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Effects of microsatellite instability on recurrence patterns and outcomes in colorectal cancers

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Background: Among colorectal cancers (CRCs), high-frequency microsatellite instability (MSI-H) is associated with a better prognosis, compared with low-frequency MSI or microsatellite stability (MSI-L/MSS). However, it is unclear whether MSI affects the prognosis of recurrent CRCs.

Methods: This study included 2940 patients with stage I–III CRC who underwent complete resection. The associations of MSI status with recurrence patterns, disease-free survival (DFS), overall survival from diagnosis to death (OS1), and overall survival from recurrence to death (OS2) were analysed.

Results: A total of 261 patients (8.9%) had MSI-H CRC. Patients with MSI-H CRC had better DFS, compared to patients with MSI-L/MSS CRC (hazard ratio (HR): 0.619, $P < 0.001$). High-frequency microsatellite instability CRC was associated with more frequent local recurrence (30.0% vs 12.0%, $P = 0.032$) or peritoneal metastasis (40.0% vs 12.3%, $P = 0.003$), and less frequent lung (10.0% vs 42.5%, $P = 0.004$) or liver metastases (15.0% vs 44.7%, $P = 0.01$). Recurrent MSI-H CRC was associated with worse OS1 (HR: 1.363, $P = 0.035$) and OS2 (HR: 2.667, $P < 0.001$). An analysis of patients with colon cancer yielded similar results.

Conclusions: Recurrence patterns differed between MSI-H CRC and MSI-L/MSS CRC, and recurrent MSI-H CRCs had a worse prognosis.

Colorectal cancers (CRCs) can be classified as cancers with microsatellite instability (MSI) and cancers with chromosomal instability but not MSI. Microsatellite instability occurs in ~10–20% of CRCs, and results from deficient DNA mismatch repair (Koopman *et al*, 2009). It is further defined as the accumulation of errors in the DNA of microsatellite regions, and is caused by epigenetic inactivation of or germline mutations in mismatch repair genes, such as *MLH1*, *MSH2*, *MSH6*, and *PMS2* (Poynter *et al*, 2008). CRCs with high-frequency MSI (MSI-H)

frequently present in the right colon and are poorly differentiated, with mucinous features and marked lymphocytic infiltration (Kim *et al*, 1994).

Most previous studies have focused on the prognostic value of MSI in early-stage CRCs, which is generally associated with a good prognosis in patients with localised disease. This is presumably because of immunosurveillance and a relatively high rate of apoptosis (Michael-Robinson *et al*, 2001a,b; Benatti *et al*, 2005; Popat *et al*, 2005). However, CRCs with MSI-H have a worse

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response to 5-fluorouracil (5-FU)-based chemotherapy, compared to CRCs with low-frequency microsatellite instability (MSI-L) or microsatellite stability (MSS), because of the reduced recognition of DNA adducts by the DNA mismatch repair system (Carethers *et al*, 1999; Ribic *et al*, 2003; Kim *et al*, 2007). Furthermore, because of their favourable prognosis, MSI-H CRC recurrence is rare. Although the recurrence patterns and outcomes have been briefly described in a few previous studies (Koopman *et al*, 2009; Sinicrope *et al*, 2011; Tran *et al*, 2011; Venderbosch *et al*, 2014), the prognostic value of MSI after recurrence is largely unknown. Therefore, in-depth investigations of the biological and clinical characteristics of recurrent MSI-H CRCs are warranted. In this study, we analysed the patterns and prognosis of recurrent CRCs in a large cohort of patients according to MSI status.

PATIENTS AND METHODS

Patients. This study evaluated 3326 patients who underwent complete resection (R0) for pathologically-confirmed stage I–III CRC at Yonsei Cancer Center between January 2002 and July 2013. However, 386 patients were excluded because of bowel obstruction at the initial presentation ($n = 231$), localised perforation ($n = 123$), synchronous cancer ($n = 17$), or confirmed metastatic lesions within 3 months after surgery ($n = 15$). Thus, 2940 patients were included in the analysis. The analysed data included patient demographics (age and sex), tumour characteristics (location, pathological stage, lymphovascular invasion, perineural invasion, number of retrieved lymph nodes, histological differentiation, and MSI status), and adjuvant chemotherapy regimen. The preoperative workup included a pathological tissue review, total colonoscopy, complete blood count, biochemical profiling, carcinoembryonic antigen (CEA) level measurement, and baseline chest and abdominopelvic computed tomography (CT). Staging was principally based on the guidelines for colon and rectal cancers in the seventh edition of the American Joint Committee on Cancer staging manual. This study was approved by the institutional review board of Yonsei Cancer Center.

