KRAS* gene.[12]

Chemotherapy treatment data were collected, with particular interest in the first-line chemotherapy fluoropyrimidine backbone: infusional 5-FU− versus capecitabine-based chemotherapy.

Outcome Measures and Statistical Analysis

Outcomes (time to chemotherapy resistance [TTCR], OS, and PFS) were defined from initiation of first-line chemotherapy. The primary outcome was TTCR, defined as time from start of first-line therapy to disease progression while receiving all drugs, including fluoropyrimidines (5-FU), irinotecan, oxaliplatin, and bevacizumab.

Secondary outcomes included OS and first-line PFS. OS was defined from start of first-line chemotherapy to date of death from any cause, and was censored at the time of last contact. PFS was calculated from the date of first-line therapy to date of disease progression, and was determined retrospectively according to computed tomographic and/or magnetic resonance imaging scans. Additional demographic, tumor-specific, and treatment-related details were collected from patients' hospital charts.

Statistical Analysis

Descriptive statistics were used to describe baseline characteristics. Survival analysis was conducted to examine the association between *KRAS* mutation status and survival outcomes. Survival curves were derived by Kaplan-Meier methods, and differences were evaluated by the log-rank test. Cox proportional hazard models were fit to describe the association of the independent variables with TTCR, OS, and PFS. Multivariate models were fit to adjust for baseline demographic and clinical factors, including age, gender, primary tumor site, presence of metastatic disease at diagnosis, and receipt of adjuvant chemotherapy. Data were analyzed by SAS 9.2 software (SAS Institute, Cary, NC).

Results

This study evaluated 223 CRC patients with known *KRAS* status. *KRAS* status influence on disease progression and TTCR before any exposure to EGFR monoclonal antibody therapy was assessed. This was possible because at the time, cetuximab and panitumumab were only available to patients with chemotherapy-refractory disease.

Table 1 Patient Characteristics

Characteristic	All Patients (N = 223)	KRAS Wild Type (N = 126)	KRAS Mutant (N = 97)	*P*
Sex				
Male	129 (57.8)	75 (60.0)	54 (55.7)	.56
Female	94 (42.2)	51 (40.0)	43 (44.3)	
Age (Y)				
Median	60	62	60	.82
Range	25-86	32-86	25-81	
Site of Primary Lesion				
Colon	139 (62.3)	74 (58.7)	65 (67.0)	.28
Rectum	63 (28.3)	41 (32.6)	22 (22.7)	
Rectosigmoid	21 (9.4)	11 (8.7)	10 (10.3)	
Metastatic Disease at Diagnosis				
Yes	121 (54.3)	62 (49.2)	59 (60.8)	.08
No	102 (45.7)	64 (50.8)	38 (39.2)	
Disease Site				
Liver	186 (83.4)	102 (81.0)	84 (86.6)	.26
Lung	111 (49.8)	61 (48.4)	50 (51.5)	.64
Other	138 (61.9)	82 (65.1)	56 (57.7)	.25
Prior Therapy				
Adjuvant chemotherapy	97 (43.5)	58 (46.0)	39 (40.2)	.38
Radiotherapy	59 (26.5)	36 (28.6)	23 (23.7)	.42

Data are presented as n (%) unless otherwise indicated.

Patient Characteristics

Patient characteristics are listed in Table 1. There were 127 patients (57%) with WT *KRAS* status and 97 patients (43%) with mutant *KRAS* status. Fifty-eight percent of patients were male. Median age was 60 years (range, 25-86 years). Ninety-seven patients (43%) had received prior adjuvant chemotherapy. Fifty-four percent of patients had metastatic disease at diagnosis. Baseline characteristics were similar among mutant *KRAS* and WT status patients. With a median follow-up of 27.2 months (standard deviation, 19.5), 64 patients (29%) were alive at the time of analysis.

