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Prognosis of stage II and III colon cancer treated with adjuvant 5-fluorouracil or FOLFIRI in relation to microsatellite status: results of the PETACC-3 trial†

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Background: Although colon cancer (CC) with microsatellite instability (MSI) has a more favorable prognosis than microsatellite stable (MSS) CC, the impact varies according to clinicopathological parameters. We studied how MSI status affects prognosis in a trial-based cohort of stage II and III CC patients treated with 5-fluorouracil (5-FU)/leucovorin or FOLFIRI.

Materials and methods: Tissue specimens of 1254 patients were tested for 10 different loci and were classified as MSI-high (MSI-H) when three or more loci were unstable and MSS otherwise. Study end points were overall survival (OS) and relapse-free survival (RFS).

Results: In stage II, RFS and OS were better for patients with MSI-H than with MSS CC [hazard ratio (HR) 0.26, 95% CI 0.10–0.65, $P = 0.004$ and 0.16, 95% CI 0.04–0.64, $P = 0.01$). In stage III, RFS was slightly better for patients with MSI-H CC (HR 0.67, 95% CI 0.46–0.99, $P = 0.04$), but the difference was not statistically significant for OS (HR 0.70, 95% CI 0.44–1.09, $P = 0.11$). Outcomes for patients with MSI-H CC were not different between the two treatment arms. RFS was better for patients with MSI-H than with MSS CC in the right and left colon, whereas for OS this was significant only in the right colon. For patients with *KRAS*- and *BRAF*-mutated CC, but not for double wild-type patients, RFS and OS were significantly better when the tumors were also MSI-H. An interaction test was statistically significant for *KRAS* and MSI status ($P = 0.005$), but not for *BRAF* status ($P = 0.14$).

Conclusions: Our results confirm that for patients with stage II CC but less so for those with stage III MSI-H is strongly prognostic for RFS and OS. In the presence of 5-FU treatment, stage II patients with MSI-H tumors maintain their survival advantage in comparison with MSS patients and adding irinotecan has no added benefit.

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Key words: colon cancer, microsatellite instability, survival, adjuvant treatment, translational research

introduction

Approximately 15% of colon cancer (CCs) are characterized by incompetence of the DNA mismatch repair (MMR) system, leading to abnormal shortening or lengthening of repeating base pair units of DNA, a phenomenon known as microsatellite instability (MSI) [1]. In sporadic CC, MSI is largely due to *MLH1* inactivation through hypermethylation of the promoter [2]. In familial CC, MSI is mostly due to inherited germline mutation of a MMR gene (notably *MLH1* and *MSH2*) [3]. In sporadic CC, MSI is more frequent in stage II (almost 20%) and III (12%) tumors than in stage IV tumors (4%) [4].

Patients with MSI-H tumors evolve more favorably than those with MSS. Several retrospective studies [1, 5, 6], a meta-analysis [7], and recent large trials [8–12] support the notion that stage-adjusted prognosis is more favorable for MSI-H than for MSS CC patients, but the difference in prognosis is larger for stage II than for stage III patients.

According to the current guidelines, adjuvant chemotherapy is the treatment of choice for stage III and a minority of high-risk stage II patients [13]. Disease stage remains the key determinant of prognosis and treatment, but more accurate prognostic and

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predictive markers are urgently needed. MSI, 18q loss of heterozygosity, *KRAS*, *BRAF*, and *TP53* mutations have been intensively investigated in this context [4, 14–16], but most are not incorporated into the treatment guidelines nor have they been confronted in large series to traditional stage II high-risk features [13] or to more recent gene expression-based prognostic signatures [17].

