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ORIGINAL RESEARCH

Prognostic value of specific KRAS mutations in patients with colorectal peritoneal metastases

M. Tonello[1], D. Baratti[2], P. Sammartino[3], A. Di Giorgio[4], M. Robella[5], C. Sassaroli[6], M. Framarini[7], M. Valle[8], A. Macrì[9], L. Graziosi[10], F. Coccolini[11,12], P. V. Lippolis[13], R. Gelmini[14], M. Deraco[2], D. Biacchi[3], M. Aulicino[4], M. Vaira[5], S. De Franciscis[15], F. D'Acapito[7], F. Carboni[8], E. Milone[9], A. Donini[10], P. Fugazzola[16], P. Faviana[17], L. Sorrentino[14], E. Pizzolato[1], C. Cenzi[18], P. Del Bianco[18] & A. Sommariva[1]*

[1]Unit of Surgical Oncology of the Esophagus and Digestive Tract, Veneto Institute of Oncology IOV-IRCCS, Padua; [2]Peritoneal Surface Malignancy Unit, Department of Surgery, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan; [3]Cytoreductive Surgery and HIPEC Unit, Department of Surgery 'Pietro Valdoni', Sapienza University of Rome, Rome; [4]Surgical Unit of Peritoneum and Retroperitoneum, Fondazione Policlinico Universitario A. Gemelli, Rome; [5]Surgical Oncology Unit, Candiolo Cancer Institute, FPO—IRCCS, Candiolo, Turin; [6]Integrated Medical Surgical Research in Peritoneal Surface Malignancy, Abdominal Oncology Department, Istituto Nazionale per lo Studio e la Cura dei Tumori Fondazione Pascale IRCCS, Naples; [7]General and Oncologic Department of Surgery, Morgagni—Pierantoni Hospital, AUSL Romagna, Forlì; [8]Peritoneal Tumours Unit, IRCCS, Regina Elena Cancer Institute, Rome; [9]Peritoneal and Retroperitonel Surgical Unit—University Hospital 'G. Martino' Messina; [10]General and Emergency Surgery Department, University of Perugia, Santa Maria Della Misericordia Hospital, Perugia; [11]General Emergency and Trauma Surgery, Bufalini Hospital, Cesena; [12]General Emergency and Trauma Surgery, Pisa University Hospital, Pisa; [13]General and Peritoneal Surgery, Department of Surgery, Hospital University Pisa (AOUP), Pisa; [14]General and Oncological Surgery Unit, AOU of Modena University of Modena and Reggio Emilia; [15]Colorectal Surgical Oncology, Abdominal Oncology Department, Istituto Nazionale per lo Studio e la Cura dei Tumori Fondazione Pascale IRCCS, Naples; [16]General surgery, Fondazione IRCCS Policlinico San Matteo, Pavia; [17]Department of Surgical, Medical, Molecular Pathology and Critical Area, University of Pisa, Pisa; [18]Clinical Research Unit, Veneto Institute of Oncology IOV-IRCCS, Padua, Italy

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Background: There is little evidence on *KRAS* mutational profiles in colorectal cancer (CRC) peritoneal metastases (PM). This study aims to determine the prevalence of specific KRAS mutations and their prognostic value in a homogeneous cohort of patients with isolated CRC PM treated with cytoreductive surgery and hyperthermic intraperitoneal chemotherapy.

Materials and methods: Data were collected from 13 Italian centers, gathered in a collaborative group of the Italian Society of Surgical Oncology. *KRAS* mutation subtypes have been correlated with clinical and pathological characteristics and survival [overall survival (OS), local (peritoneal) disease-free survival (LDFS) and disease-free survival (DFS)].

Results: *KRAS* mutations occurred in 172 patients (47.5%) out of the 362 analyzed. Two different prognostic groups of *KRAS* mutation subtypes were identified: $KRAS^{MUT1}$ (G12R, G13A, G13C, G13V, Q61H, K117N, A146V), median OS > 120 months and $KRAS^{MUT2}$ (G12A, G12C, G12D, G12S, G12V, G13D, A59E, A59V, A146T), OS: 31.2 months. $KRAS^{MUT2}$ mutations mainly occurred in the P-loop region ($P < 0.001$) with decreased guanosine triphosphate (GTP) hydrolysis activity ($P < 0.001$) and were more frequently related to size ($P < 0.001$) and polarity change ($P < 0.001$) of the substituted amino acid (AA). When $KRAS^{MUT1}$ and $KRAS^{MUT2}$ were combined with other known prognostic factors (peritoneal cancer index, completeness of cytoreduction score, grading, signet ring cell, N status) in multivariate analysis, $KRAS^{MUT1}$ showed a similar survival rate to $KRAS^{WT}$ patients, whereas $KRAS^{MUT2}$ was independently associated with poorer prognosis (hazard ratios: OS 2.1, $P < 0.001$; DFS 1.9, $P < 0.001$; LDFS 2.5, $P < 0.0001$).

Conclusions: In patients with CRC PM, different KRAS mutation subgroups can be determined according to specific codon substitution, with some mutations ($KRAS^{MUT1}$) that could have a similar prognosis to wild-type patients. These findings should be further investigated in larger series.

