JBUON 2018; 23(4): 925-935
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
E-mail: editorial_office@jbuon.com

ORIGINAL ARTICLE

Worst outcomes according to RAS mutation variants: an analysis in patients with metastatic colorectal adenocarcinoma

Pedro L.S. Usón Jr[1], Diogo D.G. Bugano[1], Fernando Moura[1], Ricardo S. Carvalho[2], Fernando C. Maluf[1,2]

[1]Oncology Department, Israelita Albert Einstein Hospital, São Paulo, Brazil; [2]Gastrointestinal Oncology Department Centro Oncológico Antônio Ermírio de Moraes, Beneficência Portuguesa de São Paulo, São Paulo, Brazil

Summary

Purpose: *Over 50% of metastatic colorectal cancers harbor RAS mutations. It is unclear if different mutation variants have an impact on survival. The purpose of this study was to evaluate the impact of these mutations on colorectal cancer survival.*

Methods: *The charts of all cases of metastatic colorectal cancer diagnosed between January 2005 and January 2016 in a tertiary hospital in Brazil were reviewed. Inclusion criteria were complete data on clinical staging, treatments received and all-RAS testing. Multivariate Cox proportional survival models were used to evaluate the impact of specific RAS variants on survival.*

Results: *There were 151 eligible patients and 61.6% had RAS alterations, the most common G12D (11.9%) and G12A (8.6%). Most patients received chemotherapy, including oxaliplatin (79%), irinotecan (53%) and bevacizumab (59%). Among RAS-wild type patients, 46% received anti-EGFR therapy. Median survival was 39.2 months for RAS-wild-type, 18.8 months for RAS G12A and 34.6 for other RAS-mutant patients (multivariate analysis for G12A vs RAS-wild type HR 1.94; 95% CI 0.83-5.51; p=0.12).*

Conclusion: *Patients with metastatic colorectal cancer who have RAS mutations have shorter overall survival. Regarding the impact of specific KRAS alterations, G12A mutations have a worse prognosis.*

Key words: *colorectal cancer, KRAS, overall survival, RAS*

Introduction

Colorectal cancer is the third most common cause of cancer death in both men and women [1]. The median survival of patients with metastatic disease is currently around 30 months, but outcomes are heterogeneous, with survival ranging from a few months to more than 5 years. Patient stratification by molecular markers may help identify patients with distinct outcomes [2,3].

Approximately 50% of tumors have RAS mutations (KRAS Exon 2-4 or NRAS Exon 2-4). All lead to similar resistance to anti-EGFR therapy and, therefore, the specific type of alteration does not currently influence clinical practice.

However, recent publications have suggested an association between type of RAS mutation and prognosis [4-10]. The limitations of these reports have been the low frequency of some alterations, heterogeneity of studied populations and lack of detailed information on surgery and chemotherapy.

We reviewed patients with metastatic colorectal cancer in Brazil, with complete information on staging and treatments received, and evaluated the prognostic impact of different RAS alterations.

Correspondence to: Pedro Luiz Serrano Usón Jr, MD. Department of Oncology, Israelita Albert Einstein Hospital, 627/701 Albert Einstein Ave, Sao Paulo, CEP 05651-901, Brazil.
Tel: +55 11 2151 1233, Fax: +55 11 37422834, E-mail: pedroluiz_uson@hotmail.com
Received: 07/11/2017; Accepted: 04/01/2018

Methods

Patient population

We retrospectively reviewed charts of all patients with metastatic adenocarcinoma of colon and rectum treated at the Israelita Albert Einstein Hospital, a tertiary general Hospital in São Paulo (Brazil) between January 2005 and January 2016. Included were only patients with complete information on RAS testing (tested by polymerase chain reaction –PCR), clinical characteristics and treatment received. Two authors (FM and RSC) contributed with data from additional patients they follow at other institutes which met the inclusion criteria. Because these institutes don´t have onsite Institutional Review Boards (IRB), this project was reviewed and approved by the IRB at the Israelita Albert Einstein Hospital (CAAE: 55880616.9.0000.0071).

