

Ascites and resistance to immune checkpoint inhibition in dMMR/MSI-H metastatic colorectal and gastric cancers

Giovanni Fucà ¹, Romain Cohen ², Sara Lonardi ³, Kohei Shitara,⁴ Maria Elena Elez,⁵ Marwan Fakih,⁶ Joseph Chao ⁶, Samuel J Klempner,^{7,8} Matthew Emmett,^{7,8} Priya Jayachandran,⁹ Francesca Bergamo,¹⁰ Marc Díez García,⁵ Giacomo Mazzoli,¹ Leonardo Provenzano,¹ Raphael Colle,² Magali Svrcek,¹¹ Margherita Ambrosini,¹ Giovanni Randon,¹ Aakash Tushar Shah,¹² Massimiliano Salati,¹³ Elisabetta Fenocchio,¹⁴ Lisa Salvatore,¹⁵ Keigo Chida,⁴ Akihito Kawazoe,⁴ Veronica Conca,^{16,17} Giuseppe Curigliano,^{18,19} Francesca Corti,¹ Chiara Cremolini,^{16,17} Michael Overman,²⁰ Thierry Andre ², Filippo Pietrantonio ¹

To cite: Fucà G, Cohen R, Lonardi S, *et al.* Ascites and resistance to immune checkpoint inhibition in dMMR/MSI-H metastatic colorectal and gastric cancers. *Journal for ImmunoTherapy of Cancer* 2022;**10**:e004001. doi:10.1136/jitc-2021-004001

► Additional supplemental material is published online only. To view, please visit the journal online (<http://dx.doi.org/10.1136/jitc-2021-004001>).

GF and RC are joint first authors.

TA and FP are joint senior authors.

Accepted 06 January 2022



© Author(s) (or their employer(s)) 2022. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

For numbered affiliations see end of article.

Correspondence to
Dr Filippo Pietrantonio;
filippo.pietrantonio@
istitutotumori.mi.it

ABSTRACT

Background Despite unprecedented benefit from immune checkpoint inhibitors (ICIs) in patients with mismatch repair deficient (dMMR)/microsatellite instability high (MSI-H) advanced gastrointestinal cancers, a relevant proportion of patients shows primary resistance or short-term disease control. Since malignant effusions represent an immune-suppressed niche, we investigated whether peritoneal involvement with or without ascites is a poor prognostic factor in patients with dMMR/MSI-H metastatic colorectal cancer (mCRC) and gastric cancer (mGC) receiving ICIs.

Methods We conducted a global multicohort study at Tertiary Cancer Centers and collected clinic-pathological data from a cohort of patients with dMMR/MSI-H mCRC treated with anti-PD-(L)1 ±anti-CTLA-4 agents at 12 institutions (developing set). A cohort of patients with dMMR/MSI-high mGC treated with anti-PD-1 agents±chemotherapy at five institutions was used as validating dataset.

Results The mCRC cohort included 502 patients. After a median follow-up of 31.2 months, patients without peritoneal metastases and those with peritoneal metastases and no ascites had similar outcomes (adjusted HR (aHR) 1.15, 95% CI 0.85 to 1.56 for progression-free survival (PFS); aHR 0.96, 95% CI 0.65 to 1.42 for overall survival (OS)), whereas inferior outcomes were observed in patients with peritoneal metastases and ascites (aHR 2.90, 95% CI 1.70 to 4.94; aHR 3.33, 95% CI 1.88 to 5.91) compared with patients without peritoneal involvement. The mGC cohort included 59 patients. After a median follow-up of 17.4 months, inferior PFS and OS were reported in patients with peritoneal metastases and ascites (aHR 3.83, 95% CI 1.68 to 8.72; aHR 3.44, 95% CI 1.39 to 8.53, respectively), but not in patients with only peritoneal metastases (aHR 1.87, 95% CI 0.64 to 5.46; aHR 2.15, 95% CI 0.64 to 7.27) when compared with patients without peritoneal involvement.

Conclusions Patients with dMMR/MSI-H gastrointestinal cancers with peritoneal metastases and ascites should be

considered as a peculiar subgroup with highly unfavorable outcomes to current ICI-based therapies. Novel strategies to target the immune-suppressive niche in malignant effusions should be investigated, as well as next-generation ICIs or intraperitoneal approaches.

INTRODUCTION

Although mismatch repair deficient (dMMR) and/or microsatellite instability high (MSI-H) advanced gastrointestinal cancers are relatively uncommon (about 4% of both metastatic colorectal cancer (mCRC) and gastric cancer (mGC)), immune checkpoint inhibitors (ICIs) have reliably demonstrated durable responses and unprecedented survival outcomes in this patients' population independent from the tumor site of origin.¹ ICIs have become a new standard of care for patients with dMMR/MSI-H cancers, and their use is nowadays supported by randomized clinical trials (RCTs).²⁻⁴ Nevertheless, about 30% of patients with MSI/dMMR advanced mCRC display intrinsic resistance to anti-PD(L)-1 monotherapy both in phase II proof-of-concept trials conducted in pretreated patients,^{5,6} and in the Keynote-177 phase III first-line trial.² A similar percentage of primary resistance was reported in a non-colorectal cohort and in post hoc analyses of RCT in mGC.⁷⁻⁹

Of note, the occurrence of early progressive disease (PD) at first disease reassessment may be explained by the phenomenon of pseudoprogression,¹⁰ or by the misdiagnosis of dMMR/MSI-H status.¹¹ However, several promising studies investigated the association of primary resistance with specific

biomarkers, including a relatively lower tumor mutational burden,^{12 13} dMMR/MSI-H heterogeneity,¹³ and elevated systemic inflammation as assessed by blood-based parameters.¹⁴

Malignant ascites is a common clinical complication in patients with peritoneal metastases from gastrointestinal cancers. Despite the well-established poor prognostic effect of both peritoneal involvement and ascites in patients with several types of advanced cancers, malignant ascites is also characterized by a pervasive immunosuppressive microenvironment.^{15 16} Indeed, serous cavities are an immunologic niche due to a variety of immunosuppressive networks, such as those driven by cavity-resident macrophages with Tim-4 overexpression.¹⁷ Drawing from these considerations, we hypothesized that patients with MSI-H cancers and coexisting malignant ascites may have poorer outcomes and high rates of primary resistance to ICI treatment. To examine this hypothesis, we assembled a large multinational cohort of patients with dMMR/MSI-H mCRC or GC receiving ICIs to investigate the prognostic role of peritoneal involvement with or without ascites.

METHODS

Patients' population

This was a retrospective, multicentric, international study that included patients with advanced dMMR/MSI-H CRC or GC treated with ICI-based therapy from June 2014 to May 2021. We assembled two cohorts of dMMR/MSI-H gastrointestinal cancers consisting of mCRC (developing set or cohort 1) and mGC (validating set or cohort 2) taking advantage of the electronic medical records of the participating centers. Inclusion criteria were: (1) pathologically/cytologically confirmed diagnosis of dMMR/MSI-H CRC or GC, with dMMR/MSI-H status confirmed by local institutional testing per international guidelines¹⁸; (2) treatment with at least one cycle of ICI-based therapy for advanced disease within clinical trials or per clinical practice; (3) age ≥ 18 years. Cohort 1 contained patients with dMMR/MSI-H mCRC treated with anti-PD-(L)1±anti CTLA-4 agents at 12 institutions worldwide. Cohort 2 contained patients with dMMR/MSI-H mGC treated with anti-PD-1±chemotherapy at five institutions worldwide. Information about clinical outcomes of ICI-based treatment (ie, progression-free survival (PFS) and overall survival (OS)) as well as the following demographic and clinico-pathological data (baseline to the initiation of the ICI-based treatment) were retrieved for all the patients included: age, sex, Eastern Cooperative Oncology Group (ECOG) Performance Status (PS), primary tumor resection, presence of synchronous metastases, number and localization of metastases, presence of peritoneal involvement and ascites, information about prior systemic treatment for metastatic disease, type of ICI-based treatment. For patients with dMMR/MSI-H mCRC the following disease-specific data were also retrieved when available: primary tumor sidedness,

RAS mutational status and *BRAF* mutational status. For patients with dMMR/MSI-H mGC the following disease-specific data were also retrieved when available: primary site of origin and histology.

The presence of peritoneal involvement and ascites was assessed by means of conventional imaging techniques (ie, abdominal CT scan or MRI) at each participating center. The presence of metastatic peritoneal involvement was coded as a polytomous categorical variable encompassing three values: no peritoneal metastases, peritoneal metastases without ascites, peritoneal metastases with ascites. Patients with evidence of peritoneal metastases with ascites were included in the 'ascites' group, whereas patients with no peritoneal metastases or peritoneal metastases without ascites were included in the 'no ascites' group.