Key words: peritoneal metastases (PM), cytoreductive surgery (CRS), HIPEC, KRAS, colorectal cancer (CRC)

INTRODUCTION

Peritoneal metastases (PM) of colorectal cancer (CRC) are the second most common site of dissemination with the most reduced life expectancy compared to other stage IV patients.[1] The main treatment for CRC PM relies on systemic chemotherapy to control disease progression.

*Correspondence to: Dr Antonio Sommariva, Unit of Surgical Oncology of Digestive Tract, Veneto Institute of Oncology IOV-IRCCS, Via dei Carpani, 16, 31033 Castelfranco Veneto (TV), Italy. Tel: +39-423421306

E-mail: antonio.sommariva@iov.veneto.it (A. Sommariva).

However, several oncological guidelines suggest cytoreductive surgery (CRS) with or without hyperthermic intraperitoneal chemotherapy (HIPEC) as a valuable option for improving disease control in selected patients when carried out in experienced centers with prolonged survival up to 40-43 months.[2] Nevertheless, the risk of recurrence remains high (up to 40% in the first year),[3] and further therapeutic strategies are needed.

In recent years, novel molecular factors (*KRAS*, *BRAF*, micro-satellite instability markers) have been investigated to improve prognostic stratification and personalize the treatment of metastatic CRC, even in patients with PM.[4] The Kristen RAt Sarcoma (*KRAS*) gene is the most common proto-oncogene mutated in human cancers.[5] KRAS protein is an intra-cytoplasmic membrane-associated guanosine triphosphatase (GTPase) linked to the epidermal growth factor receptor (EGFR) pathway, involved in cell proliferation. Mutations in the amino acid (AA) sequence can constitutively and EGFR-independently activate KRAS, leading to downstream signaling (RAF/MEK/ERK and Pi3K pathways) and pathological cell proliferation.[6] *KRAS* mutations have been found in ∼40% of CRC patients (all stages), with the majority (90%) of cases in codons 12 and 13.[7] Pivotal studies on resistance to EGFR blockade in patients with *KRAS* mutation opened the way to personalized chemotherapy.[8] Following this principle, in recent years, different biological behaviors have been described for specific codon mutations with therapeutic implications, for example, G12C KRAS mutation.[9] Moreover, *KRAS* mutation has been related to poorer prognosis in several large series of isolated PM patients treated with CRS-HIPEC,[10-13] and its prognostic role—combined with other clinical and pathological factors—is under investigation for improving the selection of PM patients for surgery. However, at present, the impact of specific codon mutation on prognosis is under-investigated in peritoneal-only stage IV CRC patients treated with radical intent.[14]

This multicentric study aims to describe the distribution and clinical-pathological correlation of specific *KRAS* codon mutations, analyzing the survival outcome in a homogenous group of CRC PM patients treated with radical surgery and HIPEC.

MATERIALS AND METHODS

Study design and patients

Patient data were collected from 13 Italian centers with PM expertise, gathered in a collaborative group of the Italian Society of Surgical Oncology (SICO). The study was approved by the Ethics Committee of the Veneto Institute of Oncology IOV-IRCCS of Padua (No 194/2019). All enrolled patients were treated according to national guidelines following multidisciplinary discussion. Surgical, preoperative and post-operative treatments have been reported previously.[13] Data on *KRAS*, *BRAF* and *NRAS* mutations were collected from each center and only patients with known AA substitution in the *KRAS* gene were included in the study. *KRAS* wild-type patients with *BRAF* and/or *NRAS* mutations and patients with double *KRAS* mutations were excluded from the analysis.

Molecular evaluation

KRAS mutation was determined at each participating center according to internal protocols for clinical purposes through forward and reverse sequencing of amplified tumor DNA. Depending on the enrollment period: before 2010, the Sanger technique was used; in the period 2010-2015 PyroSequencing; whereas in more recent cases, RT-PCR was the most frequently adopted method.[13]

In addition to clinical and pathological variables, data on specific *KRAS* mutations were collected. Amino acidic (AA) substitutions were evaluated using dimensional and physicochemical criteria: (i) dimensional comparison verified whether the new AA was the same size as the corresponding wild-type KRAS protein, grouping small and very small AA (60-117 Å) versus medium, large and very large (138-228 Å); (ii) considering AA polarity, KRAS mutations were classified according to four tiers (hydrophobic, hydrophilic uncharged, hydrophilic negatively charged/acidic and hydrophilic positively charged/basic); (iii) functional analysis of mutated KRAS was based on the position of structural domains (P-loop, switch I or II region); (iv) furthermore, according to the classification by Johnson et al., KRAS mutants were grouped into three functional classes depending on whether the specific KRAS mutation decreased GTP hydrolysis, enhanced nucleotide exchange, or presented hybrid biochemical properties.[5]

Statistical analysis

KRAS mutations were classified into two tiers ($KRAS^{MUT1}$ and $KRAS^{MUT2}$) by maximizing the discriminative ability of the Cox model through the recursive partitioning algorithm of the part package. Results were displayed as hazard ratios (HRs) with 95% confidence interval (CI). These classes were also tested for disease-free survival (DFS), local (peritoneal) DFS (LDFS) and compared to clinical and pathological variables [primary tumor sidedness and nodal status, PM disease extension, completeness of cytoreduction (CC), age, grading, presence of signet ring cells (SRC)]. Continuous variables were summarized using median and interquartile range, and their distributions among groups were compared using the Kruskal—Wallis test. Categorical variables were summarized using counts and percentages and compared using the χ^2 or Fisher's exact test, as appropriate. The nonparametric Kaplan—Meier method was used to estimate the survival probabilities, and the median time was provided along with the corresponding 95% CI estimated according to the Brookmeyer—Crowley method. The association of clinical characteristics with survival was investigated using multiple Cox proportional hazards regression models. No deviation from the proportional hazards assumption was found by the Grambsch and Therneau test statistic. Clinical factors incorporated in the models included all significant variables with significance at 10% in the univariate analysis.

