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Original Article

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Mucinous histology and resistance to immune checkpoint blockade in patients with microsatellite instability-high metastatic colorectal cancer

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Abstract

Background: **Mucinous phenotype has been associated with an immune-cold microenvironment in dMMR/MSI-H CRC. Here, we investigated the impact of mucinous histology on resistance to ICIs in patients with dMMR/MSI-H mCRC.**

Patients and methods: **We collected data on patients with dMMR/MSI-H mCRC receiving anti–PD-1 alone or with anti-CTLA-4 agents in any line from a multinational dataset. Mucinous histology was defined locally as ≥50% mucinous component. The occurrence of progression or death of disease within or after 6 months from ICIs initiation were considered innate (IR) and acquired resistance (AR). Progression-free survival (PFS)-1 and overall survival (OS)-1 were calculated using the 6-month landmark from the start of ICIs in patients without IR.**

Results: **Among 929 patients, 316 (34%) had mucinous tumors and had inferior PFS and OS. At a median follow-up of 44.7 months, the 6-month PFS rate was similar in patients with mucinous and non-mucinous tumors (71.5% and 73.0%) and a subsequent divergence of the PFS and OS curves was observed beyond this timepoint. In patients without IR, mucinous histology was independently associated with shorter PFS-1 (multivariable model HR 2.10, 95%CI 1.56–2.82; $p < 0.001$), while OS-1 was non-significantly shorter (HR 1.36, 95%CI 0.91–2.02, $p = 0.130$). Dual anti-CTLA4/PD-1 blockade was associated with longer PFS and OS regardless of mucinous histotype, but the worst outcomes were observed in patients with mucinous histology receiving single-agent anti-PD-1 (3-year PFS and OS: 39.7% and 56.1%).**

Conclusion: **Mucinous histology is associated with ICIs resistance, particularly AR, in dMMR/MSI-H mCRC. These findings may be useful to guide the management of this disease in practice.**

Key words: microsatellite instability; metastatic colorectal cancer; immune checkpoint inhibitors; mucinous histology

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Implications for Practice

In this multicenter cohort study, we have identified mucinous histology as an independent biomarker of acquired resistance to ICIs in patients with dMMR/MSI-H mCRC regardless of type of ICIs and other variables. However, patients with mucinous histology receiving treatment with anti-PD-1 alone have the lowest likelihood of achieving long-term survival. Dual PD-1/CTLA-4 blockade partially counteract the adverse effect of this resistant phenotype. Mucinous residues after treatment may be a sign of complete response, but residual viable cells may be present in those deposits and careful monitoring or locoregional approaches may be useful in the clinical practice.

Introduction

Immune checkpoint inhibitors (ICIs), either anti–programmed cell death protein (PD)-1 antibodies alone or in combination with anti-cytotoxic T-lymphocyte–associated protein 4 (CTLA-4) agents, are the standard of care for first-line treatment of patients with deficient mismatch repair (dMMR) and/or microsatellite instability-high (MSI-H) metastatic colorectal cancer (mCRC).[1,2] Some patients with metastatic disease can even be considered cured, a phenomenon attributed to immune-mediated tumor eradication.[3,4] Despite a substantial proportion of patients achieving sustained benefit from ICIs, up to 50% experience innate (IR) or acquired resistance (AR), more frequently if receiving ICI in later treatment lines or anti-PD-1 single agent.[5,6] Biological mechanisms underlying both IR and AR to ICIs remain largely unclear. A range of clinic-biological factors, such as poor performance status, the presence of liver metastases or ascites, and specific transcriptomic profiles, have been correlated with less favourable outcomes but should be mainly regarded as prognostic factors.[7–15] A mucinous differentiation is a common histopathological feature of dMMR/MSI-H CRCs, being found in up to 50% of cases.[16,17] In patients with resected tumors, the presence of mucinous histology has not been associated with differences in cancer-specific or overall survival in either dMMR or pMMR subgroups.[17] However, preclinical data suggests an association between mucinous differentiation and PD-L1 downregulation or lower T-cell infiltrate in dMMR/MSI-H CRCs.[17,18] Further, recent works from patients in both neoadjuvant and metastatic setting hint at a reduced sensitivity to ICIs of mucinous dMMR/MSI-H CRC, but these were limited by their sample size or a lack of focus on histology, thereby limiting their insight into its contribution to IR and AR.[19,20]

Therefore, we carried out a multicenter cohort study aimed at evaluating the impact of mucinous histology on the ICIs treatment outcomes and resistance patterns in patients with dMMR/MSI-H mCRC.

Patients and methods

Study population

Patients with dMMR/MSI-H mCRC treated with anti-PD-1 monotherapy or anti-CTLA-4 based combination therapy in any line were included in a multinational observational study involving multiple academic hospitals in Europe, United States and Israel. MMR and/or MSI status were locally assessed using immunohistochemistry, multiplex PCR and/or next-generation sequencing as per standard institutional practices. Collected baseline characteristics before ICI therapy were: age, sex, Eastern Cooperative Oncology Group (ECOG) Perfomance Status (PS), primary tumor sidedness (right versus left, respectively proximal and distal to the splenic flexure), primary tumor resection, histology (non-mucinous versus mucinous; mucinous histology was defined as the presence of ≥50% mucinous component assessed locally), timing of metastases (synchronous versus metachronous; synchronous metastatic disease was defined by diagnosis of metastases within 6 months from surgery/adjuvant chemotherapy or de novo diagnosis of metastatic or locally advanced, unresectable disease), number of metastatic sites (≥2 versus 1), site of metastases, ICI treatment line and ICI regimen (anti-PD-1 monotherapy versus anti-CTLA-4 + anti-PD-1 combination).

Statistical analysis

Continuous variables were expressed as the median with variation expressed as the interquartile range (IQR) and compared with Mann-Whitney test. Categorical variables are expressed as frequencies and percentages are compared using two-sided chi-square or Fisher's exact testing, when appropriate.