Data collected and primary outcomes

All data was collected from patient charts. The primary outcome was cancer-specific survival after the diagnosis of metastatic disease. Covariates were: age, gender, ECOG (Eastern Cooperative Oncology Group) performance status (PS), past medical history, tumor laterality, systemic therapy received, operations performed, NRAS, BRAF and KRAS mutations (G12D, G12V, G12C, G12S, G12A, G12F, G12R, G12T, G13D, A146T).

Statistics

Patients were divided in groups according to mutation status. Categorical data was described as absolute and relative frequencies and continuous data as means and standard deviations or medians and interquartile ranges (IQR). For survival analysis, Kaplan-Meier curves were constructed and patients were censored at last follow-up. Curves were compared using log-rank test and for covariate adjustment we used Cox proportional survival models. Unless otherwise stated, significance was set at 5%. All analyses were done using R version 3.1.3 or SPSS version 24.

Results

Patient population and treatments

The final database included 151 patients with metastatic colorectal cancer and complete information on RAS testing, clinical staging and treatments received (Figure 1). Most patients (61.6%) had RAS mutations and the most common one was G12D (11.9%) (Table1). Clinical characteristics were overall comparable in the KRAS wild-type and mutated groups, but patients with mutations were more likely to have liver metastasis, to be non-smokers and to be younger than 65 years (Supplementary Table 1).

Treatment received was also similar for both groups, with 30% of patients undergoing hepatectomy, almost 100% receiving fluorouracil, close to 80% of receiving oxaliplatin-based regimens, 50% receiving irinotecan-based regimens and 50-60% receiving bevacizumab. As expected, the only difference was treatment with anti-EGFR antibodies,

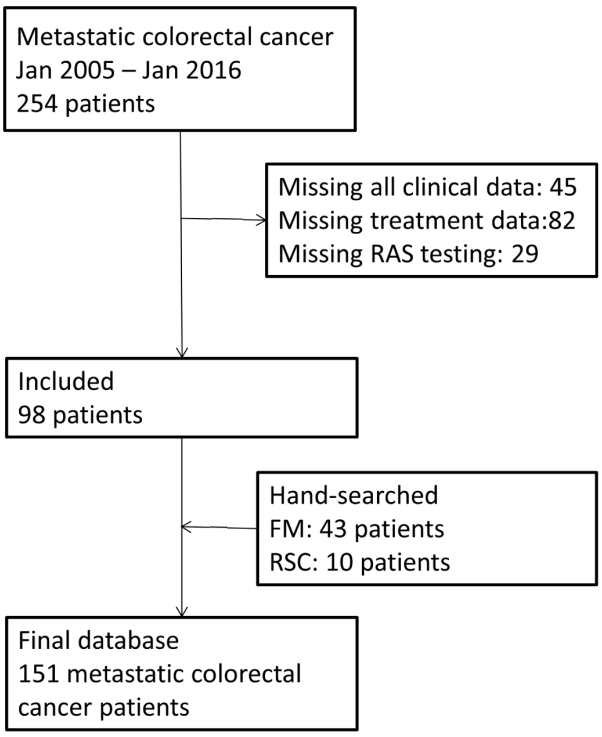


Figure 1. Patient selection chart.

Table 1. All included patient mutations

	All included patients (n=151) n (%)
Mutation	
Wild Type	58 (38.4)
KRAS	89 (58.9)
NRAS	2 (1.3)
BRAF	2 (1.3)
Type	
A146T	1 (0.7)
BRAF	2 (1.3)
G12A	13 (8.6)
G12C	17 (11.3)
G12D	18 (11.9)
G12F	1 (0.7)
G12R	1 (0.7)
G12S	5 (3.3)
G12T	1 (0.7)
G12V	13 (8.6)
G13D	9 (6.0)
NRAS codon 12	2 (1.3)
KRASmut not specified	10 (6.6)

received by 46% of patients in the KRAS wild-type group (Supplementary Table 2).

Survival analysis and impact of RAS mutations

Median follow-up was 22 months (interquartile range 9-41) and median overall survival was 34.9 months. Because of small sample sizes, it was not possible to compare survival for each individual RAS mutation; however, patients with G12A mutations had a numerically lower overall survival (18.8 months, vs 34.6 for other RAS mutations and 39.2 for wild-type patients) (Figure 2).