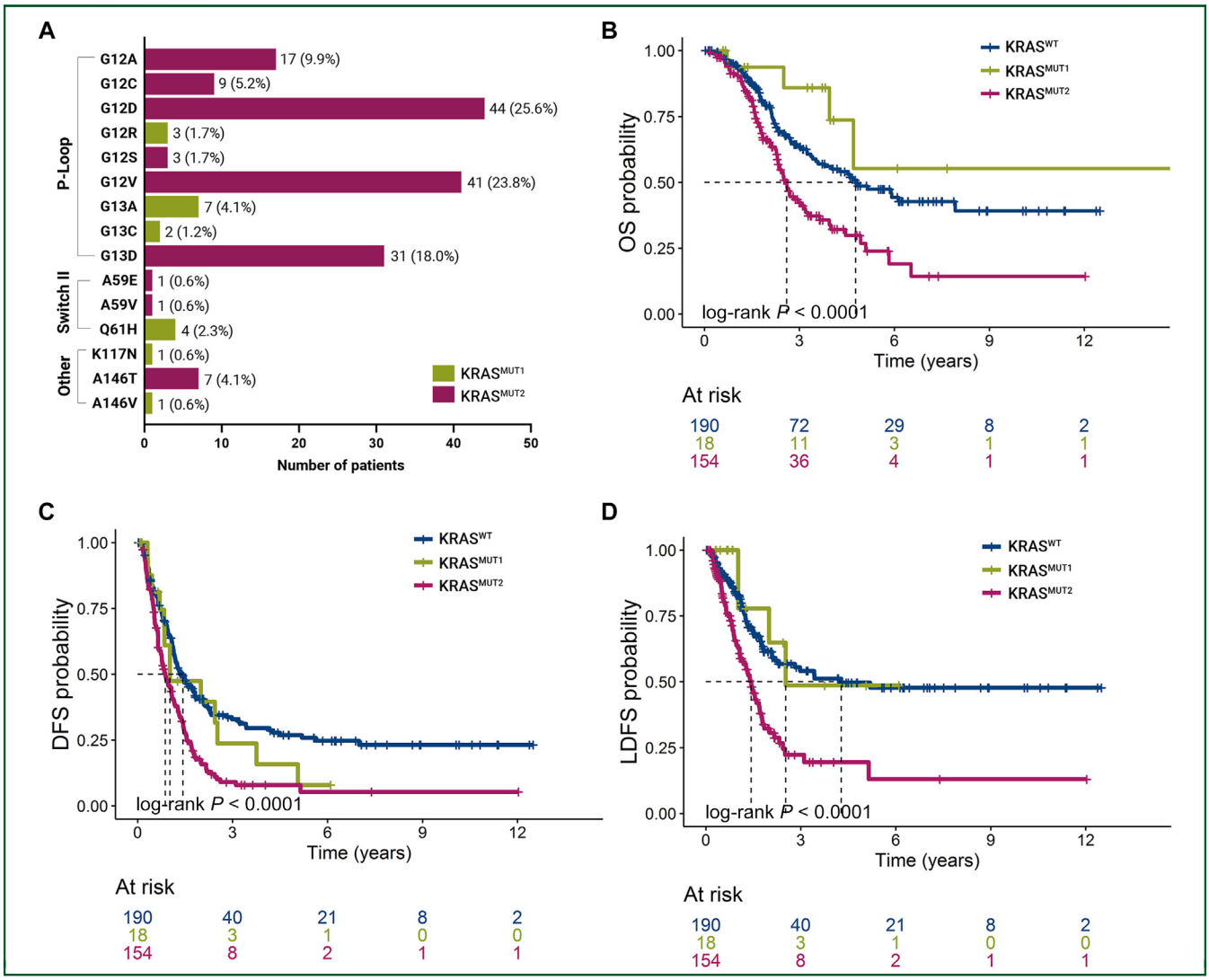

Figure 1. Frequency of KRAS mutations and survival analysis according to KRAS class. (A) Frequency of KRAS-specific mutations and classification, with mutated protein domain. (B) OS. (C) DFS. (D) LDFS.
DFS, disease-free survival; LDFS, local (peritoneal) disease-free survival; OS, overall survival.

Overall survival (OS), DFS and LDFS were the primary endpoints assessed. OS was defined as the time from CRS-HIPEC to the date of death due to any cause; DFS was the time from CRS-HIPEC to the date of a local or distant relapse or death; and LDFS was the time from CRS-HIPEC to the date of a local relapse. The last date of observation was used to censor patients who did not develop an event during the study period. All statistical tests were two-sided; a P value <0.05 was considered statistically significant. Statistical analyses were carried out using the RStudio (RStudio: Integrated Development for R. RStudio Inc., Boston, MA).