Follow-up time was estimated using the reverse Kaplan-Meier method. Progression-free survival (PFS) was defined as the time from the start of ICI treatment to the evidence of disease progression or death from any cause, whichever occurred first. Overall survival (OS) was defined as the time from the start of ICI treatment to death from any cause or last follow-up. Objective response rate (ORR) was evaluated in patients with measurable disease according to RECIST version 1.1. IR was defined as the occurrence of progressive disease (PD) or death of disease within 6 months from ICI start.[21] Conversely, patients who experienced PD or death of disease after 6 months were classified as having AR. For the cohort of patients without IR (thus, those at risk of AR), PFS and OS were calculated from the 6-month landmark and were defined as PFS-1 and OS-1, respectively. PFS and OS were estimated according to the Kaplan-Meier method. Cox proportional hazard model was used to estimate hazard ratios (HRs) and 95% confidence intervals (CI). In Cox proportional hazards regression models, all the covariates showing a statistically significant ($p < 0.05$) association with PFS and OS in the univariable model were included in the multivariable model. P values < 0.05 were considered statistically significant. The Kaplan-Meier estimator and Cox proportional hazards regression were used for survival analysis using the survival, survminer, and survMisc packages. All statistical analyses were performed using R statistical software (R V4.1.1).

Ethics committee approval

The study was approved by each investigational site's Institutional Review Board ethics committee and by Fondazione IRCCS Istituto Nazionale dei Tumori, Milano—INT 117/15 study. The research was conducted in accordance with the Declaration of Helsinki and local data protection laws. All patients were provided with written informed consent before enrolment. All data provided were anonymized in line with applicable laws and regulations.

Results

Patients and disease's characteristics according to mucinous histology

We collected data from 984 patients with dMMR/MSI-H mCRC treated with ICIs from 2014 to 2024. As detailed in the patient flowchart (Figure 1**)**, after excluding 15 patients due to missing data on mucinous histology and 40 patients without a PFS-defining event and a follow-up time <6 months, 929 patients were ultimately included in the study (main cohort), of whom 316 (34%) had mucinous tumors. Baseline features according to histology in the main cohort are summarized in Table 1. In details, patients with mucinous histology had a higher frequency of right-sided location and resection of the primary tumor, a lower frequency of liver metastases, and a numerically higher frequency of *RAS* mutations.

Prognostic impact of mucinous histology

At a median follow-up of 44.7 months (interquartile range [IQR] 27.3–67.7), patients with mucinous histology experienced inferior PFS (hazard ratio, HR, 1.48, 95% confidence interval, CI, 1.22–1.78, $p < 0.0001$) and OS (HR 1.25, 95%CI 0.99–1.57, $p = 0.053$) compared to patients with non-mucinous tumors (Figure 2). However, during the first six months the PFS and OS curves according to mucinous histology were superimposable, suggesting the association of mucinous differentiation with AR rather than IR. Consistently, the 6-month PFS rate was similar (71.5% vs 73.0% in mucinous and non-mucinous groups, respectively), and a subsequent divergence of the curves was observed beyond this timepoint: 12-, 24- and 36-month PFS rates were 63.8% vs 69.5% (Δ5.7%), 52.6% vs 64.1% (Δ11.5%) and 46.0% vs 60.0% (Δ14.0%) for mucinous and non-mucinous histology, respectively. Similarly, 12-, 24- and 36-month OS rates were 78.9% vs 81.3% (Δ2.4%), 69.2% vs 73.9% (Δ4.7), and 61.7% vs 70.7% (Δ9.0%) for mucinous and non-mucinous histology, respectively. RECIST response data were evaluable for 666/929 (72%) patients, as shown in Table 2. Patients with mucinous histology were significantly less likely to achieve an objective response compared to the non-mucinous subgroup (overall response rate [ORR] 48.0% vs 65.1%; odds ratio, OR, 0.50, 95%CI 0.36–0.69, $p < 0.001$). In addition, patients with mucinous histology had a lower complete response rate compared to those with non-mucinous histology (17.3% vs 30.9%; OR 0.47, 95%CI 0.31–0.70, $p < 0.001$). Disease control rate (DCR) was similar in the two groups (DCR 79.8% vs 81.8%; OR 0.88, 95%CI 0.58–1.34, $p = 0.540$).

