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ORIGINAL ARTICLE – PERITONEAL SURFACE MALIGNANCY

Microsatellite and RAS/RAF Mutational Status as Prognostic Factors in Colorectal Peritoneal Metastases Treated with Cytoreductive Surgery and Hyperthermic Intraperitoneal Chemotherapy (HIPEC)

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ABSTRACT

Background. Cytoreductive surgery (CRS) with hyperthermic intraperitoneal chemotherapy (HIPEC) leads to prolonged survival for selected patients with colorectal (CRC) peritoneal metastases (PM). This study aimed to analyze the prognostic role of micro-satellite (MS) status and RAS/RAF mutations for patients treated with CRS.

Methods. Data were collected from 13 Italian centers with PM expertise within a collaborative group of the Italian Society of Surgical Oncology. Clinical and pathologic variables and KRAS/NRAS/BRAF mutational and MS status were correlated with overall survival (OS) and disease-free survival (DFS).

Results. The study enrolled 437 patients treated with CRS-HIPEC. The median OS was 42.3 months [95% confidence interval (CI), 33.4–51.2 months], and the median DFS was 13.6 months (95% CI, 12.3–14.9 months).

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The local (peritoneal) DFS was 20.5 months (95% CI, 16.4–24.6 months). In addition to the known clinical factors, KRAS mutations (p = 0.005), BRAF mutations (p = 0.01), and MS status (p = 0.04) were related to survival. The KRAS- and BRAF-mutated patients had a shorter survival than the wild-type (WT) patients (5-year OS, 29.4% and 26.8% vs 51.5%, respectively). The patients with micro-satellite instability (MSI) had a longer survival than the patients with micro-satellite stability (MSS) (5-year OS, 58.3% vs 36.7%). The MSI/WT patients had the best prognosis. The MSS/WT and MSI/mutated patients had similar survivals, whereas the MSS/mutated patients showed the worst prognosis (5-year OS, 70.6%, 48.1%, 23.4%; p = 0.0001). In the multivariable analysis, OS was related to the Peritoneal Cancer Index [hazard ratio (HR), 1.05 per point], completeness of cytoreduction (CC) score (HR, 2.8), N status (HR, 1.6), signet-ring (HR, 2.4), MSI/WT (HR, 0.5), and MSS/WT-MSI/mutation (HR, 0.4). Similar results were obtained for DFS.

Conclusion. For patients affected by CRC-PM who are eligible for CRS, clinical and pathologic criteria need to be integrated with molecular features (KRAS/BRAF mutation). Micro-satellite status should be strongly considered because MSI confers a survival advantage over MSS, even for mutated patients.

Colorectal cancer (CRC) represents the third most common neoplasm and the third leading cause of death among the population of developed countries.[1] For untreated CRC metastatic patients, the median survival is shorter than 9 months, whereas with systemic chemotherapy it can be as long as 24 months.[2,3] Peritoneal metastases (PM) from CRC are estimated to develop in about 19% of patients after radical surgery and are the cause of death in more than half of CRC patients.[3–5]

Patients affected by CRC PM and treated with standard systemic chemotherapy show a shorter median survival, estimated to be about 16.3 months in isolated PM, compared with CRC patients affected at other metastatic sites (lung, liver, lymph nodes).[4]

The introduction of cytoreductive surgery (CRS) with hyperthermic intraperitoneal chemotherapy (HIPEC) leads to prolonged survival (up to 45 months) for a selected subgroup of patients.[6–9] Selection of the patients who might benefit from CRS-HIPEC procedure has always been considered crucial. Besides the extent of PM [measured as the Peritoneal Cancer Index (PCI)], and residual disease after surgery [completeness of cytoreduction (CC)], lymph node status, tumor differentiation, and signet ring histology are recognized as important risk factors in the selection process for CRS-HIPEC.[10]

Mutations in RAS and RAF kinase genes, present in up to 50% (KRAS) and up to 10% (BRAF) of CRC,[11,12] are related to impaired prognosis for liver and lung metastatic patients.[13] Recent studies have identified mutations of prognostic value in RAS and RAF genes, making their determination crucial in the selection process for surgery.[14–17] In parallel, a defective mismatch repair system (dMMR) and micro-satellite instability (MSI), found in 10% to 15% of CRC patients, are gaining an emerging role in the selection of patients who may potentially benefit from immunotherapy with checkpoint inhibitors.[18,19] However, available evidence for the prognostic role of micro-satellite (MS) status in an advanced CRC stage, especially for patients with PM, remains scant and discordant.[20–24]

This study aimed mainly to analyze the prognostic impact of MS status and RAS/RAF gene mutations in CRC patients with PM treated according to a standardized protocol of CRS.

METHODS

Data Collection and Patients

Data were retrospectively collected from 13 Italian centers with peritoneal malignancies expertise by members of a collaborative group (Peritoneal Surface Malignancies Oncoteam) in the Italian Society of Surgical Oncology (SICO). All the participating centers are referral centers certified by SICO for the surgical treatment of patients with peritoneal metastases. The study was approved by the ethics committee of the lead center (Veneto Institute of Oncology IOV Padua, nr. 194/2019).

