

Microsatellite Instability in Sporadic Colon Cancer Is Associated with an Improved Prognosis at the Population Level¹

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Abstract

Some previous studies have reported an improved prognosis in sporadic colon cancers with microsatellite instability, whereas others have not. In addition, relatively few of those reporting an improved prognosis controlled for tumor stage or were population-based. Therefore, we evaluated the relationship between microsatellite instability and prognosis, tumor stage, and other clinical variables in a population-based study of 1026 individuals. Microsatellite instability was determined by the noncoding mononucleotide repeat BAT-26 and the coding mononucleotide repeat in transforming growth factor- β receptor type II. Significant relationships were seen between microsatellite instability and proximal tumor location, female gender, young and old age at diagnosis, poor histological differentiation, and low tumor stage ($P < 0.01$). There was a significant relationship between microsatellite instability and improved prognosis, even after adjusting for stage, with a reduction in the risk of death attributable to colon cancer of ~60%. Most of this risk reduction occurred in individuals with American Joint Committee on Cancer stage III tumors, although transforming growth factor- β receptor type II mutations were associated with a significant reduction in colon cancer death in tumors with distant metastases. We conclude that microsatellite instability in sporadic colon cancer is associated with an improved prognosis at the population level.

Introduction

Microsatellite instability is seen in nearly all colon cancers from individuals with HNPCC³ (1, 2). The genetic basis for insta-

bility in these tumors is inherited germ-line mutations of mismatch repair genes, mostly involving *hMSH2* and *hMLH1* (3). Microsatellite instability is also seen in 10–15% of sporadic colon cancers; in these tumors the basis for instability is usually acquired hypermethylation of the *hMLH1* promoter with subsequent transcriptional silencing (4–8).

Previous studies (1, 9) have indicated an improved prognosis for HNPCC-associated colon cancer relative to nonfamilial colon cancer. Some studies have also reported a statistically significant improved prognosis for sporadic colon cancer with microsatellite instability (10–19), although others have not (20–23). In addition, studies reporting an improved prognosis have not always shown that this relationship was independent of tumor stage, and only one of those studies was population-based. The lone population-based study was, moreover, restricted to individuals ≤ 50 years of age (17). Because such relatively young individuals account for <10% of those with colon cancer (24), it is questionable whether the results of that study can be extrapolated to the population at large. Also, whereas that study of 607 individuals was relatively large, it had insufficient power to demonstrate a statistically significant improved survival at a given tumor stage (25), again limiting the clinical applicability of its findings (26). Therefore, we evaluated the relationship between microsatellite instability and prognosis, tumor stage, and other clinical variables in a population-based study of 1026 individuals with colon cancer, the largest sample evaluated in this manner to date. The age range of subjects (30–79 years) includes the most frequent ages of colon cancer diagnosis, additionally ensuring the applicability of results to the general population.

Materials and Methods

Study Population. Study participants were black, white, or Hispanic and were from either the Kaiser Permanente Medical Care Program of Northern California or an eight-county area in Utah (Davis, Salt Lake, Utah, Weber, Wasatch, Tooele, Morgan, and Summit counties). Microsatellite instability results from 154 individuals in the Utah sample were reported in a previous study of microsatellite instability and family history (27). In the Utah and Kaiser Permanente Medical Care Program we were able to extract normal and tumor DNA for 95.6 and 81.1%, respectively, of all of the cases diagnosed in the area, making this a truly population-based study from these geographic locations. Eligibility criteria for cases included diagnosis with first-primary incident colon cancer (International Classification of Diseases for Oncology 2nd edition codes 18.0, and 18.2–18.9) between October 1, 1991 and September 30, 1994; ages 30–79 years at time of diagnosis; and mentally competent to participate in the study. Cases with cancers of the rectosigmoid junction or rectum (defined as the first 15 cm from the anal opening) or with known familial adenomatous polyposis,

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³ The abbreviations used are: ; HNPCC, hereditary nonpolyposis colon cancer;

AJCC, American Joint Committee on Cancer; SEER, Surveillance, Epidemiology, and End Results; *TGFBRII*, transforming growth factor-beta receptor type II.