In univariate analysis, the presence of liver metastasis, bone metastasis, an ECOG PS of 3, diabetes and age older than 65 years were associated with shorter survival; treatment with any systemic chemotherapy was associated with longer survival (Supplementary Table 3). No individual mutation was statistically associated with worse survival, but, again, the G12A alteration had the

Table 2. Multivariate COX regression analysis

	Hazard ratio	*95% Confidence interval*		*p value*
		Lower limit	*Upper limit*	
Type of mutation				
Wild				
G12A	1.941	0.834	4.513	0.124
G12C	1.397	0.557	3.499	0.476
G12D	1.540	0.606	3.917	0.364
G12S	1.776	0.453	6.956	0.410
G12V	1.285	0.463	3.567	0.631
G13D	0.686	0.233	2.018	0.494
Diabetes				
No				
Yes	1.604	0.657	3.915	0.299
ECOG PS				
0				
1	0.859	0.468	1.575	0.623
2	2.673	0.931	7.673	0.068
3	11.323	1.687	75.992	**0.012**
Side				
Right				
Left	0.704	0.373	1.326	0.277
Liver metastasis				
No				
Yes	2.552	1.224	5.319	**0.012**
Carcinomatosis metastasis				
No				
Yes	1.345	0.689	2.625	0.385
Lymph nodes metastasis				
No				
Yes	1.154	0.590	2.258	0.676
Bone metastasis				
No				
Yes	6.497	2.302	18.340	**<0.001**
Lung metastasis				
No				
Yes	1.603	0.844	3.046	0.150
Age	1.475	0.800	2.720	0.214

Bold numbers denote statistical significance

Supplementary Table 1. Global descriptive analysis by presence of mutation

	Wild (n= 58) *n (%)*	*KRASmut (n= 89)* *n (%)*	*Total (n= 147)* *n (%)*
Side			
Right	21 (36.2)	21 (23.6)	42 (28.6)
Left	37 (63.8)	68 (76.4)	105 (71.4)
Liver			
No	25 (43.1)	24 (27.0)	49 (33.3)
Yes	33 (56.9)	65 (73.0)	98 (66.7)
Carcinomatosis			
No	40 (69.0)	66 (74.2)	106 (72.1)
Yes	18 (31.0)	23 (25.8)	41 (27.9)
Lymph nodes			
No	35 (60.3)	67 (75.3)	102 (69.4)
Yes	23 (39.7)	22 (24.7)	45 (30.6)
Bones			
No	54 (93.1)	85 (95.5)	139 (94.6)
Yes	4 (6.9)	4 (4.5)	8 (5.4)
Lung			
No	43 (74.1)	67 (75.3)	110 (74.8)
Yes	15 (25.9)	22 (24.7)	37 (25.2)
Brain			
No	58 (100.0)	87 (97.8)	145 (98.6)
Yes	0 (0.0)	2 (2.2)	2 (1.4)
Others			
No	58 (100.0)	87 (97.8)	145 (98.6)
Yes	0 (0.0)	2 (2.2)	2 (1.4)
Death			
No	22 (37.9)	46 (51.7)	68 (46.3)
Yes	36 (62.1)	43 (48.3)	79 (53.7)
ECOG PS			
0	16 (27.6)	35 (39.3)	51 (34.7)
1	37 (63.8)	45 (50.6)	82 (55.8)
2	5 (8.6)	6 (6.7)	11 (7.5)
3	0 (0.0)	3 (3.4)	3 (2.0)
Absent of comorbidities			
No	29 (50.0)	37 (41.6)	66 (44.9)
Yes	29 (50.0)	52 (58.4)	81 (55.1)
Smoking			
No	44 (75.9)	78 (87.6)	122 (83.0)
Yes	14 (24.1)	11 (12.4)	25 (17.0)
Alcohol consumption			
No	51 (87.9)	78 (87.6)	129 (87.8)
Yes	7 (12.1)	11 (12.4)	18 (12.2)
Cardiovascular comorbidities			
No	49 (84.5)	75 (84.3)	124 (84.4)
Yes	9 (15.5)	14 (15.7)	23 (15.6)
Diabetes			
No	53 (91.4)	83 (93.3)	136 (92.5)
Yes	5 (8.6)	6 (6.7)	11 (7.5)
Age (years)			
< 65	33 (56.9)	60 (67.4)	93 (63.3)
> 65	25 (43.1)	29 (32.6)	54 (36.7)
Sex			
female	19 (32.8)	36 (40.4)	55 (37.4)
male	39 (67.2)	53 (59.6)	92 (62.6)

strongest association (HR 1.76; 95%CI 0.84-3.69; p=0.13;Supplementary Table 4). This did not change significantly after multivariate analysis (Table 2;Supplementary Figure 1).