Statistical analyses

PFS was defined as the time from the first dose of ICIs administration to PD or death from any cause, whichever occurred first. OS was defined as the time from the first dose of ICIs administration to death from any cause. To examine baseline differences between groups, Pearson's χ^2 test or Fisher's exact test were used, as appropriate. To examine baseline differences according to the metastatic peritoneal involvement coded as a polytomous categorical variable, pairwise Fisher's exact test was used. Survival analyses were performed using the Kaplan-Meier method and Cox proportional hazards regression. Univariable Cox regression analysis was used to assess the effect of different baseline factors on PFS and OS. Variables significantly associated with survival outcomes at the univariable analysis were then fitted in a multivariable Cox proportional hazards regression model to identify independent predictors of OS and PFS. HRs with the corresponding 95% CIs were provided for Cox's proportional hazards regression models. All statistical tests were two tailed, and a $p < 0.05$ was considered statistically significant. Statistical analyses were performed using R software (V.3.5.0) and R Studio (V.1.1.447).

RESULTS

Patients' characteristics

Cohort 1 included 502 patients with dMMR/MSI-H mCRC from 12 institutions (online supplemental table 1). About 60% of the patients included in cohort 1 received the ICI-based treatment within clinical trials. Clinico-pathological and treatment characteristics are reported in [table 1](#), both overall and according to the presence or absence of ascites. Briefly, peritoneal involvement without ascites was present in 172 patients (34.3%), whereas ascites was present in 25 patients (5.0%). The presence of ascites was associated with poorer PS and *RAS* wild-type/*BRAF* mutated status. Online supplemental table 2 shows the pairwise analysis of all characteristics according to the three subgroups of patients with peritoneal involvement

Table 1 Patients and disease characteristics in the developing set (Cohort 1: dMMR/MSI-high mCRC), overall and according to the presence or absence of ascites

Characteristics	Total (N=502) N (%)	No ascites (N=477) N (%)	Ascites (N=25) N (%)	P value
Sex				0.511
Female	229 (45.6)	216 (45.3)	13 (52.0)	
Male	273 (54.4)	261 (54.7)	12 (48.0)	
Age				0.301
<70	378 (75.3)	357 (74.8)	21 (84.0)	
≥70	124 (24.7)	120 (25.2)	4 (16.0)	
ECOG PS				0.005
0	237 (47.2)	232 (48.6)	5 (20.0)	
≥1	265 (52.8)	245 (51.4)	20 (80.0)	
Primary tumor resection				>0.999
No	90 (17.9)	86 (18.0)	4 (16.0)	
Yes	412 (82.1)	391 (81.9)	21 (84.0)	
Primary tumor sidedness				0.267
Left	172 (34.3)	166 (34.8)	6 (24.0)	
Right	330 (65.7)	311 (65.2)	19 (76.0)	
NA				
RAS mutational status				0.048
All wild type	317 (63.1)	296 (62.1)	21 (84.0)	
RAS mutated	165 (32.9)	161 (33.7)	4 (16.0)	
NA	20 (4.0)	20 (4.2)	0 (0.0)	
BRAF mutational status				0.054
All wild-type	351 (69.9)	337 (70.6)	14 (56.0)	
BRAF mutated	132 (26.3)	121 (25.4)	11 (44.0)	
NA	19 (3.8)	19 (4.0)	0 (0.0)	
Synchronous metastases				0.203
No	242 (48.2)	233 (48.9)	9 (36)	
Yes	258 (51.4)	242 (50.7)	16 (64)	
NA	2 (0.4)	2 (0.4)	0 (0)	
Liver metastases				0.246
No	306 (61.0)	288 (60.4)	18 (72.0)	
Yes	196 (39.0)	189 (39.6)	7 (28.0)	
Lung metastases				0.595
No	412 (82.1)	390 (81.8)	22 (88.0)	
Yes	90 (17.9)	87 (18.2)	3 (12.0)	
Lymphnodal metastases				0.521
No	210 (41.8)	198 (41.5)	12 (48.0)	
Yes	292 (58.2)	279 (58.5)	13 (52.0)	
Bone metastases				0.320
No	479 (95.4)	456 (95.6)	23 (92.0)	
Yes	23 (4.6)	21 (4.4)	2 (8.0)	
No of metastatic sites				0.076
1	227 (45.2)	220 (46.1)	7 (28.0)	
≥2	275 (54.8)	257 (53.9)	18 (72.0)	

Continued

Table 1 Continued

Characteristics	Total (N=502) N (%)	No ascites (N=477) N (%)	Ascites (N=25) N (%)	P value
Prior treatment for metastatic disease				0.195
No	96 (19.1)	94 (19.7)	2 (8.0)	
Yes	406 (80.9)	383 (80.3)	23 (92.0)	
ICI regimen				0.525
a-PD(L)-1	332 (66.1)	314 (65.8)	18 (72.0)	
a-PD-1+a-CTLA-4	170 (33.9)	163 (34.2)	7 (8.0)	

P-values marked with bold indicate statistically significant p-values.

dMMR, mismatch repair deficient; ECOG, Eastern Cooperative Oncology Group; ICI, immune checkpoint inhibitor; mCRC, metastatic colorectal cancer; MSI-H, microsatellite instability high; NA, not available; PS, Performance Status.

with or without ascites and those without peritoneal involvement.

Cohort 2 included 59 patients with dMMR/MSI-high mGC from 5 institutions (online supplemental table 3). About 30% of the patients included in cohort 2 received the ICI-based treatment within clinical trials. Clinicopathological and treatment characteristics are reported in table 2, both overall and according to the presence or absence of ascites. Peritoneal involvement without ascites was present in 11 patients (18.6%), whereas ascites was present in 17 patients (28.8%). Of note, ascites was significantly associated with poorer PS, non-resected primary tumor, synchronous presentation of metastases and >1 sites of metastases.

Survival outcomes according to the presence of peritoneal involvement and ascites

In cohort 1, the median follow-up time was 31.2 months (IQR 15.6–46.0). Online supplemental figure 1A,B shows PFS and OS in the overall population. The presence of ascites was significantly associated with shorter PFS and OS (both $p < 0.001$; figure 1A,B). Patients without peritoneal metastases had similar PFS and OS to those with peritoneal metastases and no ascites (2-year PFS rate: 62.6% vs 60.3%; HR 1.08, 95% CI 0.80 to 1.46; 2 year OS rate: 73.2% vs 75.3%; HR 0.92, 95% CI 0.63 to 1.33), whereas poorer PFS and OS were restricted to patients with peritoneal metastases and ascites (2-year PFS rate: 30.4%; HR 2.80, 95% CI 1.65 to 4.75; 2-year OS rate: 29.7%, HR 3.58, 95% CI 2.06 to 6.22). table 3 shows the univariable and multivariable models for PFS and OS: presence of ascites had an independent effect on both survival outcomes (adjusted HR for PFS: 2.90 (95% CI 1.70 to 4.94); for OS: 3.33 (95% CI 1.88 to 5.91)), as well as ECOG PS, presence of lung metastases, prior treatment for advanced disease and ICI treatment type (anti-PD(L)-1 monotherapy or anti-CTLA-4 combination). We then investigated the impact of specific ICI approaches (combination with CTLA-4 vs PD-1 monotherapy) and observed that shorter PFS and OS outcomes were restricted to patients with ascites receiving anti-PD(L)-1 monotherapy (median and 2-year PFS: 1.6 months and 6.9%; median and 2-year OS:

5.8 months and 6.6%; figure 1C,D). Patients without peritoneal metastases had similar PFS and OS to those with peritoneal metastases and no ascites, regardless of the specific ICI approach (online supplemental figure 2).

In cohort 2, the median follow-up was 17.4 month (IQR: 11.8–51.3). Online supplemental figure 3A,B shows PFS and OS in the overall population. The presence of ascites was significantly associated with shorter PFS and OS ($p < 0.001$ and $p = 0.006$; figure 2A,B). Patients without peritoneal metastases had similar PFS and OS to those with peritoneal metastases and no ascites (2-year PFS rate: 50.2% vs 61.4%; HR 1.48, 95% CI 0.55 to 4.02; 2-year OS rate: 56.5% vs 60.6%; HR 1.69, 95% CI 0.55 to 5.18), whereas worse PFS and OS were restricted to patients with peritoneal metastases and ascites (2-year PFS rate: 17.7%; HR 4.57, 95% CI 2.07 to 10.09; 2-year OS rate: 26.5%, HR 4.18, 95% CI 1.70 to 10.26). Table 4 shows the univariable and multivariable models for PFS and OS: presence of ascites had an independent effect on survival outcomes (adjusted HR for PFS: 3.83 (95% CI 1.68 to 8.72); for OS: 3.44 (95% CI 1.39 to 8.53)).

