

The New England Journal of Medicine

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VOLUME 342

JANUARY 13, 2000

NUMBER 2



TUMOR MICROSATELLITE INSTABILITY AND CLINICAL OUTCOME IN YOUNG PATIENTS WITH COLORECTAL CANCER

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ABSTRACT

Background Colorectal cancer can arise through two distinct mutational pathways: microsatellite instability or chromosomal instability. We tested the hypothesis that colorectal cancers arising from the microsatellite-instability pathway have distinctive clinical attributes that affect clinical outcome.

Methods We tested specimens of colorectal cancer from a population-based series of 607 patients (50 years of age or younger at diagnosis) for microsatellite instability. We compared the clinical features and survival of patients who had colorectal cancer characterized by high-frequency microsatellite instability with these characteristics in patients who had colorectal cancers with microsatellite stability.

Results We found high-frequency microsatellite instability in 17 percent of the colorectal cancers in 607 patients, and in a multivariate analysis, microsatellite instability was associated with a significant survival advantage independently of all standard prognostic factors, including tumor stage (hazard ratio, 0.42; 95 percent confidence interval, 0.27 to 0.67; $P < 0.001$). Furthermore, regardless of the depth of tumor invasion, colorectal cancers with high-frequency microsatellite instability had a decreased likelihood of metastasizing to regional lymph nodes (odds ratio, 0.33; 95 percent confidence interval, 0.21 to 0.53; $P < 0.001$) or distant organs (odds ratio, 0.49; 95 percent confidence interval, 0.27 to 0.89; $P = 0.02$).

Conclusions High-frequency microsatellite instability in colorectal cancer is independently predictive of a relatively favorable outcome and, in addition, reduces the likelihood of metastases. (N Engl J Med 2000;342:69-77.)

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COLORECTAL cancer is the third most common cancer in Western society.^{1,2} Despite advances in screening, diagnosis, and treatment, it is still the second leading cause of cancer-related death in North America.^{1,2} Much has been learned over the past decade about the molecular genetic alterations that give rise to colorectal cancer. However, this knowledge has yet to affect its clinical management substantially, and pathological staging remains the basis for prognostication and decisions about therapy.³

It is now commonly believed that all cancers arise as a result of the accumulation of genetic alterations that allow the growth of neoplastic cells.^{4,5} However, the rate of random mutational events alone cannot account for the number of genetic alterations found in most cancers in humans.⁶ For this reason, it has been suggested that destabilization of the genome may be a prerequisite early in carcinogenesis.^{6,7} This “mutator phenotype” is best understood in colorectal cancer, in which there are two separate destabilizing pathways.^{8,9} The more common of these mutational pathways involves chromosomal instability^{9,10} characterized by allelic losses (loss of heterozygosity), chromosomal amplifications, and translocations in colorectal-cancer cells. In the second mutational pathway, colorectal cancers display increased rates of intragenic mutation, characterized by generalized instability of short, tandemly repeated DNA sequences

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known as microsatellites.¹¹⁻¹³ High-frequency microsatellite instability (instability at 40 percent or more of microsatellite loci) has been found in most cases of hereditary nonpolyposis colorectal cancer^{11,14} as defined by the Amsterdam criteria (which require that at least three persons from at least two successive generations have colorectal cancer and that the disease be diagnosed in at least one of these persons by the age of 50).¹⁵ In addition, high-frequency microsatellite instability occurs in approximately 15 percent of sporadic cases of colorectal cancer.¹²⁻¹⁴

Normally, mismatches of nucleotides that occur when DNA polymerase inserts the wrong bases in newly synthesized DNA are repaired by mismatch-repair enzymes. Defects in mismatch repair lead to high-frequency microsatellite instability in colorectal cancer.¹⁶⁻²⁰ Inherited germ-line mutations of mismatch-repair genes have been found in approximately 50 percent of persons with a family history that fulfills the Amsterdam criteria.^{21,22} Alterations of the *MSH2* and *MLH1* mismatch-repair genes account for more than 90 percent of these cases.^{5,21} In addition, acquired, noninherited alterations of the *MLH1* gene occur in most sporadic cases of colorectal cancer with high-frequency microsatellite instability.^{23,24}

Although colorectal cancer continues to be regarded as a single disease, it is possible that colorectal cancers with high-frequency microsatellite instability constitute a clinically distinct subtype. A number of studies have shown that high-frequency microsatellite instability occurs relatively frequently in colorectal cancers that arise proximal to the splenic flexure,^{12,13} in poorly differentiated cancers or those of the mucinous-cell type, and in cancers with peritumoral lymphocytic infiltration.²⁵ Furthermore, it has been suggested that the survival of patients with colorectal cancers that have arisen from the high-frequency microsatellite-instability pathway is longer than the survival of patients with cancers that have microsatellite stability.^{12,26-29} (The latter cases constitute the majority of colorectal cancers.) However, these results were obtained from small, uncontrolled, or potentially biased analyses. We therefore conducted a population-based study to determine whether high-frequency microsatellite instability is an independent predictor of improved survival in patients with colorectal cancer.