MSI analysis. DNA was extracted from tumours and amplified using the PCR. High-frequency microsatellite instability was identified based on the presence of at least two of the five instability markers in the Bethesda microsatellite panel (*BAT25*, *BAT26*, *MFD15*, *D2S123*, and *D5S346*). Low-frequency microsatellite instability was identified based on the presence of only one instability marker, and MSS was identified based on the absence of instability markers. The MSI-L and MSS cases were grouped together (MSI-L/MSS) because no distinct differences between them were found in the previous studies (Laiho *et al*, 2002; Benatti *et al*, 2005; Popat *et al*, 2005).

Chemotherapy. No patients with stage I disease received adjuvant chemotherapy. Patients with stage II–III disease received adjuvant chemotherapy using either 5-FU/leucovorin or 5-FU/oxaliplatin/leucovorin (FOLFOX), which was selected based on patient/physician discussions. Adjuvant chemotherapy was recommended for patients with high-risk stage II tumours that were characterised by poor differentiation, lymphovascular or perineural invasion, <12 examined lymph nodes, or positive margins. FOLFOX was recommended for patients with stage III disease, except for patients in whom oxaliplatin was contraindicated because of old age, poor performance status, or pre-existing peripheral neuropathy.

Follow-up. Patients were followed up at 3-month intervals during the first 2 years after surgery, at 6-month intervals during the next 3 years, and annually thereafter. During the regular follow-ups, the serum CEA assay was performed at each visit. Abdominopelvic CT was performed at 6-month intervals, chest CT was performed at

12-month intervals, and both were performed annually after 5 years. If recurrence was suspected, then the follow-up examinations included clinical evaluation, physical examination, serum CEA assay, chest CT, abdominopelvic CT, colonoscopy, and positron emission tomography, as appropriate. Recurrence was determined using clinical and radiological examinations or histological assessment. Resectability of the metastatic disease at the time of recurrence or after conversion chemotherapy was evaluated by a multidisciplinary team.

Outcomes. Patterns of recurrence were determined according to the involved site and organ. Recurrence was categorised as local if it is occurred at or near the anastomosis site, or as systemic if it is occurred at a distant site. Disease-free survival (DFS) was defined as the time between the initial diagnosis and the first instance of disease recurrence or death. Overall survival (OS) was defined as the time between the initial diagnosis and death (OS1) or the time between recurrence and death (OS2).

Statistical analysis. Data were analysed using SPSS software (version 22.0; SPSS Inc., Chicago, IL, USA). Fisher's exact test and the Wilcoxon rank-sum test were used to examine the associations between MSI status and the clinicopathological variables. Survival plots were generated using the Kaplan–Meier method, and differences in the survival distributions were evaluated using the log-rank test. The Cox proportional hazards model was used to assess the effects of specified risk factors on survival. A P -value of <0.05 was considered as statistically significant.

RESULTS

Baseline characteristics, treatment variables, and outcomes among patients with CRC. Among the 2940 patients, 261 patients (8.9%) had MSI-H CRC (Table 1). High-frequency microsatellite instability CRC was associated with a younger age, right colon location, early stage, less frequent lymphovascular invasion, more frequent evaluation of ≥ 12 lymph nodes, and poor differentiation or a mucinous phenotype. Patients with MSI-H CRC were less likely to receive adjuvant chemotherapy, which was largely related to their earlier tumour stage. Patients whose tumours were less advanced, located in the colon, or categorised as MSI-H had a better DFS (Figure 1A–C). Patients with MSI-H CRCs had a better DFS, compared to patient with MSI-L/MSS CRCs, regardless of stage and location (Figure 1D–I). During a median follow-up of 61 months (range: 3–168 months), 394 patients (13.4%) experienced recurrence. The incidences of overall recurrence (14.0% vs 7.7%, $P = 0.004$) and systemic recurrence (13.1% vs 6.5%, $P = 0.002$) were more frequent among patients with MSI-L/MSS CRCs, compared to patients with MSI-H CRCs.

Baseline characteristics, treatment variables, patterns of recurrence, and outcomes among patients with recurrent CRCs. Among the 394 patients who experienced recurrence, 20 patients (5.1%) had MSI-H CRCs (Table 2). Similar to primary MSI-H CRCs, recurrent MSI-H CRCs were more common among young patients and in the right colon, and were usually poorly differentiated with a mucinous phenotype. When we compared patients with MSI-H or MSI-L/MSS CRC, we did not observe any significant differences in sex, tumour stage, presence of lymphovascular or perineural invasion, number of retrieved lymph nodes, and receipt of adjuvant chemotherapy.