DISCUSSION

In this study, the presence of ascites, but not peritoneal involvement without ascites, was associated with extremely poor survival outcomes in the largest reported cohort of patients with dMMR/MSI-H mCRC and mGC treated with ICIs. The results were consistent in both a very large primary analysis dataset of mCRC and an external validation set of patients with dMMR/MSI-H mGC. Despite the association of ascites with worse performance status and other unfavorable features (such as *RAS* wild-type/*BRAF* mutated status in mCRC, and synchronous presentation/non-resected primary tumor in mGC), the presence of ascites was independently associated with both PFS and OS, and notably demonstrated a similar HR >3.0 in both dMMR/MSI-H mCRC and dMMR/MSI mGC. We acknowledge that the lack of independent, central imaging revision is a limitation of our study, since mild amounts of ascites may not be reported systematically.

Table 2 Patients and disease characteristics in the validating set (Cohort 2: dMMR/MSI-high mGC), overall and according to the presence or absence of ascites

Characteristics	Total (N=59) N (%)	No ascites (N=42) N (%)	Ascites (N=17) N (%)	P value
Sex				0.840
Female	22 (37.3)	16 (38.1)	6 (35.3)	
Male	37 (62.7)	26 (61.9)	11 (64.7)	
Age				0.304
<70	32 (54.2)	21 (50.0)	11 (64.7)	
≥70	27 (45.8)	21 (50.0)	6 (35.3)	
ECOG PS				0.047
0	18 (30.5)	16 (38.1)	2 (11.8)	
≥1	41 (69.5)	26 (61.9)	15 (88.2)	
Primary tumor resection				0.029
No	32 (54.2)	19 (45.2)	13 (76.5)	
Yes	27 (45.8)	23 (54.8)	4 (23.5)	
Primary site of origin				0.662
Gastroesophageal junction (GEJ)	6 (10.2)	5 (11.9)	1 (5.9)	
Gastric	53 (89.8)	37 (88.1)	16 (94.1)	
Histology				0.195
Intestinal	43 (72.9)	33 (78.6)	10 (58.8)	
Diffuse/other	16 (27.1)	9 (21.4)	7 (41.2)	
Synchronous metastases				0.033
No	23 (39.0)	20 (47.6)	3 (17.6)	
Yes	36 (61.0)	22 (52.3)	14 (82.4)	
Liver metastases				0.310
No	45 (76.3)	30 (71.4)	15 (88.2)	
Yes	14 (23.7)	12 (28.6)	2 (11.8)	
Lymphnodal metastases				>0.999
No	13 (22.0)	9 (21.4)	4 (23.5)	
Yes	46 (78.0)	33 (78.6)	13 (76.5)	
Lung metastases				>0.999
No	49 (83.1)	35 (83.3)	14 (82.4)	
Yes	10 (16.9)	7 (16.7)	3 (17.6)	
Bone metastases				0.620
No	54 (91.5)	39 (92.9)	15 (88.2)	
Yes	5 (8.5)	3 (7.1)	2 (11.8)	
No of metastatic sites				0.013
1	17 (28.8)	16 (38.1)	1 (5.9)	
≥2	42 (71.2)	26 (61.9)	16 (94.1)	
Prior treatment for metastatic disease				>0.999
No	7 (11.9)	5 (11.9)	2 (11.8)	
Yes	52 (88.1)	37 (88.1)	15 (88.2)	
ICI regimen				0.308
a-PD-1	54 (91.5)	37 (88.1)	17 (100)	
a-PD-1+chemotherapy	5 (8.5)	5 (11.9)	0 (0)	

P-values marked with bold indicate statistically significant p-values.

dMMR, mismatch repair deficient; ECOG, Eastern Cooperative Oncology Group; ICIs, immune checkpoint inhibitors; mGC, metastatic gastric cancer; MSI, microsatellite instability; PS, Performance Status.

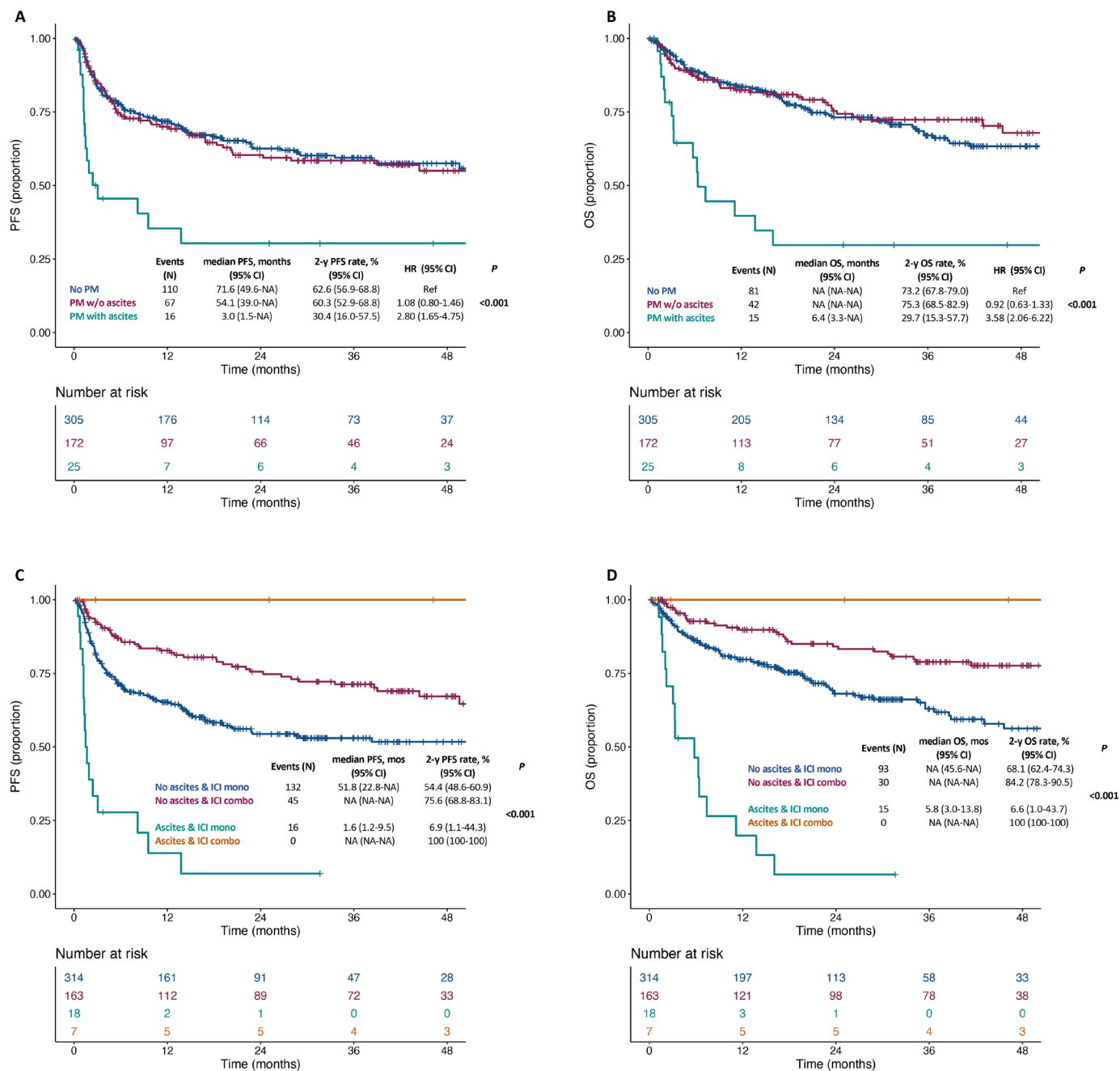


Figure 1 Kaplan-Meier curves for progression-free survival (A) and overall survival (B) in the subgroups of dMMR/MSI-H mCRC patients without peritoneal metastases, with peritoneal metastases and no ascites or with peritoneal metastases and ascites; Kaplan-Meier curves for progression-free survival (C) and overall survival (D) of patients dMMR/MSI-H mCRC according to the presence of ascites and the type of ICI regimen. dMMR, mismatch repair deficient; ICI, immune checkpoint inhibitors; mCRC, metastatic colorectal cancer; MSI-H, microsatellite instability high; NA, not available; OS, overall survival; PFS, progression-free survival.

However, the presence of peritoneal involvement and ascites was assessed at each center by expert radiologists, ensuring a high quality of the radiology reports. The reproducibility of our results confirms the agnostic role of MSI as a predictive biomarker for the efficacy of ICIs and establish the role of malignant ascites as a poor prognostic factor for dMMR/MSI-H mCRC treated with anti-PD(L)-1 agents or dMMR/MSI-H mGC treated with anti-PD-1 agents±chemotherapy independently from the anatomic tumor site of origin.