METHODS

Study Population

Through the Ontario Cancer Registry, we identified a population-based series of all newly diagnosed cases of histopathologically confirmed colorectal adenocarcinoma in patients 50 years of age or younger who were residing in Central-East Ontario (an area with a population of approximately 4.7 million) between January 1, 1989, and December 31, 1993. Identification through the Ontario Cancer Registry has been estimated to identify 96 percent of all Ontario residents with a diagnosis of colorectal cancer.³⁰ After obtaining permission to contact subjects from the physicians who treated the patients, we collected information on family his-

tory and clinical screening from the patients or their next of kin, or by reviewing medical charts.

We excluded patients from the study if they did not undergo resection of the primary colorectal adenocarcinoma or if pathological review did not confirm invasion of the tumor to at least the level of the submucosa (stage T1 or higher). In total, 640 patients treated at 41 hospitals were eligible for inclusion in the study. Specimens of colorectal cancer from 607 of the patients (95 percent) were available for retrieval and testing. The study was approved by the Human Ethics Committee of the University of Toronto.

Clinical Data Base

A clinical data base was prepared by persons with no knowledge of the results of molecular genetic testing of each patient's cancer. The date of the patient's first biopsy or resection that provided a histologic diagnosis of adenocarcinoma of the colon or rectum was recorded as the date of diagnosis of cancer.

We classified cancers according to several gross and histologic features. With the exception of the preoperative level of serum carcinoembryonic antigen, we included all College of American Pathologists category I factors (pathological stage, tumor cell type, tumor grade, and presence or absence of extramural venous invasion), which are well supported by the literature and are generally used in patient care.³¹ All specimens underwent histopathological review by a single pathologist, who was unaware of the results of molecular genetic testing. In accordance with the classification of tumors by the World Health Organization,³² we defined tumors as signet-ring cell or mucinous if 50 percent or more of the tumor displayed the specified cell type and as undifferentiated if features of tumor-cell differentiation were absent. Other tumors were classified as "adenocarcinoma, not otherwise specified" or, in rare cases, adenosquamous carcinoma, if malignant squamous and glandular components were present. Distant metastases were judged to be present if they appeared in a histopathological specimen or if they were identified by the Ontario Cancer Registry within 120 days after diagnosis. In total, 103 of the 138 cases of distant-organ metastases (75 percent) were confirmed by histopathological examination.

Radiation treatment in Ontario is provided exclusively at nine specialized oncologic-treatment centers that report to the Ontario Cancer Registry. Data on radiation treatment initiated within 120 days after diagnosis were extracted from Ontario Cancer Registry records and were available for all study patients. Chemotherapy for cancer may be administered either in oncologic-treatment centers or in other hospitals and clinics in the province. Information on chemotherapy initiated within 120 days after diagnosis was acquired from the data bases of both the Ontario Cancer Registry and the Ontario Institute for Clinical Evaluative Sciences and was available for 392 of the 607 study patients (65 percent).

DNA Preparation, Microsatellite Testing, and Analysis

Blocks of surgically resected cancerous tissue that had been fixed in formalin and embedded in paraffin were requested from the relevant pathology departments for all patients. For each specimen, regions of invasive cancer with the highest proportion of neoplastic cells (median, 80 percent; range, 40 to 100 percent) and normal tissue were microdissected, and DNA was extracted by proteinase K digestion.³³ Samples of genomic DNA were used to amplify sequences (by the polymerase chain reaction [PCR]) from 5 to 10 of the following mononucleotide and dinucleotide microsatellite loci: BAT-25, BAT-26, D5S346, D2S123, D17S250, BAT-40, TGF- β RII, D18S58, D18S69, and D17S787 (Human MapPairs, Research Genetics, Huntsville, Ala.). These specific microsatellite loci were derived from the National Cancer Institute reference and alternative loci panel in order to ensure standardized findings.³⁴ Primer sequences and conditions of the PCR assay and gel electrophoresis have been published previously.^{33,35}

The presence of additional bands in the PCR product from tumor DNA, not observed in DNA from normal tissue from the