There was no clinical condition that could characterize or differentiate patients with tumors with G12A mutation compared with other RAS status, including tumor side (Supplementary Table 5).

Discussion

We reviewed charts of 151 patients with metastatic colorectal cancer treated in a tertiary hospital in Brazil between 2005 and 2016. After correction for clinical characteristics, resections and chemotherapies received, patients with G12A alterations had numerically shorter survival (18.8 months vs 34.6 for other RAS mutations and 39.2 for RAS-wild

Supplementary Table 2. Descriptive analysis of treatments

	Wild (n= 58) *n (%)*	*KRASmut (n= 89)* *n (%)*	*Total (n= 147)* *n (%)*
Chemotherapy			
No	0 (0.0)	2 (2.2)	2 (1.4)
Yes	58 (100.0)	87 (97.8)	145 (98.6)
5-Fluorouracil / Capecitabine			
No	0 (0.0)	2 (2.2)	2 (1.4)
Yes	58 (100.0)	87 (97.8)	145 (98.6)
Oxaliplatin			
No	11 (19.0)	20 (22.5)	31 (21.1)
Yes	47 (81.0)	69 (77.5)	116 (78.9)
Irinotecan			
No	28 (48.3)	41 (46.1)	69 (46.9)
Yes	30 (51.7)	48 (53.9)	78 (53.1)
Regorafenib			
No	53 (91.4)	85 (95.5)	138 (93.9)
Yes	5 (8.6)	4 (4.5)	9 (6.1)
Bevacizumab			
No	28 (48.3)	33 (37.1)	61 (41.5)
Yes	30 (51.7)	56 (62.9)	86 (58.5)
Cetuximab/Panitumumab			
No	31 (53.4)	87 (97.8)	118 (80.3)
Yes	27 (46.6)	2 (2.2)	29 (19.7)
Mitomycin			
No	55 (94.8)	84 (94.4)	139 (94.6)
Yes	3 (5.2)	5 (5.6)	8 (5.4)
Hepatectomy			
No	45 (77.6)	58 (65.2)	103 (70.1)
Yes	13 (22.4)	31 (34.8)	44 (29.9)
Radio-frequency ablation of liver lesions			
No	49 (84.5)	76 (85.4)	125 (85.0)
Yes	9 (15.5)	13 (14.6)	22 (15.0)
Ressection of lung lesions			
No	56 (96.6)	82 (92.1)	138 (93.9)
Yes	2 (3.4)	7 (7.9)	9 (6.1)
Peritonectomy			
No	55 (94.8)	87 (97.8)	142 (96.6)
Yes	3 (5.2)	2 (2.2)	5 (3.4)