Chemotherapy Backbone and KRAS *Mutation Status*

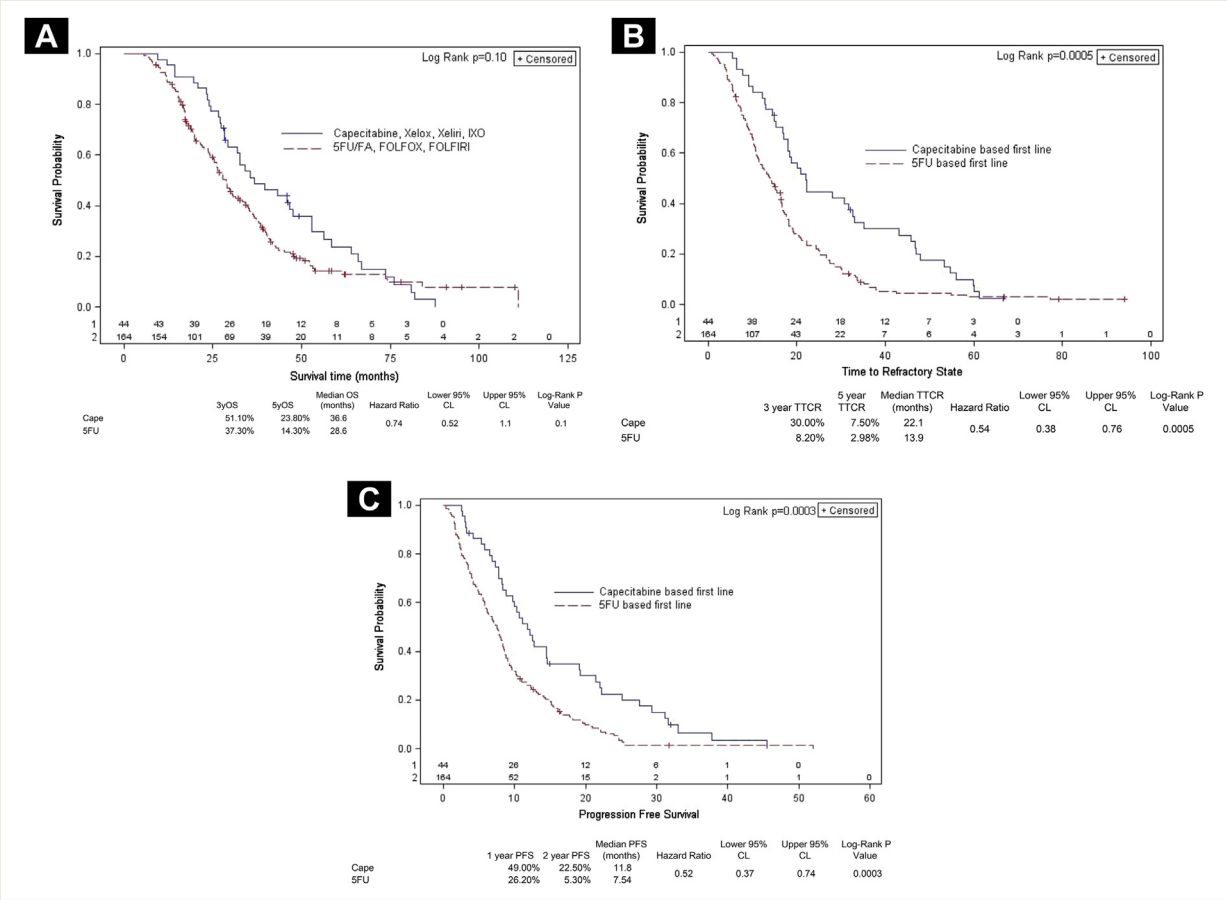
Baseline characteristics according to chemotherapy regimens are listed in Table 2. Fifty-four patients (24%) received capecitabine-based first-line chemotherapy. Treatment with a capecitabine-

Table 2 Distribution of Baseline Characteristics in 207 Patients Treated With Capecitabine-Based and 5-FU–Based First-Line Treatment

