Published in final edited form as:
N Engl J Med. 2003 July 17; 349(3): 247–257. doi:10.1056/NEJMoa022289.

Tumor Microsatellite-Instability Status as a Predictor of Benefit from Fluorouracil-Based Adjuvant Chemotherapy for Colon Cancer

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Abstract

BACKGROUND—Colon cancers with high-frequency microsatellite instability have clinical and pathological features that distinguish them from microsatellite-stable tumors. We investigated the usefulness of microsatellite-instability status as a predictor of the benefit of adjuvant chemotherapy with fluorouracil in stage II and stage III colon cancer.

METHODS—Tumor specimens were collected from patients with colon cancer who were enrolled in randomized trials of fluorouracil-based adjuvant chemotherapy. Microsatellite instability was assessed with the use of mononucleotide and dinucleotide markers.

RESULTS—Of 570 tissue specimens, 95 (16.7 percent) exhibited high-frequency microsatellite instability. Among 287 patients who did not receive adjuvant therapy, those with tumors displaying high-frequency microsatellite instability had a better five-year rate of overall survival than patients with tumors exhibiting microsatellite stability or low-frequency instability (hazard ratio for death, 0.31 [95 percent confidence interval, 0.14 to 0.72]; P=0.004). Among patients who received adjuvant chemotherapy, high-frequency microsatellite instability was not correlated with increased overall survival (hazard ratio for death, 1.07 [95 percent confidence interval, 0.62 to 1.86]; P=0.80). The benefit of treatment differed significantly according to the microsatellite-instability status (P=0.01). Adjuvant chemotherapy improved overall survival among patients with microsatellite-stable tumors or tumors exhibiting low-frequency microsatellite instability, according to a multivariate analysis adjusted for stage and grade (hazard ratio for death, 0.72 [95 percent confidence interval, 0.53 to 0.99]; P=0.04). By contrast, there was no benefit of adjuvant chemotherapy in the group with high-frequency microsatellite instability.

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CONCLUSIONS—Fluorouracil-based adjuvant chemotherapy benefited patients with stage II or stage III colon cancer with microsatellite-stable tumors or tumors exhibiting low-frequency microsatellite instability but not those with tumors exhibiting high-frequency microsatellite instability.

Colorectal cancer is the fourth most common type of cancer in Western society and the second leading cause of cancer-related death in North America.[1] Although surgical resection alone is potentially curative, local or distant recurrences develop in many patients, and those with the highest risk of recurrence are advised to receive fluorouracil-based systemic adjuvant chemotherapy, which has been shown to be beneficial in a number of cooperative-group trials and analyses.[2–12]

Traditional pathological staging systems have been useful in predicting the outcome of colorectal cancer, but it is now evident that these cancers are heterogeneous. The natural history of colorectal cancer correlates strongly with genetic alterations that occur during the progression from adenoma to carcinoma to metastatic disease.[13,14] The most common genetic alterations, occurring in approximately 85 percent of colorectal cancers, are allelic losses or loss of heterozygosity, chromosomal amplifications, and translocations.[15–19] These alterations are characteristic of the chromosomal-instability pathway, also known as the microsatellite-stability pathway. The remaining 15 percent of colorectal cancers display frame-shift mutations and base-pair substitutions that are commonly found in short, tandemly repeated nucleotide sequences known as microsatellites.[16,20–27] This form of genetic destabilization is most commonly caused by the loss of the DNA mismatch-repair function and is referred to as the microsatellite-instability pathway. The phenotype of tumors with this defect is termed the high-frequency–microsatellite-instability phenotype.[16,20,22,28–32] The chromosomal-instability phenotype and the high-frequency–microsatellite-instability phenotype do not represent alterations of single genes, but rather discrete molecular pathways involving multiple somatic genetic targets.[20]

Recently, distinct clinical and pathological features of colorectal tumors arising from these two separate mutational pathways have been identified. High-frequency microsatellite instability is observed more frequently in colorectal cancers that occur proximal to the splenic flexure. These tumors also exhibit poor differentiation, mucinous cell type, and peritumoral lymphocytic infiltration; they are usually diploid, unlike microsatellite-stable tumors, which are commonly aneuploid.[26,27,33–35] Colorectal cancers exhibiting high-frequency microsatellite instability have also been associated with a larger size of the primary tumor but a more favorable stage distribution.[22,33] Patients with colorectal cancers that exhibit high-frequency microsatellite instability have longer survival than stage-matched patients with cancers exhibiting microsatellite stability.[16,22,23,26,36–38]