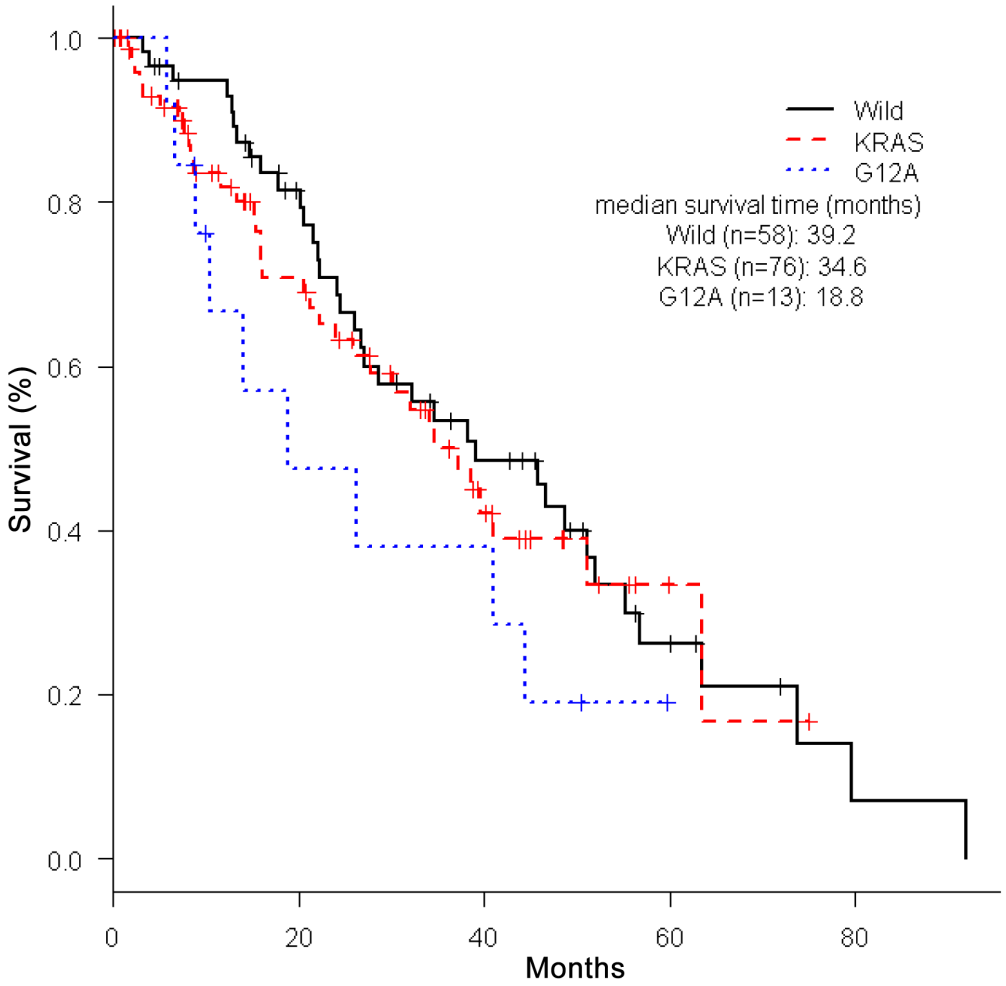
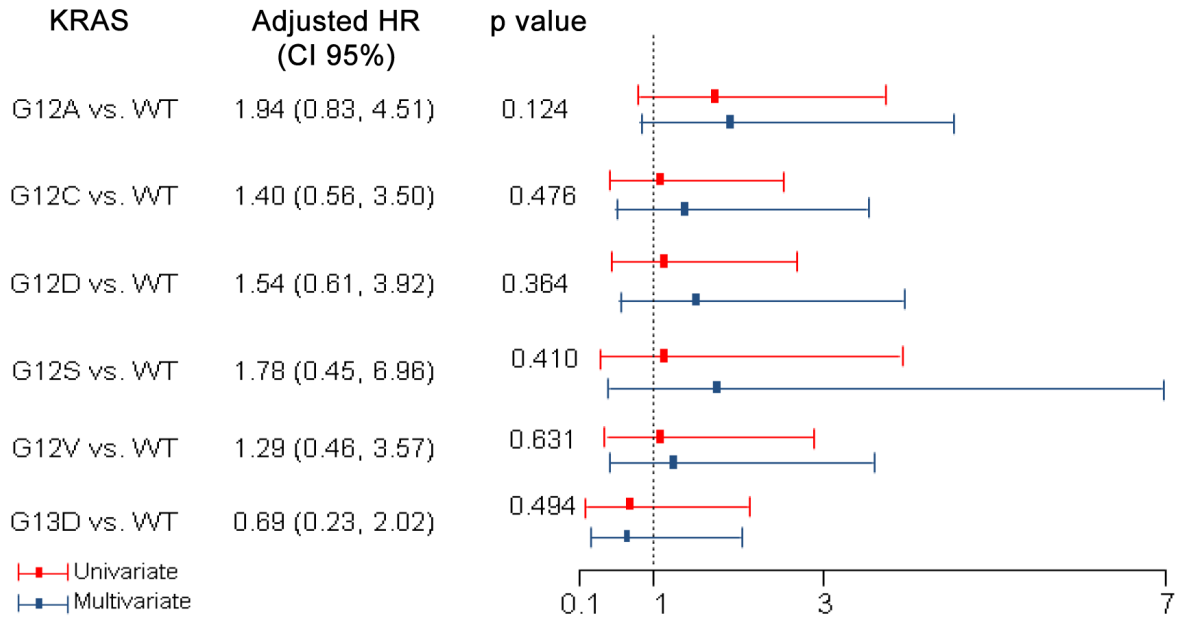