All the patients, treated according to the national guidelines for metastatic CRC, were discussed and selected for CRS-HIPEC at a multidisciplinary board meeting. All the selected patients underwent a preoperative thoracoabdominal CT scan, and when necessary, 18-fluorodeoxyglucose-positron emission tomography (^{18}FDG-PET). Cytoreductive surgery was performed according to a standardized operative procedure with the aim of eradicating all visible tumor nodules, performing en bloc resection of the affected organ or organs, and stripping the parietal peritoneum if involved.[25]

Residual disease after CRS was classified according to the grade of cytoreduction [completeness of cytoreduction (CC) grading system].[26] Tumor extent was scored at the time of laparotomy using the PCI (range, 1–39 in 13 abdominal regions).[26] Only patients with residual disease less than 2.5 mm in size (CC0 or CC1) who underwent HIPEC were considered for analysis. At the end of CRS, HIPEC was performed by a circuit connected to a pump,

supplied with a heat exchanger, using mitomycin C, oxaliplatin, or cisplatin according to center-specific protocols. Clinical and pathologic data [including patient demographics, perioperative systemic treatments, tumor-node-metastasis (TNM) staging, histology, grading, RAS/RAS and MS status, follow-up status, site and date of recurrence, date of death] were retrieved from referring hospital records.

RAS/RAF and Microsatellite Status Analysis

Analysis was performed at each center according to internal protocols for clinical purposes. In general, mutational analysis was performed on extracted tumoral DNA (in the majority of cases from primary tumor considering the high rate of concordance with PM)[27,28] through forward and reverse sequencing of amplified tumor DNA.[29] Cases analyzed before 2010 were determined predominantly by the Sanger technique,[30,31] and in the period between 2010 and 2015, by the pyrosequencing technique,[32,33] whereas in more recent cases, reverse transcription-polymerase chain reaction (RT-PCR) was the most frequently used method.[34,35] All KRAS, NRAS, and BRAF mutations were classified as binomial [mutated vs wild-type (WT)] or categorical variables (codon site and type of mutation) according to reported results. Analysis of MS status was performed with direct DNA testing on a specific gene panel for older cases (before 2015),[36,37] whereas in more recent cases, immunohistochemistry (IHC) assay of four proteins (MLH1, MSH2, MSH6 and PMS2)[38,39] and PCR analysis of mononucleotide repeat microsatellite markers were used for confirmation in doubtful cases.[40]

Statistical Analysis

In general, continuous variables were described using median and interquartile range (IQR). Categorical variables were summarized using frequency counts and percentages. Proportions were calculated on the number of patients with available data. The median follow-up time was based on the reverse Kaplan-Meier estimator. Due to the exploratory nature of the study, there was no formal hypothesis or power sample size calculation. The number of subjects was determined by the number of eligible patients from the participant clinical centers.

Overall survival (OS) was defined as the time from HIPEC to the date of death due to any cause, and disease-free survival (DFS) was defined as the time from HIPEC to the date of local or distant relapse or death. Patients without a documented event were censored at the last known date. Survival curves were estimated with the non-parametric Kaplan-Meier method, and comparisons among strata were performed using the log-rank test. Survival rates at 5 years and the corresponding 95% confidence intervals (CIs) were estimated from the Kaplan-Meier analysis. The 95% CI for the median survival was calculated according to Brookmeyer and Crowley.

The independent role of each covariate in predicting survival was verified in a multivariable Cox proportional hazard model with Efron's method of tie-handling, considering all characteristics significantly associated with the outcome in the univariate analyses. No deviation from the proportional hazards assumption was found by the numeric methods of Lin et al.[41] The hazard ratios (HRs) and their 95% CIs from the Cox model were reported.

No missing data imputation was performed or reported in tables. All statistical tests were two-sided, and p values lower than 0.05 were considered statistically significant. In the multivariable analysis, p values up to 0.08 were reported. Statistical analyses were performed using the RStudio (RStudio: Integrated Development for R, RStudio Inc., Boston, MA, USA).

RESULTS

Patient Characteristics

Data were collected from 437 patients treated with CRS and HIPEC between 2003 and 2019. The vast majority of the patients (89.5%) were treated after 2010. Actually, the median year of the CRS-HIPEC procedures was 2015 (IQR, 2013–2018). The majority of the cases (82.8%) had been treated in seven highest case-load centers.

The patients had limited peritoneal disease, with a median PCI of 9 (IQR, 5–14). The PCI was lower than 15 in 76.3% of the cases and lower than 20 in 91.3% of the cases. Surgery was without residual disease (CC0) in 84% of the cases, whereas residual disease smaller than 2.5 mm (CC1) was present in 16% of the cases. The majority of the patients (71.8%) had metachronous PM, and the median time from the primary tumor resection to the CRS procedure was 20.6 months (IQR, 13.3–32.0 months). In 85.7% of the cases, the primary tumor was located in the colon (equally distributed among left and right), and in 13.8% of the cases, it was located in the rectum. Less than 1% of the cases had multiple neoplasms. The TNM staging of the primary tumor showed that 97.4% of the patients had T3–T4 tumors, and one third had no nodal involvement (30.4% were N0). Regarding pathologic characteristics, only 6.7% of the tumors were well-differentiated (G1), whereas one third were mucinous (30.8%), and 2.5% showed signet-ring cell histology.

The majority of the patients (70%) received systemic chemotherapy before CRS-HIPEC (oxaliplatin-based for 45.6%, irinotecan-based for 32.4%, combination of