The liver was the most common site of recurrence (170 patients), and was followed by the lungs (161 patients) and peritoneum (54 patients; Table 3). Patients with recurrent MSI-H CRC more frequently exhibited local recurrence (30.0% vs 12.0%, $P = 0.032$) and peritoneal metastases (40.0% vs 12.3%, $P = 0.003$),

Table 1. Characteristics of patients with colorectal cancer according to microsatellite instability status

	All patients (n = 2940)	MSI-H (n = 261)	MSI-L/MSS (n = 2679)	P-value
Age, years				
Median	63	57	63	<0.001
Range	14–99	14–87	22–99	
Sex				
Male	1735 (59.0%)	152 (58.2%)	1583 (59.1%)	0.792
Female	1205 (41.0%)	109 (41.8%)	1096 (40.9%)	
Location				
Right colon	745 (25.3%)	158 (60.5%)	587 (21.9%)	<0.001
Left colon	1234 (42.0%)	69 (26.4%)	1165 (43.5%)	
Rectum	961 (32.7%)	34 (13.0%)	927 (34.6%)	
Stage				
I	553 (18.8%)	56 (21.5%)	497 (18.6%)	<0.001
II	1156 (39.3%)	138 (52.9%)	1018 (38.0%)	
III	1231 (41.9%)	67 (25.7%)	1164 (43.4%)	
Lymphovascular invasion				
Yes	762 (25.9%)	46 (17.6%)	716 (26.7%)	0.001
No	2178 (74.1%)	215 (82.4%)	1963 (73.3%)	
Perineural invasion				
Yes	116 (3.9%)	5 (1.9%)	111 (4.1%)	0.094
No	2824 (96.1%)	256 (98.1%)	2568 (95.9%)	
Retrieved lymph nodes				
≥ 12	2373 (80.7%)	245 (93.9%)	2128 (79.4%)	<0.001
< 12	567 (19.3%)	16 (6.1%)	551 (20.6%)	
Differentiation				
Well differentiated	626 (21.3%)	43 (16.5%)	583 (21.8%)	<0.001
Moderately differentiated	2130 (72.4%)	166 (63.6%)	1964 (73.3%)	
Poorly differentiated	121 (4.1%)	40 (15.3%)	81 (3.0%)	
Mucinous carcinoma	63 (2.1%)	12 (4.6%)	51 (1.9%)	
Adjuvant chemotherapy				
None	988 (33.6%)	105 (40.2%)	883 (33.0%)	0.005
5-FU monotherapy	1048 (35.6%)	87 (33.3%)	961 (35.9%)	
5-FU/oxaliplatin	904 (30.7%)	69 (26.4%)	835 (31.2%)	

Abbreviations: MSI-H = high-frequency microsatellite instability; MSI-L/MSS = low-frequency microsatellite instability/microsatellite stability; 5-FU = 5-fluorouracil.

compared to patients with recurrent MSI-L/MSS CRCs. In addition, MSI-H CRCs more frequently recurred as isolated peritoneal (25.0% vs 3.7%, $P = 0.001$) or intra-abdominal lymph-node metastases (15.0% vs 3.7%, $P = 0.048$). In contrast, lung (42.5% vs 10.0%, $P = 0.004$) and liver metastases (44.7% vs 15.0%, $P = 0.01$) were more frequent among patients with MSI-L/MSS CRCs. Among the non-recurrent cases, better OS1 was associated with MSI-H CRC, compared with MSI-L/MSS CRC. In contrast, poorer OS1 and OS2 were associated with recurrent MSI-H CRC (Figure 1J–L). Patients with recurrent MSI-H CRC were less likely to undergo curative resection, compared to patients with recurrent MSI-L/MSS CRC (20.0% vs 51.6%, $P = 0.01$; Table 4). Although the likelihood of curative resection at the initial recurrence was not different when we compared MSI-H and MSI-L/MSS CRCs (20.0% vs 27.8%, $P = 0.609$), patients with MSI-H CRCs did not benefit from conversion chemotherapy. Thus, palliative chemotherapy without resection was a significantly more common treatment modality for recurrent MSI-H CRC, compared with MSI-L/MSS (75.0% vs 44.1%, $P = 0.01$).

Baseline characteristics, treatment variables, patterns of recurrence, and outcomes among patients with colon cancer. To exclude the anatomical effects of tumour location on the treatment variables and outcomes, we also analysed only the colon cancer cases by excluding 961 cases of rectal cancer. Among the 1979 patients with colon cancer, 227 patients (11.5%) had MSI-H tumours (Supplementary Table 1). Similar to MSI-H CRCs, MSI-H colon tumours were associated with a younger age, right colon location, early stage, less frequent lymphovascular invasion, more

frequent evaluation of ≥ 12 lymph nodes, and poor differentiation or a mucinous phenotype. Compared to patients with MSI-L/MSS colon tumours, patients with MSI-H colon tumours were less likely to receive adjuvant chemotherapy. Although less advanced and MSI-H colon tumours were associated with better DFS, no difference in DFS was observed according to location (right vs left colon; Figure 2A–C). Patients with MSI-H tumours appeared to have a better DFS, compared to patients with MSI-L/MSS tumours, regardless of stage (Figure 2D–F).