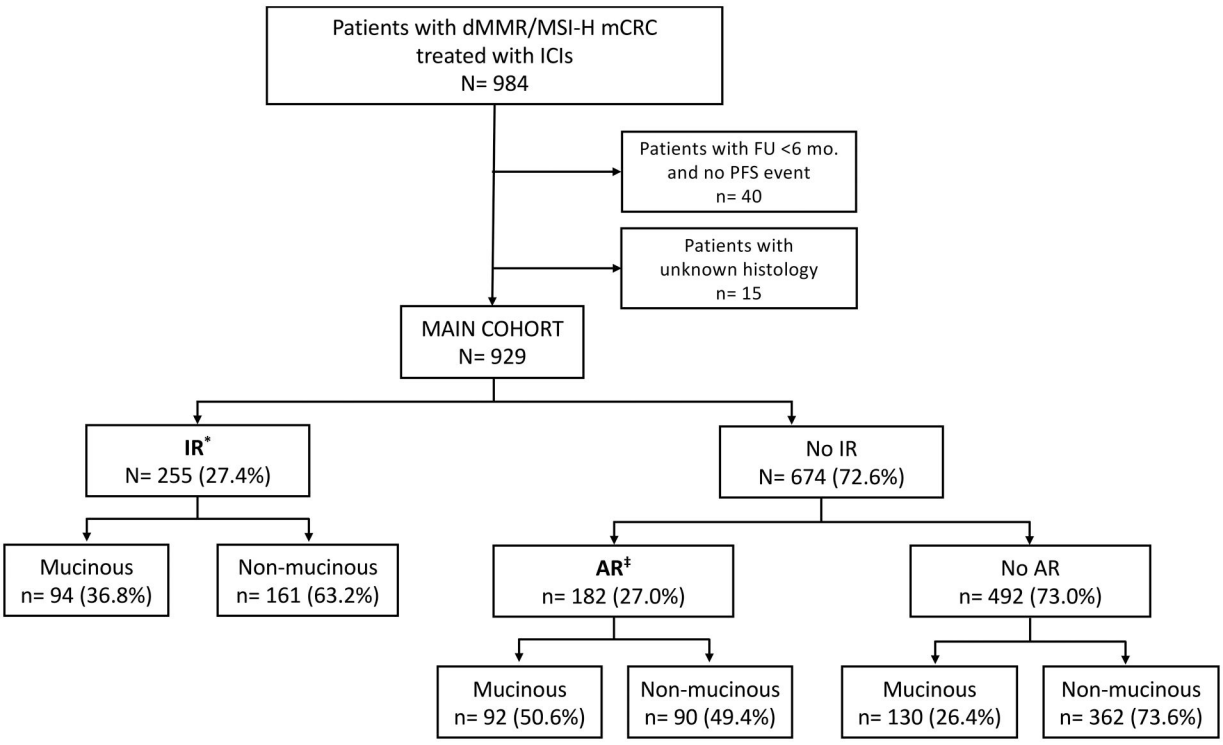

Figure 1 Study design and flow-chart of patients' inclusion. *****IR was defined as the occurrence of progressive disease (PD) or death within 6 months from ICI start. ‡AR was defined as the occurrence of PD or death after 6 months from ICI start. *List of abbreviations*. dMMR, deficient mismatch repair. MSI-H, microsatellite instability-high. mCRC, metastatic colorectal cancer. FU, follow-up. IR, innate resistance. AR, acquired resistance.

Table 1 Baseline patients and disease characteristics in the main cohort (*N* = 929) and in the cohort without innate resistance (N = 674).

Characteristics	Main cohort (N = 929)			Cohort without Innate Resistance (IR) (N = 674)		
	Non-mucinous (n = 613) n (%)	Mucinous (n = 316) n (%)	*P*	Non-mucinous (n = 452) n (%)	Mucinous (n = 222) n (%)	*P*
Sex			0.190			0.131
Male	314 (51.2)	147 (46.5)		223 (49.3)	124 (55.9)	
Female	298 (48.8)	169 (53.5)		229 (50.7)	98 (44.1)	
Age (years)			0.798			0.702
<70	419 (68.4)	214 (68.0)		314 (69.5)	151 (68.0)	
≥70	194 (31.6)	102 (32.0)		138 (30.5)	71 (32.0)	
ECOG PS			0.234			0.106
0	244 (40.5)	141 (45.3)		203 (44.9)	115 (51.8)	
1	293 (48.6)	141 (45.3)		218 (48.2)	95 (42.8)	
≥2	65 (10.8)	29 (9.4)		31 (6.8)	12 (5.4)	
NA	11	5		/	/	
Primary tumor location			**<0.001**			**0.003**
Right colon	414 (67.5)	251 (79.4)		314 (69.5)	179 (80.6)	
Left colon/rectum	199 (32.5)	65 (20.6)		138 (30.5)	43 (19.4)	
Primary tumor resection			**<0.001**			**<0.001**
Yes	471 (76.8)	285 (90.2)		352 (77.9)	202 (90.1)	
No	142 (23.2)	31 (9.8)		100 (22.1)	20 (9.9)	
Mutational status[*]			0.068			**0.039**
RAS/BRAF wild-type	219 (36.5)	95 (30.1)		160 (36.3)	64 (29.2)	
RAS mutated	174 (28.5)	109 (34.6)		131 (29.7)	86 (39.3)	
BRAF mutated	206 (33.6)	110 (34.8)		150 (34.0)	69 (31.5)	
NA	14	2		11	3	
Time to metastases			0.369			0.675
Synchronous	353 (57.6)	172 (54.4)		249 (55.0)	117 (52.7)	
Metachronous	260 (42.4)	144 (45.6)		203 (45.0)	105 (47.3)	
Metastatic sites			0.448			0.787
1	274 (44.7)	133 (42.1)		215 (47.6)	102 (45.9)	
≥2	339 (55.3)	183 (57.9)		237 (52.4)	120 (54.1)	
Liver metastases			**0.025**			0.078
Yes	239 (39.0)	100 (31.9)		165 (36.5)	66 (29.7)	
No	374 (61.0)	213 (68.1)		287 (63.5)	156 (70.3)	
Lung metastases			0.442			0.876
Yes	113 (18.4)	52 (16.5)		63 (13.9)	32 (13.6)	
No	500 (81.6)	264 (83.5)		389 (86.1)	190 (86.4)	
Peritoneal metastases			0.068			0.680
Yes	347 (56.6)	160 (50.6)		182 (40.3)	93 (41.9)	
No	266 (43.4)	156 (49.4)		270 (59.7)	129 (58.1)	
Nodal metastases			0.537			0.187
Yes	337 (55.0)	181 (57.3)		247 (54.6)	133 (60.0)	
No	276 (45.0)	135 (42.7)		205 (45.4)	89 (40.0)	
Line of ICI treatment			0.151			0.424
1st	215 (35.1)	96 (30.4)		169 (37.4)	76 (34.2)	
≥2nd	398 (74.9)	220 (69.6)		283 (62.6)	146 (65.8)	
ICI regimen			0.239			0.618
Anti-PD-1 mono	438 (71.5)	237 (75.0)		307 (67.9)	155 (70.0)	
Anti-CTLA-4 combo	175 (28.5)	79 (25.0)		145 (32.1)	67 (30.0)	

* Typical *RAS* mutations (codon 12, 13, 59, 61, 117, and 146 in *KRAS* and *NRAS*).

List of abbreviations. IR, innate resistance. IQR, inter-quartile range. ECOG PS, Eastern Cooperative Group Performance Status. ICI, immune checkpoint inhibitors.

Statistically significant (<0.05) p-values are displayed in **bold**.