Characteristic	Variable	Capecitabine-Based Treatment	5-FU-Based Treatment	*P*
Age	≤65 y	22 (50)	114 (69.5)	.0157
	>65 y	22 (50)	50 (30.5)	
	Median (range)	64 (25-84)	60 (32-83)	
Sex	Male	25 (56.8)	99 (60.4)	.67
	Female	19 (43.2)	65 (39.6)	
Primary tumor site	Colon	29 (65.9)	98 (60.1)	.75
	Rectum	11 (25)	50 (30.7)	
	Rectosigmoid	4 (9.1)	15 (9.2)	
Metastasis at presentation	Yes	25 (56.8)	92 (56.1)	
	No	19 (43.2)	72 (43.9)	
Received adjuvant treatment	Yes	16 (36.4)	68 (41.5)	.54
	No	28 (63.6)	96 (58.5)	

Data are presented as n (%) unless otherwise indicated.
Abbreviation: 5-FU = 5-fluorouracil.

Figure 1 (A) OS, (B) TTCR, and (C) PFS According to Chemotherapy Regimen, (A) OS According to Chemotherapy Administered. (B) TTCR in all Patients by Capecitabine Versus 5-FU. (C) PFS for all Patients from Date of Metastatic Diagnosis by First-line Treatment



Abbreviations: 5-FU = 5-fluorouracil; OS = overall survival; PFS = progression-free survival; TTCR = time to chemotherapy resistance.

based first-line regimen was associated with a better OS (hazard ratio [HR], 0.74; 95% confidence interval [CI], 0.52-1.1; $P = .1$) compared to patients who received infusional 5-FU–based chemotherapy (Figure 1A), with a median survival time of 36.6 months compared to 28.6 months, respectively. Receipt of capecitabine-based regimens was significantly associated with better TTCR compared to patients treated with 5-FU (HR, 0.54; 95% CI, 0.38-0.76; $P = .0004$). The median TTCR was 22.1 and 13.9 months in the capecitabine- and 5-FU–treated patients, respectively (Figure 1B). PFS was significantly longer in patients treated with capecitabine versus those treated with 5-FU–based treatments, with median PFS of 11.8 and 7.5 months, respectively (HR, 0.52; 95% CI, 0.37-0.74; $P = .0003$) (Figure 1C).

Among patients treated with a capecitabine-based regimen, median OS was 46.7 months versus 32.6 months for WT *KRAS* versus mutant disease, respectively (HR, 0.47; 95% CI, 0.23-0.948; $P < .0001$) (Figure 2A). TTCR was significantly longer in patients with WT *KRAS* status (HR, 0.49; 95% CI, 0.25-0.97; $P = .0398$) (Figure 2B). The PFS was also different, but this did not reach statistical significance (Figure 2C).