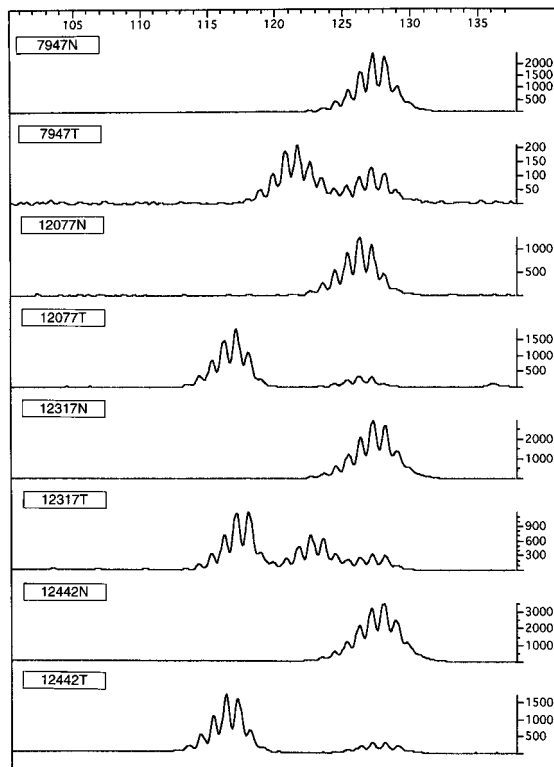


Fig. 1. Paired normal (N) and tumor (T) results for four tumors with BAT-26 microsatellite instability (*TGF β RII* results for the same tumors are shown in Fig. 2). In each tumor one or more BAT-26 alleles smaller than those seen in normal DNA are present (size in bp, scale above each repeat result; signal amplitude, scales at right).

ulcerative colitis, or Crohn's disease were not eligible. The study sample was part of a larger epidemiological-based study which excluded rectal cancers; however, microsatellite instability is mostly seen in proximal tumors (10). All of the cases were adenocarcinomas or carcinomas.

Information on age at time of diagnosis, sex, tumor site, and tumor stage were available from the Northern California Tumor Registry, the Sacramento Tumor Registry, and the Utah Cancer Registry. These registries are members of the SEER program. Proximal tumors were defined as cecum through transverse colon; tumors in the splenic flexure, descending, and sigmoid colon were defined as distal. Staging data were summarized as local, regional, or distant depending on extent of disease and node and other organ involvement as indicated by the SEER summary stage codes provided by the respective tumor registries (28). We also staged the tumors according to AJCC criteria and determined histological grade by reviewing pathology reports (29). Because we did not have access to complete medical records, AJCC stage IV tumors were identified using SEER summary stage codes to determine whether or not distant metastases were present. Vital status, date of death, primary cause of death, and two contributing causes of death were obtained from local tumor registries using death certificate information. Active follow-up of people diagnosed with cancer is done through the cancer registries on a continuous basis. Vital status as of December 30, 1999 was obtained for all of the study participants. For individuals of which the vital status or cause of death could not be determined through local tumor registries, National Death Index tapes were used. Months of

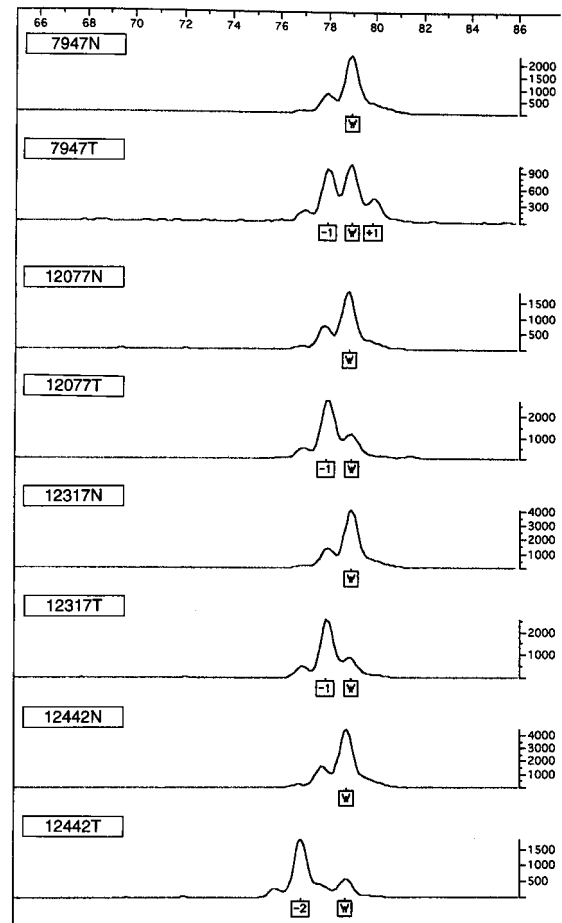


Fig. 2. Paired normal (N) and tumor (T) results for four tumors with *TGF β RII* instability (BAT-26 results for the same tumors are shown in Fig. 1). Deletions of one (-1) or two (-2) bp and/or insertions of one bp (+1) relative to wild type (W) are seen in each tumor (size in bp, scale above each repeat result; signal amplitude, scales at right).