Figure 2. Overall survival according to RAS mutation. Wild: RAS wild type, KRAS: presence of a KRAS mutation, G12A: presence of KRAS G12A mutation. Log rank, p:0.2.



Supplementary Figure 1. Forest plot of overall survival according to subgroups of mutation. This Figure demonstrates the results of investigation of mutations compared to wild type (WT) RAS by Cox proportional hazard models. In both simple (red line) and multiple (blue line) models we had no statistically significant association.

Supplementary Table 3. Univariate Cox regression analysis

	n	*Hazard ratio*	*95% Confidence interval*		*p value*
			Lower limit	*Upper limit*	
Side					
Right	44				
Left	107	0.905	0.561	1.458	0.681
Liver metastasis					
No	52				
Yes	99	1.679	1.026	2.748	**0.039**
Carcinomatosis metastasis					
No	109				
Yes	42	0.941	0.566	1.564	0.814
Lymph nodes metastasis					
No	103				
Yes	48	1.059	0.665	1.686	0.810
Bone metastasis					
No	143				
Yes	8	2.677	1.149	6.234	**0.022**
Lung metastasis					
No	113				
Yes	38	1.178	0.719	1.931	0.516
ECOG PS					
0	52				
1	85	1.040	0.626	1.726	0.880
2	11	2.230	0.954	5.216	0.064
3	3	24.851	6.644	92.943	**<0.001**
Smoking					
No	125				
Yes	26	1.548	0.921	2.603	0.099
Alcohol consumption					
No	133				
Yes	18	0.789	0.392	1.588	0.507
Cardiovascular comorbidities					
No	126				
Yes	25	0.826	0.448	1.525	0.541
Diabetes					
No	140				
Yes	11	2.380	1.219	4.646	**0.011**
Age (years)					
≤ 65	95				
> 65	56	2.058	1.323	3.201	**0.001**
Chemotherapy					
No	2				
Yes	149	0.056	0.013	0.247	**<0.001**
Bevacizumab					
No	63				
Yes	88	0.927	0.591	1.453	0.740
Hepatectomy					
No	106				
Yes	45	0.730	0.439	1.214	0.225

Bold numbers denote statistical significance

type), which was marginally significant (multivariate HR 1.94; 95% CI 0.83-4.51; p=0.12).

Our database represents a contemporary cohort, with a median survival of 34.9 months and a prevalence of KRAS mutations of 58.9%, which is consistent with the literature [8,10-12]. Regarding the individual RAS alterations, comparison with other series is challenging, because many did not include KRAS Exons 3 and 4 or NRAS mutations [3,8,13], but found a relatively higher prevalence of G12A and G12C mutations [8,9].

The exact mechanism leading to RAS mutations has not been completely elucidated, but includes both genetic and epigenetic alterations [14]. Also, the exact impact of each mutation in the function of the RAS protein is unclear. For instance, KRAS G12V alterations have been shown to induce proliferation in endodermal stem cells, while NRAS alterations have no such an impact [14, 15].

Besides RAS wild-type patients are candidates for additional therapies (anti-EGFR), RAS alterations are associated with worse survival [16,17], even in contemporary studies using multi-drug regimens such as FOLFOXIRI [18]. This is consistent with our finding that patients with RAS alterations had shorter survival (34.6 vs 39.2 months).

Other groups have looked at the impact of specific RAS mutations on survival, with conflicting results. In a pooled analysis of patients enrolled in AIO clinical trials, G12C and G13D were associated with worse outcomes [8]; in a retrospective series of patients undergoing hepatectomy for liver metastasis at Johns Hopkins University, G12C was also a marker of shorter survival [9] and in a third series in Italy the same was found for G12D mutations [19].