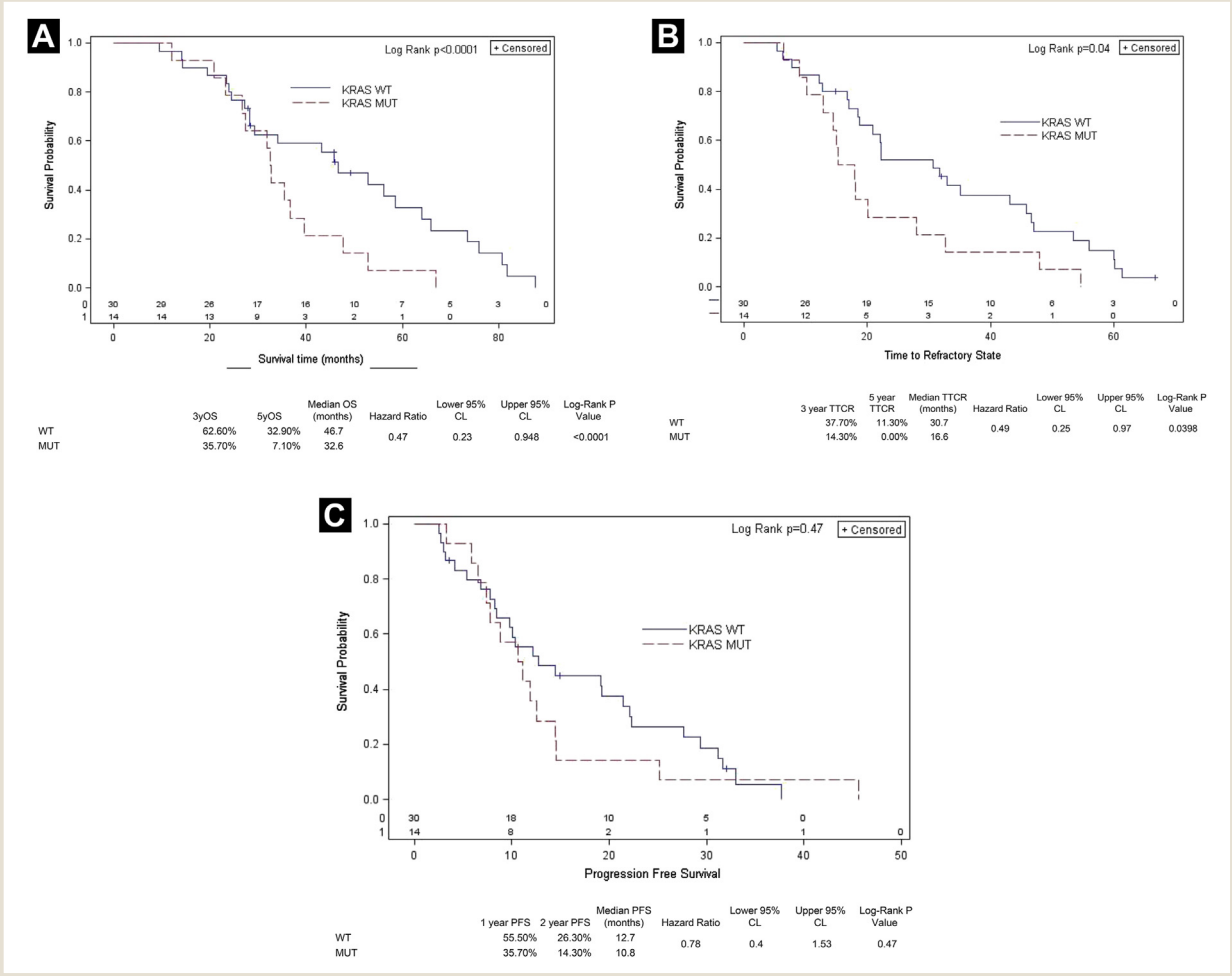
Table 3 presents a multivariate analysis of factors associated with OS, TTCR, and PFS in the WT *KRAS* patient subset. Younger age and receipt of capecitabine-based therapy were significantly associated with improved OS.

Discussion

In this study, we asked whether *KRAS* status could predict the effect of chemotherapy in mCRC patients. The data revealed that among mCRC patients with WT *KRAS*, capecitabine-based chemotherapy was more favorable than in patients with mutated *KRAS*.

The effect of *KRAS* mutation status on the efficacy of chemotherapy in patients with mCRC is controversial. Etienne-Grimaldi et al[13] showed that *KRAS* mutation status did not influence the OS of patients receiving 5-FU. Similarly, no effect of *KRAS* status on response rate, PFS, or OS in patients receiving FOLFOX was observed in a single-institute study reported by Sharma et al.[14] In 2 phase 3 studies with either FOLFOX as first-line therapy or FOLFIRI as second-line therapy, the effectiveness of the chemotherapy was not affected by *KRAS* status.[11,15]

Figure 2 (A) OS, (B) TTCR, and (C) PFS Of Patients Treated With Capecitabine-Based Regimen According to *KRAS* Status.c (A) OS by *KRAS* Status from Date of Metastatic Diagnosis for Patients Receiving First-line Capecitabine-based Regimen. (B) TTCR of Patients Receiving First-line Capecitabine-based Regimen by *KRAS* Status from Date of Diagnosis of Metastatic Disease. (C) PFS Of Patients Receiving First-line Capecitabine-based Regimen by *KRAS* Status from Date of Diagnosis of Metastatic Disease