A putative predictive role of MSI for response to 5-fluorouracil (5-FU)-based adjuvant chemotherapy has been a more contentious issue. Some reports have suggested that disease outcome after chemotherapy does not differ between patients with MSI-H and MSS CC [18], whereas others showed increased sensitivity to 5-FU for the patients with MSI-H CC [19]. Data from randomized clinical trials of 5-FU-based therapy versus surgery only, however, suggested that patients with MSI-H CC do not benefit from 5-FU-based adjuvant chemotherapy compared with surgery-alone [6]. This was confirmed in a pooled analysis [10], which added 457 cases to the previously published 570 [6]. In a recent study, however, the survival benefit of stage III MSI-H CC patients was maintained under 5-FU [11].

An issue remains the impact of MSI on the choice of adjuvant therapy. One trial initially suggested a differential effect of irinotecan-based adjuvant chemotherapy (CALGB 89803) in favor of MSI-H patients [20], but this became marginal in an updated report [8].

To clarify these controversies, we studied stage-specific prognostic effects of MSI in the homogeneous PETACC-3 trial colon cancer population treated with 5-FU or FOLFIRI. The key question was whether patients with MSI-H CC maintain their survival benefit under 5-FU treatment, when stratified for stage and treatment. In addition, we investigated how tumor site, *BRAF* and *KRAS* status, and high-risk stage II factors modulate the prognostic effect of MSI.

patients and methods

patient characteristics

All eligible patients were randomly assigned to receive 6 months of either 5-FU/leucovorin (LV) alone or with irinotecan [21]. MSI status could be determined for 1254 of the 1564 patients of whom tissue was available for analysis (89%), out of the total trial population of 3278 patients [14]. Earlier reports describe how further molecular parameters (p53 expression, SMAD4 expression, 18q LOH, *BRAF*, and *KRAS* mutation status) were obtained [4, 14, 15]. End points were overall survival (OS), defined as the time from randomization until death, and relapse-free survival (RFS), defined as the time from randomization to local, regional, or distant relapse, the occurrence of a second primary colon cancer or death.

MSI determination

MSI was evaluated at 10 different microsatellite loci containing mono- or dinucleotide repeated sequences. The panel consisted of the five markers from the Bethesda reference panel, with the addition of five markers which were also suggested during the International Workshop on HNPCC in 1997 (BAT-25, BAT-26, D2S123, D5S346, TGFBR2, BAT-40, D17S787, D18S69, D17S250, and D18S58) [22]. The amplified PCR products were analyzed using the automated ABI Prism Sequencer Model 3100 Genetic Analyzer (Applied Biosystems, Foster City, USA). A locus was called unstable if unequivocal instabilities were seen in the tumor sample in comparison with the paired normal DNA of the same patient. MSI was graded as high (MSI-H) when three or more markers were unstable, low (MSI-L) when one or two markers were unstable, and stable (MSS) when all markers were stable. For analysis, MSI-L and MSS populations were pooled to MSI-L/S.

The determination of the other markers has been described before [4, 14, 15].

statistical analyses

Survival curves were determined using Kaplan–Meier methods and compared using the log-rank test. Frequencies were compared using Fisher's exact and Pearson's χ^2 tests. Continuous variables were compared by MSI status using Wilcoxon's rank sum test. Hazard ratios (HRs) with 95% confidence intervals (CIs) were computed with uni- and multivariable proportional hazard models. Interactions were assessed by likelihood ratio tests. *P*-values are two-sided, not adjusted for multiple testing, and considered significant if <0.05. Analyses were carried out using the free R software package (www.r-project.org) version 2.13.0 or later.

results

patient and tumor characteristics in relation to MSI status

Patients and tumor characteristics by MSI status are summarized in Table 1. The MSI-H frequency was almost twice as high in node-negative, compared with node-positive, patients. The proportion of MSI-H tumors was higher with higher T-stage, in the right colon, when poorly differentiated, mutated for *BRAF* or with high thymidylate synthase (*TYMS*) expression, but lower for mucinous tumors and those with SMAD4 loss, high *TP53* expression and (weakly) a *KRAS* mutation.