TABLE 1 Patients' characteristics

	n	%[a]
Total	437	
Age (years)		
Median (IQR)		59 (50–65)
Gender		
Male	213	48.7
Female	224	51.3
PCI		
Median (IQR)		9 (5–14)
<15	332	76.3
<20	397	91.3
CC score		
CC0	367	84.0
CC1	70	16.0
Metachronous PM		
Yes	285	71.8
Interval from PM (months)		
Median (IQR)		20.6 (13.3–32.0)
Location		
Right colon	189	43.5
Left colon	183	42.2
Rectum	60	13.8
Multiple	2	0.5
T status		
T2	11	2.6
T3	197	45.8
T4	222	51.6
N status		
N0	128	30.4
N1	126	29.9
N2	167	39.7
Chemotherapy		
Pre-CRS/HIPEC	306	70
Post-CRS/HIPEC	228	52.2
Pathologic features		
G1	27	6.7
G2	198	49.0
G3	179	44.3
SRC	11	2.5
Mucinous	134	30.8
Mutational status		
WT	173	42.6
KRAS	188	46.3
BRAF	27	6.6
NRAS	12	3.0
Multiple	6	1.5
MS status		
MSS	288	86.8
MSI	44	13.2

n Number of total cases, *IQR* Interquartile range, *PCI* Peritoneal cancer index, *CC score* Completeness of cytoreduction, *PM* Peritoneal metastases, *T and N status* According to TNM classification, *CRS* Cytoreductive surgery, *HIPEC* Hyperthermic intraperitoneal chemotherapy, *SRC* Signet-ring cells present, *MSS/MSI* Micro-satellite stability/instability, *WT* All wild-type

[a]Proportions were calculated on the number of patients with available data

oxaliplatin and irinotecan for 11.5%). Adjuvant systemic chemotherapy after surgery was administered to 52.2% of the patients. Only two patients with MSI (4.5%) received checkpoint inhibitors (both before and after CRS). Detailed data are presented in Table 1.

Mutational and Microsatellite Status

About half of the patients (42.8%) had no mutations in RAS/RAF-tested genes (WT). In 46.2% of the patients, KRAS mutation was detected, mainly in codons 12 (69.4%), 13 (22.4%), 146 (4.7%), and 61 (3.5%). The study detected NRAS mutation in 3.0% and BRAF in 6.6% (V600E in 76.9%) of the cases. Multiple mutations of the RAS/RAF genes were reported in 1.5% of the cases (7 patients). Microsatellite instability was diagnosed in 13.2% of the cases (44 patients). Among the MSI patients, 13.3% also were KRAS mutated, and 20.1% were BRAF mutated (none with NRAS mutation).

Overall and Disease-Free Survival

During the follow-up period (median, 37.7 months; 95% CI, 34.4–48.8 months), 72.9% of the patients experienced recurrence (43.6% of whom presented with only extra-peritoneal metastases after surgical peritoneal eradication), and 41.5% died of disease-related causes. The median OS was 42.3 months (95% CI, 33.4–51.2 months), and the median DFS was 13.6 months (95% CI, 12.3–14.9 months). The local DFS (peritoneal recurrence only) was 20.5 months (95% CI, 16.4–24.6 months).

Prognostic Factors

In the univariate analysis, the prognostic factors for survival were PCI (considered as a continuous variable with cutoff levels of 15 and 20 points; all p values, 0.0001),

CC score ($p = 0.0001$), grading ($p = 0.0001$), signet-ring histology ($p = 0.010$), tumor location ($p = 0.02$), N status ($p = 0.0001$), KRAS ($p = 0.0052$) and BRAF ($p = 0.0171$) mutation, multiple RAS/RAF mutations ($p = 0.033$), and MS status ($p = 0.04$). Other clinical and pathologic variables (including age, gender, synchronous/metachronous PSM, chemotherapy schedule) were not significant (data omitted in Table 2).

The 5-year survival rate was 29.4% (median, 33.2 months) for the KRAS-mutated patients and 26.8% (median, 21.5 months) for the BRAF-mutated patients compared with 51.5% (median, 70.7 months) for the WT patients. The micro-satellite stability (MSS) patients had a 5-year OS of 36.7% (median, 41 months) compared with 58.3% for the MSI patients (median, 95 months) (Table 2, Fig. 1). Disease-free survival was related to PCI (continuous or 15–20 points cutoff; $p = 0.0001$, 0.0001, 0.0016, respectively), CC grade ($p = 0.0001$), N status ($p = 0.0001$), grading ($p = 0.0001$), KRAS mutation ($p = 0.0001$), BRAF mutation ($p = 0.001$), and MS status ($p = 0.0073$) (Table 2, Fig. 1).

A bivariate survival analysis of KRAS/BRAF mutation and MS status demonstrated an improved OS for the MSI patients in both the mutated and WT cases. The MSI and all-WT patients had a 5-year OS of 70.6% compared with 23.4% for the patients with MSS and KRAS/BRAF mutation. Similar survival was observed for the MSI patients with mutation and the MSS WT patients (5-year OS, 48.1%; $p = 0.0002$, log-rank; Table 3; Fig. 2). Analogous results were observed for DFS. Actually, the MSI/all-WT patients had a 5-year DFS of 62.5% compared with 3.6% for the of MSS/mutated patients ($p = 0.00001$, log-rank; Table 3; Fig. 2).

In the multivariable analysis of 337 patients, OS was related to PCI (HR, 1.05 per point; 95% CI, 1.02–1.09; $p = 0.004$), CC score (HR, 2.5; 95% CI, 1.6–3.9; $p = 0.0001$), N status (HR, 1.9; 95% CI, 1.3–2.9; $p = 0.003$), SRC histology (HR, 2.3; 95% CI, 1.1–5.0; $p = 0.04$), KRAS mutation (HR, 2.0; 95% CI, 1.3–2.9; $p = 0.0001$), and BRAF mutation (HR, 3.3; 95% CI, 1.7–6.1; $p = 0.0001$) but not to MSI ($p = 0.93$; Fig. 3).

Developing a multivariable model with a combination variable including KRAS/BRAF mutation and MS (MSI/all WT, MSS/mutation, MSI/mutation plus MSS/all WT), we observed that OS was related to PCI (HR, 1.03 per point; $p = 0.01$), CC score (HR, 2.81; $p = 0.0001$), N status (HR, 1.6; $p = 0.03$), and SRC histology (HR, 2.4; $p = 0.03$). In addition to the aforementioned clinical and pathologic factors, the MSI/WT patients (HR, 0.4; 95% CI, 0.1–1.1; $p = 0.08$) and the MSI/mutated or MSS/WT patients (HR, 0.5; 95% CI, 0.3–0.7; $p = 0.0001$) had an improved survival (Fig. 3).