Few studies have examined the effect of adjuvant treatment in colorectal cancers with high-frequency microsatellite instability.[23,24,36,39,40] Furthermore, studies that have controlled for the effects of adjuvant therapy have had small or nonrandomized study populations with potential selection biases.[24,36,39,40] We used specimens from patients with resected stage II or stage III colon cancer who were previously enrolled in prospective, randomized trials of fluorouracil-based chemotherapy. The pooled data base for these trials includes phase 3 studies with groups that received no treatment, thus permitting an analysis of the true survival advantage for patients whose tumors exhibited high-frequency microsatellite instability and who had not received adjuvant chemotherapy. In addition, studying this population of patients allowed us to analyze whether the phenotype of high-frequency microsatellite instability could be an independent predictor of a benefit from fluorouracil-based adjuvant chemotherapy.

METHODS

PATIENTS AND SPECIMENS

We studied specimens from 570 patients with colon cancer who had previously been enrolled in five phase 3 trials of adjuvant chemotherapy (Table 1). The primary objective of each of the trials was to determine whether fluorouracil-based adjuvant chemotherapy improved disease-free survival, overall survival, or both among patients who had undergone curative resection of stage II or stage III colon cancer. Three studies randomly assigned patients to fluorouracil plus leucovorin or no treatment, and two studies randomly assigned patients to fluorouracil plus levamisole or no treatment. The median duration of follow-up for all patients was 7.4 years. The current analysis was in accordance with the original informed consent signed by all patients.

Blocks of formalin-fixed, paraffin-embedded specimens were requested from the relevant pathology departments. In total, 570 specimens were included in the analysis of microsatellite instability. Collected specimens that were excluded from the analysis had low tumor cellularity (<60 percent) or could not be amplified by polymerase chain reaction (PCR).

MICROSATELLITE-INSTABILITY TESTING AND ANALYSIS

Extracted DNA was amplified by PCR with the use of 2 to 11 microsatellite loci. Specifically, specimens from the North Central Cancer Treatment Group (protocols 784852 and 874651) and Gastrointestinal Intergroup trial 0035 of the National Cancer Institute were screened with 4 to 11 dinucleotide markers, as described previously.[23,41] Nearly all specimens collected from the C.03 trial of the National Cancer Institute of Canada were amplified with 5 to 10 microsatellite loci derived from the panel of microsatellite loci defined by the National Cancer Institute, as described previously.[22,34,42,43] Specimens obtained from the trial conducted by the Fondation Française de Cancérologie Digestive, as well as five specimens from the C.03 trial of the National Cancer Institute of Canada, were screened only with mononucleotide markers BAT25 and BAT26, since non-neoplastic control tissue was not available.[44]

The presence of additional bands observed in the PCR products from tumor DNA that were not observed in DNA from normal tissue from the same patient was scored as instability at that particular locus. In accordance with consensus definitions of the National Cancer Institute, tumor samples were classified as displaying high-frequency microsatellite instability (instability at 30 percent or more of the loci screened), low-frequency microsatellite instability (instability at less than 30 percent of the loci screened), or microsatellite stability (stability at all the loci tested).[34,42] Since extensive data indicate that tumors with low-frequency microsatellite instability are not biologically distinct from those exhibiting microsatellite stability, these two molecular phenotypes were grouped together in all analyses.[23,45]

The microsatellite-instability status of tumors from patients without available corresponding normal tissue was analyzed with the use of the BAT25 and BAT26 markers, without the need for amplified normal DNA, as described previously.[40,46] Specifically, samples with instability at both markers were scored as exhibiting high-frequency microsatellite instability, whereas samples with no instability at these markers were scored as microsatellite-stable. No specimen exhibited instability at only one of the two mononucleotide markers.

CLINICAL DATA BASE

A common clinical data base had previously been established and verified by investigators from centers in the International Multicentre Pooled Analysis of Colon Cancer Trials. This data base was maintained by the Clinical Trials Group of the National Cancer Institute of Canada and was recently merged with the clinical data bases of Gastrointestinal Intergroup trial 0035 of the National Cancer Institute and protocols 784852 and 874651 of the North Central Cancer Treatment Group for combined analysis. All data bases were prepared and managed by persons with no knowledge of the molecular data.