survival were calculated by subtracting the date of last contact or death from the date of diagnosis. Deaths from any cause as well as deaths attributed to colon cancer were assessed. All of the aspects of this study were approved by the University of Utah Institutional Review Board.

Microsatellite Instability. Colon cancer tissue was microdissected and DNA extracted from formalin-fixed paraffin-embedded tissue blocks as described previously (30). The respective normal DNA from each individual was extracted from peripheral blood or from paraffin blocks of normal colonic mucosa. Each tumor was evaluated for microsatellite instability with the noncoding mononucleotide repeat BAT-26 and the coding mononucleotide repeat in codons 125 to 128 of *TGF β RII* (31). The primer sequences and PCR conditions were as described previously (32, 33). As before, primers were labeled with fluorescent dye, and the sizes of PCR products were evaluated on an ABI machine. Also, a 5' tail (GTTTCT) was added to the reverse primer to complete the addition of an extra adenosine to PCR products (34).

Both tumoral DNA and normal DNA were PCR amplified with the above primer sets. Microsatellite instability for a given primer set was defined as the appearance of one or more new PCR products either smaller or larger than those produced from normal DNA. For BAT-26 a deletion of at least 4 bp was

Table 1 Description of population by microsatellite instability status

	BAT-26			TGFβRII		
	BAT-26 S ^a n (%)	BAT-26 U ^b n (%)	OR (95% CI) ^c	TGFβRII S ^a n (%)	TGFβRII U ^b n (%)	OR (95% CI) ^c
Age						
<55	165 (85.0)	29 (15.0)	1.00	175 (87.9)	24 (12.1)	1.00
55–64	291 (91.8)	26 (8.2)	0.42 (0.22–0.80)	297 (92.8)	23 (7.2)	0.47 (0.24–0.92)
65–70	279 (89.7)	32 (10.3)	0.80 (0.45–1.44)	285 (91.0)	28 (9.0)	1.01 (0.55–1.84)
71–79	477 (83.1)	97 (16.9)	1.24 (0.73–2.10)	499 (85.0)	88 (15.0)	1.20 (0.69–2.10)
<i>P</i>		<0.01			<0.01	
Sex						
Male	651 (90.0)	72 (10.0)	1.00	672 (91.2)	65 (8.8)	1.00
Female	561 (83.4)	112 (16.6)	1.75 (1.21–2.55)	584 (85.6)	98 (14.4)	1.90 (1.28–2.82)
<i>P</i>		<0.01			<0.01	
Tumor site						
Proximal	528 (76.9)	159 (23.1)	1.00	556 (79.8)	141 (20.2)	1.00
Distal	649 (96.7)	22 (3.3)	0.12 (0.07–0.20)	664 (97.1)	20 (2.9)	0.14 (0.08–0.24)
<i>P</i>		<0.01			<0.01	
SEER summary stage						
Local	377 (84.9)	67 (15.1)	1.00	403 (88.2)	54 (11.8)	1.00
Regional	573 (85.3)	99 (14.7)	0.76 (0.52–1.13)	595 (86.1)	96 (13.9)	0.94 (0.62–1.43)
Distant	240 (94.5)	14 (5.5)	0.30 (0.13–0.67)	239 (96.4)	9 (3.6)	0.47 (0.21–1.02)
<i>P</i>		<0.01			<0.01	
AJCC stage						
1	269 (85.9)	44 (14.1)	1.00	276 (88.5)	36 (11.5)	1.00
2	320 (82.5)	68 (17.5)	1.30 (0.86–1.96)	340 (84.4)	63 (15.6)	1.42 (0.92–2.20)
3	338 (86.5)	53 (13.5)	0.96 (0.62–1.48)	353 (87.4)	51 (12.6)	1.11 (0.70–1.75)
4	240 (94.5)	14 (5.5)	0.36 (0.19–0.67)	239 (96.4)	9 (3.6)	0.29 (0.14–0.61)
<i>P</i>		<0.01			<0.01	
Differentiation						
Well	124 (91.9)	11 (8.1)	1.00	131 (95.6)	6 (4.4)	1.00
Moderate	827 (89.4)	98 (10.6)	1.34 (0.70–2.56)	845 (90.3)	91 (9.7)	2.35 (1.01–5.49)
Poor	172 (72.6)	65 (27.4)	4.26 (2.16–8.41)	188 (75.8)	60 (24.2)	6.97 (2.92–16.6)
<i>P</i>		<0.01			<0.01	