Disease-free survival was related to the CC score (HR, 1.5; 95% CI, 1.0–2.2 $p = 0.04$), N status (HR, 1.7; 95% CI, 1.2–2.2; $p = 0.0001$), KRAS (HR, 1.8; 95% CI, 1.3–2.3; $p = 0.0001$), and BRAF (HR, 3.4; 95% CI, 2.1–5.4; $p = 0.0001$), with PCI showing a tendency to DFS correlation (HR, 1.02; 95% CI, 1.0–1.05; $p = 0.09$), but not MS ($p = 0.12$). In the second model with the combination variable of KRAS/BRAF mutation and MS, DFS was related to PCI (HR, 1.02 per point; $p = 0.09$), CC (HR, 1.56; $p = 0.02$), and N-status (HR, 1.45; $p = 0.01$). In addition, DFS was higher for the MSI/WT patients (HR, 0.3; 95% CI, 0.1–0.6; $p = 0.003$) and the MSI/mutated or MSS/WT patients (HR, 0.5; 95% CI, 0.4–0.7; $p = 0.0001$) than for the MSS/mutated patients (Fig. 3).

DISCUSSION

Despite remarkable and constant progress in systemic treatments, patients who have isolated PM of colorectal origin treated with cytotoxic/targeted agents show a significantly worse survival (16,3 months) than patients with isolated non-peritoneal sites (liver, lung, lymph nodes).[2,4] For selected patients treated in high-volume referral centers, CRS-HIPEC provides a long-term survival of up to 43 months. Even as the real value of HIPEC over CRS alone still is debated, surgery for PM is widely adopted worldwide, especially in the presence of limited disease (PCI <15/20) and when CC can be obtained.[9,42–45]

Our study showed that CRS-HIPEC is a valid option for a selected group of patients affected by isolated PM of colorectal origin, achieving a median survival of 42.3 months, quite identical to recent high-level evidence-reported data.[9,16] Moreover, our results, obtained in high-volume centers with shared selection and treatment protocols, are in line with already established clinical and pathologic prognostic factors for PM patients treated with CRS-HIPEC.[10]

The current study focused on KRAS/BRAF mutational and MS status as prognostic factors for patients with CRC peritoneal metastases treated with radical surgery. The study confirmed that KRAS and BRAF mutations have a negative prognostic impact affecting both OS and DSF.[16,17,23] In addition, we observed for the first time that MS instability is a relevant prognostic factor that can mitigate the detrimental effect of KRAS/BRAF mutation. Therefore, MS status should be considered as a new factor for risk stratification of patients eligible for CRS-HIPEC.

In the vast field of research on metastatic CRC, few data exist on the role of systemic chemotherapy for patients affected by isolated PM compared with other metastatic sites such as liver, lung, and lymph nodes. A study analyzing a large sample of previously untreated patients

TABLE 2 Univariate overall and disease-free survival analysis

	Events/ *n*	5-Year OS % (95% CI)	Median OS (m) (95%CI)	*p* value (log-rank)	Events/ *n*	5-Year DFS % (95% CI)	Median DFS (m) (95%CI)	*p* value (log-rank)
PCI								
< 15	123/ 331	45.4 (38.0–52.6)	54.3 (42.3–66.2)	< 0.001	236/ 331	18.1 (13.5–23.3)	14.1 (13.1–17.2)	< 0.001
≥ 15	59/101	20.3 (11.0–31.7)	25.1 (18.9–28.0)		84/101	8.6 (3.6–16.5)	9.6 (7.2–12.9)	
CC score								
CC0	131/ 364	46.5 39.2–53.4	55.2 (42.8–70.0)	< 0.001	258/ 364	18.7 (14.2–23.8)	14.0 (12.7–16.6)	< 0.001
CC1	51/70	10.8 (3.9–21.7)	20.7 (17.4–26.3)		62/70	3.8 (0.7–11.2)	11.3 (7.5–13.1)	
Location								
Right colon	85/187	33.1 (23.9–42.5)	32.4 (27.3–41.0)	0.02	138/ 187	13.2 (7.8–20.0)	12.8 (10.8–14.5)	0.5
Left colon	74/182	45.3 (35.8–54.2)	48.3 (40.3–70.7)		135/ 182	18.2 (12.3–25.1)	14.8 (12.8–18.2)	
Rectum	20/60	49.6 (29.7–66.8)	54.3 (29.6–NE)		43/60	19.1 (8.8–32.3)	13.1 (10.0–15.8)	
Multiple	1/2	NE	20.2 (NE)		½	NE	13.1 (NE)	
N status								
N0	40/128	54.8 (43.1–65.2)	95.0 (41.0–NE)	< 0.001	82/128	27.6 (19.1–36.7)	17.7 (15.1–21.5)	< 0.001
N+	137/ 290	32.6 (25.2–40.3)	35.6 (31.2–44.3)		227/ 290	8.7 (4.4–14.9)	11.5 (9.3–13.5)	
Grading								
G1	5/27	75.8 (50.8–89.3)	NE	< 0.001	16/27	34.8 (16.7–53.6)	17.2 (10.1–NE)	< 0.001
G2	74/198	45.9 (36.0–55.3)	53.5 (39.2–70.7)		144/ 198	17.3 (11.6–24.0)	13.2 (11.6–15.1)	
G3	85/177	29.6 (20.9–38.8)	31.3 (27.5–38.9)		134/ 177	12.1% (6.9–18.7)	13.7 (10.7–15.8)	
SRC								
No	174/ 423	41.3 (34.9–47.6)	43.1 (35.6–55.2)	0.010	310/ 423	16.8 (12.9–21.2)	13.5 (11.7–14.8)	0.98
Yes	8/11	NE	27.1 (12.8–32.7)		10/11	NE	17.9 (12.5–28.0)	
Mucinous								
No	132/ 299	39.6 (32.3–46.8)	38.9 (32.4–51.3)	0.5	221/ 299	16.3 (11.7–21.5)	13.4 (12.3–14.5)	0.5
Yes	49/133	40.8 (28.4–52.8)	43.7 (35.6–103.0)		97/133	16.3 (9.5–24.9)	14.3 (9.6–17.6)	
MS status								
MSS	116/ 288	36.7 (28.8–44.6)	41.0 (33.9–51.3)	0.04	216/ 288	12.7 (8.4–17.9)	14.1 (13.1–15.7)	0.0073
MSI	13/44	58.3 (37.7–74.2)	95.0 (36.5;NE)		24/44	38.7 (22.6–54.6)	19.2 (12.6–NE)	
Mutation								
WT	61/173	51.5 (41.5–60.5)	70.7 (41.0–NE)	Ref	111/ 173	27.1 (19.7–35.0)	17.6 (14.2–22.1)	Ref
KRAS	84/188	29.4 (19.8–39.7)	33.2 (29.9–43.7)	0.0052	152/ 188	6.9 (3.2–12.4)	11.5 (9.8–13.8)	< 0.001
BRAF	14/27	26.8 (8.1–50.1)	21.5 (18.9–NE)	0.0171	24/27	NE	10.5 (7.0–13.9)	< 0.001
NRAS	4/12	NE	32.3 (13.8–NE)	0.5433	10/12	NE	10.4 (3.6–13.8)	0.0174
Multiple	4/6	NE	27.5 (10.1–NE)	0.033	4/6	NE	11.1 (6.7–NE)	0.4868