The frequency of malignant ascites was much higher in patients with mGC (28.8%), consistent with the higher frequency of peritoneal involvement in this tumor type, compared with those with mCRC (5%).¹⁹ Despite the potential generalizability of our results to all patients with MSI-high advanced solid tumors, caution should be adopted in specific tumor types with extremely low frequency of dMMR/MSI-H—such as cholangiocarcinoma and pancreatic cancers. In fact, non-randomized trial cohorts showed lower than expected response rates

Table 3 Univariable and multivariable Cox proportional hazards regression models for PFS and OS in the developing set (Cohort 1: dMMR/MSI-high mCRC)

Characteristics	PFS		OS	
	Univariable	Multivariable	Univariable	Multivariable
	HR (95% CI)	P value	HR (95% CI)	P value
Sex		0.673		0.644
Female	Ref		Ref	
Male	1.06 (0.80 to 1.41)		1.08 (0.77 to 1.52)	
Age (years)		0.723		0.588
<70	Ref		Ref	
≥70	1.061 (0.77 to 1.47)		1.11 (0.76 to 1.63)	
ECOG S		0.001		0.003
0	Ref		Ref	
≥1	1.608 (1.20 to 2.15)		1.56 (1.16 to 2.08)	
Primary tumor resection		0.802		0.317
No	Ref		Ref	
Yes	1.05 (0.72 to 1.54)		1.281 (0.79 to 2.08)	
Primary tumor sidedness		0.917		0.342
Left	Ref		Ref	
Right	1.02 (0.75 to 1.37)		0.85 (0.60 to 1.20)	
RAS mutational status		0.724		0.459
All wild-type	Ref		Ref	
RAS mutated	0.95 (0.70 to 1.28)		0.87 (0.61 to 1.25)	
BRAF mutational status		0.196		0.151
All wild-type	Ref		Ref	
BRAF mutated	1.23 (0.90 to 1.67)		1.303 (0.91 to 1.87)	
Synchronous metastases		0.737		0.460
No	Ref		Ref	
Yes	1.05 (0.79 to 1.39)		1.14 (0.81 to 1.59)	
Liver metastases		0.221		0.105
No	Ref		Ref	
Yes	1.20 (0.90 to 1.59)		1.32 (0.94 to 1.85)	
Lung metastases		<0.001		<0.001
No	Ref		Ref	
Yes	1.812 (1.31 to 2.50)		1.79 (1.29 to 2.48)	
Lymphnodal metastases		0.249		0.885
No	Ref		Ref	
Yes	0.85 (0.64 to 1.13)		1.03 (0.73 to 1.45)	
Bone metastases		0.085		0.011
No	Ref		Ref	
Yes	1.67 (0.93 to 3.01)		2.22 (1.20 to 4.12)	
No of metastatic sites		0.078		0.013
1	Ref		Ref	
≥2	1.30 (0.97 to 1.74)		1.56 (1.10 to 2.22)	

Continued



Table 3 Continued

Characteristics	PFS				OS			
	Univariable		Multivariable		Univariable		Multivariable	
	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value
Prior systemic treatment for metastatic disease		0.001		0.015		0.007		0.0563
No	Ref		Ref		Ref		Ref	
Yes	2.061 (1.31 to 3.24)		1.76 (1.12 to 2.79)		2.13 (1.23 to 3.70)		1.73 (0.99 to 3.03)	
ICI regimen		<0.001		<0.001		<0.001		<0.001
a-PD(L)-1	Ref		Ref		Ref		Ref	
a-PD-1+a-CTLA-4	0.46 (0.33 to 0.64)		0.46 (0.33 to 0.64)		0.41 (0.27 to 0.62)		0.41 (0.27 to 0.62)	
Peritoneal metastases		<0.001		0.002		<0.001		0.001
No	Ref		Ref		Ref		Ref	
Yes, without ascites	1.08 (0.80 to 1.46)		1.15 (0.85 to 1.56)		0.92 (0.63 to 1.33)		0.96 (0.65 to 1.42)	
Yes, with ascites	2.80 (1.65 to 4.75)		2.90 (1.70 to 4.94)		3.58 (2.06 to 6.22)		3.33 (1.88 to 5.91)	

P-values marked with bold indicate statistically significant p-values.

dMMR, mismatch repair deficient; ECOG, Eastern Cooperative Oncology Group; ICIs, immune checkpoint inhibitors; mCRC, metastatic colorectal cancer; MSI, microsatellite instability; OS, overall survival; PFS, progression-free survival; PS, Performance Status.

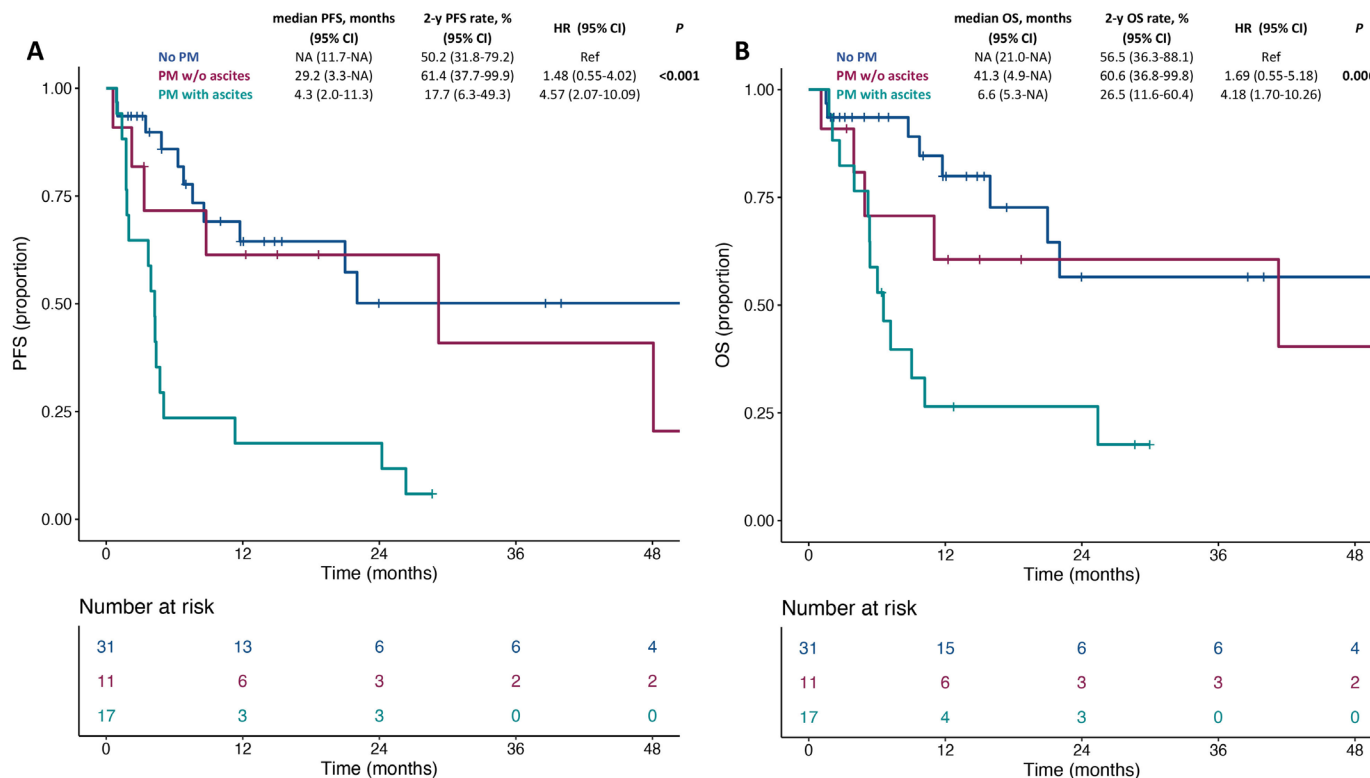


Figure 2 Kaplan-Meier curves for progression-free survival (A) and overall survival (B) in the subgroups of dMMR/MSI-H mGC patients without peritoneal metastases, with peritoneal metastases and no ascites or with peritoneal metastases and ascites. dMMR, mismatch repair deficient; mGC, metastatic gastric cancer; MSI-H, microsatellite instability high; NA, not available; OS, overall survival; PFS, progression-free survival.

