Original Contribution

Microsatellite Instability Is a Predictive Marker for Survival Benefit from Adjuvant Chemotherapy in a Population-Based Series of Stage III Colorectal Carcinoma

Hany Elsaleh[1,2] and Barry Iacopetta[1]

Abstract

Tumors with the microsatellite instability (MSI) phenotype appear to comprise a biologically and clinically distinct group of colorectal carcinomas (CRC). MSI+ has been associated with favorable prognosis; however, it is not clear whether this is because MSI+ tumors are inherently less aggressive or because they are more sensitive to chemotherapy. We investigated the prognostic and predictive significance of this molecular alteration along with its association with nodal burden in a large, population-based cohort of stage III CRC patients. Eight hundred seventy-six stage III CRC patients with long median follow-up (76 months) were included in the study. MSI status was determined by screening for deletions in the BAT-26 mononucleotide repeat. Systemic adjuvant fluoropyrimidine-based chemotherapy was delivered to 266 patients (30%). MSI+ was more common in tumors from female patients and tumors that originated in the proximal colon. It was predictive of excellent survival benefit from chemotherapy but was not associated with better prognosis for patients who did not receive treatment. Lower nodal burden was a prognostic factor for improved survival. MSI+ was associated with lower nodal burden in the overall group ($P = 0.02$, χ^2 test) but not for patients who received chemotherapy. In stage III CRC, MSI+ was not prognostic in nonadjuvant-treated patients, suggesting that the biological behavior of MSI+ tumors in the absence of chemotherapy is the same as MSI- tumors. Tumors with the MSI+ phenotype appear to be more sensitive to chemotherapy, as observed by improved survival for patients receiving this treatment. MSI along with other molecular markers could be used in the future for a more refined selection of CRC patients to receive fluoropyrimidine-based chemotherapy.

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Key words: Microsatellite instability, Stage III colon carcinoma, Adjuvant chemotherapy, Predictive molecular markers, Nodal burden

Introduction

The microsatellite instability (MSI) phenotype is characterized by a subgroup of colorectal cancer (CRC) with distinct morphological and clinical features.[1-3] These tumors have defective DNA mismatch repair resulting from the inactivation of genes involved in the repair process. The majority of individuals with hereditary nonpolyposis colon cancer (HNPCC) syndrome have a germ-line mutation in 1 of these genes, whereas inactivation in most sporadic CRCs is thought to result from transcriptional silencing of the *hMLH1* repair gene brought about by DNA methylation.[4] MSI+ CRCs are located predominantly in the right-sided colon, often have a mucinous and poorly differentiated histology, and frequently show a Crohn's-like lymphoid reaction.[2,5] Many,[6-9] but not all[10,11] workers have reported a better prognosis for MSI+ tumors. The predictive value of this molecular alteration has been examined in a number of studies, but stage heterogeneity and incomplete adjuvant treatment information have confounded the results.[8,12] We recently reported excellent survival for MSI+ stage III CRC patients treated with fluoropyrimidine-based adjuvant chemotherapy.[13] This finding has been confirmed by an independent, albeit smaller study.[14] The latter did not report results for survival of nonadjuvant-treated MSI+ patients. Therefore, it is still unclear whether the excellent survival observed for MSI+ patients treated with chemotherapy is because these tumors are inherently less aggressive or because they are more chemosensitive. MSI+ tumors show a tendency to remain more localized,[15] but it is not known if they have a different clinical course than MSI- tu-

[1]Department of Surgery, University of Western Australia, Nedlands, Australia
[2]Department of Radiation Oncology, Sir Charles Gairdner Hospital, Nedlands, Australia

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Address for correspondence: Barry Iacopetta, PhD, Department of Surgery, University of Western Australia, Crawley 6009, Australia
Fax: 61-8-9346-2416; e-mail: bjiac@cyllene.uwa.edu.au

mors once they have metastasized to regional nodes (ie, stage III CRC).

In order to further investigate these issues, we have increased our original series by inclusion of stage III CRC patients from a preadjuvant chemotherapy era (1985-1990). These serve as controls for the original, more contemporary series that may have potential confounding factors because of selection for adjuvant chemotherapy in a nonrandomized setting. We also examined the extent of nodal burden associated with MSI+ tumors to determine whether this factor was related to our previous observation.