prognostic value of MSI varies according to stage

After a median follow-up of 69.1 months, RFS (HR 0.48, 95% CI 0.34–0.69, $P < 0.001$) as well as OS (HR 0.47, 95% CI 0.31–0.72, $P < 0.001$) were better for patients with MSI-H than with MSI-L/S CC. This was most striking in patients with stage II CC with a strong effect of MSI status on RFS and OS, still significant but weaker for RFS in stage III patients but not significant for OS in stage III (Figure 1A and B). A statistically significant interaction between stage and MSI status was found for OS ($P = 0.047$), still borderline significant for RFS ($P = 0.06$).

the prognostic value of MSI is not affected by 5-FU/LV versus FOLFIRI treatment

For stage II 5-FU/LV- as well as FOLFIRI-treated patients, RFS and OS were better for MSI-H than for MSI-L/S CC (Figures 1C and D, and 2).

For stage III 5-FU/LV-treated patients, the MSI-H effect was weaker compared with stage II 5-FU/LV-treated patients. For stage III FOLFIRI-treated almost no difference was found by MSI status, neither on RFS nor on OS (Figures 1E and F, and 2). An interaction test between treatment and MSI status within stage III patients, however, was not significant ($P = 0.31$ for RFS and $P = 0.18$ for OS).

When patients were stratified according to MSI status, RFS and OS were similar in both treatment arms. We could not confirm the benefit suggested by Bertagnolli et al. [8] for irinotecan addition in MSI-H tumors (supplementary Table S1, available at *Annals of Oncology* online).

Table 1. Association between MSI status, patients' characteristics, and tumours' molecular characteristics

Patients' and molecular tumors' characteristics	MSI-L/S patients *n* (%)	MSI-H *n* (%)	*P*-value
Total number of cases (*n* = 1254)	1064 (84.9)	190 (15.1)	
Stage			
II	309 (78.2)	86 (21.8)	<0.001
III	755 (87.9)	104 (12.1)	
Treatment group			
5-FU/FA	533 (84.2)	100 (15.8)	0.53
FOLFIRI	531 (85.5)	90 (14.5)	
N-stage			
N0	309 (78.2)	86 (21.8)	<0.001
N1	495 (88.4)	65 (11.6)	
N2	260 (87.0)	39 (13.0)	
T-stage			
T1/2	69 (93.2)	5 (6.8)	0.03
T3	815 (85.1)	143 (14.9)	
T4	180 (81.1)	42 (18.9)	
Grade			
G-1/2	993 (87.8)	138 (12.2)	<0.001*
G-3/4	63 (55.3)	51 (44.7)	
No result	8	1	
Mucinous features			
Yes	898 (88.6)	115 (11.4)	<0.001*
No	158 (68.1)	74 (31.9)	
No result	8	1	
Primary tumor location			
Left	707 (93.9)	46 (6.1)	<0.001
Right	357 (71.3)	144 (28.7)	
SMAD4 expression status			
No loss	799 (81.8)	178 (18.2)	<0.001*
Any loss	249 (95.8)	11 (4.2)	
No result	16	1	
BRAF mutation status			
Wt	1002 (87.5)	143 (12.5)	<0.001*
mut	53 (54.6)	44 (45.4)	
No result	9	3	
KRAS mutation status			
Wt	626 (83.0)	128 (17.0)	0.03*
mut	421 (87.7)	59 (12.3)	
No result	17	3	
TP53 expression status			
≤45% cells positive	641 (78.7)	173 (21.3)	<0.001*
>45% cells positive	410 (96.5)	15 (3.5)	
No result	13	2	
TYMS expression status			
>75% cells positive	254 (67.7)	121 (32.3)	<0.001*
<75% cells positive	708 (92.2)	60 (7.8)	
No result	102	9	
Age, median (range)	61 (21–76)	54 (25–75)	<0.001

MSI, microsatellite instability; MSI-L/S, MSI-low/stable, MSI-H, MSI-high; *TYMS*, thymidylate synthase.

*Missing values have not been considered for the calculation of *P*-values. Except for *TYMS* expression, there was no significant difference in terms of missingness between MSI-H and MSI-L/S tumors.