^a S, microsatellite stable tumor.

^b U, microsatellite unstable tumor.

^c OR, odds ratio and 95% CI (confidence intervals).

required for a designation of instability (35). Instability in *TGFβRII* was indicated by deletions of one or two bases or an insertion of one base (33).

Most of the microsatellite evaluation in this study was performed before the publication of the Bethesda consensus panel (36). However, instability in BAT-26, a component of the consensus panel, has been shown to be highly correlated with generalized dinucleotide repeat instability (35) and with the Bethesda panel itself in the Utah subset of the current study (37).

Statistical Analyses. Differences in microsatellite instability were determined for age, tumor site, tumor grade, gender, and disease stage using χ^2 statistics. Crude survival was evaluated using Kaplan-Meier survival curves for both overall mortality and mortality from colon cancer alone. Associations between survival and microsatellite instability were determined using Cox proportional hazards models, adjusting for age at time of diagnosis, disease stage, tumor grade, and site. Using these multivariate models, we estimated the likelihood of dying from any cause as well as from colon cancer alone. Median follow-up was 62 months. Five-year survival (Table 2) was determined for individuals who had complete 5-year follow-up data. Of the total sample, 89 people were censored alive before 5 years of follow-up and were excluded from the analyses shown in Table 2.

Results

Microsatellite instability was identified in 12.8% (128/1000) of colon cancers with BAT-26 and 11.6% (114/986) with *TGFβRII*. Representative electropherograms demonstrating al-

terations in these repeats in four unstable tumors are shown in Figs. 1 and 2. Instability in the coding mononucleotide repeat of *TGFβRII* consisted of one or two bp deletions (–1 and –2) or one bp insertion (+1).

The relationship of microsatellite instability to age, sex, tumor location, tumor grade (differentiation), and stage is delineated in Table 1. Instability was significantly related to age at diagnosis, because it was more common in the relatively young (<55) and older (71–79) ages. Microsatellite instability also was significantly more common in tumors from women, proximal tumors, in tumors of relatively low stage, and in poorly differentiated tumors. All of these statistically significant relationships were seen with both measures (BAT-26 and *TGFβRII*) of instability.

Univariate relationships between microsatellite instability, clinical variables, and survival are indicated in Table 2. Microsatellite instability, low tumor stage, and distal tumor location were all significantly related to improved 5-year survival, whereas poor differentiation was associated with a decreased 5-year survival; there was a borderline significant relationship between age and survival. The statistically significant ($P < 0.01$) relationship between microsatellite instability and prognosis was seen with both measures of instability.

The relationship between microsatellite instability and prognosis is additionally delineated in the results of a multivariate analysis shown in Table 3. Individuals with microsatellite unstable tumors were significantly less likely to die of any cause or of colon cancer in the first 8 years after diagnosis; this relationship was independent of age, stage, tumor site, tumor