	3yOS	5yOS	Median OS (months)	Hazard Ratio	Lower 95% CL	Upper 95% CL	Log-Rank P Value
WT	62.60%	32.90%	46.7	0.47	0.23	0.948	<0.0001
MUT	35.70%	7.10%	32.6				

	3 year TTCR	5 year TTCR	Median TTCR (months)	Hazard Ratio	Lower 95% CL	Upper 95% CL	Log-Rank P Value
WT	37.70%	11.30%	30.7	0.49	0.25	0.97	0.0398
MUT	14.30%	0.00%	16.6				

	1 year PFS	2 year PFS	Median PFS (months)	Hazard Ratio	Lower 95% CL	Upper 95% CL	Log-Rank P Value
WT	55.50%	26.30%	12.7	0.78	0.4	1.53	0.47
MUT	35.70%	14.30%	10.8				

However, in the COIN study, treatment with oxaliplatin/5-FU combinations in patients with WT *KRAS* resulted with a higher median PFS compared to that observed in the mutated *KRAS* cohort.[4-7,16-18] Likewise, ORR was lower in mutated *KRAS* patients receiving chemotherapy with oxaliplatin only (41% vs. 50%).

A meta-analysis found that the effect of *KRAS* status on the chemotherapy effectiveness could not be definitively resolved with the available evidence.[19]

The COIN study was a negative study, with no added benefit found from cetuximab- to oxaliplatin-based chemotherapy. Specifically, no difference in the OS of patients with known *KRAS* mutation status receiving oxaliplatin and capecitabine or infused 5-FU with or without cetuximab was observed. The negative results of the study raised the possibility that the chemotherapy backbone of anti-EGFR may have an impact on treatment efficacy. Indeed, further analysis of the backbone of each combination therapy revealed that the worst outcomes were observed in the group that was treated with capecitabine as the backbone. However, this is in contrast to our results, which demonstrated that capecitabine-based chemotherapy yielded better outcomes, especially in *KRAS* WT but also in mutated *KRAS* mCRC. It is important to note that the patients in our analysis did not receive anti-EGFR with the chemotherapy. Thus, it might be that the COIN results represent negative cross talk between anti-EGFR and capecitabine.

The differential effect of chemotherapy according to the *KRAS* mutation status was further supported by the subanalysis from the study of Hurwitz et al,[20] which found that patients with *KRAS* gene mutation had lower overall PFS and OS. In this study examining the effect of different *KRAS* mutations on various anticancer treatments, it was demonstrated that median PFS was lower in patients with codon 13 mutations receiving infusional 5-FU, compared to a higher median PFS demonstrated in patients treated with capecitabine. Patients with codon 12 mutations showed a trend toward the opposite effect—that is, median PFS was lower while receiving capecitabine and oxaliplatin therapy, while a higher

Table 3 Multivariate Analysis of Factors Associated With OS, TTCR, and PFS in WT *KRAS* Subset

Factor	Hazard Ratio	Lower Confidence Limit	Upper Confidence Limit	*P*
Multivariate for OS				
Age <65 y (vs. ≥65 y)	0.51	0.33	0.81	.004[a]
Sex female (vs. male)	1.34	0.88	2.05	.171
Capecitabine (XELOX, IXO, XELIRI)	0.56	0.35	0.92	.0219[a]
Prior adjuvant therapy: yes	0.64	0.34	1.19	.158
Prior RT: yes	1.44	0.80	2.60	.227
Metastatic disease at presentation	1.05	0.53	2.10	.885
Multivariate for TTRC				
Age <65 y (vs. ≥65 y)	0.60	0.40	0.91	.016[a]
Sex female (vs. male)	0.85	0.57	1.27	.422
Capecitabine (XELOX, IXO, XELIRI)	0.36	0.22	0.58[a]	<.0001[a]
Prior adjuvant therapy: yes	0.60	0.29	1.20	.147
Prior RT: yes	0.99	0.59	1.65	.960
Metastatic disease at presentation	1.28	0.61	2.68	.510
Multivariate for PFS				
Age <65 y (vs. ≥65 y)	0.76	0.51	1.14	.180
Sex female (vs. male)	1.20	0.81	1.78	.365
Fluoropyrimidine backbone in first line (capecitabine vs. 5-FU)	0.44	0.27	0.71	.0008[a]
Prior adjuvant therapy: yes	0.83	0.42	1.64	.589
Prior RT: yes	0.81	0.49	1.35	.427
Metastatic disease at presentation	0.93	0.45	1.89	.835