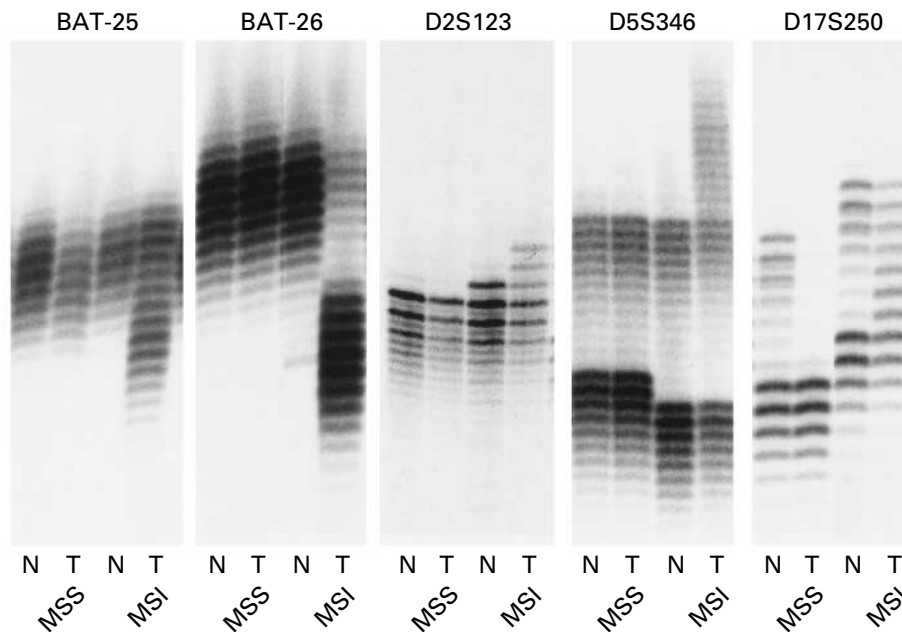


Figure 1. Colorectal Cancers with High-Frequency Microsatellite Instability (MSI) and Microsatellite Stability (MSS).

The MSI colorectal cancer displays shifted bands in tumor DNA (T) as compared with normal DNA (N) at the BAT-25, BAT-26, D2S123, D5S346, and D17S250 microsatellite loci. The MSS colorectal cancer has identical bands in tumor and normal DNA at the BAT-25, BAT-26, D2S123, and D5S346 microsatellite loci. In addition, the MSS colorectal cancer displays loss of heterozygosity at the D17S250 locus — that is, a loss of the top (larger) allele in tumor DNA as compared with normal DNA.

same patient, was scored as instability at that particular locus. In accordance with the National Cancer Institute consensus on microsatellite instability,³⁴ any pair of samples of normal DNA and tumor DNA that displayed instability at two or more of five loci was scored as having high-frequency microsatellite instability, whereas a sample pair with no instability at five loci was scored as having microsatellite stability. Any sample pair observed to have instability at one of five microsatellite loci underwent a second test at that locus. If instability was confirmed, additional loci, up to a maximum of 10, were tested to determine whether the phenotype of the sample was low-frequency microsatellite instability — instability at 1 to 3 of 10 loci assayed — or high-frequency microsatellite instability — instability at 4 or more loci.

Statistical Analysis

The primary outcome of this study was overall survival, measured from the date of histologic diagnosis of colorectal cancer. The study was designed to determine the prognostic importance of high-frequency microsatellite instability in addition to known prognostic factors. Because the genetic basis of low-frequency microsatellite instability remains poorly understood,³⁴ and because the incidence of low-frequency microsatellite instability was too low in our series to allow for meaningful statistical testing, we excluded from the study 20 patients (3 percent) with colorectal cancers characterized by low-frequency microsatellite instability before we performed the statistical analysis.

The univariate associations between the presence or absence of high-frequency microsatellite instability and base-line prognostic factors were analyzed with a chi-square test for categorical variables and an unpaired Student's *t*-test for continuous factors. The associations of microsatellite status and the depth of tumor invasion with metastases to regional lymph nodes and distant organs

were evaluated with multivariate logistic regression. Survival curves were prepared according to the method of Kaplan and Meier,³⁶ and univariate survival distributions were compared with use of the log-rank test. All patients were followed from diagnosis until death or until data were censored (and the patient considered to be alive) as of September 30, 1998. A multivariate survival analysis was evaluated according to the Cox proportional-hazards model.³⁷ A model obtained with step-down variable selection, in which all prognostic factors were initially entered into the model and in which nonsignificant factors ($P > 0.1$) were successively rejected, was compared with the primary model, which included all prognostic factors regardless of their measured significance. All factors were treated as simple categorical variables with the exception of age at diagnosis, which was analyzed as a continuous variable. All reported *P* values are two-sided, and *P* values of less than 0.05 were considered to indicate statistical significance.

RESULTS

Clinical Characteristics Associated with High-Frequency Microsatellite Instability

Of the 607 specimens of colorectal cancer that we tested, 102 (17 percent) were characterized by high-frequency microsatellite instability, 20 (3 percent) had low-frequency microsatellite instability, and 485 (80 percent) had microsatellite stability (Fig. 1). Colorectal cancers with high-frequency microsatellite instability were more likely to be poorly differentiated and located proximal to the splenic flexure than were cancers with microsatellite stability (Table 1). The pa-