RESULTS

The study group comprised 362 patients with available and complete data, selected from 447 cases gathered in a SICO collaborative database. Median year of CRS-HIPEC procedure was 2015 (interquartile range 2013-2018) and 90% of gene mutation analyses were made with RT-PCR or Pyro-Sequencing. Of the remaining 362 patients, 172 (47.5%) were *KRAS* mutated. The most frequent mutations occurred in the P-loop region (codons 12 and 13, 91.3% of cases); G12D, G12V and G13D were the most frequent AA substitutions (Figure 1A).

$KRAS^{MUT1}$ affected 18 patients (10.5% of mutated cases) with G12R, G13A, G13C, G13V, Q61H, K117N and A146V substitutions; the $KRAS^{MUT1}$ patients had very long OS (>120 months, 95% CI not estimable). $KRAS^{MUT2}$ included most mutated cases (154 patients, 89.5%) with G12A, G12C, G12D, G12S, G12V, G13D, A59E, A59V and A146T substitutions; the median OS for $KRAS^{MUT2}$ patients was 31.2 months (95% CI 27.6-37.9 months). The $KRAS^{WT}$ patients had a median OS of 57.3 months (95% CI 42.3-95.0 months; log-rank $P < 0.0001$) (Figure 1B). A similar stratification was also observed in DFS between the two class of patients (median DFS: 12.3 months for $KRAS^{MUT1}$, 10.5 months for $KRAS^{MUT2}$, $P = 0.0001$) and in LDFS (median LDFS: 30.3 months for $KRAS^{MUT1}$, 17.2 months for $KRAS^{MUT2}$, $P < 0.001$) (Figure 1B and C).

Table 1. Patient characteristics

	KRAS^{WT} (n = 190)	KRAS^{MUT1} (n = 18)	KRAS^{MUT2} (n = 154)	Total (N = 362)	P value (WT versus MUT)	P value (MUT1 versus 2)
Age, years median (IQR)	59.00 (48.75-66.00)	56.50 (51.25-62.00)	57.00 (48.00-64.50)	59.00 (48.00-65.00)	0.4700	0.9610
Gender, n (%)						
Male	87 (45.8)	8 (44.4)	77 (50.0)	172 (47.5)	0.4900	0.6560
Female	103 (54.2)	10 (55.6)	77 (50.0)	190 (52.5)		
Primary tumor, n (%)						
Right colon	71 (37.6)	8 (44.4)	73 (47.4)	152 (42.1)	**0.0170**	0.8130
Left colon	83 (43.9)	9 (50.0)	67 (43.5)	159 (44.0)		
Rectum	35 (18.5)	1 (5.6)	14 (9.1)	50 (13.9)		
N-Miss	1	0	0	1		
Grading, n (%)						
G1	11 (6.2)	2 (12.5)	12 (8.2)	25 (7.4)	0.4890	0.3660
G2	83 (46.9)	10 (62.5)	71 (48.6)	164 (48.4)		
G3	83 (46.9)	4 (25.0)	63 (43.2)	150 (44.2)		
N-Miss	13	2	8	23		
Mucinous, n (%)						
No	137 (72.9)	8 (44.4)	106 (68.8)	251 (69.7)	0.1740	**0.0380**
Yes	51 (27.1)	10 (55.6)	48 (31.2)	109 (30.3)		
CC grade, n (%)						
0	154 (81.1)	16 (88.9)	130 (84.4)	300 (82.9)	0.3340	0.6160
1	36 (18.9)	2 (11.1)	24 (15.6)	62 (17.1)		
PCI, n (%)						
≤15	150 (79.8)	16 (88.9)	125 (81.2)	291 (80.8)	0.5980	0.4200
>15	38 (20.2)	2 (11.1)	29 (18.8%)	69 (19.2)		
N-Miss	2	0	0	2		
SRC histology, n (%)						
No	179 (94.2)	18 (100.0)	154 (100.0)	351 (97.0)	**0.0010**	-
Yes	11 (5.8)	0 (0.0)	0 (0.0)	11 (3.0)		
Nodal status, n (%)						
N0	59 (32.1)	7 (46.7)	40 (26.3)	106 (30.2)	0.4240	0.0940
N+	125 (67.9)	8 (53.3)	112 (73.7)	245 (69.8)		
N-Miss	6	3	2	11		
T, n (%)						
2	4 (2.2)	0 (0.0)	3 (1.9)	7 (2.0)	0.1140	0.6490
3	76 (41.3)	11 (61.1)	79 (51.3)	166 (46.6)		
4	104 (56.5)	7 (38.9)	72 (46.8)	183 (51.4)		
N-Miss	6	0	0	6		

Bold values are significant ($P < 0.05$).
KRAS^{MUT1} mutations: A146V, G12R, G13A, G13C, Q61H, K117N.
KRAS^{MUT2} mutations: A146T, A59E, A59V, G12A, G12C, G12D, G12S, G12V, G13D.
CC, completeness of Cytoreduction; IQR, interquartile range; PCI, peritoneal cancer index; SRC, signet ring cells; WT, wild-type.