MSI is prognostic in both the right and left colon

Right-sided carcinomas were almost five times more often MSI-H than left-sided carcinomas. More precisely, we found a gradual pattern of MSI-H incidence as reported elsewhere [23]. RFS was better for patients with MSI-H than for MSI-L/S CC, regardless of side. In the right colon, OS was statistically significantly different between MSI-H and MSI-L/S CC, but not in the left. Of note is the similarity of the two HRs (Figure 2), with a non-significant interaction. As left CCs are less frequently MSI-H than right CCs ($n = 46$versus 144), the power of tests in this subgroup is lower.

For patients with a stage II carcinoma in the left colon, RFS was similar for MSI-H and MSI-L/S CC, whereas in the right colon, RFS was significantly better for patients with MSI-H CC. For patients with stage II carcinomas in the left colon, MSI status had no effect on OS. There was no event for the 64 MSI-H CC in the right colon. For stage III patients, RFS and OS tended to be better for MSI-H carcinomas, irrespective of site, but this trend was not significant. These observations were confirmed in multivariable models including *BRAF* mutation status and gender.

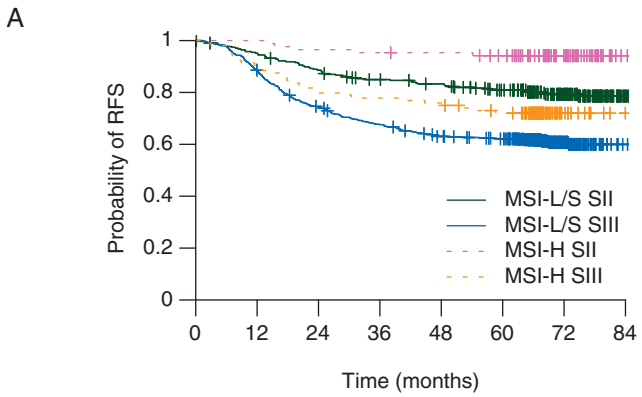
MSI status and BRAF/KRAS status

BRAF-mutated CCs were almost four times more often MSI-H than *BRAF* wild-type CC. In contrast, *KRAS*-mutated CCs were 1.5 times less often MSI-H than *KRAS* wild-type CC (Table 1). In patients with a double wild-type CC, MSI status had no effect on RFS or OS (Figure 2). For patients with a *KRAS*-mutated CC, however, RFS and OS were clearly better. An interaction test for *KRAS* and MSI status was significant ($P = 0.005$). For *BRAF*-mutated carcinomas, the CIs were larger, but the effect was still significant for both RFS and OS. A test for interaction between MSI and *BRAF* status, however, was not significant ($P = 0.14$). Conversely, *BRAF* status was not prognostic in patients with a MSI-H CC (RFS: HR = 1.26, 95% CI 0.59–2.70, $P = 0.55$; OS: HR = 1.53, 95% CI 0.63–3.70, $P = 0.35$). Similar results were obtained in multivariable analyses when stratified by stage (supplementary Table S2, available at *Annals of Oncology* online).