Patients and Methods

A consecutive series of 876 margin-negative, stage III CRC patients diagnosed between January 1986 and December 1998 was identified from the histopathology database of the Sir Charles Gairdner Hospital in Nedlands, Australia. The study group comprised patients treated surgically in public hospitals, the majority (80%) at the Sir Charles Gairdner Hospital and the remainder in local and regional centers throughout the state of Western Australia. Ethics approval was granted from the 3 metropolitan teaching hospitals (Sir Charles Gairdner, Royal Perth, and Fremantle) as well as from the Health Department of Western Australia. Information on disease-specific patient survival was obtained from the West Australian Health Department Death Register. The median patient follow-up time was 76 months (range, 11-162 months). Only patients whose primary cause of death was CRC were considered as events in the survival analysis. Data from patients dying from other causes were censored at the time of death. At the end of the study period (July 1999), 474 patients (54%) had died as a result of recurrence of their disease and 65 (7%) had died of other causes. Right-sided tumors were defined as those originating proximal to the splenic flexure and left-sided cases as those arising distal to this site. Tumors with positive circumferential margins were not included in the study.

Adjuvant Chemotherapy

During the study period, adjuvant chemotherapy was being progressively introduced by the oncology service at Sir Charles Gairdner Hospital and by treating physicians throughout the state of Western Australia. Standard therapy was administered according to the Mayo Clinic regimen.[16,17] One third of patients received adjuvant chemotherapy on an intention-to-treat basis. In 85% of these patients, at least 6 monthly cycles of intravenous fluoropyrimidine-based treatment were given, while the remainder received between 1 and 5 monthly cycles.

Microsatellite Instability Status

The MSI status for each tumor was determined by nonisotopic polymerase chain reaction–single-stranded conformation polymorphism (PCR-SSCP) analysis of the BAT-26 mononucleotide repeat as described recently by our laboratory.[18] Deletions within the quasimonomorphic BAT-26 allele have been shown in Caucasian populations to establish the MSI status of tumors with greater than 99% accuracy.[19] DNA for PCR-SSCP analysis was obtained following proteinase-K digestion of 10 µm sections cut from formalin-fixed, paraffin-embedded tumor blocks.[18] These were estimated from histological examination to comprise at least 25% tumor cells. One µL of the tissue digest was added to 12 µL PCR reaction mixes, and 5 µL of the PCR product was then used for SSCP analysis. All suspected MSI+ cases were confirmed at least once by separate PCR and SSCP runs.

Pathology

Lymph node involvement was determined by retrospective review of pathology reports of patients from 1990 onwards and was completed in 674 of the 876 cases. The number of nodes examined as well as the number involved with metastatic carcinoma was recorded for each specimen. Tumors were divided into 2 groups: those with 1 or 2 involved nodes and those with 3 or more involved nodes. This allowed division of the cohort into approximately equal sized subgroups for the purpose of analysis.

Statistical Analysis

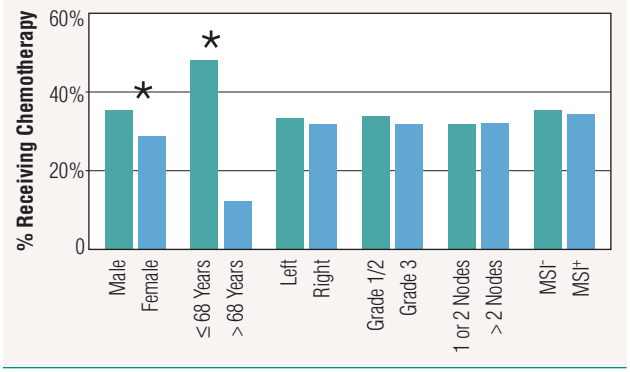
Univariate survival analysis was conducted using the method of Kaplan and Meier, with the difference between survival curves evaluated by the log-rank test. Cox's proportional hazard univariate analysis was used to determine hazard ratios and 95% confidence intervals. Multivariate analysis was performed with stepwise forward selection of independent variables based on the likelihood ratio. Statistical significance was considered as a P value of < 0.05. All analyses were carried out using the Statistical Package for Social Sciences (SPSS) software.