OS Overall survival, *DFS* Disease-free survival, *CI* Confidence interval, *(m)* Median OS and DFS in months, *Events/n* Events/no. of total cases, *PCI* Peritoneal cancer index, *CC score* Completeness of cytoreduction, *NE* Not estimable, *N status* N0: no nodal involvement verus positive (N1–N3) according to TNM classification, *SRC* Signet-ring cells present, *MS* Micro-satellite, *MSS/MSI* Micro-satellite stability/instability, *WT* All wild-type, *m* Months, *Ref* Reference

Only variables with $p < 0.05$ have been reported.

enrolled in 14 randomized trials demonstrated that patients with peritoneal metastases have a worse prognosis than other stage 4 patients with a single metastatic site.[2,4] Several possible explanations have been advocated for this difference. Compared with other patients, PM patients could have a higher tumor burden because radiologic detection of small peritoneal nodules is more difficult than radiologic detection of liver or nodal metastases.[46] The severity of PM symptoms (from early onset of cachexia due to malnutrition to bowel occlusion) may lead to reduced therapy adherence or administration, although this seems to be refuted by a retrospective analysis of two CAIRO randomized trials, in which worst prognosis could have been due to relative resistance of peritoneal metastases, even if adequately treated.[47] Finally, the so-called "sanctuary effect" could be responsible for the 10% to 20% response rate reduction of peritoneal metastases compared with liver metastases.[48]

In the last two decades, the role of peritoneal surgery has progressively but steadily gained in importance, achieving results similar to those for surgical treatment of liver and lung metastases.[49–52] Currently, CRS combined in a multimodal treatment strategy of perioperative systemic chemotherapy is considered the best therapeutic option and the only potentially curative treatment for PM patients with limited disease.[42–45]

Our study confirmed that surgery provides a survival advantage for patients treated in referral centers according to a standardized protocol and in a setting of multimodal treatment with systemic chemotherapy. The median survival obtained for our patients (42.3 months) was quite identical to that reported in other studies,[9,16] confirming the pivotal role of surgery performed for PM.

Addition of HIPEC to CRS has been questioned during the last few years, after results of randomized controlled trials in a proactive/prophylactic (patients at risk for the development of PM)[53,54] or curative ("adjuvant" treatment after surgery) setting.[9] Of relevance, reported results of a still unpublished PRODIGE 7 randomized trial showed a notable median OS survival in both arms, but failed to demonstrate a survival advantage of CRS+HIPEC with oxaliplatin over CRS alone, reporting a higher rate of complication in the HIPEC arm.[9] Although publication of the full study is needed for any final conclusion to be drawn, no substantial evidence for advantage of oxaliplatin-based HIPEC after CRS (except for patients with