During a median follow-up of 60 months (range: 3–168 months), 221 patients with colon cancer (11.2%) experienced recurrence, who included 17 patients (17/227, 7.5%) with MSI-H tumours and 204 patients (204/1752, 11.6%) with MSI-L/MSS tumours. Similar to their primary counterparts, recurrent MSI-H colon cancers were more frequently observed among young patients (Supplementary Table 2) and tended to recur locally (23.5% vs 6.4% for MSI-H vs MSI-L/MSS, respectively; $P = 0.031$; Supplementary Table 3). Compared to patients with recurrent MSI-L/MSS tumours, patients with MSI-H tumours more frequently experienced recurrence as peritoneal metastases (47.1% vs 18.1%, $P = 0.009$) and less frequently experienced recurrence as liver (17.6 vs 53.9%, $P = 0.005$) or lung metastases (5.9% vs 30.4%, $P = 0.046$). Isolated peritoneal metastasis was more common in MSI-H tumours, compared with MSI-L/MSS tumours (29.4% vs 6.4%, $P = 0.007$). Among non-recurrent cases, better OS1 was associated with MSI-H tumours, compared with MSI-L/MSS tumours. In contrast, OS2 was worse for recurrent MSI-H tumours, although there was no significant difference in OS1 (Figure 2G–I). Similar to the recurrent CRC cohort, patients with