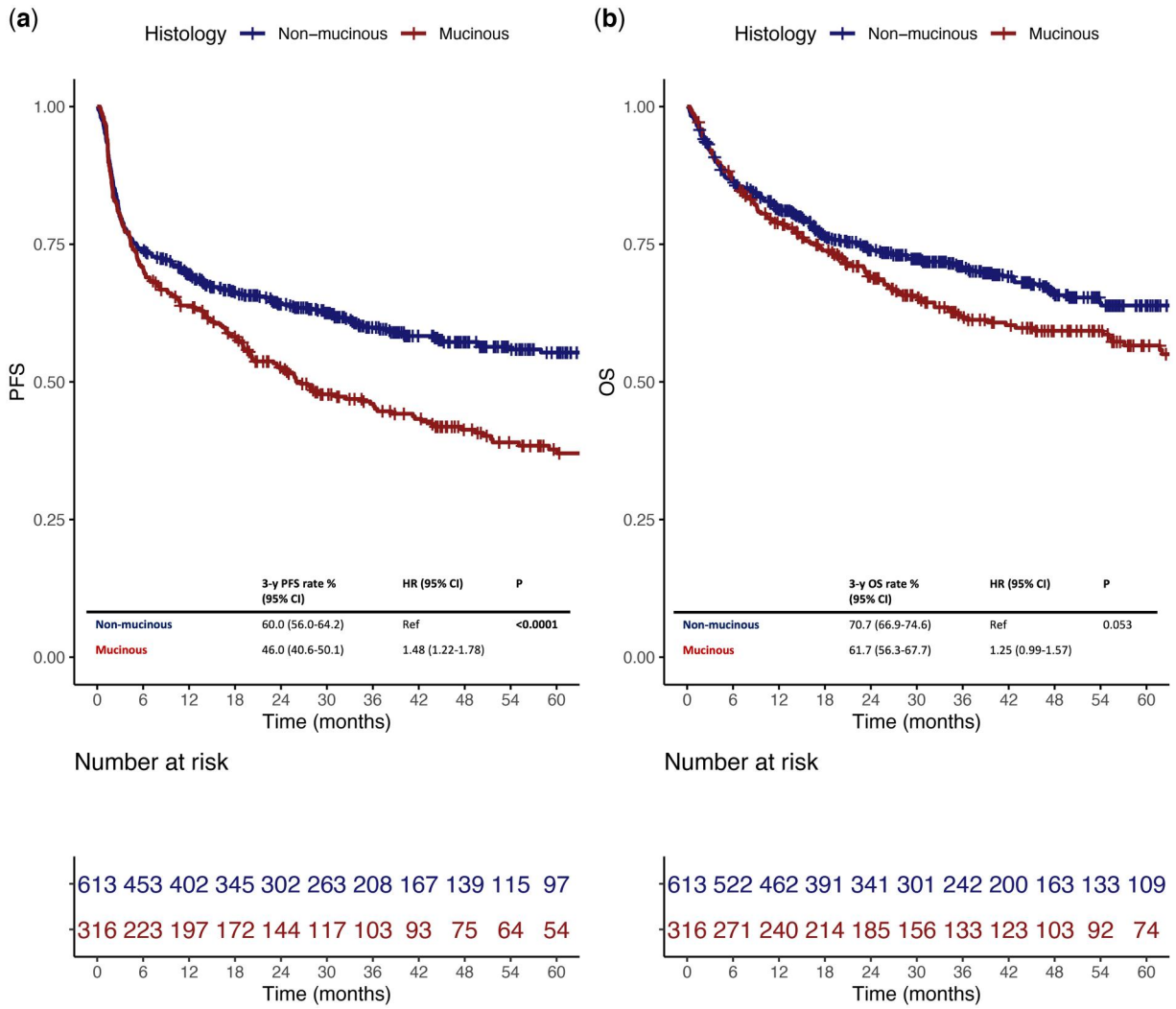

Figure 2 Kaplan-Meier analysis of progression-free survival (a) and overall survival (b) according to histology (mucinous vs non-mucinous) in patients with dMMR/MSI-H mCRC ($N = 929$). *List of abbreviations*. PFS, progression-free survival. OS, overall survival. dMMR, mismatch repair deficient. MSI-H, microsatellite instability high. mCRC, metastatic colorectal cancer.

Impact of mucinous histology in mediating acquired resistance

Among 929 patients in the main cohort, 255 patients showed IR and were therefore excluded from analyses on AR to ICIs. The baseline patients and disease's characteristics of the 674 patients without IR are summarized according to histology in Table 1. In line with the main cohort, patients with mucinous disease were enriched for right-sided primary tumor location, resection of primary tumor, and *RAS* mutations.

One hundred and eighty-two (27.0%) patients developed AR, of whom 92 (41.4%) and 90 (19.9%) had mucinous and non-mucinous histology, respectively. Patients with mucinous histology had inferior PFS-1 compared to those with non-mucinous histology (3-year rate: 63.6% vs 81.1%; HR 2.06, 95%CI 1.54–2.75, $p < 0.001$; Figure 3a). In addition, patients with mucinous histology showed numerically lower OS-1 (3-year OS-1 rate: 83.0% vs 89.2%; HR 1.36, 95%CI 0.91–2.02, $p = 0.130$; Figure 3b). Table 3 shows uni- and multivariable analyses for PFS-1 and OS-1. In the multivariable models, mucinous histology was associated with inferior PFS-1 (HR 2.10, 95%CI 1.56–2.82, $p < 0.001$), with non-significant association in terms of OS.

Predictive impact of mucinous histology for dual immune checkpoint inhibition

We next explored the outcomes according to both histology and type of ICI treatment (anti-PD-1 agent with or without anti-CTLA-4) in both the main cohort (Figure 4a,b) and the one without IR (Figure 4c,d). In the main cohort, the anti-CTLA-4/anti-PD-1 combination was associated with a longer PFS and OS both in mucinous and non-mucinous tumors (Figure 4a,b). However, patients with mucinous tumors receiving anti-PD-1 monotherapy had the worst PFS and OS (3-year PFS and OS rates of 39.7% and 56.1%, respectively). Similar results were observed in the cohort of patients without IR (Figure 4c,d).

Table 2 Best tumor response per RECISTv1.1 criteria according to histology among patients with measurable disease and available tumor response (*N* = 666), with odds ratios for objective response rate and disease control rate for mucinous vs non-mucinous disease.

	Non-mucinous (n = 418) n (%)	Mucinous (n = 248) n (%)	OR (95% CI)	p*
Overall response rate	272 (65.1)	119 (48.0)	0.50 (0.36–0.69)	**<0.001**
Disease control rate	342 (81.8)	198 (79.8)	0.88 (0.58–1.34)	0.540
Best overall response				**<0.001**
CR	129 (30.9)	43 (17.3)		
PR	143 (34.2)	76 (30.6)		
SD	70 (16.7)	79 (31.9)		
PD	76 (18.2)	50 (20.2)		

* *P*-value of the Odds Ratio from Fisher's Exact test.