Table 4 Univariable and multivariable Cox proportional hazards regression models for PFS and OS in the validating set (Cohort 2: dMMR/MSI-high mGC)

Characteristics	PFS				OS			
	Univariable		Multivariable		Univariable		Multivariable	
	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value
Sex		0.643				0.870		
Female	Ref		Ref		Ref		Ref	
Male	0.84 (0.41 to 1.73)				0.94 (0.41 to 2.11)			
Age		0.695				0.483		
<70	Ref		Ref		Ref		Ref	
≥70	0.87 (0.44 to 1.73)				0.76 (0.35 to 1.65)			
Eastern Cooperative Oncology Group (ECOG) Performance Status (PS)		0.018		0.410		0.022		0.301
0	Ref		Ref		Ref		Ref	
≥1	2.94 (1.20 to 7.18)		1.54 (0.55 to 4.31)		3.49 (1.20 to 10.2)		1.84 (0.58 to 5.88)	
Primary tumor resection		0.003		0.133		0.009		0.066
No	Ref		Ref		Ref		Ref	
Yes	0.30 (0.13 to 0.66)		0.46 (0.17 to 1.27)		0.30 (0.12 to 0.74)		0.37 (0.13 to 1.07)	
Primary site of origin		0.353				0.419		
Gastroesophageal junction (GEJ)	Ref		Ref		Ref		Ref	
Gastric	0.60 (0.21 to 1.75)				0.60 (0.18 to 2.05)			
Histology		0.506				0.575		
Intestinal	Ref		Ref		Ref		Ref	
Diffuse/other	1.29 (0.61 to 2.71)				1.27 (0.56 to 2.93)			
Synchronous metastases		0.460				0.855		
No	Ref		Ref		Ref		Ref	
Yes	1.33 (0.63 to 2.81)				0.93 (0.42 to 2.06)			
Liver metastases		0.294				0.201		
No	Ref		Ref		Ref		Ref	
Yes	0.60 (0.23 to 1.56)				0.46 (0.14 to 1.52)			
Lymphnodal metastases		0.767				0.947		
No	Ref		Ref		Ref		Ref	
Yes	1.14 (0.49 to 2.62)				0.97 (0.39 to 2.42)			
Lung metastases		0.789				0.802		
No	Ref		Ref		Ref		Ref	

Continued

Table 4 Continued

Characteristics	PFS				OS			
	Univariable		Multivariable		Univariable		Multivariable	
	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value
Yes	0.88 (0.34 to 2.28)				0.87 (0.30 to 2.53)			
Bone metastases		0.039		0.065		0.057		
No	Ref		Ref		Ref			
Yes	3.13 (1.06 to 9.27)		2.94 (0.94 to 9.20)		3.36 (0.98 to 11.64)			
No of metastatic sites		0.175				0.165		
1	Ref				Ref			
≥2	1.74 (0.78 to 3.87)				1.92 (0.76 to 4.80)			
Prior treatment for metastatic disease		0.792				0.719		
No	Ref				Ref			
Yes	0.87 (0.30 to 2.48)				0.80 (0.24 to 2.68)			
ICI regimen		0.722				0.908		
a-PD-1	Ref				Ref			
a-PD-1 +chemotherapy	0.77 (0.18 to 3.24)				1.10 (0.27 to 4.63)			
Peritoneal metastases		<0.001		0.005		0.006		0.023
No	Ref		Ref		Ref		Ref	
Yes, without ascites	1.48 (0.55 to 4.02)		1.87 (0.64 to 5.46)		1.69 (0.55 to 5.18)		2.15 (0.64 to 7.27)	
Yes, with ascites	4.57 (2.07 to 10.09)		3.83 (1.68 to 8.72)		4.18 (1.70 to 10.26)		3.44 (1.39 to 8.53)	

P-values marked with bold indicate statistically significant p-values.

dMMR, mismatch repair deficient; ICIs, immune checkpoint inhibitors; mGC, metastatic gastric cancer; MSI, microsatellite instability; OS, overall survival; PFS, progression-free survival.

to PD-1 blockade in these specific cancers, consistent with a highly immune-suppressive tumor microenvironment that may counterbalance the positive effect of hypermutation.⁷

Our study cannot clarify if malignant ascites is just a poor prognostic factor (which is well established for patients with advanced GI cancers^{20,21}) or if it is predictive of resistance to current ICI-based strategies. However, several works highlighted the immunosuppressive environment of serous human cavities and malignant effusions.^{15–17,22–25} Contrary to the more intuitive theory that peritoneum and pleura could represent an immune-excluded milieu because of the difficulty of immune system to penetrate the effusion fluids, recent studies supported the idea of serous cavities as an immune-enriched environment with high concentration of immune-suppressive cells such as macrophages, myeloid-derived suppressive cells and T regulatory cells.^{15–17} Also, a recent work showed that cavity-resident Tim-4⁺ macrophages populate the pleural and peritoneal microenvironment and can induce an immune-suppressed microenvironment by impairing CD8⁺ T cells proliferation.¹⁷ In this context, dual Tim-4/PD-1 blockade was synergic in murine models.¹⁷ In attempt to clinically validate these results, the authors analyzed a monocentre cohort of 61 patients with MSI-high mCRC treated with ICIs, reporting worse outcomes in those with peritoneal metastases, with unadjusted HRs for PFS and OS of 2.69 and 3.59, respectively. However, given the relatively low prevalence of ascites in their cohort of mCRC, it was not possible to investigate the prognostic impact of peritoneal metastases in a multivariable model and according to the presence or absence of malignant effusion.

Recently, the immune-suppressed microenvironment of ascites was demonstrated also in patients with mGC, irrespective of MMR status.²² Consistent with these findings, similar data were found in other immune-sensitive tumors.^{23,24} In a multicentre retrospective series, patients with metastatic non-small cell lung cancer and pleural effusion treated with anti-PD(L)-1 agents showed poorer survival outcomes and higher early death rates, even in the subgroup with high PD-L1 expression.²⁵

Our work has several potential implications for clinical practice. First, a subset of patients may sometimes develop ascites as a late-stage complication of peritoneal metastases. Based on the recent results of the Keynote-177 first-line mCRC trial,² and considering that our study showed that no prior treatment for advanced disease was independently associated with good outcomes, ICIs should be offered as early as possible in patients with peritoneal metastases and hopefully prior to the development of malignant ascites. Second, we showed that patients with ascites treated with anti-PD(L)-1 monotherapy had worse outcomes and almost all were dead by the 2-year time point, whereas no PFS and OS events were reported in patients treated with anti-CTLA-4 plus anti-PD-1 combination. The peculiar immune microenvironment of the serous cavities in the presence of ascites might confer a particular susceptibility to anti-CTLA-4-mediated priming

of T cells, explaining (at least in part) why we observed extremely good outcomes in mCRC patients with ascites treated with the combination of anti-CTLA-4 plus anti-PD-1 combination. Despite the greater efficacy reported with dual immune checkpoint blockade in non-randomized studies and in our series, the potential benefit of adding anti-CTLA-4 agents to an anti-PD(L)-1 backbone in patients with dMMR/MSI-H mCRC and malignant ascites should be interpreted with caution considering the low number of patients included in this analysis. Notably our results are aligned with the observation that dual checkpoint inhibition yielded relatively better outcomes in patients with poor prognostic features such as those with elevated systemic inflammation and poorer PS.^{14,26–28} Based on these considerations, the use of more aggressive therapies such as dual CTLA-4/PD-1 inhibition and chemoimmunotherapy in patients with dMMR/MSI-H advanced GI cancers and ascites may be warranted. Additional novel strategies including intraperitoneal delivery of immunomodulatory agents and exploration of checkpoints enriched in peritoneal metastases (eg, VISTA, LAG3, and TIM3) are worthy of further exploration.^{29–31}

Prospective validation of the potential predictive role of ascites with regard to the intensification of ICI-based regimens may be possible from post-hoc analyses of ongoing randomized trials, for instance, the ongoing COMMIT trial investigating FOLFOX/bevacizumab/atezolizumab vs atezolizumab (NCT02997228) or the ongoing CA209-8HW trial with ipilimumab–nivolumab versus nivolumab across multiple treatment lines (NCT04008030). The same concept may be applied to patients with dMMR/MSI-H mGC. In fact, although the outcomes of these subjects were not different when receiving anti-PD-1 alone or with chemotherapy,^{4,8,32} the addition of chemotherapy to ICI should be further investigated in the relevant subgroup of patients with dMMR/MSI-H mGC and malignant ascites.

In conclusion, patients with dMMR/MSI-H mCRC or mGC with peritoneal metastases and ascites have inferior outcomes with ICI therapy and should be viewed as a clinical subgroup requiring particular attention. Importantly, peritoneal involvement without ascites may not impart the same unfavorable outcomes and should not be regarded as a clinical factor associated with failure of ICIs.