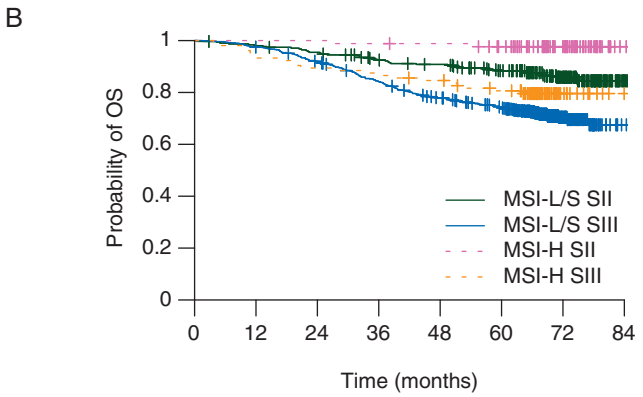
MSI status and stage II risk factors

As shown in supplementary Table S3, available at *Annals of Oncology* online, the distribution of MSI-H and MSI-L/S according to stage II risk factors was similar in both groups, except for poorly differentiated CC, which are rare (5.3% of all stage II tumors) but more often MSI-H. Small patient numbers allowed only univariate analyses of these risk factors in the MSI-H group. T stage (HR = 5.28, 95% CI 0.88–31.64, $P = 0.07$), positive margins (HR = 11.53, 95% CI 1.28–104.15, $P = 0.03$), and perforation (HR = 8.40, 95% CI 1.40–50.38, $P = 0.02$) were prognostic but with high uncertainty due to small patient numbers.

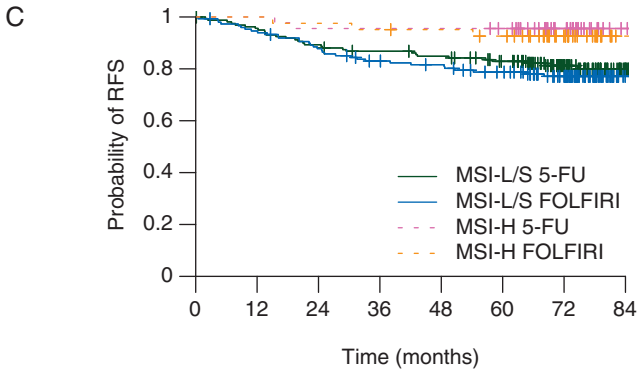
When combining risk factors into a high-risk group with at least one risk factor and a low-risk group without any risk factor, the HRs of patients with MSI-L/S CC were higher than those of high-risk patients both in RFS (HR = 3.63, 95% CI 1.46–9.04, $P = 0.006$ versus HR = 2.40, 95% CI 1.28–4.47, $P = 0.006$) and OS (HR = 6.03, 95% CI 1.46–24.91, $P = 0.01$ versus HR = 2.80, 95% CI 1.25–6.28, $P = 0.01$). This suggests that the prognostic value of MSI status is stronger than that of the combined classical risk factors for stage II patients. In a



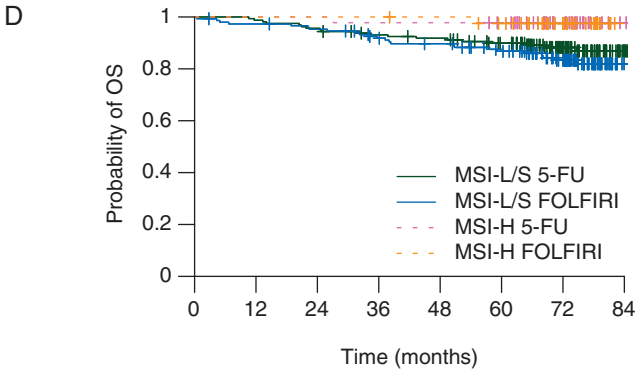
# at risk								
MSI-L/S SII	309	292	271	255	247	227	153	34
MSI-L/S SIII	755	667	559	506	467	451	98	6
MSI-H SII	86	86	83	82	81	77	47	11
MSI-H SIII	104	93	83	81	79	72	11	0



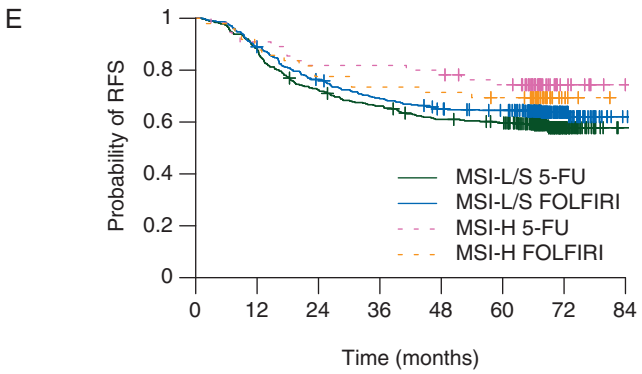
# at risk								
MSI-L/S SII	309	302	293	277	269	247	164	36
MSI-L/S SIII	755	735	691	629	577	535	107	7
MSI-H SII	86	86	86	85	84	79	49	11
MSI-H SIII	104	97	93	90	87	80	12	0