List of abbreviations. OR, odds ratio. CI, confidence interval. CR, complete response. PR, partial response. SD, stable disease. PD, progressive disease. Statistically significant (<0.05) p-values are displayed in **bold**.

Discussion

Clinical and molecular drivers of resistance to ICIs in patients with dMMR/MSI-H mCRC remain largely elusive. Here, we first observed that mucinous histology was associated with worse PFS and OS in patients receiving ICIs. Then, we isolated the relative contribution of mucinous histology to adverse prognosis, finding that it was mainly driven by its independent association with AR. In fact, the PFS curves displayed a distinctive pattern, with a clear separation between mucinous and non-mucinous groups emerging only after 6 months of treatment. In line with these findings, mucinous tumors were not enriched for any baseline clinical features, including those previously associated with poor outcomes upon ICIs, especially early treatment failure, such as worse ECOG performance status, high tumor burden, peritoneal metastases or the use of ICIs in later lines of treatment, among others.[8–10] Additionally, when looking at the tumor response dynamics, lower overall response rate and complete response rate mucinous tumors, suggesting a propensity for disease persistence despite initial disease control, which may eventually lead to AR over time.[22] Overall, these findings support the hypothesis that mucinous histology reduces the activity and capability of ICIs to achieve tumor eradication, rather than simply representing a negative prognostic factor. If the effect were exclusively prognostic, a comparable impact would be expected in the setting of IR, which was not observed.

Preclinical data correlated mucinous-rich tumor microenvironment to immunosuppression which may explain the failure of establishing an effective antitumor immunity upon ICI treatment. Specifically in dMMR colon tumors, extracellular mucus and MUC1 expression were associated with lower T-cell densities compared to non-mucinous counterpart.[17] Additionally, among dMMR/MSI-H CRCs mucinous histology was associated with PD-L1 downregulation compared to non-mucinous counterpart.[18] Similarly, in patients with lung adenocarcinoma, mucinous histology had lower intratumor $CD8^+$, $PD\text{-}1^+$, $CD8^+PD\text{-}1^+$, and $FOXP3^+$ infiltrate and inferior outcomes to ICIs or combinations of chemotherapy and ICIs.[23] These preclinical findings have been paralleled by consistent clinical results in CRC both in early stage and metastatic settings. Dostarlimab achieved a clinical complete response and organ preservation rate of 100% in a proof-of-concept phase 2 trial conducted in patients with locally advanced dMMR rectal cancer. However, a recent report on individual patients experiencing disease progression while on dostarlimab highlighted that a feature shared by all cases was mucinous differentiation.[24] Then, a higher prevalence of mucinous histology has been described in patients with dMMR colorectal tumors undergoing consolidation surgery after ICIs and exhibiting no pathological complete response (pCR), suggesting a reduced chance of tumor eradication even in responder patients.[19,25] Additionally, mucinous histology has been independently associated with worse PFS and OS after ICIs in dMMR/MSI-H mCRC.[20]

Taken together, these data are relevant to the clinical practice. First, among patients with mucinous tumors, Kaplan–Meier curves for PFS and OS showed an early and persistent separation over time between those treated with dual CTLA-4/PD-1 blockade and those receiving single-agent anti-PD-1. At the 6-month landmark analysis restricted to patients without IR, this separation was maintained, supporting a sustained benefit of adding anti–CTLA-4 as upfront therapy. A similar pattern was observed in patients with non-mucinous tumors, suggesting that both groups may derive benefit from dual immune checkpoint inhibition. However, the clinical implications appeared more pronounced in the mucinous cohort since patients with mucinous tumors treated with anti-PD-1 monotherapy experienced the worst outcomes in terms of PFS and OS both either in the main cohort and in the cohort without IR. Although our retrospective design precludes definitive conclusions regarding treatment selection, these findings suggest that dual PD-1/CTLA-4 blockade partially counteract the adverse effect of this resistant phenotype. Intriguingly, exploratory analyses from the CheckMate 142 trial suggested that the addition of ipilimumab may partially rescue the adverse prognostic impact of a immune-depleted tumor microenvironment which is frequently observed in mucinous tumors, as discussed above.[26,27] In the context of contemporary management, first line ipilimumab and