STATISTICAL ANALYSIS

For the outcome analysis, patients were classified according to the presence of high-frequency microsatellite instability, low-frequency microsatellite instability, or microsatellite stability in the tumor specimens. The primary outcomes were overall survival and disease-free survival. Overall survival was defined as the time from study entry to death. Disease-free survival was defined as the time from study entry to the first confirmed relapse or death, whichever occurred first. Data on overall and disease-free survival were censored at eight years from the date of randomization. Survival curves were generated according to the method of Kaplan and Meier, and univariate survival distributions were compared with the use of the log-rank test.[47] Hazard ratios and 95 percent confidence intervals for univariate and multivariate models were computed with the use of Cox proportional-hazards regression.[48] P values for tests of interaction were computed with the use of the likelihood-ratio statistic in comparisons between a model including main effects but no interaction and the same model with the inclusion of the term for interaction.

Differences in base-line prognostic factors according to the microsatellite-instability status of the patients' tumors were tested for statistical significance with the use of a chi-square test for categorical variables or an unpaired Student's t-test for continuous variables. The use of randomized clinical trials comparing fluorouracil-based adjuvant treatment with no adjuvant treatment permitted us to test directly for an effect of chemotherapy. In addition, a test for interaction between microsatellite-instability status and treatment effect was performed with the use of Cox proportional-hazards regression. The probability that chemotherapy is associated with a 5 percent or greater increase in the rate of five-year survival among patients with colon cancers exhibiting high-frequency microsatellite instability was analyzed with the use of both a simulation based on a Weibull survival model and a standard bootstrap technique.

All time-to-event analyses were stratified according to the type of treatment protocol (levamisole or leucovorin in addition to fluorouracil-based adjuvant chemotherapy). Specifically, the C.03 trial of the National Cancer Institute of Canada, the trial of the Fondation Française de Cancérologie Digestive, and protocol 874651 of the North Central Cancer Treatment Group were treated as one stratum, and protocol 784852 of the North Central Cancer Treatment Group and Gastrointestinal Intergroup trial 0035 of the National Cancer Institute were treated as a separate stratum for univariate analyses. Additional analyses were further stratified according to the stage of disease, as indicated. All reported P values are two-sided, and P values of less than 0.05 were considered to indicate statistical significance.

RESULTS

CHARACTERISTICS OF THE PATIENTS AND MICROSATELLITE-INSTABILITY STATUS

Of 570 tumor samples tested for microsatellite instability, 95 (16.7 percent) demonstrated high-frequency microsatellite instability, 60 (10.5 percent) demonstrated low-frequency

microsatellite instability, and 415 (72.8 percent) were microsatellite-stable. High-frequency microsatellite instability was associated with localization of the tumor to a site proximal to the splenic flexure (P<0.001) and a high histologic tumor grade (P<0.001) (Table 2). In other respects, the patients with tumors exhibiting high-frequency microsatellite instability were similar to the patients with tumors exhibiting microsatellite stability or low-frequency microsatellite instability (Table 2).

RELATION BETWEEN MICROSATELLITE-INSTABILITY STATUS AND SURVIVAL

In total, 185 of the 570 patients (32.5 percent) died during a median follow-up period of 7.4 years. In a pooled analysis that did not control for the use or nonuse of adjuvant chemotherapy, the rate of five-year disease-free survival among patients with tumors exhibiting high-frequency microsatellite instability (75.3 percent) was significantly greater than that among patients with tumors exhibiting low-frequency microsatellite instability or microsatellite stability (64.1 percent; P=0.04) (Table 3). In univariate analyses, there was no significant difference in five-year overall survival between these groups of patients (P=0.07) (Table 3). In multivariate models adjusted for the stage of disease and tumor grade, high-frequency microsatellite instability was significantly associated with overall survival (hazard ratio for death, 0.61 [95 percent confidence interval, 0.38 to 0.96]; P=0.03) (Table 4).

Among patients who had not received adjuvant chemotherapy, patients with tumors exhibiting high-frequency microsatellite instability had longer overall survival (Fig. 1A) and higher rates of five-year disease-free survival (Table 3) than patients with tumors exhibiting low-frequency microsatellite instability or microsatellite stability. Multivariate analysis controlled for the stage of disease and tumor grade also demonstrated that high-frequency microsatellite instability in patients not receiving fluorouracil-based adjuvant chemotherapy was significantly and independently associated with survival (hazard ratio for death, 0.32 [95 percent confidence interval, 0.14 to 0.75]; P=0.008) (Table 4). However, the analysis of patients who did receive adjuvant therapy failed to show significant differences in overall or disease-free survival according to microsatellite-instability status (Table 3 and Fig. 1B).