Comparing the two classes of mutations (*KRAS*^{MUT1}/*KRAS*^{MUT2}), there were no differences in the main clinical and pathological variables (age, gender, grading, tumor T stage, peritoneal disease extension and CC) between *KRAS*^{MUT1} and *KRAS*^{MUT2}, except for a higher prevalence of mucinous histotype in *KRAS*^{MUT1} (55.6% versus 31.2%, $P = 0.038$) (Table 1).

At univariate survival analysis, *KRAS*^{MUT1}/*KRAS*^{MUT2}, tumor sidedness, grading, peritoneal cancer index (PCI), SRC, CC and nodal status were correlated with OS. *KRAS*^{MUT} classification, PCI, CC score and nodal status were correlated with DFS and LDFS (Table 2).

Multivariable analysis confirmed the prognostic role of already-known factors (PCI > 15: HR 2.0; CC score 1: HR 2.6; grading G3: HR 2.6, N+: HR 1.8) and further demonstrated that *KRAS*^{MUT1} had similar OS to *KRAS*^{WT} (HR 0.4, $P = 0.163$), whereas *KRAS*^{MUT2} had a worse prognosis (HR 2.1, $P < 0.001$). Analogous results were obtained for DFS (*KRAS*^{MUT1} HR 1.2, $P = 0.469$; *KRAS*^{MUT2} HR 1.93, $P < 0.001$) and LDFS (*KRAS*^{MUT1} HR 0.9, $P = 0.858$; *KRAS*^{MUT2} HR 2.49, $P < 0.001$) (Table 3).

AA substitutions were analyzed using dimensional and physicochemical criteria (Figure 2A). The dimensional analysis between *KRAS*^{MUT1} and *KRAS*^{MUT2} showed differences in the size of substituted AA compared to wild-type KRAS (size changed in 87% of *KRAS*^{MUT2} versus 13% of *KRAS*^{MUT1}, $P < 0.001$) (Figure 2B). Regarding AA polarity, a negatively charged/acidic AA substitution was more frequent in *KRAS*^{MUT2} patients (49.7% versus 0%, $P < 0.001$). In contrast, a higher number of mutations characterized by positively charged/basic substituted AA was observed in the *KRAS*^{MUT1} class (31.2% versus 0%, $P < 0.001$). A similar prevalence of polar uncharged and nonpolar AA substitution was found in both classes (Figure 2B). Looking at polarity conservation, only 18.8% of *KRAS*^{MUT1} has a polarity change compared to 61.4% of *KRAS*^{MUT2}; $P = 0.01$ (Figure 2B). The functional analysis of mutated KRAS revealed 94.2% of *KRAS*^{MUT2} with mutation in the P-loop region (compared to 66.7% of *KRAS*^{MUT1}) with decreased GTP hydrolysis (74.0%, compared to 16.7% of *KRAS*^{MUT1}); both $P < 0.001$ (Figure 2B).

Table 2. Univariable analysis

	Overall survival			Disease-free survival			Local disease-free survival		
	Median (95%CI)	HR (95% CI)	*P* value	Median (95%CI)	HR (95% CI)	*P* value	Median (95%CI)	HR (95% CI)	*P* value
KRAS mutation									
Wild-type	57.3 (42.3-95.0)	Ref		17.8 (14.1-21.8)	Ref		51.3 (25.1-)	Ref	
MUT1	—	0.57 (0.19-1.31)	0.2033	12.3 (8.4-30.3)	1.33 (0.72-2.25)	0.3372	30.3 (12.3-)	0.87 (0.28-2.01)	0.7637
MUT2	31.2 (27.6-37.9)	1.86 (1.35-2.57)	**0.0001**	10.6 (8.8-13.0)	1.92 (1.49-2.46)	**<0.001**	17.2 (13.0-20.4)	2.33 (1.67-3.27)	**0.0000**
Gender									
Male	51.3 (36.5-78.3)	Ref		14.4 (11.6-16.0)	Ref		24.1 (17.2-)	Ref	
Female	37.9 (30.0-54.3)	1.35 (0.99-1.86)	0.0579	13.5 (11.6-17.6)	1.07 (0.84-1.36)	0.5896	24.0 (20.2-35.8)	1.05 (0.76-1.46)	0.7510
Primary tumor									
Right colon	32.5 (27.6-47.4)	Ref		13.2 (11.2-15.7)	Ref		21.1 (15.7-27.5)	Ref	
Left colon	48.3 (38.9-70.7)	0.70 (0.50-0.97)	**0.0325**	15.3 (12.8-18.5)	0.93 (0.72-1.20)	0.5583	25.1 (20.4-41.2)	0.84 (0.60-1.19)	0.3294
Rectum	95.0 (30.5-)	0.60 (0.33-1.01)	0.0532	13.7 (9.7-25.2)	0.85 (0.57-1.23)	0.3931	62.3 (16.8-)	0.67 (0.37-1.13)	0.1330
Grading									
G1	—	Ref		14.1 (7.2-)	Ref		18.0 (9.7-)	Ref	
G2	54.3 (39.2-73.6)	2.11 (0.97-5.71)	0.0618	13.7 (12.0-15.9)	1.19 (0.73-2.07)	0.4920	35.8 (18.6-)	0.83 (0.46-1.64)	0.5681
G3	31.3 (27.6-40.3)	3.17 (1.46-8.57)	**0.0020**	14.7 (12.5-19.2)	1.20 (0.74-2.10)	0.4742	21.8 (19.2-27.5)	1.02 (0.57-2.02)	0.9395
Mucinous									
No	40.3 (31.9-56.2)	Ref		13.6 (12.2-15.3)	Ref		24.1 (20.5-37.3)	Ref	
Yes	54.3 (35.6-)	0.84 (0.59-1.19)	0.3385	15.7 (10.2-20.0)	0.98 (0.75-1.27)	0.8921	21.9 (17.3-41.1)	1.18 (0.82-1.64)	0.3781
CC grade									
0	56.5 (43.7-71.0)	Ref		15.1 (12.8-17.7)	Ref		30.3 (22.1-62.3)	Ref	
1	22.7 (18.0-26.9)	3.21 (2.25-4.52)	**<0.001**	12.8 (7.7-14.0)	1.70 (1.25-2.28)	**0.0009**	14.4 (12.3-17.6)	2.53 (1.74-3.62)	**<0.001**
PCI									
≤15	55.2 (42.3-70.0)	Ref		15.4 (13.5-18.0)	Ref		29.3 (21.5-51.3)	Ref	
>15	20.9 (15.7-27.6)	3.11 (2.16-4.40)	**<0.001**	9.0 (6.5-12.8)	1.82 (1.35-2.42)	**0.0002**	13.8 (9.3-17.6)	2.45 (1.67-3.51)	**<0.001**
SRC histology									
No	47.3 (36.5-58.2)	Ref		13.7 (12.2-15.7)	Ref		24.0 (20.5-33.8)	Ref	
Yes	27.1 (19.4-)	2.26 (1.04-4.26)	**0.0404**	19.0 (12.5-28.0)	1.08 (0.55-1.91)	0.7984	19.0 (12.8-)	1.41 (0.61-2.73)	0.3875
Nodal status									
N0	95.0 (41.0-)	Ref		18.6 (17.2-26.9)	Ref		61.7 (26.0-)	Ref	
N+	38.4 (31.2-51.3)	1.99 (1.38-2.94)	**0.0001**	12.8 (10.5-14.0)	1.84 (1.40-2.45)	**<0.001**	20.5 (16.0-25.1)	1.82 (1.26-2.68)	**0.0010**