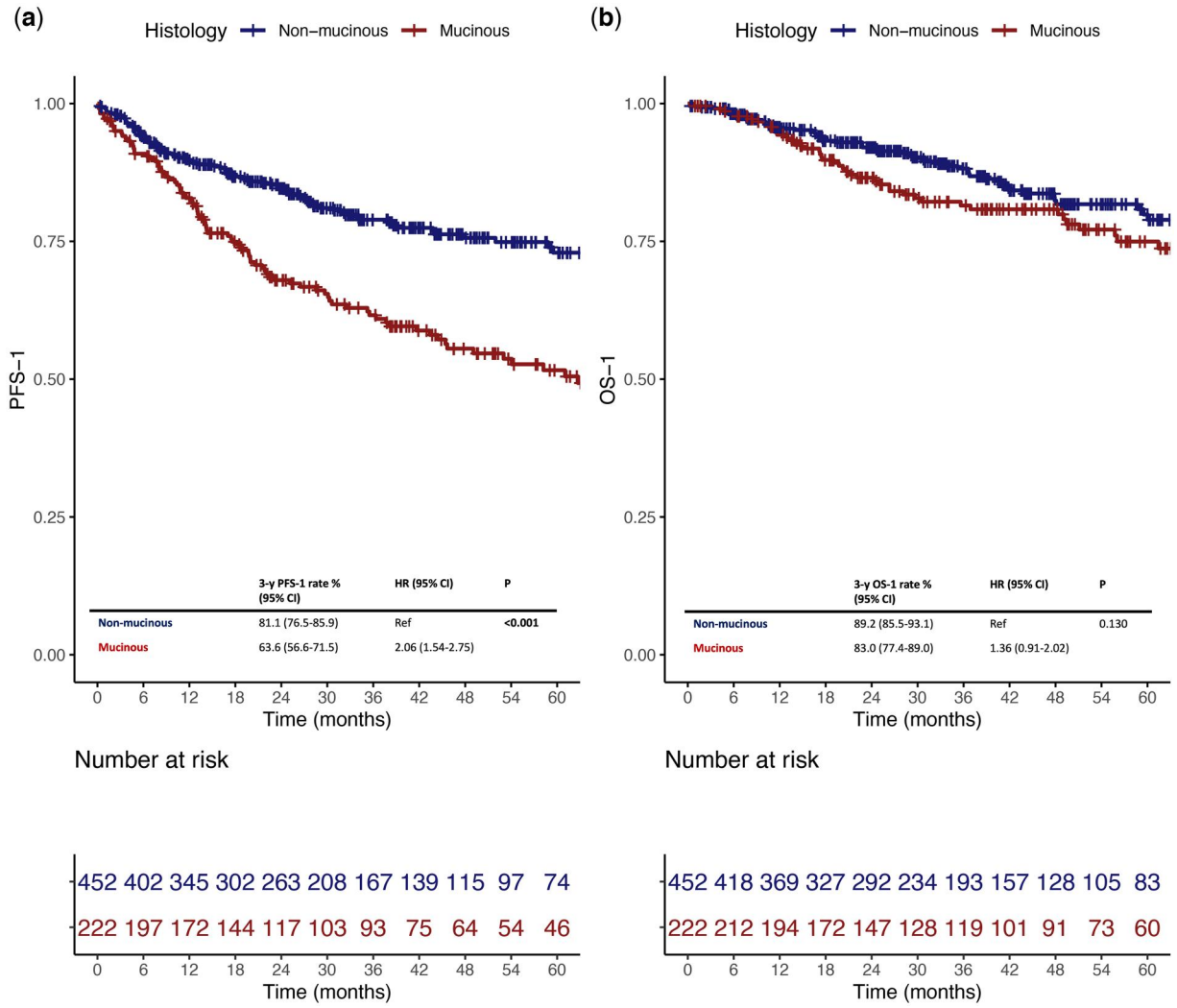

Figure 3 Kaplan-Meier curves of progression-free survival (PFS)-1 (a) and overall survival (OS)-1 (b) along with 3-year PFS-1 (a) and 3-year OS-1 (b) rates according to histology (mucinous vs non-mucinous) in patients with dMMR/MSI-H mCRC without IR and at risk for AR ($n = 674$). *List of abbreviations*. PFS, progression-free survival. OS, overall survival. dMMR, mismatch repair deficient. MSI-H, microsatellite instability high. mCRC, metastatic colorectal cancer.

nivolumab significantly improved PFS compared nivolumab in patients with dMMR/MSI-H mCRC in the CheckMate-8HW trial and should be regarded as the treatment of choice.[2] However, careful evaluation of adverse prognostic features such as mucinous histology should be carried out when balancing the use of anti-PD-1 monotherapy, for instance in less fit patients.

While a relevant proportion of patients with dMMR/MSI-H mCRC achieve a pCR after ICIs even in presence of residual lesions detected by conventional imaging, clinicians should be aware of the substantial risk of disease progression or residual viable tumor in patients with mucinous histology even after an initial benefit from this treatment.[3, 25] In this context, assessing tumor response is even more challenging since acellular mucin, indicating an ongoing treatment-related tissue response, and mucin containing residual tumor cells may appear similar on magnetic resonance and computed tomography imaging.[28] As a result, accurate evaluation of a clinical CR can be difficult, particularly when mucinous histology was not clearly identified on baseline biopsy specimens. The integration of functional imaging techniques (e.g., positron emission tomography) or liquid biopsy assays to track circulating tumor DNA changes and minimal residual disease are promising options that can help clarify tumor viability and treatment response.[29–31] Therefore, patients with residual disease after long-term disease stabilization should be carefully evaluated within multidisciplinary tumor boards for locoregional therapies aimed at eradicating residual disease, if needed.[32,33]

Then, the dampened sensitivity to ICIs of patients with mucinous histology should also prompt the investigation of novel treatment strategies, such as vaccines, novel immune checkpoint inhibitors and adoptive-cell therapy, and should encourage physicians to actively seek clinical trials that assess the activity of these new treatments in this subgroup.[34–37] Additionally, with growing retrospective studies addressing the

Table 3 Association of baseline patient and disease's characteristics and progression-free survival-1 (PFS-1) and overall survival-1 (OS-1).