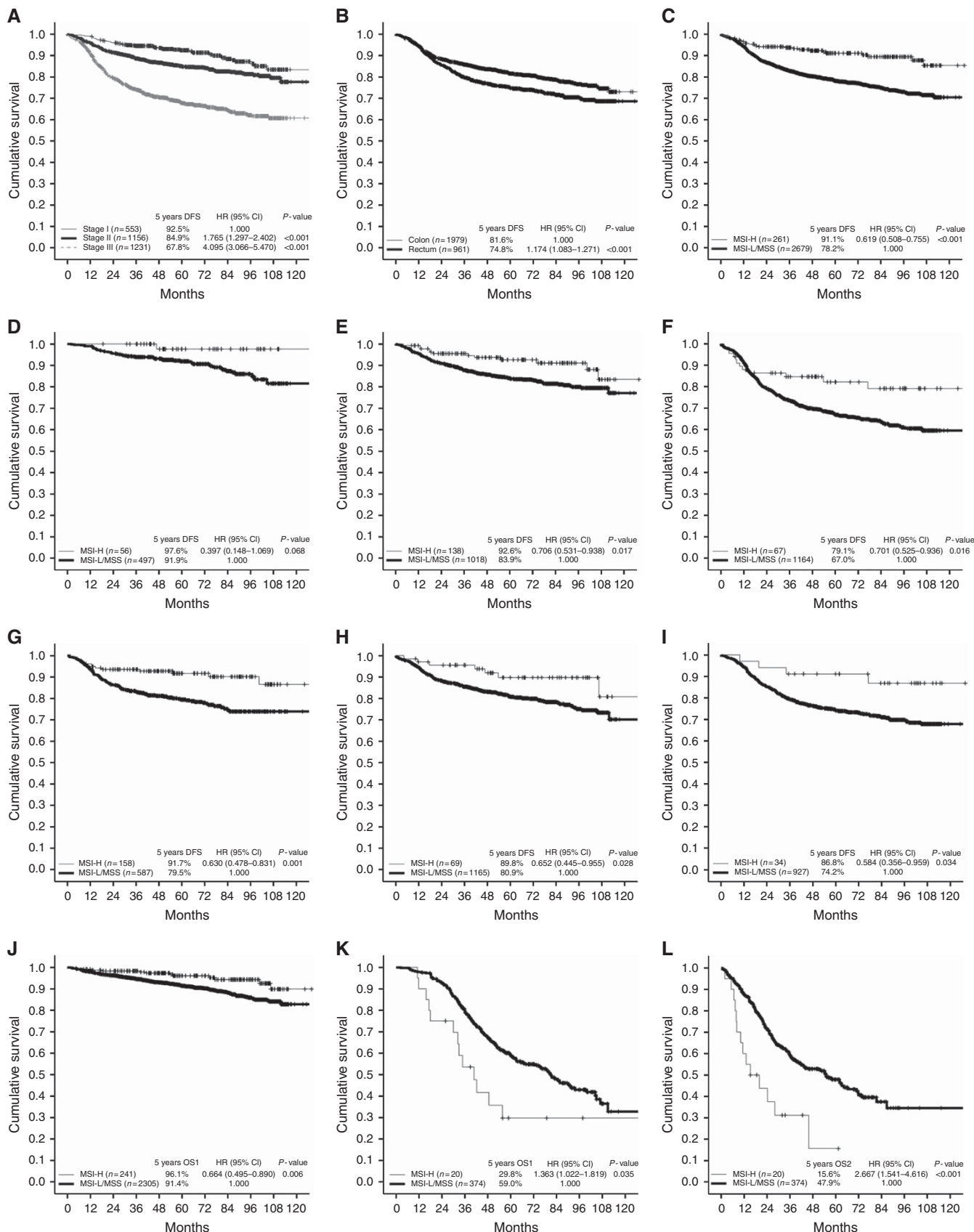


Figure 1. Kaplan–Meier analysis of survival among patients with colorectal cancer. Disease-free survival (DFS) according to stage (A), location (B), and microsatellite instability (MSI) status (C); DFS according to MSI status and stage (stages I–III in D–F, respectively) or location (right colon, left colon, and rectum in G, H, and I, respectively). Overall survival from diagnosis to death (OS1) according to MSI status in patients without (J) and with recurrence (K). Overall survival from recurrence to death (OS2) according to MSI status in patients with recurrence (L). Abbreviations: CI, confidence interval; HR, hazard ratio.

Table 2. Characteristics of patients with recurrent colorectal cancer according to microsatellite instability status

	All patients (n = 394)	MSI-H (n = 20)	MSI-L/MSS (n = 374)	P-value
Age, years				
Median	62	49	63	0.001
Range	14–86	14–72	28–86	
Sex				
Male	240 (60.9%)	13 (65.0%)	227 (60.7%)	0.816
Female	154 (39.1%)	7 (35.0%)	147 (39.3%)	
Location				
Right colon	87 (22.1%)	10 (50.0%)	77 (20.6%)	0.004
Left colon	134 (34.0%)	7 (35.0%)	127 (34.0%)	
Rectum	173 (43.9%)	3 (15.0%)	170 (45.5%)	
Stage				
I	21 (5.3%)	1 (5.0%)	20 (5.3%)	1
II	108 (27.4%)	5 (25.0%)	103 (27.5%)	
III	265 (67.3%)	14 (70.0%)	251 (67.1%)	
Lymphovascular invasion				
Yes	172 (43.7%)	12 (60.0%)	160 (42.8%)	0.166
No	222 (56.3%)	8 (40.0%)	214 (57.2%)	
Perineural invasion				
Yes	32 (8.1%)	0 (0.0%)	32 (8.6%)	0.391
No	362 (91.9%)	20 (100.0%)	342 (91.4%)	
Retrieved lymph nodes				
≥ 12	298 (75.6%)	17 (85.0%)	281 (75.1%)	0.427
< 12	96 (24.4%)	3 (15.0%)	93 (24.9%)	
Differentiation				
Well differentiated	64 (16.2%)	3 (15.0%)	61 (16.3%)	0.038
Moderately differentiated	296 (75.1%)	12 (60.0%)	284 (75.9%)	
Poorly differentiated	20 (5.1%)	3 (15.0%)	17 (4.5%)	
Mucinous carcinoma	14 (3.6%)	2 (10.0%)	12 (3.2%)	
Adjuvant chemotherapy				
None	64 (16.2%)	3 (15.0%)	61 (16.3%)	0.46
5-FU monotherapy	165 (41.9%)	6 (30.0%)	159 (42.5%)	
5-FU/oxaliplatin	165 (41.9%)	11 (55.0%)	154 (41.2%)	

Abbreviations: MSI-H = high-frequency microsatellite instability; MSI-L/MSS = low-frequency microsatellite instability/microsatellite stability; 5-FU = 5-fluorouracil.

MSI-H colon cancer were less likely to undergo curative resection after recurrence (17.6% vs 48.0% for MSI-H vs MSI-L/MSS, respectively; $P = 0.021$; Supplementary Table 4). In addition, conversion chemotherapy was more successful for patients with MSI-L/MSS (22.1% vs 0.0% for MSI-L/MSS vs MSI-H, respectively; $P = 0.027$), and palliative chemotherapy without curative resection was the main therapeutic approach for patients with MSI-H (76.5% vs 45.6% for MSI-H vs MSI-L/MSS, respectively; $P = 0.021$).

DISCUSSION

The present study examined the effects of MSI on recurrence patterns and outcomes among patients with CRC. We found that MSI-H CRCs usually recurred locally, and that pulmonary and hepatic metastases were relatively uncommon. Although MSI-H was generally a good prognostic marker (higher DFS and OS1 rates), it was a poor prognostic marker in recurrent cases (lower OS1 and OS2 rates). Similar results were observed in our analysis of only colon cancers.