Table 2 Univariate relationships with survival

	<i>P</i> ^a	Status at end of follow-up		
		Dead <i>n</i> (%)	Alive <i>n</i> (%)	5-yr survival (% alive)
Age at time of diagnosis				
≤55		93 (44.7)	115 (55.3)	54.8
56–64		133 (39.8)	201 (60.2)	60.2
65–70		137 (41.6)	192 (58.4)	61.4
71–79		317 (51.5)	298 (48.5)	52.9
<i>P</i>	0.05			
Sex				
Male		364 (47.2)	407 (52.8)	55.8
Female		316 (44.2)	399 (55.8)	57.6
<i>P</i>	0.48			
Tumor site				
Proximal		353 (48.3)	378 (51.7)	54.0
Distal		306 (42.9)	408 (57.1)	60.2
<i>P</i>	0.02			
SEER summary stage				
Local		115 (24.1)	363 (75.9)	79.5
Regional		309 (43.2)	407 (56.8)	60.5
Distant		247 (93.2)	18 (6.8)	7.3
<i>P</i>	<0.01			
AJCC stage				
1		67 (20.4)	261 (79.6)	83.6
2		138 (33.0)	280 (67.0)	71.1
3		210 (50.0)	210 (50.0)	53.4
4		247 (93.2)	18 (6.8)	7.3
<i>P</i>	<0.01			
Differentiation				
Well		39 (27.3)	104 (72.7)	77.6
Moderate		446 (45.5)	534 (54.5)	56.8
Poor		146 (56.6)	112 (43.4)	45.7
<i>P</i>	<0.01			
Microsatellite instability status				
BAT-26 stable		580 (47.9)	632 (52.1)	54.3
BAT-26 unstable		64 (34.8)	120 (65.2)	70.5
<i>P</i>	<0.01			
<i>TGFβRII</i> stable		593 (47.2)	663 (52.8)	54.7
<i>TGFβRII</i> unstable		54 (33.1)	109 (66.9)	72.4
<i>P</i>	<0.01			

^a *P* is χ^2 statistic for differences in 5-yr survival.

Table 3 Hazard rate ratios (HRR) and 95% confidence intervals (CI) for likelihood of dying during follow-up comparing unstable to stable tumors (as classified by BAT-26 or *TGFβRII*)

	Age-adjusted Unstable vs. stable HRR (95% CI)	Age, AJCC stage, grade, and tumor site- adjusted unstable vs. stable HRR (95% CI)
All-cause mortality		
BAT-26	0.63 (0.49–0.82)	0.61 (0.45–0.86)
<i>TGFβRII</i>	0.59 (0.45–0.78)	0.53 (0.39–0.82)
Colon cancer mortality		
BAT-26	0.46 (0.31–0.68)	0.43 (0.28–0.69)
<i>TGFβRII</i>	0.44 (0.30–0.67)	0.36 (0.22–0.46)

grade, and the method used for assessing instability. The age, stage, grade, and site-adjusted reduction in risk of colon cancer death associated with microsatellite instability was ~60%.

The relationship between microsatellite instability and prognosis for each stage of colon cancer is shown in Table 4. Most of the decreased risk of colon cancer death is seen in AJCC stage III tumors and SEER regional stage, with risk reductions in stage III tumors of approximately 60–66% de-

pending on the microsatellite measurement. Interestingly, a statistically significant nearly 60% reduction in risk of colon cancer death is seen in tumors with distant metastases that harbor *TGFβRII* mutations.

The relationship between *TGFβRII* mutations and prognosis is also demonstrated in Kaplan-Meier survival curves stratified by AJCC stage (Figs. 3 and 4). In Fig. 3, all of the causes of mortality are taken into account, whereas in Fig. 4 only mortality from colon cancer is considered. Microsatellite instability in *TGFβRII* does not appear to be related to colon cancer survival in stage I or II tumors. *TGFβRII* mutations are associated with a significantly improved prognosis in stage III and IV tumors ($P < 0.01$; Mantel Cox statistic); interestingly, the survival curve for stage III unstable tumors is very similar to that for stage II stable tumors for colon cancer mortality (Fig. 4) and to stage I and II tumors for overall mortality (Fig. 3).