Characteristics	PFS-1				OS-1			
	Univariable models		Multivariable model		Univariable models		Multivariable model	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Sex		**0.007**		**0.008**		0.061		
Female	Ref		Ref		Ref			
Male	1.51 (1.12–2.03)		1.52 (1.12–2.07)		1.46 (0.98–2.18)			
Age (years)		0.590				**0.004**		0.267
<70	Ref				Ref		Ref	
≥70	1.09 (0.79–1.50)				1.80 (1.20–2.68)		1.30 (0.82–2.05)	
ECOG PS		**0.015**		**0.008**		**<0.001**		**0.022**
0	Ref.		Ref		Ref.		Ref	
≥1	1.45 (1.08–1.95)		1.51 (1.11–2.05)		2.14 (1.41–3.24)		1.39 (1.05–1.85)	
Primary tumor location		0.133				0.630		
Left colon/Rectum	Ref.				Ref.			
Right colon	1.29 (0.93–1.80)				1.11 (0.72–1.72)			
Primary tumor resection		0.634				0.799		
No	Ref.				Ref.			
Yes	0.91 (0.62–1.33)				2.07 (0.62–1.83)			
Mutational status		0.500				**0.010**		0.806
RAS/BRAF *wild-type*	Ref				Ref		Ref	
RAS *mut*	1.14 (0.80–1.64)				0.99 (0.60–1.67)		0.96 (0.57–1.61)	
BRAF *mut*	1.23 (0.86–1.77)				1.84 (1.15–2.94)		1.56 (0.95–2.58)	
Histology		**<0.001**		**<0.001**		0.130		
Non-mucinous	Ref		Ref		Ref			
Mucinous	2.06 (1.54–2.75)		2.10 (1.56–2.82)		1.36 (0.91–2.02)			
Time to metastases		0.479				0.810		
Metachronous	Ref.				Ref.			
Synchronous	0.90 (0.68–1.20)				1.05 (0.71–1.55)			
Metastatic sites		0.061				**0.037**		0.194
1	Ref.				Ref.		Ref	
≥2	1.33 (0.99–1.78)				1.54 (1.03–2.32)		1.34 (0.86–2.07)	
Liver metastasis		0.955				0.937		
No	Ref				Ref			
Yes	0.99 (0.73–1.34)				0.99 (0.66–1.48)			
Lung metastasis		**0.037**		0.11		**0.004**		0.083
No	Ref.		Ref		Ref.		Ref	
Yes	1.48 (1.02–2.14)		1.36 (0.93–1.99)		1.95 (1.24–3.06)		1.57 (0.94–2.62)	
Peritoneal metastasis		0.757				0.755		
No	Ref				Ref			
Yes	1.05 (0.78–1.41)				1.07 (0.72–1.58)			
Nodal metastasis		0.890				0.698		
No	Ref				Ref			
Yes	1.02 (0.76–1.37)				0.93 (0.62–1.37)			
Line of ICI treatment		**0.003**		**0.04**		0.475		
1st	Ref		Ref		Ref			
≥2nd	1.22 (1.02–1.47)		1.21 (1.01–1.46)		1.09 (0.86–1.39)			
ICI regimen		**0.002**		**0.002**		**0.003**		**0.007**
Anti-PD-1 monotherapy	Ref		Ref		Ref		Ref	
Anti-CTLA4 combination	0.59 (0.42–0.83)		0.58 (0.41–0.82)		0.48 (0.30–0.77)		0.51 (0.31–0.83)	

List of abbreviations. ECOG PS, Eastern Cooperative Group Performance Status. ICI, immune checkpoint inhibitors.Statistically significant (<0.05) *p*-values are displayed in **bold**.

issue of treatment duration in patients with dMMR/MSI-H mCRC,[22,38–40] paralleling ongoing trials in other cancer types (NCT02821013, ISRCTN15837212, NCT05255302), the identification of features associated with increased risk of later relapse, such as mucinous histology, should be considered in the context of de-escalation strategies.

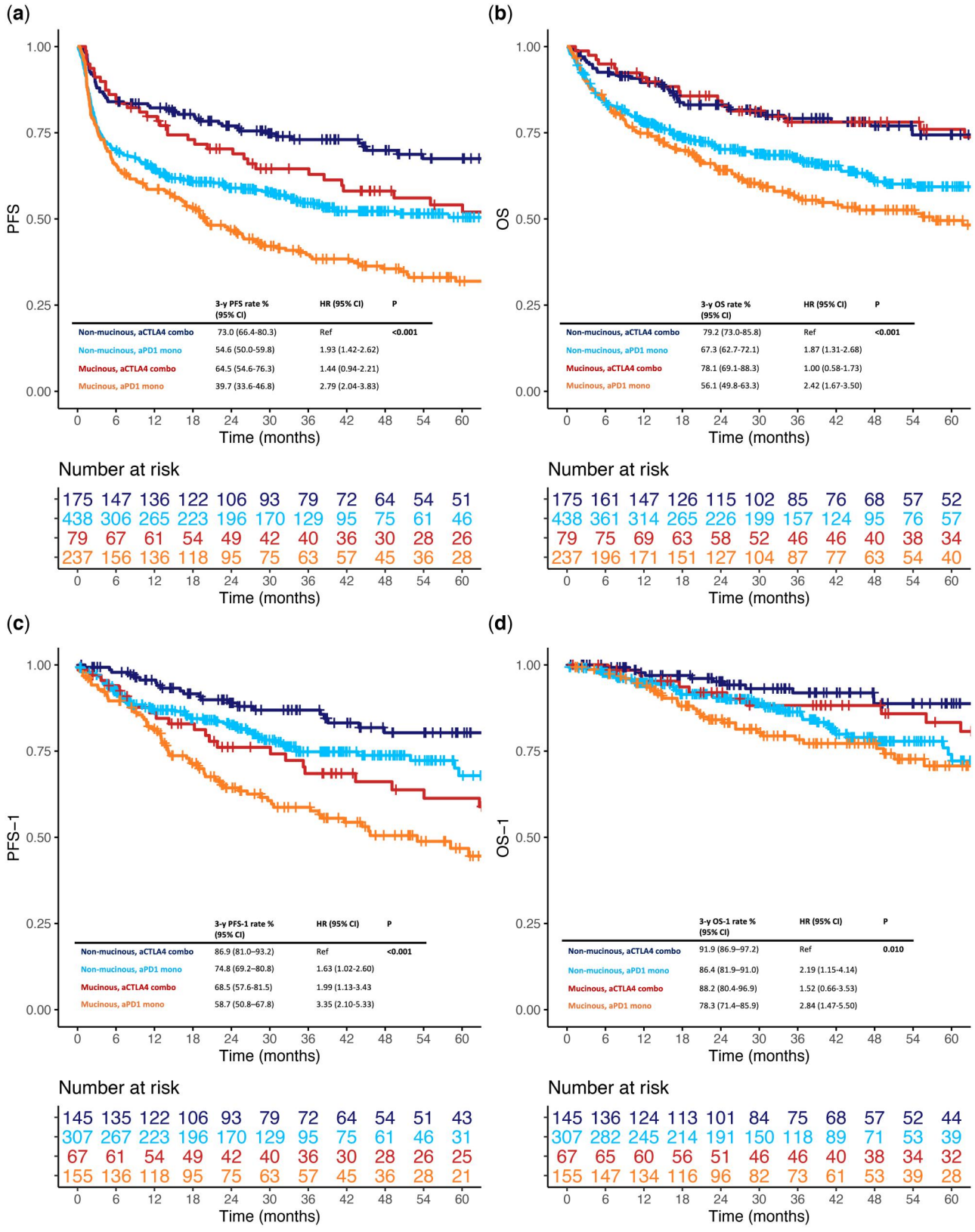

Figure 4 Kaplan-Meier curves of progression-free survival (PFS) (a), overall survival (OS) (b), PFS-1 (c) and OS-1 (d) according to histology and type of immune checkpoint inhibitor treatment (anti-PD-1 single agent or anti-PD-1 with anti-CTLA-4) in the main cohort ($N = 929$) and in the cohort without IR and at risk for AR ($n = 674$). *List of abbreviations*. PFS, progression-free survival. OS, overall survival. dMMR, mismatch repair deficient. MSI-H, microsatellite instability high. mCRC, metastatic colorectal cancer. IR, innate resistance. AR, acquired resistance.