Bold values are significant ($P < 0.05$).
CC, completeness of cytoreduction; CI, confidence interval; HR, hazard ratio; PCI, peritoneal cancer index; SRC, signet ring cells.

DISCUSSION

Colorectal PM are a dismal clinical condition with the worst prognosis as compared to other stage IV non-peritoneal sites.[1] During recent decades, modern chemotherapy regimens and targeted agents have gradually improved the prognosis of patients with CRC PM, reaching a median survival of up to 24 months.[1] In selected cases (isolated PM, limited disease, radical surgery), the combination of systemic therapy with cytoreductive surgery, with possible locoregional treatment (HIPEC), has led to prolonged median survival (up to 42-43 months).[2] However, a significant number of patients who could potentially benefit from surgery continue to be excluded and treated with palliative systemic chemotherapy alone.[15]

Since cytoreductive surgery for CRC PM is a relatively high-morbidity and costly procedure, the selection of patients who can benefit from the treatment is crucial. Referral centers for peritoneal cancer surgery have, over the years, identified established clinical and pathological prognostic factors that can guide the selection pathway, such as peritoneal disease burden (measured as PCI), CC (surgical radicality, such as CC score), nodal status of primary tumor (N), grading (G) and the presence of SRC.[3,16] More recently, oncologists dealing with CRC PM have turned their attention toward molecular prognostic factors (mainly RAS- and RAF-mutated oncogenes and micro-satellite instability markers) that can be applied to personalize treatment and possibly improve patient selection for surgery. In particular, the mutation of the *KRAS* oncogene in CRC is of therapeutic and prognostic relevance because of the impaired response to anti-EGFR-targeted therapy.[17,18] Moreover, despite the contradictory nature of the results reported for early stages,[19] *RAS* mutations have been found to have a negative prognostic role in patients with liver and lung CRC metastases.[20,21] In CRC PM, the mutational status of *KRAS* is reported in several large retrospective studies as a negative independent prognostic factor in patients treated with CRS-HIPEC.[10,12,13] However, two reports have demonstrated similar survival rates in $KRAS^{MUT}$ and $KRAS^{WT}$ peritoneal-only stage IV patients, both with a reduced sample size (110 patients), and one study also included appendiceal adenocarcinoma cases.[22,23] In 2019, *KRAS* mutation was included in a score (BIOSCOPE) developed from a large series of patients, including other prognostic scores (also *BRAF* mutation), recently validated on an Italian patient cohort.[24]

Having established the prognostic value of *KRAS* mutation in CRC PM, our study focused on the incidence and prognostic role of mutational subtypes of the KRAS protein. *KRAS* is a proto-oncogene encoding an intracellular protein