Results

Chemotherapy in Clinicopathologically Defined Subgroups

Figure 1 shows the percentage of CRC patients who re-

Figure 1 Use of Adjuvant Chemotherapy by Subgroup



Use of adjuvant chemotherapy in stage III colorectal cancer patient subgroups defined by clinicopathological and molecular features. Significant differences were observed within age and sex subgroups.
* $P < 0.05$

ceived chemotherapy in each of the clinicopathologically defined subgroups (sex, age, tumor site, histological grade, nodal involvement). As expected, younger patients received chemotherapy more frequently than older patients. Slightly fewer females received chemotherapy compared to males, perhaps relating to the fact that the median age of female CRC patients in this series was 4 years older than males (70 vs. 66 years). No difference was observed for the use of chemotherapy in patient groups defined by MSI status. These results demonstrate that patient groups treated by surgery alone or by surgery plus chemotherapy were well matched for all features except age and sex. The incidence of MSI$^+$ in right-sided tumors was 18% (58/320) and in left-sided tumors was 1% (5/395). Overall, 92% (58/63) of MSI$^+$ tumors were right-sided in origin ($P < 0.0001$). This phenotype was almost twice as common in females compared to males (11% vs. 6%, $P = 0.0029$). Right-sided tumors were more common in female patients (59% vs. 41%), whereas left-sided tumors were more frequent in male patients (56% vs. 44%, $P < 0.0001$). Of the 63 MSI$^+$ tumors, only 7 (11%) arose in patients aged less than 50 years at diagnosis, suggesting that the large majority of MSI$^+$ cases in our cohort were sporadic rather than familial. The median age of patients with MSI$^+$ tumors was 70 years and with MSI$^-$ tumors was 68 years.

Cox's univariate analysis revealed that older patient age, right-sided tumor origin, poor histological grade, and increased nodal involvement were each significant prognostic factors for worse overall survival (Table 1). Patients who received chemotherapy had significantly better 5-year survival than those who did not (48% vs. 36%, $P < 0.0001$) (Table 1 and Figure 2). These patients were younger, with a median age of 60 years (range, 19-80 years) compared to 71 years (range, 25-93 years) for those not receiving chemotherapy.

Survival According to MSI Status

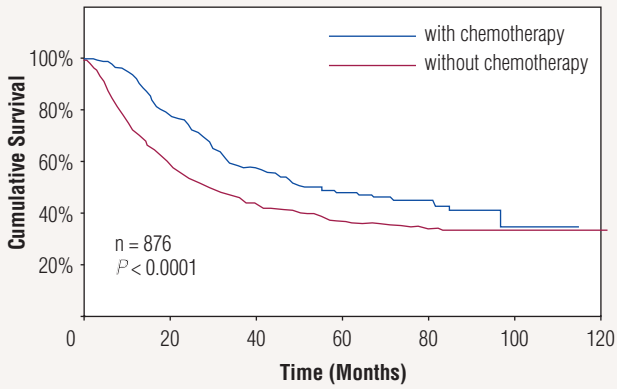
In the overall patient group, MSI$^+$ was weakly associated with better 5-year survival (55% vs. 40%, $P = 0.047$), compared to CRCs without this genetic alteration (Table 2 and Figure 3A). Interestingly, however, no association was observed (43% vs. 36%, $P = 0.644$) in the nonadjuvant-treated group (Table 2 and Figure 3B), whereas a significant association (85% vs. 44%, $P = 0.012$) was seen for the chemotherapy-treated group (Table 2 and Figure 3C). At the end of the study period, only 2 of 21 (10%) MSI$^+$ patients who received chemotherapy had died of their disease compared to 106 of 237 (45%) of those who were MSI$^-$. The median age of patients who received chemotherapy was 58 years for MSI$^+$ and 61 years for MSI$^-$ patients. Multivariate analysis carried out on the adjuvant-treated group revealed that MSI$^+$ (hazard ratio [HR] = 0.14; 95% confidence interval [CI]: 0.34-0.57; $P = 0.006$), gender (HR = 1.98; 95% CI: 1.31-2.99; $P = 0.001$), and tumor grade (HR = 1.58; 95% CI: 1.14-2.18; $P = 0.006$) were independent predictors of survival, but not nodal involvement, tumor site, or patient age.