Approximately 10–20% of CRCs are diagnosed as MSI-H (Young *et al*, 2001; Giacomini *et al*, 2005). However, the reported prevalence varies according to ethnicity and the microsatellite markers that were used for the detection (Popat *et al*, 2005; Poynter *et al*, 2008). High-frequency microsatellite instability CRCs accounted for only 5.5% of CRCs among Korean patients and for 4.5–5.9% of CRCs among Japanese patients (Asaka *et al*, 2009; Yamada *et al*, 2010; Oh *et al*, 2012). In the present study, which

was conducted in Korea, 8.9% of the patients had MSI-H CRCs. These percentages are lower than those from Western countries and slightly higher than those from other Eastern countries. Similar to the previous studies, MSI-H CRC was associated with a younger age, proximal location, early stage, absence of lymphovascular invasion, a larger number of retrieved lymph nodes, and poor differentiation. In addition, patients with resected MSI-H CRCs had favourable outcomes, with fewer recurrences at distant sites (Benatti *et al*, 2005; Sinicrope *et al*, 2011; Klingbiel *et al*, 2015).

The different recurrence patterns that were observed in our study may be relevant to postoperative surveillance strategies. For example, local recurrences and peritoneal metastases were relatively common among patients with MSI-H CRCs. In addition, the peritoneum was the most common site of MSI-H CRC recurrence, whereas the liver and lungs were the most common sites of MSI-L/MSS CRC recurrence. Notably, extra-abdominal recurrence was infrequent among patients with MSI-H CRC, which agrees with the findings of a previous study (Sinicrope *et al*, 2011). Furthermore, lung metastases were observed in < 1% (2 out of 261) of patients with MSI-H CRCs during a median follow-up of 61 months, and in < 0.5% (1 out of 227) of patients with MSI-H colon cancers. The recently published follow-up guidelines endorsed by the National Comprehensive Cancer Network (Benson *et al*, 2014; Benson *et al*, 2015), the American Society of Clinical Oncology (Meyerhardt *et al*, 2013), and the European Society of Medical Oncology (Glimelius *et al*, 2013; van de Velde *et al*, 2014) recommend chest CT for patients who have undergone surgical resection for CRC. However, the low incidence of lung metastases among patients with MSI-H CRC or colon cancer may

Table 3. Recurrence patterns in patients with colorectal cancer according to microsatellite instability status

	All patients (n = 394)	MSI-H (n = 20)	MSI-L/MSS (n = 374)	P-value
Number of involved organs				0.935
1	273 (69.3%)	15 (75.0%)	258 (69.0%)	
2	85 (21.6%)	4 (20.0%)	81 (21.7%)	
3 or more	36 (9.1%)	1 (5.0%)	35 (9.4%)	
Local recurrence	51 (12.9%)	6 (30.0%)	45 (12.0%)	0.032
Systemic recurrence	368 (93.4%)	17 (85.0%)	351 (93.9%)	0.137
Extra-abdominal recurrence	180 (45.7%)	3 (15.0%)	177 (47.3%)	0.005
Lung	161 (40.9%)	2 (10.0%)	159 (42.5%)	0.004
Extra-abdominal LN	19 (4.8%)	0 (0.0%)	19 (5.1%)	0.613
Bone	14 (3.6%)	0 (0.0%)	14 (3.7%)	1
Muscle	4 (1.0%)	1 (5.0%)	3 (0.8%)	0.189
Brain	1 (0.3%)	0 (0.0%)	1 (0.3%)	1
Intra-abdominal recurrence	256 (65.0%)	14 (70.0%)	242 (64.7%)	0.811
Liver	170 (43.1%)	3 (15.0%)	167 (44.7%)	0.01
Peritoneum	54 (13.7%)	8 (40.0%)	46 (12.3%)	0.003
Intra-abdominal LN	48 (12.2%)	4 (20.0%)	44 (12.8%)	0.286
Ovary	17 (4.3%)	0 (0.0%)	17 (4.5%)	1
Ureter	7 (1.8%)	1 (5.0%)	6 (1.6%)	0.308
Abdominal wall	6 (1.5%)	1 (5.0%)	5 (1.3%)	0.27
Adrenal gland	3 (0.8%)	0 (0.0%)	3 (0.8%)	1
Spleen	2 (0.5%)	0 (0.0%)	2 (0.5%)	1
Pancreas	1 (0.3%)	0 (0.0%)	1 (0.3%)	1

Abbreviations: LN = lymph node; MSI-H = high-frequency microsatellite instability; MSI-L/MSS = low-frequency microsatellite instability/microsatellite stability.

Table 4. Treatment strategies after recurrence in patients with colorectal cancer according to microsatellite instability status

	All patients (n = 394)	MSI-H (n = 20)	MSI-L/MSS (n = 374)	P-value
Curative resection	197 (50.0%)	4 (20.0%)	193 (51.6%)	0.01
Upfront resection	108 (27.4%)	4 (20.0%)	104 (27.8%)	0.609
Upfront chemotherapy	89 (22.6%)	0 (0.0%)	89 (23.8%)	0.01
Palliative chemotherapy alone	180 (45.7%)	15 (75.0%)	165 (44.1%)	0.01
No further treatment	17 (4.3%)	1 (5.0%)	16 (4.3%)	0.595

Abbreviations: MSI-H = high-frequency microsatellite instability; MSI-L/MSS = low-frequency microsatellite instability/microsatellite stability.

indicate that chest CT has limited clinical benefits in this population.