In our series, G12A alterations were associated with a shorter overall survival (18.8 months vs 34.6

Supplementary Table 4. Comparison between wild and mutated types

	n	*Hazard ratio*	*95% Confidence interval*		*p value*
			Lower limit	*Upper limit*	
Type of mutation					
Wild	58				0.829
G12A	13	1.762	0.840	3.695	0.134
G12C	17	1.117	0.492	2.533	0.792
G12D	18	1.171	0.513	2.670	0.708
G12S	5	1.183	0.360	3.882	0.782
G12V	13	1.122	0.436	2.888	0.811
G13D	9	0.757	0.268	2.138	0.599
Type of mutation					
MT	80				
G13D	9	0.649	0.228	1.845	0.418
Type of mutation					
MT	76				
G12A	13	1.519	0.724	3.185	0.269
Type of mutation					
MT	72				
G12C	17	0.880	0.390	1.982	0.757
Type of mutation					
MT	71				
G12D	18	0.920	0.407	2.078	0.840
Type of mutation					
MT	84				
G12S	5	0.954	0.283	3.214	0.939
Type of mutation					
MT	76				
G12V	13	0.914	0.358	2.329	0.850

Supplementary Table 5. Cox regression analysis accounting for interaction with tumor side

	Hazard ratio	*95% Confidence interval*		*p value*
		Lower limit	*Upper limit*	
Type of mutation				
Wild				
KRAS	0.868	0.385	1.958	0.733
Side				
Right				
Left	0.699	0.350	1.398	0.312
Interaction (KRAS * Left)	1.666	0.617	4.502	0.314
Type of mutation				
Wild				
G12	1.020	0.426	2.446	0.964
Side				
Right				
Left	0.678	0.337	1.366	0.277
Interaction (G12 * Left)	1.406	0.478	4.138	0.536
Type of mutation				
G13D				
G12	2.932	0.364	23.615	0.312
Side				
Right				
Left	2.701	0.274	26.648	0.395
Interaction (G12 * Left)	0.332	0.029	3.844	0.378
Type of mutation				
Wild				
G13D	0.312	0.041	2.375	0.261
Side				
Right				
Left	0.703	0.352	1.406	0.320
Interaction (G13D* Left)	4.029	0.377	43.105	0.249
Type of mutation				
MT				
G13D	0.333	0.042	2.642	0.298
Side				
Right				
Left	0.942	0.441	2.011	0.878
Interaction (G13D* Left)	2.849	0.252	32.252	0.398
Type of mutation				
MT				
G12C	1.029	0.217	4.885	0.971
Side				
Right				
Left	1.169	0.528	2.585	0.701
Interaction (G12C* Left)	0.812	0.131	5.053	0.823
Type of mutation				
MT				
G12D	1.110	0.234	5.275	0.895
Side				
Right				
Left	1.177	0.533	2.597	0.687
Interaction (G12D* Left)	0.780	0.126	4.827	0.789
Type of mutation				
MT				
G12S	0.486	0.055	4.293	0.516
Side				
Right				
Left	1.007	0.478	2.123	0.984
Interaction (G12S * Left)	3.341	0.242	46.166	0.368
Type of mutation				
MT				
G12V	0.870	0.109	6.953	0.895
Side				
Right				
Left	1.122	0.529	2.380	0.764
Interaction (G12V * Left)	1.061	0.103	10.964	0.960

*shows interaction

months for other RAS alterations). In a pooled analysis from 3 randomized studies, Peeters et al. [20] also showed that, among patients receiving exclusive supportive care, those with G12A mutations had worse survivals. The same group also showed that while adding Panitumumab to patients with RAS alterations had no impact on survival, adding it to patients with G12A mutations was detrimental. Furthermore, another study also indicated that G12A and G12V KRAS mutations were prognostic biomarkers for inferior progression-free survival and overall survival in patients treated with bevacizumab [21].

In our study, there was no clinical or anatomical condition that could characterize or differentiate patients with tumors with G12A mutation from the other cases. More studies are needed to understand the biological action of these mutations in the function of the RAS protein [14].

Conclusion

Patients with metastatic colorectal cancer who have RAS mutations have shorter overall survival. Regarding the impact of specific KRAS alterations, G12A mutations have a worse prognosis.

Conflict of interests

The authors declare no conflict of interests.

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