TABLE 1. CHARACTERISTICS OF 587 PATIENTS WITH COLORECTAL CANCER EVALUATED FOR MICROSATELLITE INSTABILITY.*

CHARACTERISTIC	ALL PATIENTS (N=587)	PATIENTS WITH MSS (N=485)	PATIENTS WITH MSI (N=102)	P VALUE†	CHARACTERISTIC	ALL PATIENTS (N=587)	PATIENTS WITH MSS (N=485)	PATIENTS WITH MSI (N=102)	P VALUE†
Patient					Histologic features — no. (%)				
Sex — no. (%)				0.71	Cell type				0.14
Male	295 (50)	242 (50)	53 (52)		Adenocarcinoma, not otherwise specified	530 (90)	444 (92)	86 (84)	
Female	292 (50)	243 (50)	49 (48)		Mucinous	28 (5)	21 (4)	7 (7)	
Mean (±SE) age at diagnosis — yr	43.1±0.3	43.5±0.3	41.3±0.7	0.004	Signet ring	19 (3)	13 (3)	6 (6)	
Coexisting illness — no. (%)					Undifferentiated	8 (1)	5 (1)	3 (3)	
Inflammatory bowel disease				0.60	Adenosquamous	2 (<1)	2 (<1)	0	<0.001
No	571 (97)	471 (97)	100 (98)		Grade				
Yes	16 (3)	14 (3)	2 (2)		Well differentiated	53 (9)	45 (9)	8 (8)	
Familial adenomatous polyposis				0.19	Moderately differentiated	405 (69)	354 (73)	51 (50)	
No	579 (99)	477 (98)	102 (100)		Poorly differentiated	129 (22)	86 (18)	43 (42)	
Yes	8 (1)	8 (2)	0		Extramural venous invasion				0.62
Extracolonic cancer				0.70	No	552 (94)	455 (94)	97 (95)	
No	566 (96)	467 (96)	99 (97)		Yes	35 (6)	30 (6)	5 (5)	
Yes	21 (4)	18 (4)	3 (3)		Tumor invasion¶				0.006
Synchronous or metachronous colorectal cancer				0.006	T1	34 (6)	30 (6)	4 (4)	
No	556 (95)	465 (96)	91 (89)		T2	65 (11)	53 (11)	12 (12)	
Yes	31 (5)	20 (4)	11 (11)		T3	462 (79)	387 (80)	75 (74)	
Site of tumor relative to splenic flexure — no. (%)					T4	26 (4)	15 (3)	11 (11)	
Colon				<0.001‡	Pathological stage¶				0.001
Proximal	198 (34)	126 (26)	72 (71)		I	73 (12)	59 (12)	14 (14)	
Distal	186 (32)	177 (36)	9 (9)		II	173 (29)	127 (26)	46 (45)	
Total	384 (65)	303 (62)	81 (79)	0.001§	III	207 (35)	180 (37)	27 (26)	
Rectum	203 (35)	182 (38)	21 (21)		IV	134 (23)	119 (25)	15 (15)	
Treatment — no. (%)					Outcome — no. (%)				
Resection					Alive				
Segmental colectomy					315 (54)				
Subtotal colectomy					272 (46)				
Chemotherapy					242 (50)				
No					73 (72)				
Yes					29 (28)				
Not known									
Radiotherapy									
No									
Yes									

*Twenty patients who had colorectal cancers with low-frequency microsatellite instability were excluded from the analysis. MSS denotes colorectal cancer with microsatellite stability, and MSI colorectal cancer with high-frequency microsatellite instability.

†We used the chi-square test to compare all variables except mean age at diagnosis, for which we used an unpaired t-test.

‡The P value is for the comparison of the proximal colon, distal colon, and rectum.

§The P value is for the comparison of the colon and rectum.

¶Tumor invasion and pathological stage were classified according to the criteria of the American Joint Committee on Cancer.³ Tumor invasion was classified as follows: T1, tumor invading submucosa; T2, tumor invading muscularis propria; T3, tumor invading through the muscularis propria; and T4, tumor invading other organs or perforating the visceral peritoneum.

tients with colorectal cancer with high-frequency microsatellite instability were more likely to have multiple synchronous or metachronous colorectal cancers and received a diagnosis at a younger age than the patients with colorectal cancers with microsatellite stability.

Although colorectal cancers with high-frequency microsatellite instability were diagnosed at a significantly greater depth of tumor invasion, these tumors had a significantly lower overall pathological stage than cancers with microsatellite stability (Table 1). Multivariate logistic regression demonstrated that

both high-frequency microsatellite instability and a lesser depth of tumor invasion were independently associated with a decreased likelihood of metastases to either regional lymph nodes or distant organs (Table 2).

To ensure that treatment did not differ in an era when the benefits of adjuvant therapy were still being established, we compared the use of chemotherapy and radiation therapy in patients with colorectal cancer with high-frequency microsatellite instability with their use in patients whose cancers had micro-

TABLE 2. MULTIVARIATE ANALYSIS OF PREDICTIVE FACTORS FOR METASTASES TO REGIONAL LYMPH NODES OR DISTANT ORGANS IN 587 PATIENTS WITH COLORECTAL CANCER.*

VARIABLE	METASTASES		ODDS RATIO (95% CI)†	P VALUE‡
	ABSENT	PRESENT		
	no. of patients			
Lymph-node metastases				
Microsatellite status				<0.001
MSS§	206	279	1.00	
MSI	66	36	0.33 (0.21–0.53)	
Tumor invasion¶				<0.001
T1§	31	3	1.00	
T2	44	21	5.29 (1.45–19.3)	
T3	190	272	16.1 (4.86–53.5)	
T4	7	19	43.4 (9.67–195)	
Distant-organ metastases				
Microsatellite status				0.02
MSS§	366	119	1.00	
MSI	87	15	0.49 (0.27–0.89)	
Tumor invasion¶				0.003
T1	34	0	NA	
T2§	61	4	1.00	
T3	340	122	5.45 (1.94–15.3)	
T4	18	8	8.08 (2.15–30.5)	

*CI denotes confidence interval, MSS colorectal cancer with microsatellite stability, MSI colorectal cancer with high-frequency microsatellite instability, and NA not assessed.