Table 3. Multivariable analysis

	Overall survival		Disease-free survival		Local disease-free survival	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
KRAS mutation						
$KRAS^{WT}$	Ref		Ref		Ref	
$KRAS^{MUT1}$	0.45 (0.09-1.32)	0.1630	1.26 (0.67-2.17)	0.4490	0.92 (0.3-2.14)	0.8578
$KRAS^{MUT2}$	2.14 (1.50-3.07)	**<0.001**	1.94 (1.50-2.51)	**<0.001**	2.49 (1.77-3.52)	**0.0000**
Gender						
Male	Ref		—	—	—	—
Female	1.42 (1.00-2.03)	0.0505	—	—	—	—
Primary tumor						
Right colon	Ref		—	—	—	—
Left colon	0.55 (0.39-0.79)	**0.0013**	—	—	—	—
Rectum	0.72 (0.37-1.29)	0.2801	—	—	—	—
Grading						
G1	Ref		—	—	—	—
G2	1.83 (0.83-4.99)	0.1456	—	—	—	—
G3	2.36 (1.06-6.45)	**0.0335**	—	—	—	—
CC grade						
0	Ref		Ref		Ref	
1	2.63 (1.70-3.99)	**<0.001**	1.43 (1.00-2.04)	0.0564	2.12 (1.34-3.3)	**0.0017**
PCI						
≤15	Ref		Ref		Ref	
>15	2.05 (1.30-3.17)	**0.0023**	1.50 (1.04-2.13)	**0.0306**	1.71 (1.07-2.68)	**0.0252**
SRC histology						
No	Ref		—	—	—	—
Yes	1.47 (0.61-3.11)	0.3620	—	—	—	—
Nodal status						
N0	Ref		Ref		Ref	
N+	1.824 (1.23-2.78)	**0.0027**	1.72 (1.30-2.30)	**<0.001**	1.61 (1.11-2.38)	**0.0114**

Bold values are significant ($P < 0.05$).
CC, completeness of cytoreduction; CI, confidence interval; HR, hazard ratio; PCI, peritoneal cancer index; SRC, signet ring cells.

involved in the signaling of many extracellular stimuli, including growth factors. *KRAS* mutations have been described in roughly 50% of CRC patients and are associated with the alteration of GTPase activity with incongruous activation of RAS/RAF signaling.[7,25] More recently, there has been increasing interest in the biological behavior of different *KRAS* mutations with the hope of identifying potentially targetable mutations, such as the target drug sotorasib, which was approved for clinical use in patients with KRAS G12C mutation.[7,9,25] Besides G12C mutation, differential treatment according to specific *KRAS* mutations has been tested in several clinical trials.[8,26,27] From the

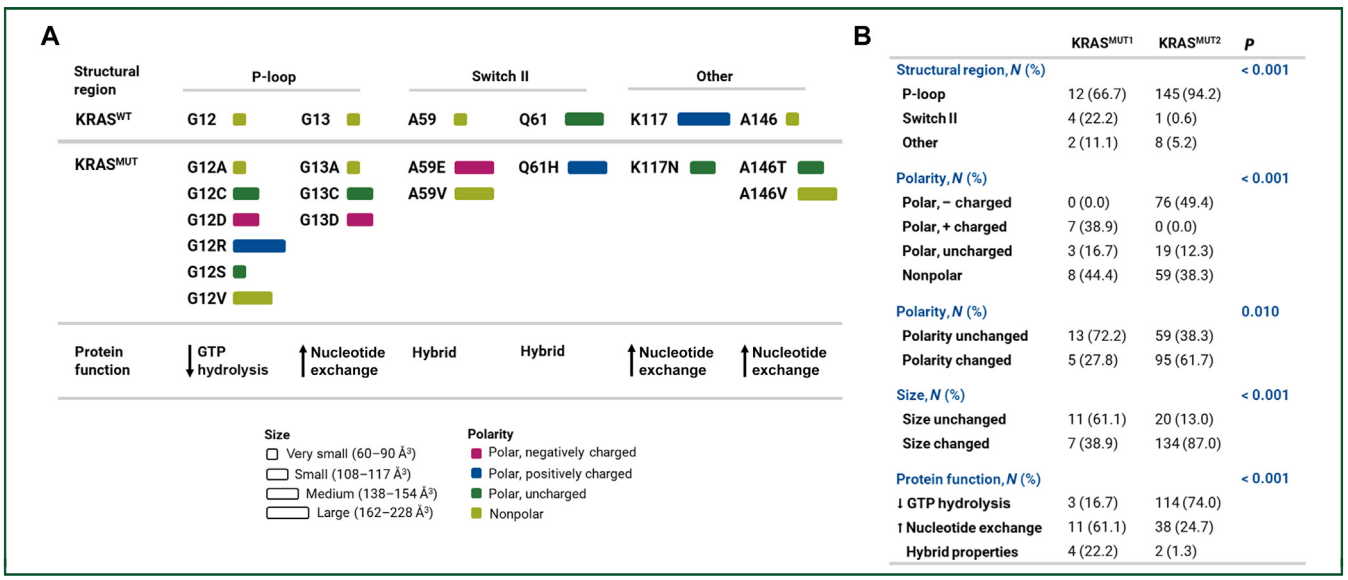

Figure 2. Amino acid characteristics and relation with $KRAS^{MUT}$ class. (A) Summary of protein function, site of mutation and amino acid characteristics (polarity, size). (B) Analysis of relation with $KRAS^{MUT}$ class.
GTP, guanosine triphosphate.