Abbreviations: IXO = irinotecan, capecitabine, oxaliplatin; OS = overall survival; PFS = progression-free survival; RT = radiotherapy; TTCR = time to chemotherapy resistance; WT = wild type; XELIRI = irinotecan, capecitabine; XELOX = capecitabine, oxaliplatin.
[a]Statistically significant at $P < .0008$.

median PFS was observed in patients receiving 5-FU and oxaliplatin.[21]

Another recent study evaluated *KRAS* status in 201 patients receiving a second-line chemotherapy with oxaliplatin and infusional 5-FU. Mutation of *KRAS* gene at codons 12 or 13 was significantly associated with tumor response and PFS in mCRC patients treated with 5-FU/oxaliplatin. Thus, patients with codon 13 mutations seem to benefit more in terms of PFS from the oral capecitabine-based protocols.[22] Unfortunately, we could not retrieve the data of the specific *KRAS* mutation.

A plausible explanation for the different efficacy of capecitabine according to *KRAS* status is the ability of cells' harboring *KRAS* mutation to override the different G_1 checkpoints of the cell cycle. G_1 is a checkpoint in which the cells are susceptible to capecitabine. It has been shown that depleting cells from glutamine makes the *KRAS*-mutated cell sensitive to capecitabine. However, this is quite difficult; as long as there is glutamine, the mutated *KRAS* cells evade capecitabine cell arrest.[23]

It is important to note that in the current study, some of the patients receiving capecitabine were included in a clinical trial assessing triple chemotherapy with capecitabine, oxaliplatin, and irinotecan, thereby offering an aggressive chemotherapeutic regimen. This group also had a lower percentage of *KRAS* mutations.

Limitations

The limitations in this analysis relate to the fact that this was a retrospective study and therefore prone to potential bias, including selection bias. For instance, patients with good performance status were more likely to receive triple therapy than those with worse performance status who received single agents, which might affect the OS.

Lack of Eastern Cooperative Oncology Group performance status data limited our ability to adjust this parameter to the outcome. There may also have been confounding that could not be controlled for this study, such as *BRAF* status, as well as treatment details that could have affected the results, such as doses, number of treatments, and adverse events.

The analysis was performed using the status of *KRAS* mutations only in exons 12; samples were not evaluated for *NRAS* and other lower-frequency *RAS* mutations.

Conclusion

Our findings, taken together, lead us to conclude that patients with WT *KRAS* experience more benefit from capecitabine-based chemotherapy treatment then patients with mutated *KRAS*, and regimens including capecitabine should be more favorably considered in this group of patients.

Clinical Practice Points

- It is well known RAS status is predictive of the anti-EGFR treatment; however, there is no substantial evidence regarding the predictive value of *KRAS* on chemotherapy treatment.
- This study has shown the effect of *KRAS* status on chemotherapy efficacy. Among those treated with capecitabine-based regimens, survival was longer for patients with *KRAS* WT status (HR, 0.47; 95% CI, 0.23-0.95; $P < .0001$) when compared with those with mutant status. The median OS was 46.7 versus 32.6 months for patients with *KRAS* WT versus mutant status, respectively.
- If further supported, these findings might guide clinicians to treat patients with *KRAS* WT tumors with capecitabine rather than intravenous 5-FU.

Disclosure

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

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