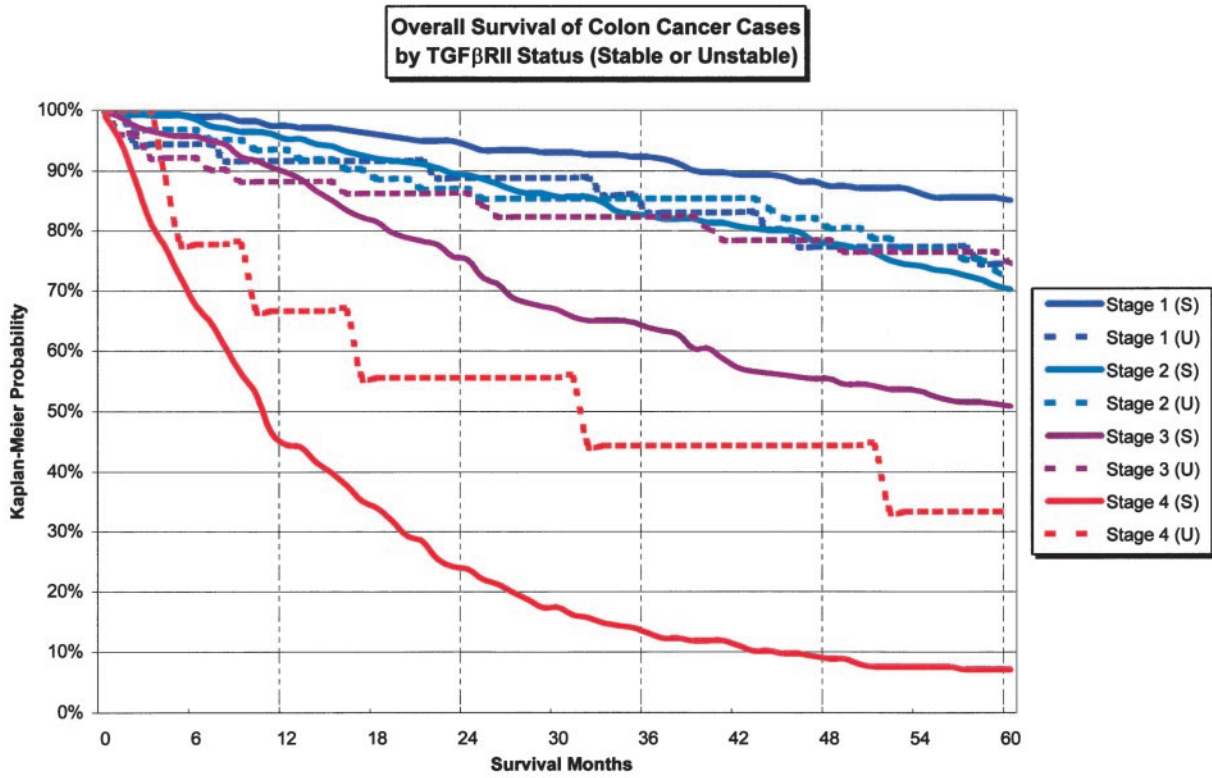
Discussion

This population-based study of >1000 individuals with sporadic colon cancer identified significant relationships between microsatellite instability and proximal tumor location, female gender, young and old age at diagnosis, poor histological differentiation, and low tumor stage. The relationships to proximal tumor site (10, 38, 39) and poor histological differentiation (15, 17, 38, 39) have been seen by numerous previous studies. The relationship to female gender was seen in two previous studies (8, 18), and one of those studies (8) as well as an additional study (17) reported a relationship with low tumor stage. The relationship to age was seen in a previous smaller study (27), which included some of the individuals in the current analysis, and a relationship to young age at diagnosis was seen in two other studies (19, 23). Several other studies (10, 12, 13, 20, 21, 39) have failed to identify significant relationships between microsatellite instability and age, gender, and tumor stage, but most of these studies were of relatively small numbers of tumors and may have lacked sufficient power to detect statistically significant relationships. Notably, most of the studies in addition to ours which did show relationships to gender and stage evaluated >500 individuals with colon cancer.

In the current study, microsatellite instability, low tumor stage, low tumor grade, and distal tumor location were all significantly related to improved prognosis in univariate analyses (Table 2). A multivariate analysis (Table 3) revealed that the relationship between instability and improved prognosis was independent of stage, site, tumor grade, and age and was associated with a 60% decrease in death attributable to colon cancer. Several previous studies (10–19) have reported a relationship between instability and prognosis, although others (20–23) have not. Studies that failed to show this association were usually of relatively small numbers of individuals and, therefore, evaluated very few unstable tumors and probably lacked the power to show a statistically significant relationship. Of the studies that did show a relationship between instability and improved prognosis, in only five was this association shown to be independent of tumor stage (12, 15–18), an important consideration given the relationship we and others have observed between instability and low tumor stage. Of these five studies, only one was population-based, and that study was restricted to individuals ≤50 years of age (17), and, therefore, would be predicted to account for <10% of all colon cancer (24). Thus, our current study, the largest to date, is the first population-based study of sporadic colon cancer that includes the most frequent ages at diagnosis and demonstrates a stage-independent improved prognosis associated with microsatellite instability. These features indicate that our findings, in contrast to those of