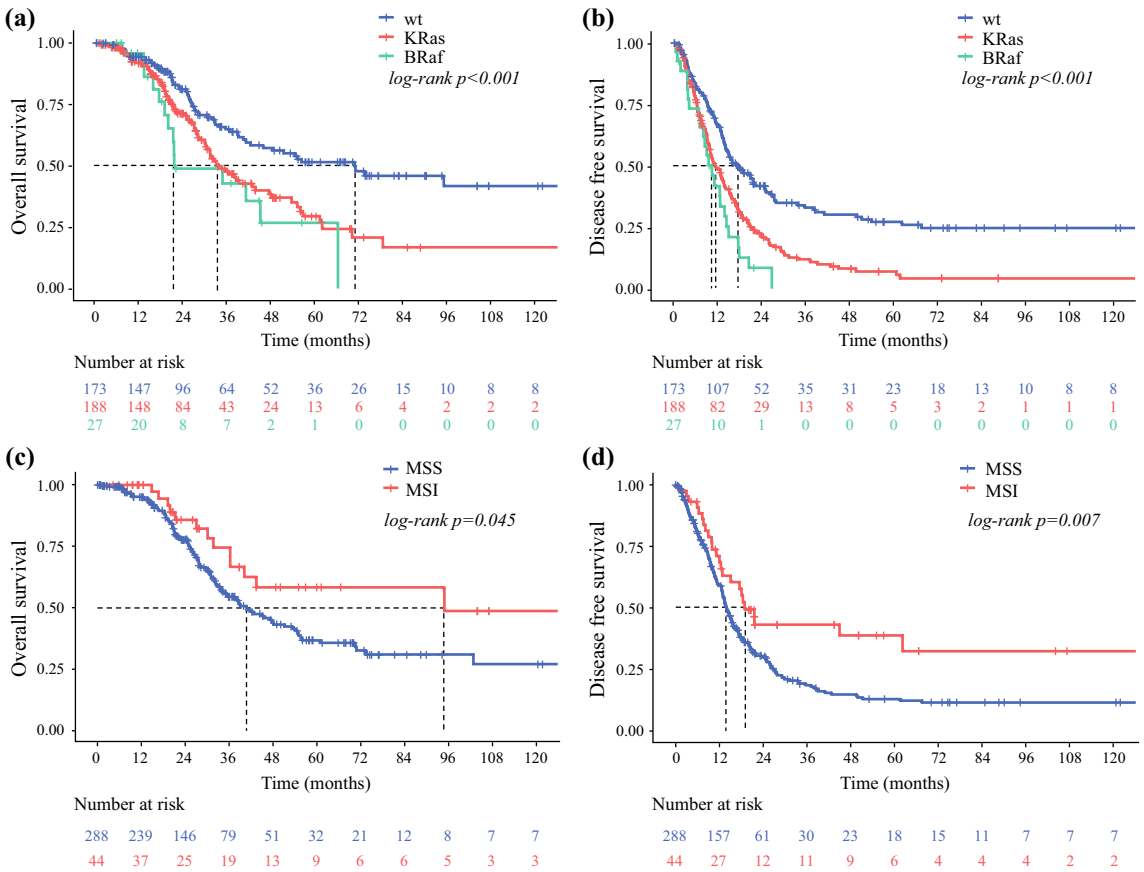

FIG. 1 Survival curves according to mutational (KRAS/BRAF) and micro-satellite (MS) status. WT, all wild-type; MSS/MSI, micro-satellite stability/instability

TABLE 3 Bivariate analysis of mutational and micro-satellite (MS) status

		Events/ *n*	5-Year OS % (95% CI)	Median OS (m) (95% CI)	*p* value (log-rank)	Events/ *n*	5-Year DFS % (95% CI)	Median DFS (m) (95%CI)	*p* value (log-rank)
WT	MSS	48/134	48.1 (36.6–58.6)	55.2 (38.9–NE)	0.0002	89/134	22.6 (14.8–31.5)	16.0 (14.0–22.1)	< 0.0001
WT	MSI	5/18	70.6 (38.9–88.0)	NE		7/18	62.5 (34.0–81.5)	NE	
Mutated	MSS	68/154	23.4 (13.2–35.3)	34.4 (29.9–41.0)		127/ 154	3.6 (1.0–9.1)	12.8 (10.2–14.5)	
Mutated	MSI	8/26	NE	43.7 (27.3–NE)		17/26	NE	15.3 (10.1–19.2)	

OS Overall survival, *DFS* Disease-free survival, *CI* Confidence interval, *(m)* Median OS and DFS in months, *Events/n* Events/no. of total cases, *WT* All wild-type, *MSS/MSI* Micro-satellite stability/instability, *mutated* KRAS or BRAF mutated (NRAS and multiple mutations excluded), *NE* Not estimable

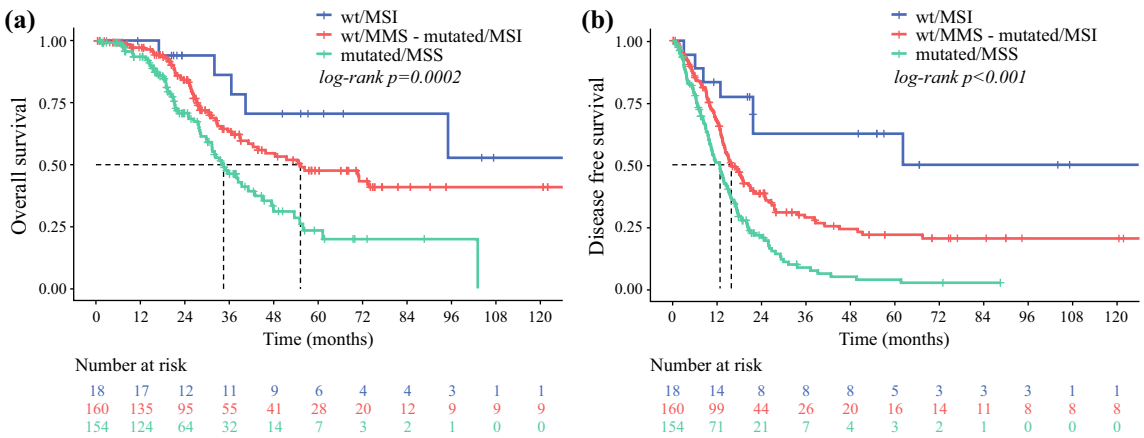

FIG. 2 Survival curves according to mutational/micro-satellite (MS) status. WT, all wild-type; MSS/MSI, micro-satellite stability/instability; mutated, KRAS- or BRAF-mutated (NRAS and multiple mutations excluded)

limited-extent disease in a subgroup analysis) has been found. However, the role of HIPEC after CRS in colorectal cancer remains an open question, considering that a recent randomized controlled trial showed a survival advantage of 11.8 months in the HIPEC arm compared with CRS alone for ovarian PM.[55]