Table 1 Univariate Analysis of Clinicopathological Features and Patient Survival

Feature*	Total	Hazard Ratio (95% CI)	*P* Value
Patient Sex	876	1.02 (0.86-1.23)	0.78
Patient Age	876	1.30 (1.09-1.56)	0.004
Tumor Site	876	1.32 (1.10-1.58)	0.003
Histological Grade	876	1.37 (1.34-1.84)	< 0.0001
Nodal Involvement	674	1.94 (1.55-2.42)	< 0.0001
Chemotherapy†	876	0.63 (0.51-0.78)	< 0.0001

* The groups shown in Figure 1 were compared.
† Survival of patients receiving chemotherapy was compared to survival of patients treated by surgery alone.
Abbreviation: CI = confidence interval

Figure 2 Survival According to Treatment



Kaplan-Meier survival analysis for stage III colorectal cancer patients treated with or without chemotherapy

Lymph Node Involvement in Stage III CRC

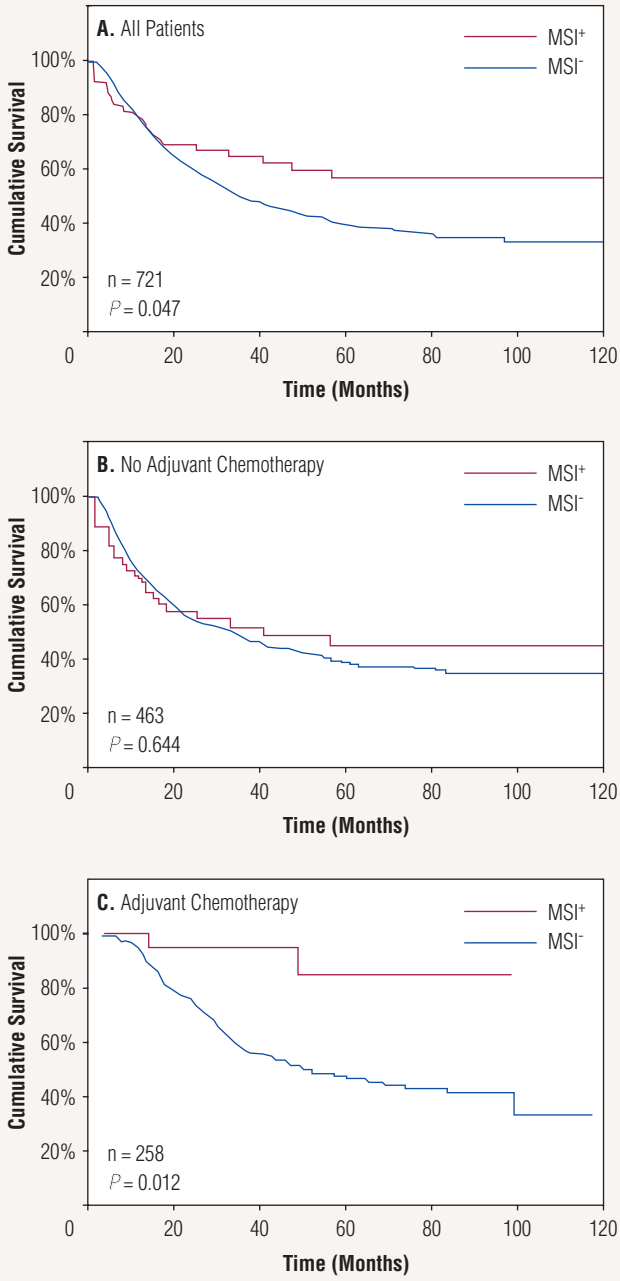
The extent of nodal involvement was examined in 674 tumors, including 53 of the 63 MSI$^+$ cases (Table 3). Tumors from older patients showed significantly less nodal involvement, whereas poorly differentiated tumors showed more nodal involvement. Interestingly, there was no difference in the extent of nodal involvement between adjuvant-treated and nontreated patients, suggesting this factor did not influence the selection of patients to receive chemotherapy. MSI$^+$ was

Table 2 Prognostic Significance of MSI$^+$ in Stage III CRC Patients Treated with or Without Chemotherapy

Treatment Group*	MSI$^+$	MSI$^-$	Number of Patients	Hazard Ratio (95% CI)	*P* Value
Total	63	658	721	0.66 (0.44-0.99)	0.047
Surgery + Chemotherapy	21	237	258	0.17 (0.04-0.67)	0.012
Surgery Alone	42	421	463	0.90 (0.58-1.40)	0.644