Table 4 Stage-specific age-adjusted hazard rate ratios (HRR) and 95% confidence intervals for risk of dying of colon cancer comparing microsatellite unstable to stable tumors (as classified by BAT-26 or *TGFβRII*)

	HRR (95% CI)	HRR (95% CI)	HRR (95% CI)	
SEER stage	Local	Regional	Distant	
BAT-26	0.65 (0.20–2.16)	0.46 (0.26–0.79)	0.79 (0.41–1.56)	
<i>TGFβRII</i>	1.09 (0.32–2.63)	0.50 (0.29–0.84)	0.42 (0.18–0.94)	
AJCC stage	1	2	3	4
BAT-26	0.59 (0.81–4.63)	0.79 (0.39–1.59)	0.40 (0.20–0.79)	0.79 (0.41–1.56)
<i>TGFβRII</i>	1.47 (0.32–6.67)	0.84 (0.41–1.70)	0.34 (0.17–0.71)	0.42 (0.18–0.94)



# at Risk	0	6	12	18	24	30	36	42	48	54	60
Stage 1 U	36	33	31	30	27	25					
Stage 1 S	276	267	256	248	234	217					
Stage 2 U	62	58	53	52	50	42					
Stage 2 S	340	324	298	268	249	211					
Stage 3 U	51	45	44	42	40	39					
Stage 3 S	353	317	263	224	188	163					
Stage 4 U	9	6	5	4	4	3					
Stage 4 S	239	111	57	33	21	15					

Fig. 3. Kaplan-Meier survival curves stratified by colon cancer stage and microsatellite instability (as determined by *TGFβRII*). Microsatellite instability was associated with a significantly improved survival in both stage III and stage IV tumors. (Fig. 3 takes into account all causes of mortality; Figure 4 considers mortality from color cancer alone. S, stable; U, unstable.

most previous studies, are directly applicable and clinically relevant to the population at large.

Most of the reduction of risk of colon cancer death associated with microsatellite instability occurred in AJCC stage III tumors (Table 4). This is similar to a previous report, which identified an improved prognosis in Dukes' C tumors with microsatellite instability but not in Dukes' B tumors (15). The only previous popu-

lation-based study (17) demonstrated a survival benefit of microsatellite instability, which was independent of tumor stage in a multivariate analysis but had insufficient power to show a statistically significant survival advantage at a particular stage (25).

The improved prognosis in stage III tumors, cancers with lymph node metastases, was quite dramatic in our study, because microsatellite instability essentially "downstaged" such tumors to