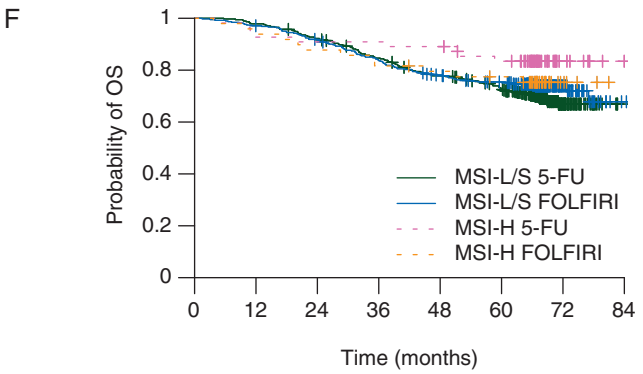
# at risk								
MSI-L/S 5-FU	160	152	142	136	132	121	71	18
MSI-L/S FOLFIRI	149	140	129	119	115	106	82	16
MSI-H 5-FU	45	45	43	43	43	41	25	7
MSI-H FOLFIRI	41	41	40	39	38	36	22	4



# at risk								
MSI-L/S 5-FU	160	158	153	146	143	131	78	19
MSI-L/S FOLFIRI	149	144	140	131	126	116	86	17
MSI-H 5-FU	45	45	45	44	44	42	26	7
MSI-H FOLFIRI	41	41	41	41	40	37	23	4



# at risk								
MSI-L/S 5-FU	373	328	269	245	224	216	48	3
MSI-L/S FOLFIRI	382	339	290	261	243	235	50	3
MSI-H 5-FU	55	50	45	45	44	39	7	0
MSI-H FOLFIRI	49	43	38	36	35	33	4	0



# at risk								
MSI-L/S 5-FU	373	365	343	312	285	260	54	4
MSI-L/S FOLFIRI	382	370	348	317	292	275	53	3
MSI-H 5-FU	55	51	50	50	49	44	7	0
MSI-H FOLFIRI	49	46	43	40	38	36	5	0

Figure 1. Kaplan–Meier plots showing outcome according to tumor stage, treatment, and microsatellite status. (A) Relapse-free survival (RFS), (B) overall survival (OS), (C) RFS in stage II, (D) OS in stage II, (E) RFS in stage III, (F) OS in stage III. SII, stage II; SIII, stage III; MSI, microsatellite instable; MSI-H, MSI-high; MSI-L/S, MSI-low/stable.

	RFS HR (95% CI)	OS HR (95% CI)
Overall	0.48 (0.34–0.69)	0.47 (0.31–0.72)
Stage II	0.26 (0.10–0.65)	0.16 (0.04–0.64)
Stage III	0.67 (0.46–0.99)	0.70 (0.44–1.09)
Left	0.46 (0.23–0.94)	0.53 (0.23–1.20)
Right	0.50 (0.33–0.77)	0.41 (0.24–0.68)
Stage II, left	0.39 (0.09–1.62)	0.61 (0.15–2.57)
Stage II, right	0.23 (0.07–0.77)	0.00 (0.00–Inf)
Stage III, left	0.61 (0.27–1.36)	0.60 (0.22–1.61)
Stage III, right	0.71 (0.45–1.11)	0.63 (0.38–1.07)
5-FU/FA	0.41 (0.25–0.69)	0.37 (0.19–0.70)
5-FU/FA, SII	0.22 (0.05–0.91)	0.18 (0.02–1.32)
5-FU/FA, SIII	0.56 (0.32–0.96)	0.51 (0.26–1.00)
FOLFIRI	0.57 (0.35–0.93)	0.58 (0.33–1.04)
FOLFIRI, SII	0.30 (0.09–0.96)	0.14 (0.02–1.03)
FOLFIRI, SIII	0.82 (0.48–1.40)	0.94 (0.52–1.72)
Double wt	0.72 (0.45–1.16)	0.58 (0.30–1.10)
KRAS mut	0.19 (0.08–0.47)	0.27 (0.11–0.67)
BRAF mut	0.40 (0.19–0.86)	0.31 (0.13–0.72)