†An odds ratio of less than 1.00 represents a decreased likelihood of metastases, whereas an odds ratio greater than 1.00 represents an increased likelihood of metastases.

‡The P values resulted from the hypothesis that the odds ratio as determined by multivariate logistic regression equaled 1.0.

§Patients in this category served as the reference group.

¶Tumor invasion was classified according to the American Joint Committee on Cancer, as described in a footnote to Table 1.

satellite stability. Although a trend toward more frequent use of chemotherapy and radiation treatment was evident in the care of patients whose cancers had microsatellite stability (Table 1), we found no significant differences in treatment patterns after controlling for pathological stage (P=0.60 for chemotherapy and P=0.14 for radiation therapy, according to logistic-regression analysis).

High-Frequency Microsatellite Instability and Standard Clinical Prognostic Factors for Survival

In total, 272 of the 587 patients (46 percent) died during a mean follow-up period of 7.2±0.1 years after diagnosis. The survival of patients with colorectal cancers with high-frequency microsatellite instability (mean [±SE] five-year survival, 76±4 percent) was significantly better than that of patients with cancers with microsatellite stability (five-year survival, 54±2 percent; P<0.001) (Fig. 2). Colorectal cancers with mucinous, signet-ring, and undifferentiated cell types, poorer grade, higher pathological stage, or extramu-

ral venous invasion were associated with significantly lower survival (Table 3).

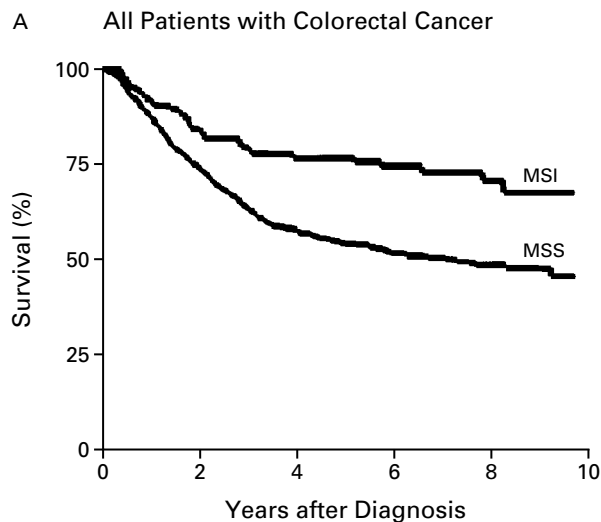
Information on family history was available for 84 (82 percent) of the 102 patients who had colorectal cancer with high-frequency microsatellite instability, including 21 of the 29 patients (72 percent) who died during follow-up. In total, 13 of these 84 patients (15 percent) had family histories that fulfilled the Amsterdam criteria for hereditary nonpolyposis colorectal cancer. Among the patients who had cancer with high-frequency microsatellite instability, no significant difference in survival was found between those who fulfilled the Amsterdam criteria (five-year survival, 77±12 percent) and those who did not (five-year survival, 78±5 percent; P=0.41). Of the 84 patients, only 1 (whose family history did not fulfill the Amsterdam criteria) was asymptomatic when a diagnosis was made by clinical screening.

In a step-down multivariate analysis, the microsatellite status, pathological stage, tumor grade, and histologic type of the cancer were found to be significantly and independently associated with survival (Table 4). The survival advantage of high-frequency microsatellite instability over microsatellite stability was similar in the model that included all 12 prognostic variables listed in Table 3, regardless of their measured significance (hazard ratio, 0.42; 95 percent confidence interval, 0.27 to 0.67; P<0.001). The proportionality of the survival advantage associated with high-frequency microsatellite instability can be seen in Kaplan–Meier survival curves stratified according to disease stage (Fig. 2).