RELATION BETWEEN MICROSATELLITE-INSTABILITY STATUS AND BENEFIT OF ADJUVANT CHEMOTHERAPY

Analyses were performed to determine whether the effect of treatment, microsatellite-instability status, or both differed according to the stage of disease. Neither interaction was found to be significant (P=0.48 and P=0.21, respectively), and therefore, patients were pooled regardless of stage for the analyses examining treatment effect and microsatellite-instability status. When the entire group of 570 patients was analyzed, we found no significant difference between those who were treated with fluorouracil-based adjuvant chemotherapy and those who were not in the rates of five-year overall survival (P=0.12) or five-year disease-free survival (P=0.06) (Table 3). However, a significant interaction was observed between microsatellite-instability status and the benefit of treatment (P=0.01). This interaction remained significant after stratification according to the stage of disease (P=0.02). Among patients with tumors exhibiting low-frequency microsatellite instability or microsatellite stability, adjuvant chemotherapy was associated with significant increases in the duration of overall survival (Fig. 2A) and the rates of five-year disease-free survival (Table 3). This survival benefit was also seen in a multivariate analysis controlled for stage and grade (Table 4). There was no evidence of a three-way interaction among treatment effect, microsatellite-instability status, and stage of disease (P=0.39).

Conversely, among patients with tumors exhibiting high-frequency microsatellite instability, fluorouracil-based chemotherapy did not improve the outcome as compared with no chemotherapy (Table 3 and Fig. 2B). The probability that fluorouracil-based chemotherapy

was associated with an increase of at least 5 percent in the rate of five-year survival among patients with tumors exhibiting high-frequency microsatellite instability was less than 1 percent.

These trends were consistently maintained in analyses of subgroups defined according to the stage of disease. Treatment was associated with an improved outcome among patients with stage II or stage III cancers with low-frequency microsatellite instability or microsatellite stability (hazard ratio for death among treated patients as compared with untreated patients, 0.67 [95 percent confidence interval, 0.39 to 1.15] among patients with stage II cancer and 0.69 [95 percent confidence interval, 0.47 to 1.01] among patients with stage III cancer). In contrast, among patients with tumors exhibiting high-frequency microsatellite instability, treatment was associated with a worse outcome for both stage II and stage III cancer (hazard ratio for death, 3.28 [95 percent confidence interval, 0.86 to 12.48] among patients with stage II cancer and 1.42 [95 percent confidence interval, 0.36 to 5.56] among patients with stage III cancer).

DISCUSSION

Recent studies of colorectal cancer have identified two molecular pathways leading to the malignant phenotype — the pathway of high-frequency microsatellite instability and that of microsatellite stability — which respond differently to DNA damage. It is unlikely that tumors with these distinct pathways would respond similarly to chemotherapeutic agents that damage DNA. Since it may be unethical to withhold chemotherapy in a clinical trial for potentially curable advanced-stage colon cancer, we used samples from previous multicenter, prospective, randomized, controlled trials to determine whether microsatellite-instability status could serve as a predictor of a survival benefit with fluorouracil-based adjuvant chemotherapy.

Our results in patients with stage II or stage III colon cancer confirm previous reports of a survival benefit for patients with tumors exhibiting high-frequency microsatellite instability.[22,23,26,33,35–38] In a univariate analysis that did not control for the possible effect of chemotherapy, high-frequency microsatellite instability was associated with improved five-year disease-free survival among patients with stage II or stage III colon cancer. We also found that patients with tumors exhibiting microsatellite stability or low-frequency microsatellite instability tended to benefit from fluorouracil-based adjuvant chemotherapy, whereas such chemotherapy did not benefit patients with high-frequency microsatellite instability and may in fact have led to worse outcomes among such patients. These results remained consistent in models that adjusted for the stage of disease and in models stratified according to stage, and they held true for both patients with stage II cancer and patients with stage III cancer.