The recurrence pattern also affects the treatment strategy after recurrence. In our study, post-recurrence survival was worse among patients with MSI-H tumours, compared to patients with MSI-L/MSS tumours, and more than twice as many patients with MSI-L/MSS tumours experienced recurrent disease that was amenable to curative resection, compared to patients with MSI-H tumours. Similarly, previous studies have revealed a decrease in the OS rate among patients with advanced MSI-H CRC. For example, the Dutch Colorectal Cancer Group performed the CAIRO study of 515 patients with stage IV diseases, and found a tendency towards inferior outcomes among patients with MSI-H CRC (Koopman *et al*, 2009). However, those authors were not able to draw definite conclusions regarding this trend, based on the small number of patients with MSI-H CRC. In a retrospective analysis that was conducted at the Royal Melbourne Hospital and University of Texas MD Anderson Cancer Center, MSI-H CRC was associated with poorer survival in cases of metastatic disease (Tran *et al*, 2011). That study also compared the patterns of metastatic spread and frequency of metastasectomy according to MSI status, and found higher rates of liver metastases and metastasectomy among patients with MSI-L/MSS CRC, which is similar to our findings. Moreover, a recently published pooled analysis of three prospective trials revealed that the poor prognosis of metastatic MSI-H CRCs appeared to be driven by *BRAF* mutations (Venderbosch *et al*, 2014).

There are two possible explanations for the worse outcomes among patients with recurrent MSI-H CRC. First, most patients with MSI-H tumours were not eligible for curative resection after recurrence. However, the surgical removal of oligometastases, especially liver and lung metastases, is generally considered as beneficial (Kanas *et al*, 2012; Salah *et al*, 2012), and metastasectomy can improve OS among patients with metastatic MSI-H CRCs (Goldstein *et al*, 2014). In addition, chemotherapeutic conversion to resectability was better among patients with MSI-L/MSS CRC, compared to patients with MSI-H CRC (23.8% vs 0.0%, $P = 0.01$), which reflects the intrinsic chemoresistance of MSI-H CRCs. Second, recurrent MSI-H tumours have developed mechanisms to overcome immunosurveillance. In early-stage MSI-H CRCs, tumour-specific neo-antigens induce strong local and systemic anti-tumour immune responses that correlate with a favourable prognosis (Buckowitz *et al*, 2005). Thus, the suppression or evasion of these responses is required for MSI-H CRC progression, which substantially increases the malignant potential, and the evolution of these immunoselective mechanisms has been associated with poor prognosis in several cancers (Seliger *et al*, 2002; Kloor *et al*, 2010). In recent studies, MSI-H tumours have been associated with expression of the immunosuppressive programmed death-1 receptor ligand (PD-L1) and sensitivity to PD-1 blockade (Rizvi *et al*, 2015; Schumacher and Schreiber, 2015). Similarly, a phase 2 study found that advanced MSI-H CRCs were more responsive to PD-1 blockade, compared with MSI-L/MSS CRCs (Le *et al*, 2015). Therefore, we expect that the inferior outcomes that are observed