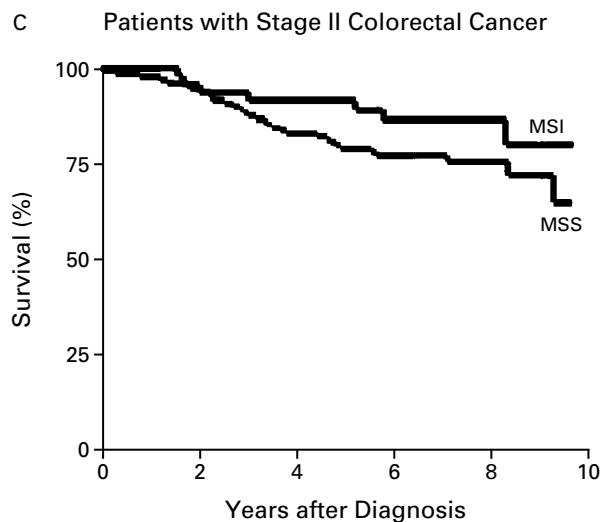
DISCUSSION

Because most cancers are thought to arise from an accumulation of genetic alterations, it is not surprising that cancers that emerge from different mutational pathways should differ clinically. We have found this to be the case for the subgroup of colorectal cancers that are characterized by high-frequency microsatellite instability. In our population-based series, high-frequency microsatellite instability was associated with prolonged survival independently of classic clinical prognostic factors, including the disease stage. Eighty-five percent of the patients who had cancer with high-frequency microsatellite instability did not have a family history suggestive of hereditary nonpolyposis colorectal cancer. For this reason, the considerable survival advantage conferred by high-frequency microsatellite instability appears to be applicable to both heritable and sporadic types of colorectal cancer. Furthermore, in only one of the patients whose cancer had high-frequency microsatellite instability was the cancer diagnosed by clinical screening when he was asymptomatic; this fact eliminates lead-time bias as a likely cause of the survival advantage.

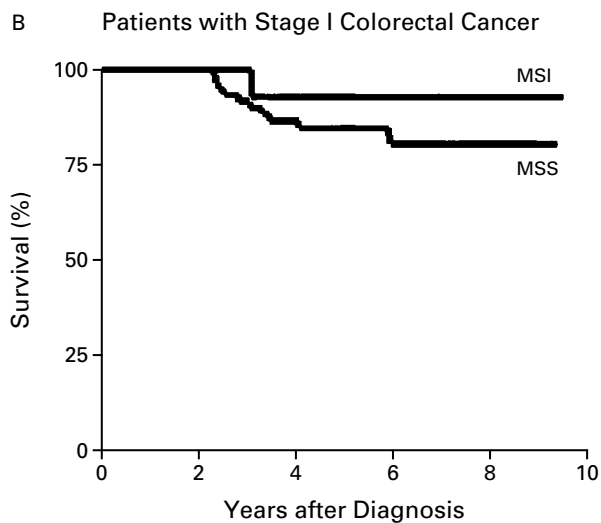
The association of high-frequency microsatellite instability with improved clinical outcome has been



No. AT RISK		0	2	4	6	8	10
MSI	102	86	78	53	29	0	0
MSS	485	358	280	183	75	0	0



No. AT RISK		0	2	4	6	8	10
MSI	46	44	42	25	15	0	0
MSS	127	120	105	72	28	0	0



No. AT RISK		0	2	4	6	8	10
MSI	14	14	13	9	5	0	0
MSS	59	59	51	35	13	0	0

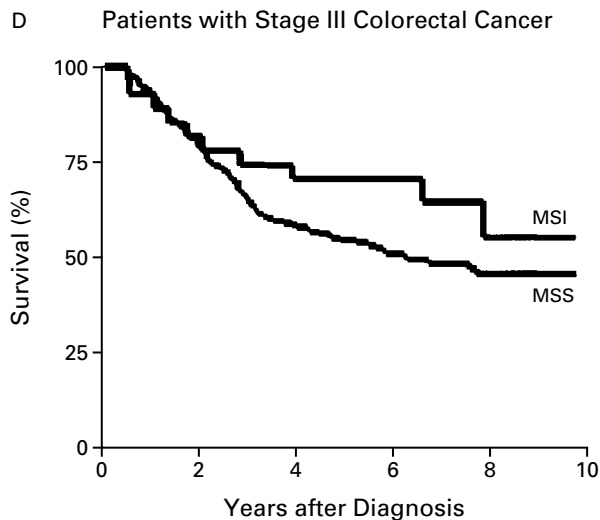
Figure 2. Kaplan–Meier Survival Curves for Patients with Colorectal Cancer, Stratified According to Microsatellite Status.

Panel A shows that the survival of patients who had colorectal cancer with high-frequency microsatellite instability (MSI) was significantly better than that of patients who had cancers with microsatellite stability (MSS) ($P < 0.001$). Panels B, C, D, and E show survival curves for patients with colorectal cancer according to the microsatellite status of the cancer and according to the American Joint Committee on Cancer disease stage. The survival of patients with cancers with high-frequency microsatellite instability was better than that for patients with cancers with microsatellite stability at all disease stages.