perspective of prognosis, the role of specific mutations (such as G12C or G12S) has been associated with reduced survival in stage IV CRC patients.[28-31] To our knowledge, the only work on PM treated with CRS-HIPEC is of an Australian group that reported the negative prognostic role of G12V compared to other *KRAS* mutations.[14]

To our knowledge, this is the first large series (362 cases) of peritoneal-only CRC stage IV patients treated homogeneously to analyze the relationship between clinical outcome and specific *KRAS* mutations. The two classes of mutated KRAS proteins ($KRAS^{MUT1}$ and $KRAS^{MUT2}$) were clustered considering OS and validated using multivariate analysis with already-known prognostic factors (such as disease extension and surgical radicality). The study showed that a small subgroup of patients with *KRAS* mutation ($KRAS^{MUT1}$) have a similar survival rate to $KRAS^{WT}$ patients ($P = 0.2$). Most *KRAS* patients belong to the $KRAS^{MUT2}$ group with reduced survival (HR 2.1, $P < 0.001$) as compared to $KRAS^{WT}$ patients. Interestingly, some of the mutations in the unfavorable class $KRAS^{MUT2}$ have already been reported as detrimental in stage IV patients (such as G12C, G12V and G12S).[14,28,29] The interesting finding is that a small but significant group of patients with specific *KRAS* mutations have a good prognosis and should be considered the same as $KRAS^{WT}$ patients before CRS-HIPEC.

Different behaviors of *KRAS* subtypes might explain their different prognostic roles, as described in a biochemical study based on HRAS G12V mutation.[32] Different mutations in different sites (exons 12, 13, 61 and 146 are the most commonly involved in KRAS) are associated with different enzyme activity due to modifications of the tertiary structure of the protein (change in polarity, steric hindrance, affinity for substrates and downstream effector proteins).[32,33] For example, RAS G13D-mutated proteins showed decreased hydrolytic activity compared to $KRAS^{WT}$.[34] Q61L KRAS mutation altered the rigidity of the protein structure, reflecting an increased interaction with *RAF* protein and, therefore, the *RAS* signaling activity.[35]

These preclinical results suggest that *KRAS* subtype mutations have specific consequences on protein activity and could be related to the clinical outcome. Our study showed that the two prognostic mutational clusters ($KRAS^{MUT1}$ and $KRAS^{MUT2}$) presented a different pattern of biochemical and physical characteristics based on substituted AAs. $KRAS^{MUT2}$ has a higher degree of polarity change ($P = 0.010$), AA substitution ($P < 0.001$), with mutation occurring in the P-loop region ($P < 0.001$), and decrease in GTP hydrolytic activity ($P < 0.001$), compared to $KRAS^{MUT1}$. This result is in line with previous studies, which showed that mutations clustered in $KRAS^{MUT2}$ seem associated with greater changes to protein structure and function.[32,33]

There are few studies comparing specific *KRAS* mutations and survival rates, including tumors of different origin (pancreas, lung and CRC) and different TNM (tumor—node-metastasis) stages. The vast majority of mutations enclosed in $KRAS^{MUT1}$ in our study are also related to good prognosis in pancreatic, CRC and lung neoplasms, with some contradictory results in a minority of specific mutations (two or three mutations according to different studies).[36-38]

Patients harboring $KRAS^{MUT1}$ have similar survival rates to patients with $KRAS^{WT}$, possibly because changes in KRAS protein have less effect on cell proliferation compared to $KRAS^{MUT2}$ patients. Therefore, $KRAS^{MUT1}$ could be considered silent mutations or, at least, *KRAS* isoforms observed in a cancer-affected population. Schirripa and colleagues proposed a similar classification for *BRAF*, demonstrating that a minority of patients with non-V600E mutations (class III mutations in their study) had similar survival rates to patients with *BRAF* wild-type.[39]

Our results, as previously reported on the *BRAF* gene, show that 'benign' mutations are rarer than detrimental ones ($KRAS^{MUT2}$, BRAF-V600E). Some authors, using an 'epidemiologic and ethological' approach, consider rare mutations diagnosed in a cancer-affected population to be like mutations with a reduced oncogenic capability. In terms of biological sciences, rare characteristics of a population are less represented because of an intrinsic reduced ability to fulfill a specific environmental request (in this case, the ability to induce cell proliferation in a population of CRC patients).[40]

A better understanding of the factors underlying this relationship between biochemical and physical characteristics of mutated KRAS protein is needed and thus requires further investigation. This would pave the way for future progress in this area, such as developing targeted therapies and differential treatment according to specific mutations.

The present study had limitations: it was a retrospective analysis based on a multicentric data collection; $KRAS^{MUT1}$ had a reduced sample size, considering the relative rarity of such mutations. On the other hand, its strengths included: the large overall sample size; complete pathological/molecular data with mutation in the *KRAS* gene only; a long follow-up period; and homogenous treatment in referral centers.

Conclusions

Different *KRAS* mutation subgroups can be determined according to specific codon substitution and some mutations could have similar prognosis to patients with the *KRAS* wild-type. Additional, larger studies are required to define the role of specific *KRAS* mutations in patients affected by colorectal PM treated with CRS-HIPEC.

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DISCLOSURE

The authors have declared no conflicts of interest.

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