In vitro studies have shown that colon-cancer cell lines displaying high-frequency microsatellite instability are less responsive than microsatellite-stable cell lines to fluorouracil.[49–54] However, our findings contrast with those of a large, selected case series of patients with stage III colon cancer, which demonstrated a significant association between an increased duration of survival and high-frequency microsatellite instability among patients receiving adjuvant chemotherapy.[40] But this nonrandomized case series has the potential for bias. For example, patients not receiving chemotherapy were, on average, 13 years older than those who received adjuvant fluorouracil therapy. Increasing age has been demonstrated to be significantly and independently associated with a poor outcome among patients with colorectal cancer, after adjustment for the microsatellite-instability status of the tumor.[24] A significantly older mean age also makes it likely that the presence of coexisting

disease was an important reason why some patients in this nonrandomized study were not offered adjuvant treatment.[40]

Although the results of our analysis and previous data from in vitro studies suggest that fluorouracil-based adjuvant chemotherapy is not beneficial in patients with colon cancer exhibiting high-frequency microsatellite instability, other drugs, such as the topoisomerase-I inhibitor camptothecin, have been shown to kill mismatch-repair–deficient cancer cells exhibiting high-frequency microsatellite instability.[55] It would therefore seem important to conduct molecular analyses of specimens from recent clinical trials of non–fluorouracil-based chemotherapies and to ensure that future trials include analyses of molecular pathways.[56]

In our retrospective analysis, the finding that fluorouracil-based adjuvant chemotherapy does not significantly increase, and may potentially decrease, overall and disease-free survival among patients with tumors exhibiting high-frequency microsatellite instability raises several provocative issues regarding postoperative management of stage II and stage III colon cancer. However, we would urge caution and not advocate altering clinical decision making on the basis of our findings. If confirmed by other analyses of previous, well-designed clinical trials or by future prospective, randomized, controlled studies, however, our findings would indicate that microsatellite-instability testing should be conducted routinely and the results used to direct rational adjuvant chemotherapy in colon cancer.

Acknowledgments

Supported in part by grants from the National Cancer Institute of Canada (012200) and the National Cancer Institute, National Institutes of Health (CA25224, CA60100, CA21115, CA62924, CA23318), and fellowship funding from the American Society of Colon and Rectal Surgeons.

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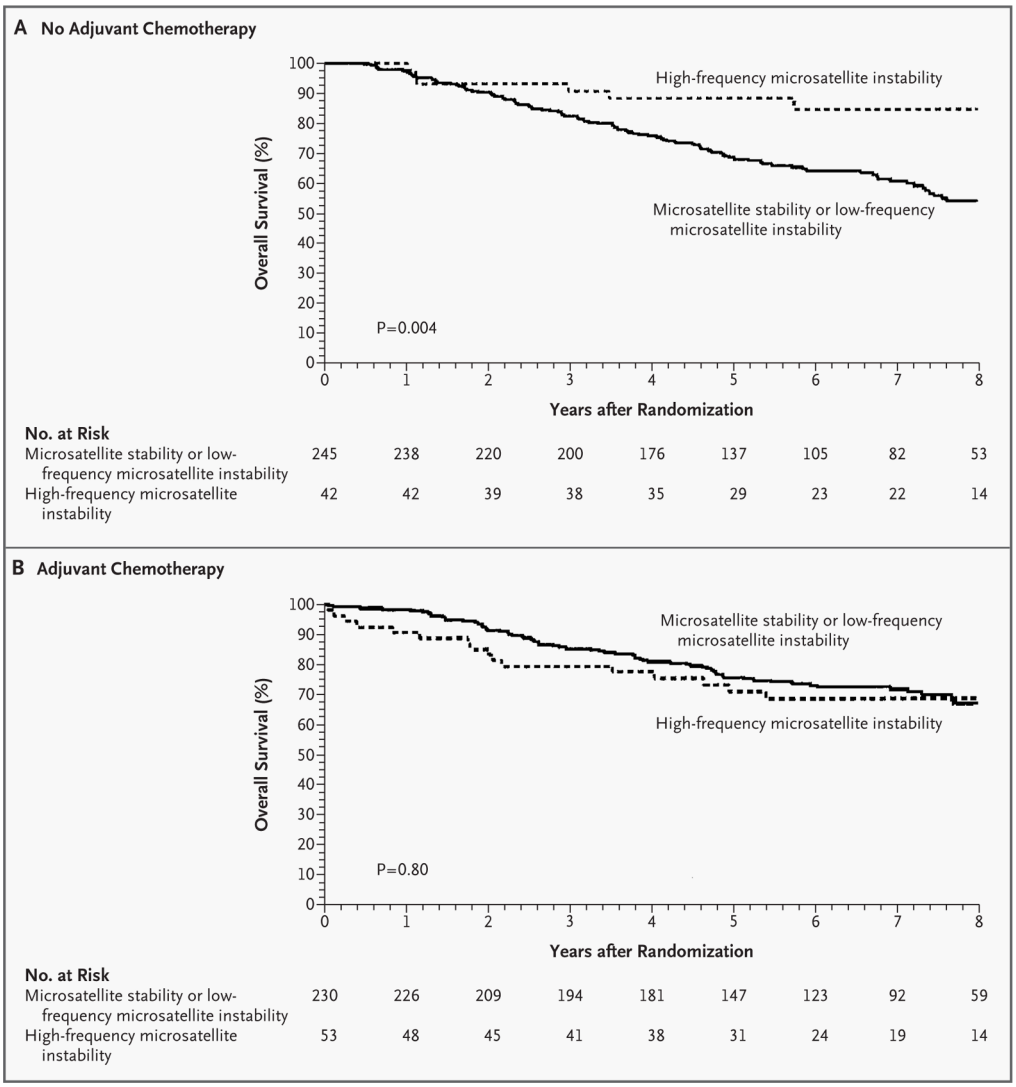