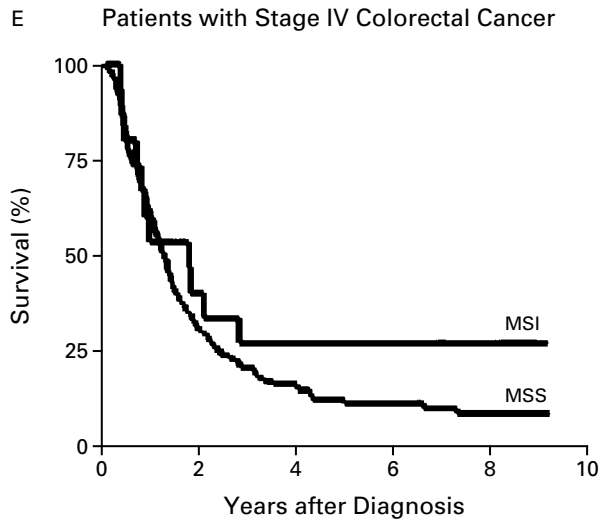
suggested previously.^{12,26-29} In other studies, however, no survival advantage was detected,³⁸⁻⁴¹ and a recent National Cancer Institute workshop concluded that microsatellite instability had not yet been shown conclusively to be an independent predictor of prognosis.³⁴ Furthermore, since the first descriptions of high-frequency microsatellite instability,¹¹⁻¹³ the literature has been complicated by inconsistent and confusing definitions of this molecular phenotype.³⁴ The

term “high-frequency microsatellite instability” is meant to describe a generalized (not occasional) instability of microsatellite DNA in cancers that almost always lack the ability to repair mismatched bases in DNA. For this reason, the National Cancer Institute has defined high-frequency microsatellite instability, low-frequency microsatellite instability, and microsatellite stability in colorectal cancer in terms of how many microsatellite loci and which specific loci need to be tested and shown to be altered.³⁴ In our study we used these consensus definitions.

We found a 17 percent incidence of high-frequency microsatellite instability, but in a recent large series reported by Aaltonen et al.,¹⁴ a 12 percent incidence was found. There was a similar difference in incidence among patients whose family histories fulfilled the Amsterdam criteria for hereditary nonpolyposis colorectal cancer (15 percent in our series and 11 percent in the study by Aaltonen et al.¹⁴). Thus, the differences noted are likely to reflect the fact that our popu-



No. AT RISK		0	2	4	6	8	10
MSI	27	22	19	15	6	0	0
MSS	180	143	105	66	30	0	0



No. AT RISK		0	2	4	6	8	10
MSI	15	6	4	4	3	0	0
MSS	119	36	19	10	4	0	0

lation was relatively young (all received a diagnosis at 50 years of age or younger) and thus may have included a greater proportion of patients with hereditary nonpolyposis colorectal cancer. Despite their relatively young age, less than 10 percent of the patients in our cohort had colorectal cancer associated with hereditary nonpolyposis colorectal cancer, familial adenomatous polyposis, or inflammatory bowel disease.

Previous case-control studies reported that 58 percent⁴² and 47 percent²⁸ of colorectal cancers in patients 35 years of age or younger and 40 years of age or younger, respectively, had high-frequency microsatellite instability. These results highlight the need for unbiased methods of case ascertainment to use as a basis for calculating accurate frequencies of molecular markers such as high-frequency microsatellite instability.

In addition to high-frequency microsatellite instability, we found that the pathological stage of colorectal cancer was an independent and powerful predictor of clinical outcome. This is not surprising, because the pathological stage is the main determinant of outcome for most cancers.³ The fact that high-frequency microsatellite instability was strongly associated with a lower stage of cancer, even after we controlled for the depth of tumor invasion, is intriguing. These results indicate that high-frequency microsatellite instability contributes to improved survival in two separate ways. First, high-frequency microsatellite instability is prognostic of improved survival independently of other prognostic factors, including pathological stage. Second, high-frequency microsatellite instability is in-

dependently predictive of lower pathological stage, thus further contributing to the improved survival through tumor down-staging.

The mechanism by which high-frequency microsatellite instability influences clinical outcome is unknown, but it may be related to the kinds of mutations or the genetic targets involved in colorectal cancers that are deficient in DNA-mismatch repair. For example, colorectal cancers with high-frequency microsatellite instability have fewer mutations of the adenomatous polyposis coli (*APC*)⁴³ and p53^{13,43} genes and more frequent mutations of the β -catenin (*CTNNB1*)^{44,45} and transforming growth factor β receptor type II⁴⁶ genes than colorectal cancers with microsatellite stability. Distinct clinical and pathological features, such as the intense lymphocytic infiltrates observed in tumors with high-frequency microsatellite instability,²⁵ may result from these unique genetic alterations and contribute to the less aggressive nature of these cancers.

In addition, the therapeutic effects of DNA-damaging chemotherapeutic agents, such as fluorouracil, are likely to be influenced by the underlying mutational mechanism. In vitro, cell lines with high-frequency microsatellite instability are less responsive than cell lines with microsatellite stability to various chemotherapeutic agents.⁴⁷ Furthermore, the targeting of DNA cells that are deficient in mismatch repair may offer a specific intervention that does not affect normal tissues that retain mismatch-repair function.⁴⁸

In conclusion, we detected high-frequency microsatellite instability in 17 percent of colorectal-cancer