Author affiliations

¹Department of Medical Oncology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy

²Sorbonne Université, Department of Medical Oncology, Hôpital Saint-Antoine, AP-HP and INSERM, Unité Mixte de Recherche Scientifique 938, Centre de Recherche Saint-Antoine, Equipe Instabilité des Microsatellites et Cancer, Equipe labellisée par la Ligue Nationale contre le Cancer, Paris, France

³Medical Oncology 3 and Medical Oncology 1, Istituto Oncologico Veneto IOV-IRCCS, Padua, Italy

⁴Department of Gastroenterology and Gastrointestinal Oncology, National Cancer Center Hospital East, Kashiwa, Japan

⁵Department of Medical Oncology, Vall d'Hebron Barcelona Hospital Campus, Vall d'Hebron Institute of Oncology (VHIO), Universitat Autònoma de Barcelona, Barcelona, Spain

⁶Department of Medical Oncology and Therapeutic Research, City of Hope Comprehensive Cancer Center, Duarte, California, USA

⁷Mass General Cancer Center and Department of Medicine, Massachusetts General Hospital, Boston, Massachusetts, USA

- ⁸Harvard Medical School, Boston, Massachusetts, USA
⁹Division of Medical Oncology, Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, Los Angeles, California, USA
¹⁰Medical Oncology 1, Istituto Oncologico Veneto IOV-IRCCS, Padua, Italy
¹¹Sorbonne Université, Department of Pathology, Hôpital Saint-Antoine, AP-HP, and INSERM, Unité Mixte de Recherche Scientifique 938, Centre de Recherche Saint-Antoine, Equipe Instabilité des Microsatellites et Cancer, Equipe labellisée par la Ligue Nationale contre le Cancer, Paris, France
¹²Baylor College of Medicine, Houston, Texas, USA
¹³Division of Oncology, Department of Oncology and Hematology, University Hospital of Modena, PhD Clinical and Experimental Medicine (CEM), University of Modena and Reggio Emilia, Modena, Italy
¹⁴Multidisciplinary Outpatient Oncology Clinic, Candiolo Cancer Institute FPO-IRCCS, Candiolo, Italy
¹⁵Department of Medical Oncology, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy
¹⁶Unit of Medical Oncology 2, Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy
¹⁷Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy
¹⁸European Institute of Oncology (IEO), IRCCS, Milan, Italy
¹⁹Department of Oncology and Hemato-oncology, University of Milan, Milan, Italy
²⁰Department of Gastrointestinal Oncology, University of Texas MD Anderson Cancer Center, Houston, Texas, USA

Contributors Conception and design: GF and FP. Acquisition of data: RC, SL, KS, MEE, MF, JC, SJK, ME, PJ, FB, MDG, LP, RC, MS, MA, GR, ATS, SM, EF, LS, KC, AK, VC, GC, FC, CC and MO. Analysis and interpretation of data: GF, SM, LP and FP. Manuscript drafting: GF, RC, LP, GM and FP. Manuscript revision: SL, KS, MEE, MF, JC, SJK, ME, PJ, FB, MDG, RC, MS, MA, GR, ATS, SM, EF, LS, KC, AK, VC, GC, FC, CC, MO and TA. Final approval: GF, RC, SL, KS, MEE, MF, JC, SJK, ME, PJ, FB, MDG, GM, LP, RC, MS, MA, GR, ATS, SM, EF, LS, KC, AK, VC, GC, FC, CC, MO, TA and FP. Author responsible for the overall content as the guarantor: FP.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests KS paid consulting or advisory roles for Astellas, Lilly, Bristol-Myers Squibb, Takeda, Pfizer, Ono, MSD, Taiho, Novartis, AbbVie, GlaxoSmithKline, Daiichi Sankyo, Amgen, and Boehringer Ingelheim; honoraria from Novartis, AbbVie, and Yakult; and research funding from Astellas, Lilly, Ono, Sumitomo Dainippon, Daiichi Sankyo, Taiho, Chugai, MSD, Medi Science and Eisai. JC reports grants and personal fees from Merck, Amgen, MacroGenics, Ono Pharmaceuticals, Foundation Medicine, Daiichi-Sankyo and Bristol Myers Squibb. AK reports honoraria from Ono Pharmaceutical, Bristol-Myers Squibb, Daiichi-Sankyo, Taiho Pharmaceutical, and Eli Lilly outside the submitted work. CC received honoraria from Amgen, Bayer, Merck, Roche, and Servier; has consulting or advisory role at Amgen, Bayer, MSD, and Roche; was a speakers bureau member at Servier; received research funding from Bayer, Merck, and Servier; and received travel and accommodation expenses from Roche and Servier. MO: consulting for Merck Sharp & Dohme, AbbVie, Agilvax, Acrotech Biopharma, and Novartis Pharmaceuticals Corp; and scientific/ advisory committee member of Takeda Pharmaceuticals (Japan). AK reports honoraria from Ono Pharmaceutical, Bristol-Myers Squibb, Daiichi-Sankyo, Taiho Pharmaceutical, and Eli Lilly outside the submitted work. FP received honoraria from Amgen, Merck-Serono, Sanofi, Lilly, Bayer, Servier, Astrazeneca; research grants from Astrazeneca and BMS. All the remaining authors declared no conflict of interest.

Patient consent for publication Not applicable.

Ethics approval The study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of the Fondazione IRCCS Istituto Nazionale dei Tumori of Milan (INT 117/15).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Supplemental material This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability

of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See <http://creativecommons.org/licenses/by-nc/4.0/>.

Author note TA and FP are joint last authors.

ORCID iDs

Giovanni Fucà <http://orcid.org/0000-0002-1560-2253>
 Romain Cohen <http://orcid.org/0000-0001-9602-5162>
 Sara Lonardi <http://orcid.org/0000-0002-7593-8138>
 Joseph Chao <http://orcid.org/0000-0002-1809-504X>
 Thierry Andre <http://orcid.org/0000-0002-5103-7095>
 Filippo Pietrantonio <http://orcid.org/0000-0002-8530-8420>

REFERENCES

- Le DT, Durham JN, Smith KN, *et al.* Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science* 2017;357:409–13.
- André T, Shiu K-K, Kim TW, *et al.* Pembrolizumab in microsatellite-instability-high advanced colorectal cancer. *N Engl J Med* 2020;383:2207–18.
- Janjigian YY, Shitara K, Moehler M, *et al.* First-line nivolumab plus chemotherapy versus chemotherapy alone for advanced gastric, gastro-oesophageal junction, and oesophageal adenocarcinoma (CheckMate 649): a randomised, open-label, phase 3 trial. *Lancet* 2021;398:27–40.
- Pietrantonio F, Randon G, Di Bartolomeo M, *et al.* Predictive role of microsatellite instability for PD-1 blockade in patients with advanced gastric cancer: a meta-analysis of randomized clinical trials. *ESMO Open* 2021;6:100036.
- Overman MJ, McDermott R, Leach JL, *et al.* Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): an open-label, multicentre, phase 2 study. *Lancet Oncol* 2017;18:1182–91.
- Le DT, Kim TW, Van Cutsem E, *et al.* Phase II open-label study of pembrolizumab in treatment-refractory, microsatellite instability-high/mismatch repair-deficient metastatic colorectal cancer: KEYNOTE-164. *J Clin Oncol* 2020;38:11–19.
- Marabelle A, Cassier PA, Fakih M, *et al.* Pembrolizumab for previously treated advanced anal squamous cell carcinoma: pooled results from the KEYNOTE-028 and KEYNOTE-158 studies. *JCO* 2020;38:4020.
- Shitara K, Van Cutsem E, Bang Y-J, *et al.* Efficacy and safety of pembrolizumab or pembrolizumab plus chemotherapy vs chemotherapy alone for patients with first-line, advanced gastric cancer: the KEYNOTE-062 phase 3 randomized clinical trial. *JAMA Oncol* 2020;6:1571–80.
- Shitara K, Özgüroğlu M, Bang Y-J, *et al.* Pembrolizumab versus paclitaxel for previously treated, advanced gastric or gastro-oesophageal junction cancer (KEYNOTE-061): a randomised, open-label, controlled, phase 3 trial. *Lancet* 2018;392:123–33.
- Colle R, Radzik A, Cohen R, *et al.* Pseudoprogression in patients treated with immune checkpoint inhibitors for microsatellite instability-high/mismatch repair-deficient metastatic colorectal cancer. *Eur J Cancer* 2021;144:9–16.
- Cohen R, Hain E, Buhard O, *et al.* Association of primary resistance to immune checkpoint inhibitors in metastatic colorectal cancer with misdiagnosis of microsatellite instability or mismatch repair deficiency status. *JAMA Oncol* 2019;5:551–5.
- Schrock AB, Ouyang C, Sandhu J, *et al.* Tumor mutational burden is predictive of response to immune checkpoint inhibitors in MSI-high metastatic colorectal cancer. *Ann Oncol* 2019;30:1096–103.
- Kwon M, An M, Klemperer SJ, *et al.* Determinants of response and intrinsic resistance to PD-1 blockade in microsatellite instability-high gastric cancer. *Cancer Discov* 2021;11:1–18.
- Corti F, Lonardi S, Intini R, *et al.* The pan-immune-inflammation value in microsatellite instability-high metastatic colorectal cancer patients treated with immune checkpoint inhibitors. *Eur J Cancer* 2021;150:155–67.
- Donnenberg AD, Luketich JD, Dhupar R, *et al.* Treatment of malignant pleural effusions: the case for localized immunotherapy. *J Immunother Cancer* 2019;7:110.