Figure 1. Kaplan–Meier Estimates of Overall Survival among Patients with Stage II or Stage III Colon Cancer According to the Microsatellite-Instability Status of the Tumor

In the absence of adjuvant chemotherapy, the patients with tumors displaying high-frequency microsatellite instability had significantly longer overall survival than patients with tumors exhibiting microsatellite stability or low-frequency microsatellite instability (hazard ratio for death, 0.31 [95 percent confidence interval, 0.14 to 0.72]; P=0.004)(Panel A). When the analysis was limited to the group receiving adjuvant chemotherapy, patients with tumors exhibiting high-frequency microsatellite instability did not have a significant increase in overall survival as compared with patients with tumors exhibiting microsatellite stability or low-frequency microsatellite instability (hazard ratio for death, 1.07 [95 percent confidence interval, 0.62 to 1.86]; P=0.80)(Panel B). The analysis included data for eight years from the date of randomization.

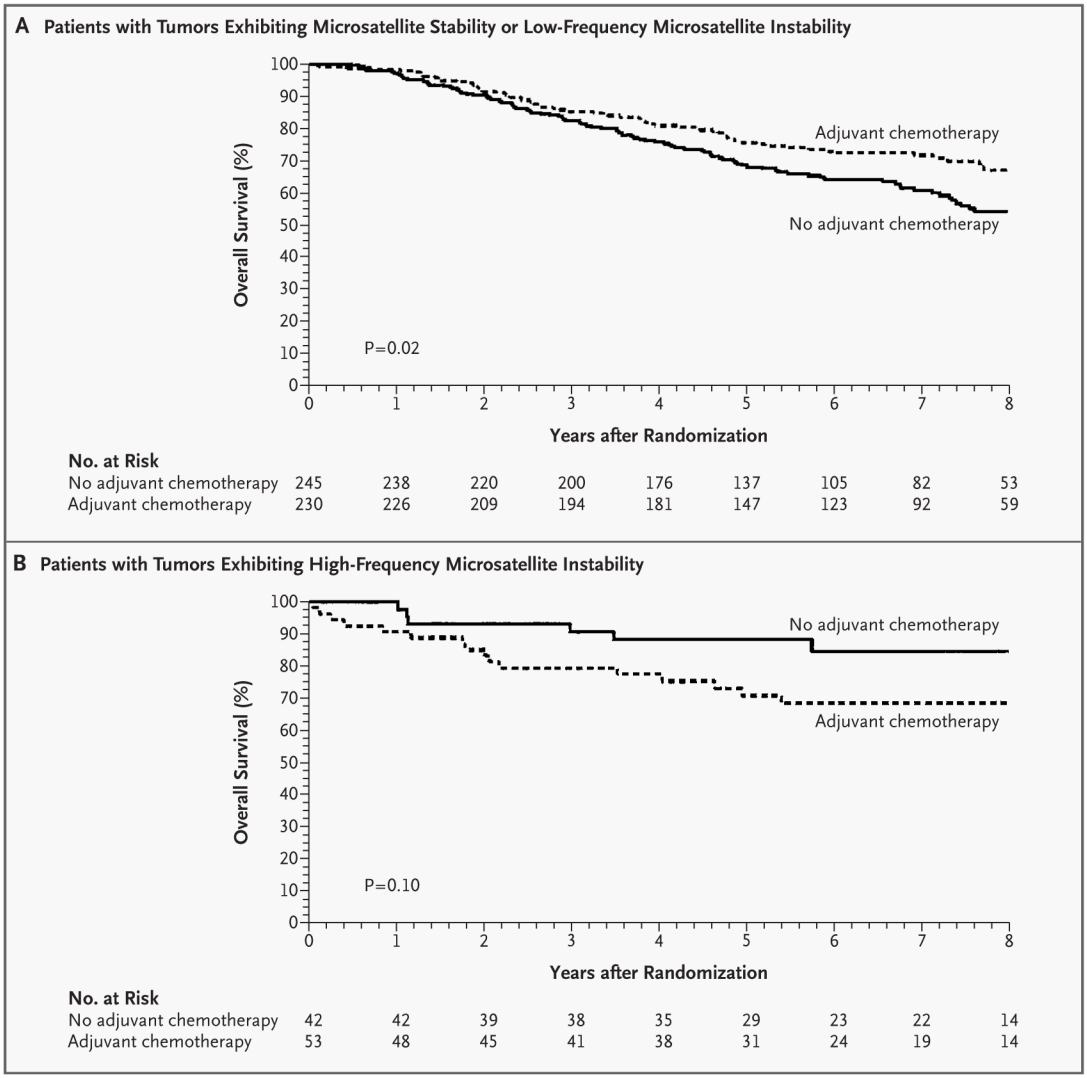

Figure 2. Kaplan–Meier Estimates of Overall Survival among Patients with Stage II or Stage III Colon Cancer According to Treatment Status

Patients with tumors exhibiting microsatellite stability or low-frequency microsatellite instability who received adjuvant chemotherapy had a significant increase in overall survival as compared with patients who received no adjuvant chemotherapy (hazard ratio for death, 0.69 [95 percent confidence interval, 0.50 to 0.94]; P=0.02) (Panel A). Among patients with tumors exhibiting high-frequency microsatellite instability, there was no significant difference in the duration of overall survival between patients who received adjuvant chemotherapy and those who did not (hazard ratio for death, 2.17 [95 percent confidence interval, 0.84 to 5.55]; P=0.10) (Panel B). The analysis included data for eight years from the date of randomization.

N Engl J Med. Author manuscript; available in PMC 2013 February 28.

Table 1

Characteristics of the Trials.*

Characteristic	Clinical Trials Group, National Cancer Institute of Canada	North Central Cancer Treatment Group		Gastrointestinal Intergroup, National Cancer Institute	Fondation Française de Cancérologie Digestive