* For each group, the survival of MSI$^+$ patients is compared to that of MSI$^-$ patients.
Abbreviations: CI = confidence interval; CRC = colorectal cancer; MSI = microsatellite instability

Figure 3 Survival According to MSI Status



Kaplan-Meier survival analysis of stage III colorectal cancer patients according to microsatellite instability (MSI) status. (**A**) Total cohort, (**B**) nonadjuvant treated, and (**C**) adjuvant treated

Table 3 Extent of Lymph Node Involvement in Stage III CRC: Associations with Clinicopathological Features and with MSI+

Feature	Number of Patients	1-2 Nodes Involved	> 2 Nodes Involved	*P* Value
Total	674	326 (48%)	348 (52%)	
Sex				
Male	330	155 (47%)	175 (53%)	0.312
Female	326	161 (49%)	165 (51%)	
Age				
< 68 years	330	146 (44%)	184 (56%)	0.036
≥ 68 years	344	180 (52%)	164 (48%)	
Tumor Site				
Left-sided	384	190 (49%)	194 (51%)	0.723
Right-sided	256	123 (48%)	133 (52%)	
Differentiation				
Well differentiated	111	58 (52%)	53 (48%)	0.006
Moderately differentiated	385	200 (52%)	185 (48%)	
Poorly differentiated	144	53 (37%)	91 (63%)	
Chemotherapy				
No chemotherapy	389	195 (50%)	194 (50%)	0.448
Chemotherapy	257	121 (47%)	136 (53%)	
MSI				
MSI-	587	279 (48%)	308 (52%)	0.020
MSI+	53	34 (64%)	19 (36%)	

Abbreviation: CRC = colorectal cancer; MSI = microsatellite instability

associated with significantly lower nodal burden compared to patients with MSI- tumors (Table 3). Closer examination revealed this was due primarily to lower nodal involvement for MSI+ tumors in the nonadjuvant-treated group (Table 4). Although we used a staging system of 1 or 2 and greater than 2 involved nodes, essentially the same results were obtained when a staging system of 1 to 3 and 4 or more involved nodes (International Union Against Cancer, American Joint Committee on Cancer) was used (results not shown).

Discussion

Patients with stage III colorectal cancer who are treated with fluoropyrimidine-based chemotherapy derive significant long-term survival benefit from this treatment.[16,17] Tumor-specific factors thought to influence the response to chemotherapy include p53 and K-*ras* mutations,[20] MSI,[13] and thymidylate synthase expression.[21] As genetic mutation in p53 and/or K-*ras* has been shown to dramatically affect the in vitro properties of human CRC cell lines, it is not surprising these genetic alterations should also be directly or indirectly associated with tumor aggressiveness and/or response to chemotherapy in the clinical setting. It is hoped that the incorporation of such molecular markers in clinical trials will in the near future allow identification of chemoresponsive tu-

Table 4 MSI+ and Lymph Node Involvement in Adjuvant Treated and Nontreated Patients

Patient Group	Number of Patients	1-2 Nodes Involved	> 2 Nodes Involved	*P* Value
Chemotherapy				
MSI+	21	11 (52%)	10 (48%)	0.596
MSI-	233	108 (46%)	125 (54%)	
No Chemotherapy				
MSI+	32	23 (72%)	9 (28%)	0.011
MSI-	354	171 (48%)	183 (52%)	

Abbreviation: MSI = microsatellite instability

mors with a high degree of accuracy, thereby leading to a dramatic improvement in patient management.

Although MSI+ is generally considered to be a marker of good prognosis in CRC,[6-9,13] research in this field has been hampered by a number of issues. First, some workers have used instability in dinucleotide repeats to define MSI+; however, it is becoming apparent that only the instability observed in mononucleotide repeats such as BAT-26 is of clinical and perhaps also biological significance.[22] Second, MSI+ occurs in only 8%-10% of population-based CRC,[8,13,14] making it difficult for single institutes to obtain sufficiently large numbers of MSI+ tumors for study. Partially because of this, most reports on MSI+ have been on tumor series comprising mixed stages. Finally, with the wider introduction of fluoropyrimidine-based therapies during the early 1990s, most reported tumor series are also heterogeneous with respect to the use of this treatment. This issue is critical for the delineation of prognostic from predictive value of MSI+.