- 16 Buechler MB, Kim K-W, Onufer EJ, *et al.* A stromal niche defined by expression of the transcription factor WT1 mediates programming and homeostasis of cavity-resident macrophages. *Immunity* 2019;51:119–30.
- 17 Chow A, Schad S, Green MD, *et al.* Tim-4+ cavity-resident macrophages impair anti-tumor CD8+ T cell immunity. *Cancer Cell* 2021;39:973–88.
- 18 Luchini C, Bibeau F, Ligtenberg MJL, *et al.* ESMO recommendations on microsatellite instability testing for immunotherapy in cancer, and its relationship with PD-1/PD-L1 expression and tumour mutational burden: a systematic review-based approach. *Ann Oncol* 2019;30:1232–43.
- 19 Cortés-Guiral D, Hübner M, Alyami M, *et al.* Primary and metastatic peritoneal surface malignancies. *Nat Rev Dis Primers* 2021;7:91.
- 20 Pietrantonio F, Miceli R, Rimassa L, *et al.* Estimating 12-week death probability in patients with refractory metastatic colorectal cancer: the colon life nomogram. *Ann Oncol* 2017;28:555–61.
- 21 Pietrantonio F, Barretta F, Fanotto V, *et al.* Estimating survival probabilities of advanced gastric cancer patients in the second-line setting: the gastric life nomogram. *Oncology* 2018;95:344–52.
- 22 Tanaka Y, Chiwaki F, Kojima S, *et al.* Multi-omic profiling of peritoneal metastases in gastric cancer identifies molecular subtypes and therapeutic vulnerabilities. *Nat Cancer* 2021;2:962–77.
- 23 Porcel JM, Gasol A, Bielsa S, *et al.* Clinical features and survival of lung cancer patients with pleural effusions. *Respirology* 2015;20:654–9.
- 24 Aarnink A, Fumet JD, Favier L, *et al.* Role of pleural and peritoneal metastasis in immune checkpoint inhibitors efficacy patients with non-small cell lung cancer: real-world data from a large cohort in France. *J Cancer Res Clin Oncol* 2020;146:2699–707.
- 25 Epailard N, Benitez JC, Gorria T, *et al.* Pleural effusion is a negative prognostic factor for immunotherapy in patients with non-small cell lung cancer (NSCLC): the pluide study. *Lung Cancer* 2021;155:114–9.
- 26 Pietrantonio F, Loupakis F, Randon G, *et al.* Efficacy and safety of immune checkpoint inhibitors in patients with microsatellite instability-high end-stage cancers and poor performance status related to high disease burden. *Oncologist* 2020;25:803–9.
- 27 Fucà G, Corti F, Ambrosini M, *et al.* Prognostic impact of early tumor shrinkage and depth of response in patients with microsatellite instability-high metastatic colorectal cancer receiving immune checkpoint inhibitors. *J Immunother Cancer* 2021;9:e002501.
- 28 Pietrantonio F, Lonardi S, Corti F, *et al.* Nomogram to predict the outcomes of patients with microsatellite instability-high metastatic colorectal cancer receiving immune checkpoint inhibitors. *J Immunother Cancer* 2021;9:e003370.
- 29 Principe N, Kidman J, Lake RA, *et al.* Malignant pleural effusions-A window into local anti-tumor T cell immunity? *Front Oncol* 2021;11:14–22.
- 30 Murthy P, Ekeke CN, Russell KL, *et al.* Making cold malignant pleural effusions hot: driving novel immunotherapies. *Oncoimmunology* 2019;8:e1554969.
- 31 Wang R, Song S, Harada K, *et al.* Multiplex profiling of peritoneal metastases from gastric adenocarcinoma identified novel targets and molecular subtypes that predict treatment response. *Gut* 2020;69:18–31.
- 32 Chao J, Fuchs CS, Shitara K, *et al.* Assessment of pembrolizumab therapy for the treatment of microsatellite Instability-High gastric or gastroesophageal junction cancer among patients in the KEYNOTE-059, KEYNOTE-061, and KEYNOTE-062 clinical trials. *JAMA Oncol* 2021;7:895–902.

Supplemental Table 1. Centers' list and contribution to cohort 1 (dMMR/MSI-H mCRC).

Center	Patients included (N=502) N (%)
Hôpital Saint-Antoine, Paris, France	171 (34.0)
Istituto Oncologico Veneto IOV-IRCCS, Padua, Italy	89 (17.7)
University of Texas MD Anderson Cancer Center, Houston, Texas, USA	82 (16.3)
Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy	34 (6.8)
Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain	30 (6.0)
University Hospital of Modena, Modena, Italy	22 (4.4)
Candiolo Cancer Institute FPO-IRCCS, Candiolo, Italy	20 (4.0)
Norris Comprehensive Cancer Center, Los Angeles, California, USA	20 (4.0)
City of Hope Comprehensive Cancer Center, Duarte, California, USA	17 (3.4)
Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy	9 (1.8)
European Institute of Oncology (IEO), Milan, Italy	5 (1.0)
Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy	3 (0.6)

Supplemental Table 2. Pairwise analysis of the clinico-pathological and treatment characteristics according to the presence metastatic peritoneal involvement with or without ascites in the developing set of patients with MSI-high/dMMR mCRC (Cohort 1).

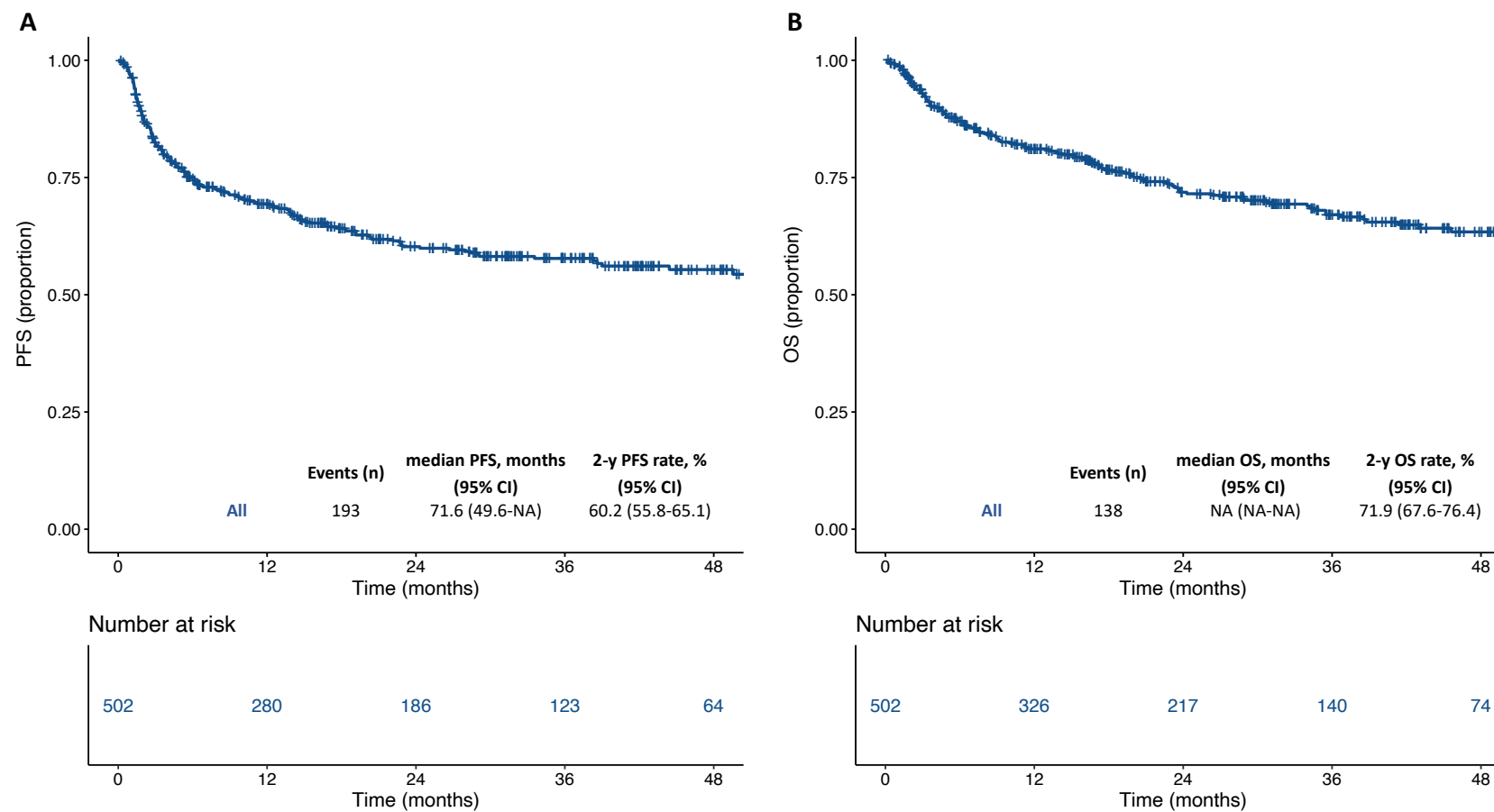
Characteristics	A - No peritoneal metastases (N=305) N (%)	B - Peritoneal metastases without ascites (N=172) N (%)	C - Peritoneal metastases with ascites (N=25) N (%)	B vs A <i>p</i> *	C vs A <i>p</i> *	C vs B <i>p</i> *
Sex				0.567	0.532	0.674
Female	135 (44.3)	81 (47.1)	13 (52.0)			
Male	170 (55.7)	91 (52.9)	12 (48.0)			
Age (years)				0.323	0.468	0.236
<70	233 (76.4)	124 (72.1)	21 (84.0)			
≥70	72 (23.6)	48 (27.9)	4 (16.0)			
ECOG PS				0.924	0.006	0.009
0	149 (48.9)	83 (48.3)	5 (20.0)			
≥1	156 (51.1)	89 (51.7)	20 (80.0)			
Primary tumor resection				0.084	0.796	0.762
No	62 (20.3)	24 (14.0)	4 (16.0)			
Yes	243 (79.7)	148 (86.0)	21 (84.0)			
Primary tumor sidedness				0.021	0.197	0.813
Left	118 (38.7)	48 (27.9)	6 (24.0)			
Right	187 (61.3)	124 (72.1)	19 (76.0)			
NA						
RAS mutational status				0.414	0.048	0.107
Wild-type	186 (61.0)	110 (64.0)	21 (84.0)			
RAS mutated	108 (35.4)	53 (30.8)	4 (16.0)			
NA	11 (3.6)	9 (5.2)	0 (0.0)			
BRAF mutational status				0.321	0.056	0.166
Wild-type	221 (72.5)	116 (67.4)	14 (56.0)			
BRAF mutated	73 (23.9)	48 (27.9)	11 (44.0)			
NA	11 (3.6)	8 (4.67)	0 (0.0)			
Synchronous metastases				0.390	0.302	0.198
No	145 (47.5)	88 (51.2)	9 (36.0)			
Yes	160 (52.5)	82 (47.6)	16 (64.0)			
NA	0 (0.0)	2 (1.2)	0 (0.0)			
Liver metastases				<0.001	0.060	0.625
No	157 (51.5)	131 (76.2)	18 (72.0)			
Yes	148 (48.5)	41 (23.8)	7 (28.0)			
Lung metastases				0.048	0.437	>0.999
No	241 (79.0)	149 (86.6)	22 (88.0)			
Yes	64 (21.0)	23 (13.3)	3 (12.0)			
Lymph nodal metastases				<0.001	0.187	0.520
No	101 (33.1)	97 (56.4)	12 (48.0)			
Yes	204 (66.9)	75 (43.6)	13 (52.0)			
Bone metastases				0.352	0.636	0.218
No	289 (94.8)	167 (97.1)	23 (92.0)			
Yes	16 (5.2)	5 (2.9)	2 (8.0)			