TABLE 3. UNIVARIATE ANALYSIS OF PREDICTIVE FACTORS FOR SURVIVAL IN 587 PATIENTS WITH COLORECTAL CANCER.*

PROGNOSTIC FACTOR	NO. OF PATIENTS	5-Yr SURVIVAL (%)	P VALUE†
Patient			
Sex			
Male	295	54±3	0.29
Female	292	60±3	
Age at diagnosis			
≤35 yr	78	53±6	0.87
36–40 yr	88	56±5	
41–45 yr	184	59±4	
46–50 yr	237	56±3	
Coexisting illness			
Inflammatory bowel disease			
No	571	58±2	0.86
Yes	16	57±12	
Familial adenomatous polyposis			
No	579	58±2	0.84
Yes	8	50±17	
Extracolonic cancer			
No	566	58±2	0.83
Yes	21	55±11	
Synchronous or metachronous colorectal cancer			
No	556	58±2	0.47
Yes	31	55±9	
Site of tumor relative to the splenic flexure			
Colon			
Proximal	198	57±4	0.96‡
Distal	186	59±4	
Total	384	58±3	
Rectum	203	55±3	0.92§
Histologic features			
Cell type			
Adenocarcinoma, not otherwise specified	530	60±2	<0.001
Mucinous	28	29±9	
Signet ring	19	16±8	
Undifferentiated	8	38±17	
Adenosquamous	2	NA	
Grade			
Well differentiated	53	75±6	<0.001
Moderately differentiated	405	59±2	
Poorly differentiated	129	42±4	
Extramural venous invasion			
No	552	59±2	<0.001
Yes	35	37±8	
Pathological stage¶			
I	73	85±4	<0.001
II	173	81±3	
III	207	55±3	
IV	134	13±3	
Microsatellite status			
MSS	485	54±2	<0.001
MSI	102	76±4	

*Plus-minus values are means ±SE. MSS denotes colorectal cancer with microsatellite stability, MSI colorectal cancer with high-frequency microsatellite instability, and NA not assessed.

†The log-rank test was used to calculate P values.

‡The P value is for the comparison of the proximal colon, distal colon, and rectum.

§The P value is for the comparison of the colon and rectum.

¶The pathological stage was classified according to the criteria of the American Joint Committee on Cancer.³

TABLE 4. SIGNIFICANT PREDICTIVE FACTORS FOR SURVIVAL IN A COX PROPORTIONAL-HAZARDS ANALYSIS OF 587 PATIENTS WITH COLORECTAL CANCER.*

PROGNOSTIC FACTOR	HAZARD RATIO (95% CI)†	P VALUE‡
Molecular genetics		
MSI vs. MSS	0.45 (0.30–0.68)	<0.001
Cell type		
Mucinous vs. adenocarcinoma, not otherwise specified	1.37 (0.85–2.22)	0.001
Signet ring vs. adenocarcinoma, not otherwise specified	2.58 (1.47–4.52)	
Undifferentiated vs. adenocarcinoma, not otherwise specified	3.19 (1.33–7.65)	
Adenosquamous vs. adenocarcinoma, not otherwise specified	NA	
Grade		
Moderately differentiated vs. well differentiated	1.67 (0.95–2.95)	0.02
Poorly differentiated vs. well differentiated	2.27 (1.23–4.17)	
Pathological stage§		
II vs. I	1.42 (0.74–2.71)	<0.001
III vs. I	3.30 (1.81–6.03)	
IV vs. I	12.4 (6.78–22.7)	

*CI denotes confidence interval, MSI colorectal cancer with high-frequency microsatellite instability, MSS colorectal cancer with microsatellite stability, and NA not assessed.

†Hazard ratios less than 1.00 represent a decreased risk of death, whereas hazard ratios greater than 1.00 represent an increased risk of death.

‡The P values result from the hypothesis that the hazard ratio as determined by a Cox proportional-hazards analysis equaled 1.0.

§The pathological stage was classified according to the criteria of the American Joint Committee on Cancer.³

specimens from a population-based series of relatively young patients. In most of these patients, there was no family history suggestive of hereditary non-polyposis colorectal cancer. High-frequency microsatellite instability was found to be an independent predictor of improved survival, and tumors with this genetic phenotype were less likely to metastasize than those characterized by microsatellite stability.

Supported by the National Cancer Institute of Canada with funds provided by the Canada Cancer Society. Dr. Gryfe is a Research Fellow of the National Cancer Institute of Canada, with funds provided by the Terry Fox Run, and was supported by the American Society of Colon and Rectal Surgeons through the Leon Hirsch Surgical Research Fellowship. Dr. Bull is a National Health Research Scholar of the National Health Research Development Program.

We are indebted to the treating physicians and participating departments of pathology for their cooperation; to Darlene Dale, Nelson Chong, Lisa Madlensky, Dr. Andrew Smith, Dr. Malcolm Moore, and Scott Mackay for assistance in data retrieval; to Dr. Susan Bondy and Marc Theriault of the Institute for Clinical Evaluative Sciences for assistance in the analysis of chemotherapy data; to Kazy Hay and Susie Tjan for assistance in specimen handling; and to Dr. Robin McLeod, Dr. Steven Narod, Dr. Michelle Cotterchio, and Dr. Allan Detsky for helpful discussions.

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