Also, the role of perioperative systemic chemotherapy for patients selected to undergo surgery remains debated. Despite the lack of high-quality evidence, systemic chemotherapy currently is administered before or after CRS. A survival benefit of neoadjuvant (and perioperative) therapy may be suggested.[56,57] Our data reflect this clinical attitude, with 70% of patients receiving systemic chemotherapy before CRS versus 52.2% of patients treated with adjuvant therapy. The regimens used among centers do not differ, but we observed that a combination of neoadjuvant oxaliplatin and irinotecan was administered preferentially in most recent cases (2017 was the median administration year for FOLFOXIRI vs 2015 for the other regimens; $p = 0.04$), possibly reflecting a treatment shift after publication of the TRIBE trial subgroup analysis.[58]

A constant effort is being made to identify prognostic factors to drive the multidisciplinary decision of this dismal-prognosis subset of patients. Historically, the PCI (a tumor burden surrogate) and completeness of cytoreduction (a score of surgical radicality) have been used as selection and prognostic criteria.[7,46–58]

Our results clearly showed the independent role of PCI (HR 1.03 per increasing point) and completeness of surgery (CC score) (HR, 2.8 for complete vs suboptimal cytoreduction) in predicting survival and disease relapse. Indeed, patients with a PCI lower than 15 have a median survival twice as long as patients with a higher PCI (55.0 vs 25.1 months). The main limitation of PCI and the CC score is the difficulty of having a reliable radiologic PCI and predictive criteria for an optimal cytoreduction before surgery.[59,60]

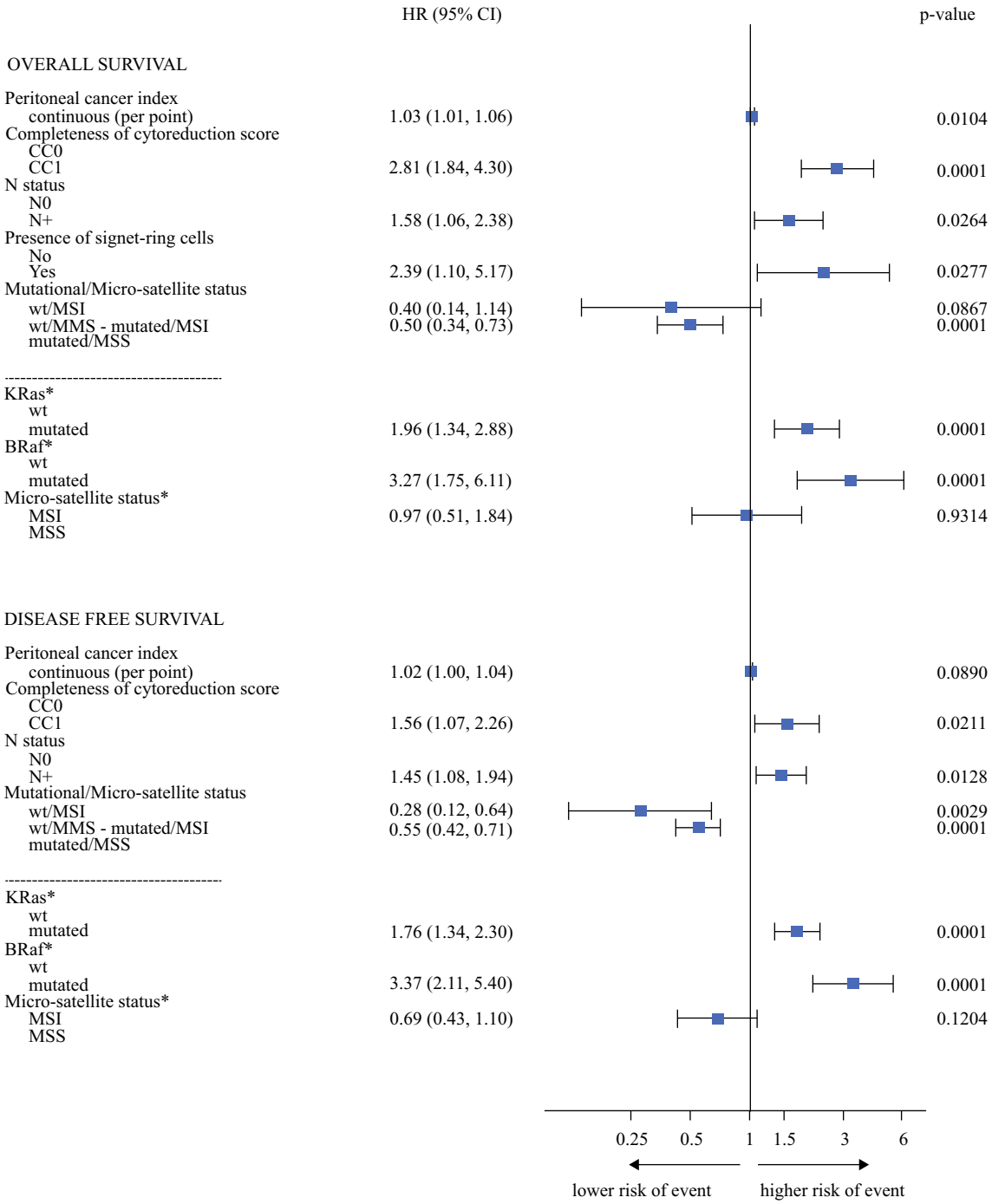

FIG. 3 Multivariable analysis. *Results obtained with multivariable model including Peritoneal Cancer Index (PCI), completeness of cytoreduction (CC) score, N status, and SRC (data on modelling reported in the Survival Analysis section). WT, all wild-type; MSS/MSI, micro-satellite stability/instability; mutated, KRAS- or BRAF-mutated (NRAS and multiple mutations excluded)