Number of metastatic sites				<0.001	0.021	0.654
1	162 (53.1)	58 (33.7)	7 (28.0)			
≥2	143 (46.9)	114 (66.3)	18 (72.0)			
Prior systemic treatment for metastatic disease				0.281	0.128	0.380
No	65 (21.3)	29 (16.9)	2 (8.0)			
Yes	240 (78.7)	143 (83.1)	23 (92.0)			
ICI regimen				0.045	>0.999	0.279
a-PD(L)-1	211 (69.2)	103 (59.9)	18 (72.0)			
a-PD-1 + a-CTLA-4	94 (30.8)	69 (40.1)	7 (28.0)			
*Pairwise Fisher's exact test						

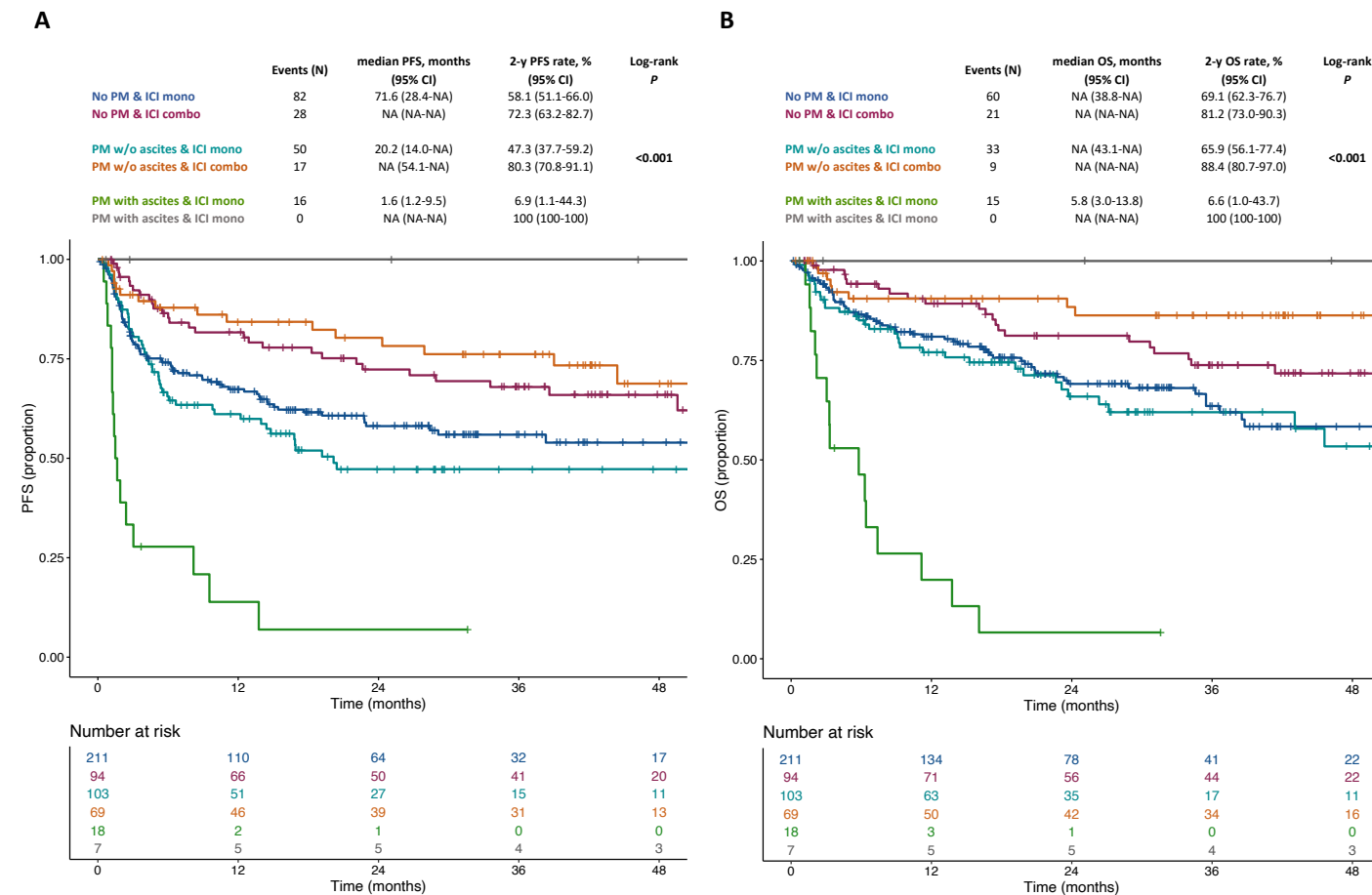
Supplemental Table 3. Centers' list and contribution to cohort 2 (dMMR/MSI-H mGC).

Center	Patients included (N=59) N (%)
National Cancer Center Hospital East, Kashiwa, Japan	18 (30.5)
Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy	13 (22.1)
City of Hope Comprehensive Cancer Center, Duarte, California, USA	10 (16.9)
Massachusetts General Hospital, Boston, Massachusetts, USA	10 (16.9)
Istituto Oncologico Veneto IOV-IRCCS, Padua, Italy	8 (13.6)

Supplemental Figure 1. Kaplan Meier curves for progression-free survival (PFS, panel A) and overall survival (OS, panel B) in the overall population of patients with dMMR/MSI-H mCRC treated with immune checkpoint inhibitors (Cohort 1).



Supplemental Figure 2. Kaplan Meier curves for progression-free survival (panel A) and overall survival (panel B) in dMMR/MSI-H mCRC patients according to the type of peritoneal involvement (no peritoneal metastases vs peritoneal metastases and no ascites vs peritoneal metastases and ascites) and to the type of ICI regimens (anti-PD(L)-1 monotherapy vs dual anti-CTLA-4/PD1 combination).



Supplemental Figure 3. Kaplan Meier curves for progression-free survival (PFS, panel A) and overall survival (OS, panel B) in the overall population of patients with dMMR/MSI-high mGC treated with immune checkpoint inhibitors (Cohort 2).