		Protocol 784852	Protocol 874651		
Details of trial					
Date of first randomization	May 1987	May 1978	February 1988	January 1985	October 1982
Total no. of patients randomized	370	267	111	936	268
Total no. of samples collected and analyzed for microsatellite-instability status	292	66	34	143	35
Eligibility criteria					
Age limit	None	None	None	None	≤75 yr
Minimum no. of days between surgery and beginning of chemotherapy	56	35	35	35	35
Adjuvant chemotherapy					
Dose of fluorouracil (mg/m^2)†	370	450	425	450	400
Dose of leucovorin (mg/m^2)†	200	—	20	—	200
Dose of levamisole (mg/m^2)†	—	50	—	50	—
Duration of therapy (mo)	6	12	6	12	6
Median follow-up (yr)	6.2	11.6	7.8	8.8	4.9

*Modified from the International Multicentre Pooled Analysis of Colon Cancer Trials (IMPACT) Investigators.[4]

†The doses are given as milligrams per square meter of body-surface area.

Table 2

Characteristics of the 570 Patients with Colon Cancer.*

Characteristic	All Eligible Patients (N=570)	Patients with Tumors Exhibiting High-Frequency Microsatellite Instability (N=95)	Patients with Tumors Exhibiting Microsatellite Stability or Low-Frequency Microsatellite Instability (N=475)	P Value
Treatment — no. (%)				0.19
Adjuvant chemotherapy	283 (50)	53 (56)	230 (48)	
No adjuvant chemotherapy	287 (50)	42 (44)	245 (52)	
Age — yr	59.8±11.2	60.7±13.0	59.7±10.8	0.13
Sex — no. (%)				0.45
Male	326 (57)	51 (54)	275 (58)	
Female	244 (43)	44 (46)	200 (42)	
Stage of disease — no. (%)				0.18
II	312 (55)	58 (61)	254 (53)	
III	258 (45)	37 (39)	221 (47)	
Site of tumor — no. (%)†				<0.001
Proximal	257 (45)	84 (89)	173 (36)	
Distal	305 (54)	9 (10)	296 (62)	
Multiple	6 (1)	1 (1)	5 (1)	
Tumor grade — no. (%)†				<0.001
Well differentiated (G1)	97 (17)	8 (9)	89 (19)	
Moderately differentiated (G2)	376 (66)	49 (53)	327 (69)	
Poorly differentiated (G3)	65 (11)	24 (26)	41 (9)	
Undifferentiated (G4)	28 (5)	12 (13)	16 (3)	
No. of positive nodes — no. (%)				0.21
0	312 (55)	58 (61)	254 (53)	
1–4	128 (22)	15 (16)	113 (24)	
>4	130 (23)	22 (23)	108 (23)	
Vital status at 8 yr — no. (%)				0.03
Alive	385 (68)	73 (77)	312 (66)	
Dead	185 (32)	22 (23)	163 (34)	