Among the pathologic factors, nodal involvement of the primary tumor (N status), grading, and the presence of signet-ring cells (SRC) are related to survival. In the multivariate analysis, only N status (HR, 1.6) and signet-ring histology (HR, 2.4) remained related to OS (Fig. 3). These results are consistent with previously reported data showing an increased risk of disease-related death in the case of lymph-node involvement[16] and signet-ring histology. The latter represents a contraindication to CRS in some referral centers.[61–63]

Currently, RAS/RAF mutational status is part of the standard clinical evaluation since demonstration that constitutive activation of the RAS pathway leads to an impaired response to anti-epidermal growth factor receptor

(EGFR) targeted therapy, which is an important therapeutic option for CRC metastatic patients.[64,65] It is widely reported that RAS and RAF mutations have a negative effect on the survival of stage 4 CRC patients treated with chemotherapy[66,67] and that they also represent a negative prognostic factor after surgery for liver or lung metastases resection.[13,15,68,69] Currently, strong evidence exists that RAS/RAF mutations also act as negative prognostic factors for PM patients treated with surgery.[16,17] According to our results, the rates of RAS (46.2%) and RAF (6.6%) mutations are comparable with already reported data on PM patients.[16,17,23] For patients with KRAS and BRAF, mutations are related to a worse prognosis than for WT patients [33.2 and 21.5 months ($p = 0.005$) vs 70.1 months ($p = 0.01$) for WT patients]. In the multivariable analysis, the KRAS- and BRAF-mutated patients showed survival HRs of 2.0 ($p = 0.0001$) and 3.3 ($p = 0.0001$), respectively, compared with the WT patients. Also, these results are almost identical to those of a recent series reporting the same analysis, confirming the relevance of mutational status for PM patients.[16,17]

To date, the prognostic role of MS status in CRC has not been clearly defined. In some studies, MSI is related to an improved prognosis in American Joint Committee on Cancer (AJCC) stages 2 and 3 patients.[70–72] Conversely, stage 4 MSI patients show a reduced OS.[21,22] In addition, neither incidence (estimated to be <15%) nor prognostic relevance of MS for patients with peritoneal metastasis has been defined to date because the vast majority of data derived from studies are focused on liver or lung stage 4 patients.[73–77] According to the few data on MS status for patients with PM, the MSI detection rate is similar to our results (13.2%).[18,78,79] The univariate analysis showed that MSI is related to a remarkably improved survival (median OS, 95 months vs 41 MSS, $p = 0.04$). This result is consistent with reported data on stages 1 to 3 CRC[70–72,77] and stage 4 for peritoneal malignancies,[23] but seems to be in contradiction to results obtained in other series of stage 4 patients.[21,22]

The multivariable analysis failed to demonstrate a direct correlation between MS status and survival, possibly because of the relatively small sample of MSI patients compared with MSS patients. In addition, only 4.5% of the MSI patients (2 cases) had received immune checkpoint inhibitors, whereas 95.5% had been treated with 5-fluorouracil (5-FU) and cytotoxic drugs, which have a postulated detrimental effect on survival.[80,81] Considering these factors, our results could possibly have underestimated the survival of MSI patients, reflecting a reduced power in the multivariable analysis.

Although KRAS and BRAF mutations play a major role in determining the prognosis for the whole PM population, MSI seems to have protective effects for mutated patients. In our series, the KRAS- or BRAF-mutated MSI patients had a significantly better prognosis than the MSS-mutated patients (median OS, 43.7 vs 34.4 months; $p = 0.002$). Even if for a different subset of patients, similar results had been reported for a large group of MSI patients receiving nivolumab plus ipilimumab (CheckMate 142 trial), in which objective response rates (ORR) were similar independently from KRAS and BRAF status.[19,82] The multivariable analysis confirmed the MSI survival advantage by using a combination variable of mutations and MS status. The prognosis of MSI (mutated or not) and MSS/WT was significantly better than the prognosis of the MSS-mutated cases [HR, 0.4 ($p = 0.08$) and 0.5 ($p = 0.0001$), respectively].

The main limitation of our study was its retrospective nature and lack of centralized specimen analysis for mutational and MS status, which were unavoidably related to a certain degree of missing data in the series. However, the study results demonstrate the same mutational/MS rates and survival outcomes, showing prognostic stratification factors identical to those of previous studies, indirectly confirming the homogeneity of the study population.

This study represents the largest series analyzing MS status in a homogeneous peritoneal-only stage 4 population with similar disease extension (91.3% of cases had a PCI < 20) treated with radical surgery accordingly with a shared protocol. These results will be useful for improving patient selection, but further large, prospective studies are required to consolidate the role of MS as a prognostic factor in colorectal peritoneal metastases. In the near future, it may be possible to expand surgical eligibility to MSI patients with negative prognostic factors or contraindications such as high tumor burden (PCI > 20) or pathologic features (SRC).

CONCLUSIONS

The role of clinical and pathologic criteria in the selection pathway for the surgery of patients affected by CRC PM needs to be integrated constantly with tumor molecular features (KRAS and BRAF mutations). Based on our results, MS status also should be strongly considered in the selection process for patients potentially eligible for CRS because MSI confers a significant survival advantage over the survival of stable patients, even in the group with KRAS/BRAF mutation.

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