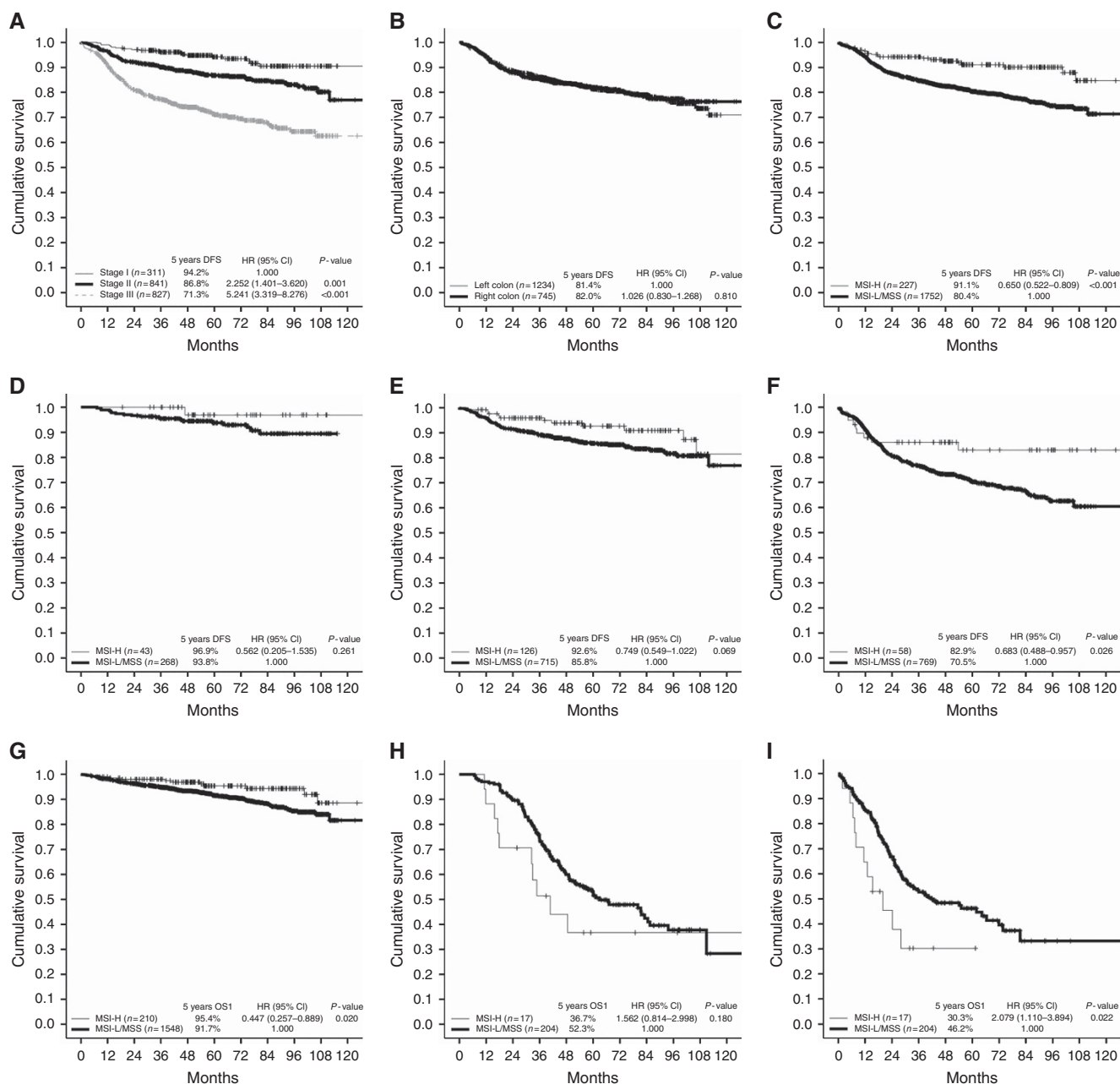


Figure 2. Kaplan–Meier analysis of survival among patients with colon cancer. Disease-free survival (DFS) according to stage (A), and microsatellite instability (MSI) status (C); DFS according to MSI status and stage (stages I–III in D–F, respectively). Overall survival from diagnosis to death (OS1) according to MSI status in patients without (G) and with recurrence (H). Overall survival from recurrence to death (OS2) according to MSI status in patients with recurrence (I). Abbreviations: CI, confidence interval; HR, hazard ratio.

in patients with recurrent MSI-H CRCs could be overcome using immunotherapeutic approaches.

This study has two limitations. First, we did not fully evaluate the prognostic roles of *BRAF* and germline mutations in *MLH1*, *MSH2*, *MSH6*, and *PMS2*, despite previous findings that MSI-H tumours with *BRAF* mutations or *MLH1* promoter methylation have a poor prognosis (French *et al*, 2008; Sinicrope *et al*, 2011). Previous studies have found an association between *BRAF* mutations and MSI-H through the high-level CpG island methylation phenotype (Dahlin *et al*, 2010; Lao and Grady, 2011; Tran *et al*, 2011; Venderbosch *et al*, 2014). In addition, *BRAF* mutations are independently associated with increased recurrence and poor prognosis in resected CRC (Ogino *et al*, 2012; Schirripa *et al*, 2015; Seppala *et al*, 2015). However, the limited number of patients with known *BRAF* mutations in the present study

precluded a more detailed molecular classification analysis. Second, this study was performed at a single centre, and the possibility of selection bias cannot be excluded. For example, curative resection was performed more frequently in the present study, especially after recurrence, compared with the previous studies (Pozzo *et al*, 2004; Alberts *et al*, 2005; Van Cutsem *et al*, 2006). Therefore, multicentre prospective studies are needed to further elucidate the characteristics of MSI-H CRC.

CONCLUSION

The present study identified different patterns of recurrence in patients with MSI-H CRC and MSI-L/MSS CRC. These findings

suggest that modification of the current postoperative surveillance strategies should be considered. We also found an association between recurrent MSI-H CRC and a poor prognosis. Thus, investigation of the biological mechanisms and clinical implications of these findings will facilitate better patient counselling and provide an in-depth understanding of the MSI-H phenotype.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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