*Plus–minus values are means ± SD. P values for the comparison between patients with tumors exhibiting high-frequency microsatellite instability and patients with tumors exhibiting microsatellite stability or low-frequency microsatellite instability were calculated by the chi-square test for all variables except the mean age at diagnosis, for which an unpaired t-test was used. Because of rounding, not all percentages total 100.

†Data pertaining to the site of the tumor and the tumor grade are not available for all 570 patients.

Table 3

Univariate Analysis of Survival among Patients with Stage II or Stage III Colon Cancer According to Microsatellite-Instability Status or Adjuvant-Chemotherapy Status.*

Analysis	No. of Patients	Patients Surviving at 5 Yr *% (95% CI)*	P Value	Patients Surviving without Disease at 5 Yr *% (95% CI)*	P Value
According to microsatellite-instability status					
All patients	570				
Stability or low-frequency instability	475	71.9 (67.9–76.1)	0.07	64.1 (59.9–68.6)	0.04
High-frequency instability	95	78.4 (70.3–87.3)		75.3 (67.0–84.6)	
Patients receiving no adjuvant chemotherapy	287				
Stability or low-frequency instability	245	68.4 (62.7–74.6)	0.004	58.7 (52.8–65.3)	0.004
High-frequency instability	42	88.0 (78.7–98.4)		82.9 (72.1–95.3)	
Patients receiving adjuvant chemotherapy	283				
Stability or low-frequency instability	230	75.5 (70.1–81.4)	0.66	69.8 (64.1–76.0)	0.85
High-frequency instability	53	70.7 (59.2–84.5)		69.3 (57.8–83.1)	
According to adjuvant-chemotherapy status					
All patients	570				
Adjuvant chemotherapy	283	74.6 (69.6–80.0)	0.12	69.7 (64.5–75.3)	0.06
No adjuvant chemotherapy	287	71.2 (66.1–76.8)		62.3 (56.9–68.2)	
Stability or low-frequency instability	475				
Adjuvant chemotherapy	230	75.5 (70.1–81.4)	0.02	69.8 (64.1–76.0)	0.01
No adjuvant chemotherapy	245	68.4 (62.7–74.6)		58.7 (52.8–65.3)	
High-frequency instability	95				
Adjuvant chemotherapy	53	70.7 (59.2–84.5)	0.07	69.3 (57.8–83.1)	0.11
No adjuvant chemotherapy	42	88.0 (78.7–98.4)		82.9 (72.1–95.3)	

*P values are for the comparison between the rate in the given subgroup and that in the subgroup below it. CI denotes confidence interval.

Table 4

Hazard Ratios for Death with Adjustment for Stage of Disease and Tumor Grade.*

Analysis	Hazard Ratio for Death (95% CI)	P Value
According to microsatellite-instability status		
All patients	0.61 (0.38–0.96)	0.03
Patients receiving no adjuvant chemotherapy	0.32 (0.14–0.75)	0.008
According to adjuvant-chemotherapy status		
All patients	0.81 (0.60–1.08)	0.15
Patients with tumors exhibiting microsatellite stability or low-frequency microsatellite instability	0.72 (0.53–0.99)	0.04
Patients with tumors exhibiting high-frequency microsatellite instability	2.14 (0.83–5.49)	0.11

* Data are from Cox survival modeling. Hazard ratios in the analysis according to microsatellite-instability status are for patients with tumors exhibiting high-frequency microsatellite instability as compared with patients with tumors exhibiting microsatellite stability or low-frequency microsatellite instability; hazard ratios in the analysis according to adjuvant-chemotherapy status are for patients who received adjuvant chemotherapy as compared with patients who did not. CI denotes confidence interval.