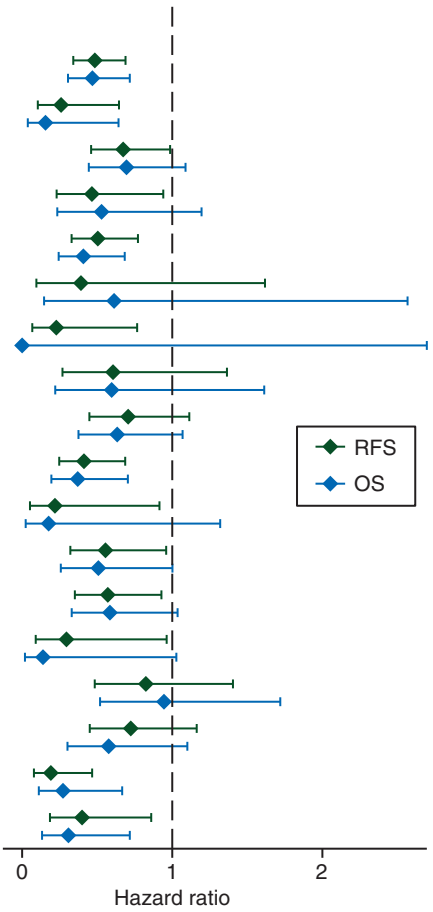


Figure 2. Forest plots of the prognostic value of MSI status in selected patient groups. RFS, relapse-free survival; OS, overall survival; HR, hazard ratio; 95% CI, 95 percent confidence interval; SII, stage II; SIII, stage III; wt, wild type; mut, mutated.

multivariable model, T stage alone was stronger than all other risk factors combined and equaled the effect of MSI status (supplementary Figure S1 and Table S4, available at *Annals of Oncology* online).

discussion

Our results confirm earlier reports [1, 5–8, 10, 18] that MSI-H colon cancer patients have a better survival than those with MSI-L/S tumors (supplementary Table S5, available at *Annals of Oncology* online). Furthermore, we confirm MSI-H tumors to be more often stage II, located in the proximal colon, and of poor or undifferentiated histology, in line with previous reports [8, 10, 11, 24].

We found the MSI-H effect on RFS and OS to be stronger in stage II than in stage III patients [9]. The striking effect in stage II, even though in both arms patients were treated with 5-FU, suggests that patients with MSI-H tumors have a good prognosis, even when treated. Earlier work of Sargent et al. [10] has suggested a lack of benefit from 5-FU-based chemotherapy, but in the absence of an untreated control arm, however, our dataset cannot assess directly the effect of 5-FU on MSI-H patients.

Furthermore, we have shown that MSI-H colon cancer patients treated with FOLFIRI do not fare better than those treated with only 5-FU/LV [12], in contrast to an earlier report of Bertagnolli et al. [20]. More in line with our data, Bertagnolli et al. [8] recently reported, in a larger patient cohort, an only marginally significant increase in RFS for 5-FU-/irinotecan-treated MSI-H patients, when all other risk factors were taken into account. We found no evidence for stage-specific or overall interactions between treatment and MSI status. Nevertheless, in the above-mentioned two trials, different irinotecan-based regimens were used. These differences must be taken into consideration in cross-trial comparisons.

We confirm the high frequency of *BRAF* mutations in the MSI-H population [25]. As a novel observation, we find MSI to be prognostic in *KRAS*- and *BRAF*-mutated, but not in double wild-type, patients. *BRAF*, however, had no prognostic impact in MSI-H patients, possibly limited by sample size [26].