The present CRC series comprised a large number of cases obtained from a single institution over a 13-year period. The status of adjuvant therapy as it relates to standard fluoropyrimidine-based chemotherapy was known for each patient, allowing us to evaluate both the prognostic and predictive significance of MSI+. With the exception of patient age and sex, the adjuvant-treated and nontreated patients in this nonrandomized, retrospective CRC series were well matched (Figure 1). Similar to results from randomized clinical trials,[16,23] a 10% absolute survival benefit from the use of chemotherapy was seen in this series (Figure 2). The existence of potentially confounding factors inherent to any retrospective study should be borne in mind, however (eg, the depth of tumor invasion). We also cannot exclude that other mismatch repair defects implicated in the cellular response to 5-fluorouracil may not be detected when using the BAT-26 marker alone.

Similar to previous studies,[6-9] patients with MSI+ tumors showed improved prognosis (Table 2 and Figure 3A). The large number of cases in this series along with their known adjuvant therapy status allowed us to analyze the prognostic value of MSI+ within treatment subgroups (Table 2, Figures 3B and 3C). No prognostic significance was seen in the nontreatment group, whereas a striking difference in survival between MSI+ and MSI- cases was apparent in the chemotherapy-treated group. Nearly identical survival curves for the latter group were recently published in a prospective series by Hemminki et al.[14] Our results suggest that in the absence of chemotherapy, the clinical behavior of stage III MSI+ tumors is the same as that of MSI- tumors (Figure 3B). Comparison of survival between adjuvant-treated and nontreated MSI+ patient groups suggests instead that patients with these tumors derive substantial benefit from chemotherapy. If validated in prospective studies, this marker could have major clinical importance for selection of patients to receive fluoropyrimidine-based chemotherapy. In vitro data on the sensitivity of MSI+ colon tumor cell lines to 5-fluorouracil are contradictory, with 1 study suggesting these cells are chemoresistant[24] whereas another found they were more sensitive in comparison to MSI- cell lines.[25]

The extent of lymph node involvement was strongly prognostic in univariate analysis (Table 1). MSI+ was associated with a lower nodal burden in the overall patient group (Table 3) but not in the chemotherapy group (Table 4). Therefore, it is unlikely that nodal burden was a confounding factor for survival benefit from chemotherapy in the MSI+ patient group. The association between MSI+ and extent of nodal involvement has not previously been reported in stage III CRC. While MSI+ tumors are thought to remain localized for longer

Table 5 Features of MSI in Stage III CRC

Feature	General Observations	Current Series
Frequency	10% of sporadic CRC	9% in population-based series
Presentation	Mostly seen in sporadic CRC	90% of MSI+ in patients > 50 years of age
Sex	Female predominance	2:1 female-to-male ratio
Age	Older age	MSI+ median age 2 years older than MSI-
Site	Proximal location	20x more frequent in proximal vs. distal colon
Grade	Higher differentiation	90% > G1
Nodal Involvement	Lower nodal burden	MSI+ associated with less nodal burden
p53 Mutation	Inverse correlation	Only 1% of MSI+ had p53 mutation*
Prognosis	Generally good	Only in chemotherapy-treated patients
Survival Benefit from Chemotherapy	Unknown	MSI+ has strong predictive value for good response to 5-fluorouracil–based chemotherapy

* Inverse correlation of MSI and p53 presented at American Society of Clinical Oncology 2000 Meeting.[26]
Abbreviations: CRC = colorectal cancer; MSI = microsatellite instability

periods of time before developing regional or distant metastasis, their clinical behavior has not been evaluated in the context of nodal burden. It is intriguing that while nodal burden was significantly lower for MSI+, nonadjuvant treated patients (Table 4), this factor did not appear to influence survival (Figure 3B).

The generally accepted features of MSI+ tumors are summarized in Table 5. Our findings in stage III CRC suggest that MSI+ tumors are not less aggressive than MSI- tumors, but rather more sensitive to fluoropyrimidine-based chemotherapy. It is likely that the mechanisms governing response to chemotherapy are more complex than MSI status alone. However, together with other molecular markers, in the future MSI could allow more refined selection of CRC patients to receive chemotherapy.

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