In an earlier work, our group proposed a "cure" model for patients with dMMR/MSI-H mCRC treated with ICIs but mucinous histotype was not selected through the random forest model as a key prognostic variable in the final nomogram.[7] However, it should be noted that the nomogram estimated survival from the initiation of ICIs, thereby encompassing both patients with IR and AR. As a result, the specific contribution of mucinous histology to AR may have been confounded in that setting. Additionally, the dataset used for that nomogram had a shorter median follow-up and this issue is particularly relevant in the context of investigating AR, which, per definition, may occur late in the clinical disease course.

We acknowledge that our work has some limitations. Despite using a 50% cutoff to define mucinous tumor, the pathological assessment was carried out locally and lacks an external or centralized review, therefore we cannot exclude potential misclassification or inconsistencies among different centers. In this sense, the integration of artificial intelligence-based tools may provide a way not to refine pathology assessment.[41] Then, to confirm the predictive role of mucinous histology for AR to ICIs, a cohort with ICI-untreated patients would be necessary, possibly from a randomized controlled trial (RCT), like the CheckMate-8HW[42] or the Keynote-177[43] trials. It is unknown whether this information is available for most patients, as previously published subgroup analyses of these trials did not mention mucinous histotype. Due to the reduced sample size within subgroups, we reported data on treatment with ICIs across all lines rather than restricting first-line therapy. Nonetheless, the prognostic impact of mucinous histology remained significant in the multivariable analysis adjusted for treatment line. Lastly, mucinous histology had not been independently associated with OS in patients with no IR. In fact, a longer follow-up may be needed to detect a significant impact of histology on OS in this patient population. Additionally, an oligoprogressive pattern may be observed in just under 50% of patients with dMMR/MSI-H mCRC with AR to ICIs and these cases are typically managed with ablative therapies with curative intent.[33] This may have contributed to masking a significant impact of mucinous differentiation on OS. Given the study was purely clinical, it cannot elucidate the mechanisms governing the attenuated immunotherapeutic sensitivity of mucinous disease, warranting further preclinical studies. However, due to the rarity of these cases and to the technical difficulties in obtaining valid preclinical models (e.g., patient-derived organoids)[44] that faithfully recapitulate this subgroup, we do not foresee these experiments to be easily accomplishable in the near future. Establishing a multinational biobank of samples from patients with resistance to ICI may overcome these limitations and foster the research for these rarer patient subgroups where ICIs are not sufficient for achieving cure.

Conclusion

Our work proposes mucinous histology as a biomarker for AR to ICI in patients with dMMR/MSI-H mCRC, likely due to tumor-intrinsic factors rather than an association with unfavorable baseline clinical characteristics. Our results should prompt physicians to consider anti-CTLA-4-based combinations in case of mucinous histology, but also highlight the need for integrated, multidisciplinary care and for new therapeutic strategies to truly eradicate disease in this population.

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Author contributions

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Conflicts of interest

RC: Honoraria from Amgen, AstraZeneca/Daiichi Sankyo, Bristol Myers Squibb, Exeliome Bioscience, Merck, MSD Oncology, Servier; travel fees and congress invitations from Bristol Myers Squibb, Enterome, MSD Oncology, Servier.

JR: personal speaker honoraria from Sanofi, Pfizer, Takeda, Merck and Amgen, and accommodation expenses from Pierre-Fabre, Pfizer, Servier, Amgen and Merck.

MJO: consulting roles at 3D Medicines, Array BioPharma, Bristol Myers Squibb, Eisai, Gritstone Bio, Janssen, MedImmune, Merck, Novartis, Pfizer, Roche/Genentech, and Simcere; and

research funding from Bristol Myers Squibb, MedImmune, Merck, and Roche.

TA: consulting or advisory roles in Aptitude health, Abbvie, Astellas Pharma, Biocartis, Bristol Myers Squibb, Gritstone Bio, GlaxoSmithKline, MSD Oncology, Medicenna, Nimbus, Nordic Bioscience, Sanofi, Servier, Pfizer, Takeda/Lundbeck; receiving honoraria from Bristol Myers Squibb, MSD Oncology, Merck Serono, GlaxoSmithKline, Roche/Genentech, Sanofi, Seagen, Servier DMC member role: Inspirna; and travel and/or accommodation expenses from Bristol Myers Squibb, MSD Oncology, and Takeda.

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FP: Research funding (to Institution) from Lilly, BMS, Incyte, AstraZeneca, Amgen, Agenus, Rottapharm, Johnson&Johnson, GSK, Tempus, BeOne. Personal honoraria as an invited speaker from BeOne, Daiichi-Sankyo, Seagen, Astellas, Ipsen, AstraZeneca, Servier, Bayer, Takeda, Johnson&Johnson, BMS, MSD, Amgen, Merck-Serono, Pierre-Fabre, Incyte, AstraZeneca. Advisory/Consultancy from BMS, MSD, Amgen, Pierre-Fabre, Johnson&Johnson, Servier, Bayer, Takeda, Astellas, GSK, Daiichi-Sankyo, Pfizer, BeOne, Jazz Pharmaceuticals, Incyte, Rottapharm, Merck-Serono, Italfarmaco, Gilead, AstraZeneca, Agenus, Revolution Medicines, 3 T Biosciences. Travel expenses from Amgen, Merck-Serono, Pierre-Fabre, Servier, Astellas, Incyte, Johnson&Johnson.

Data availability statement

To minimize the risk of patient re-identification, de-identified individual patient-level clinical data are available under restricted access. Upon review by INT Committee, requests for data access can be obtained via INT which will contact the corresponding author. Data requests will also require signing a data access agreement with the INT.

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