A still unanswered question involves the potential impact of the site of the primary tumor, for which we report novel data on the PETACC3 cohort. We found no difference in RFS, between right- and left-sided MSI-H carcinomas. OS of patients with a right-sided MSI-H CC was significantly better compared with those with a right-sided MSI-S/L CC, as previously reported [27]. Our data, however, do not provide convincing evidence in favor of or against a benefit for patients with a left-sided MSI-H CC. In a recent publication, Sinicrope et al. [28] reported that, although patients with a MSI-H right-sided CC have a statistically significant DFS advantage, the outcome of those with a left-

sided MSI-H CC was worse, which we did not find. A larger validation series is needed to settle this question, especially given the evidence for stage-specific effects. The improvement in RFS and OS for stage II patients with a MSI-H CC seemed stronger in the right than in the left colon, with the notable observation that out of 64 patients not a single patient with a stage II right-sided MSI-H CC had died and only three had relapsed. Such differences were not found in stage III patients. Another hypothesis emerging from our data, but in need of validation, is the potential interaction between *KRAS* mutation and MSI status.

An open issue is how useful MSI status is as a marker for stage II patients considered for 5-FU chemotherapy. We compared MSI status with conventionally applied high-risk factors [29]. In terms of RFS and OS, MSI-H status was slightly stronger than the combination of high-risk factors. Among high-risk factors, T-stage was the strongest. Comparison between T-stage and MSI status resulted in a similar effect on outcome, as we previously reported [15]. Previously published data from the Sargent group [10, 30] advocated that MSI-H patients might be spared adjuvant treatment. The lack of untreated patients in our study prevents a direct comparison, but we found that stage II patients with T3 and MSI-H CC fare very well. Given the modest treatment effect of 5-FU in this population, they seem to represent the best candidate group for omitting adjuvant treatment. Conversely, patients with MSI-S/L T4 CC fared much worse, even though they received chemotherapy. For the intermediate-risk patient with a MSI-H T4 or a MSI-S/L T3 CC, other factors need to be considered before a conclusion can be reached.

In conclusion, our results confirm that MSI-H is strongly prognostic for RFS and OS for stage II patients, and less so for stage III patients. In the presence of 5-FU treatment, stage II patients with MSI-H tumors maintain their survival advantage in comparison with MSI-L/S patients and adding irinotecan has no added benefit. Additional parameters (including gene expression profiling, ploidy, methylation, and microRNA expression) have to be explored in order to more accurately define stage II patients who require adjuvant treatment and to predict which patients will respond. Based on new emerging information, further exploratory analyses in large patients' cohorts looking also at the impact of site, mutation profile, and genomic signatures will be necessary to further appreciate the molecular and prognostic impact of MSI status in colon cancer.

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disclosure

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Abituzumab combined with cetuximab plus irinotecan versus cetuximab plus irinotecan alone for patients with *KRAS* wild-type metastatic colorectal cancer: the randomised phase I/II POSEIDON trial

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Background: Integrins are involved in tumour progression and metastasis, and differentially expressed on colorectal cancer (CRC) cells. Abituzumab (EMD 525797), a humanised monoclonal antibody targeting integrin αν heterodimers, has demonstrated preclinical activity. This trial was designed to assess the tolerability of different doses of abituzumab in combination with cetuximab and irinotecan (phase I) and explore the efficacy and tolerability of the combination versus that of cetuximab and irinotecan in patients with metastatic CRC (mCRC) (phase II part).

Methods: Eligible patients had *KRAS* (exon 2) wild-type mCRC and had received prior oxaliplatin-containing therapy. The trial comprised an initial safety run-in using abituzumab doses up to 1000 mg combined with a standard of care (SoC: cetuximab plus irinotecan) and a phase II part in which patients were randomised 1 : 1 : 1 to receive abituzumab

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