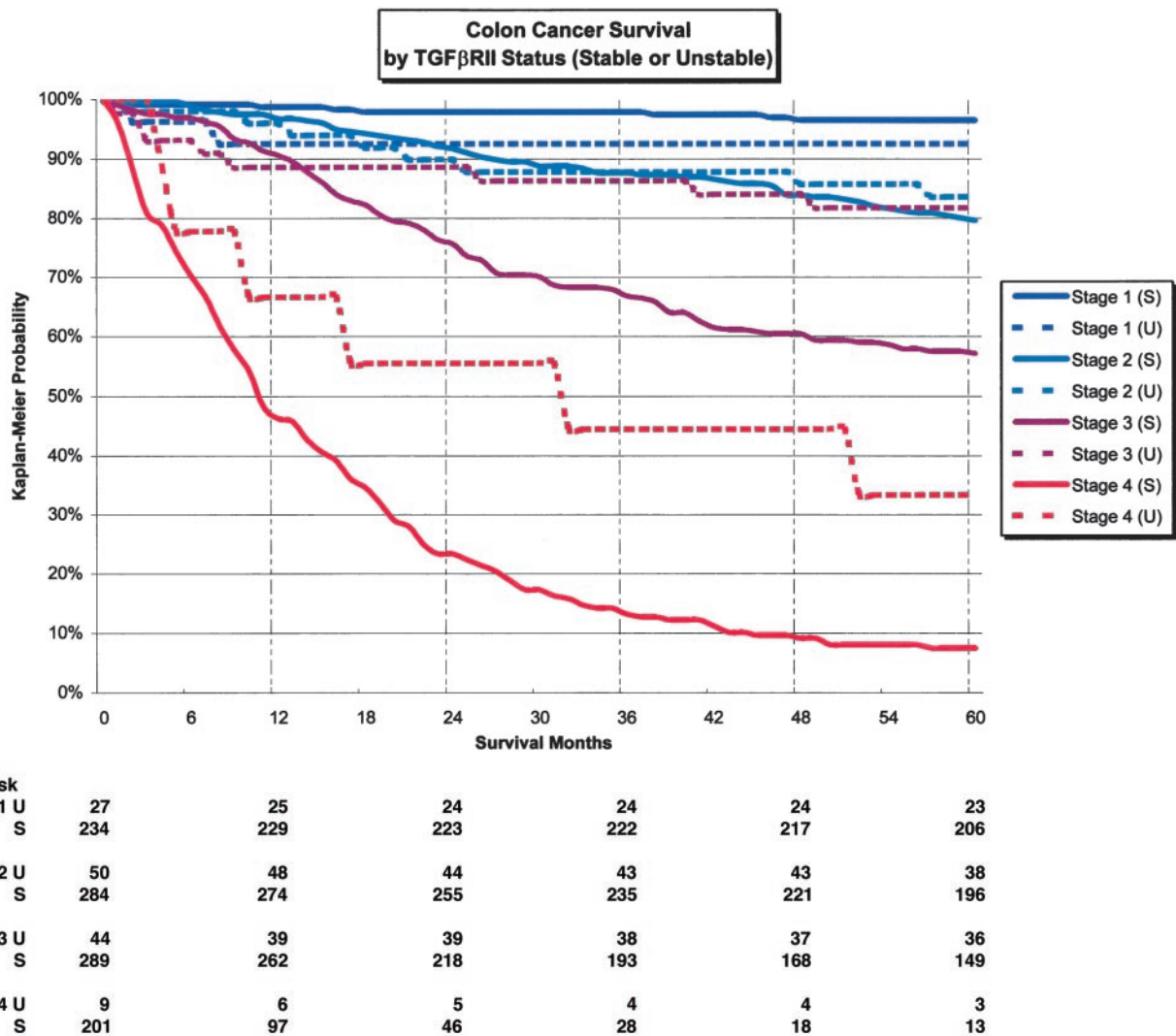


Fig. 4. Kaplan-Meier survival curves stratified by colon cancer stage and microsatellite instability (as determined by *TGF β R2*). Microsatellite instability was associated with a significantly improved survival in both stage III and stage IV tumors. (Fig. 3 takes into account all causes of mortality; Figure 4 considers mortality from color cancer alone. S, stable; U, unstable.

the colon cancer mortality of stage II tumors, cancers without nodal metastases (Fig. 4). An improved prognosis was also seen in stage IV tumors with *TGF β R2* mutations (Table 4; Figs. 3 and 4), although the significance of this latter finding is somewhat uncertain because of the small numbers of individuals in this category. Still, it is of interest that 33% (3 of 9) of individuals with stage IV, *TGF β R2* unstable tumors survived colon cancer, compared with only 6.3% (15 of 239) of individuals with stage IV, *TGF β R2* stable tumors. Additional studies with larger numbers of individuals with unstable stage IV tumors will be necessary to determine the generalizability of this finding.

Besides its utility as an indicator of prognosis, microsatellite instability could also be clinically important if it could be used to predict the response to therapy. One retrospective, nonrandomized study suggested that the survival benefit associated with microsatellite instability was only seen in individuals who received chemotherapy (18), whereas another such study found no relationship between instability and chemotherapeutic response (15). A third study (40) reported that chemotherapy was efficacious in

individuals with unstable tumors, but nearly half of those patients had HNPCC, and, therefore, those results are of questionable relevance for the majority of individuals of which the unstable tumors are not attributable to an inherited predisposition (4–8). Information regarding chemotherapy or radiation therapy was not readily accessible on the individuals in our study, although from the time period of colon cancer diagnosis (October 1, 1991 to September 30, 1994), one would expect that most individuals with stage III disease received adjuvant chemotherapy. There is also some experimental evidence that tumors with microsatellite instability show different degrees of responsiveness to different classes of chemotherapeutic agents (41). It is possible that future prospective, randomized studies that stratify patients according to tumor instability may identify a treatment regimen specifically suited to unstable tumors and/or to what extent the improved prognosis in unstable tumors is related to a better response to chemotherapy.

The microsatellite instability analysis in this study was performed before the National Cancer Institute workshop, which developed the Bethesda Consensus Panel (36). Our re-

sults with BAT-26, one of the consensus panel repeats, should be a fairly good indicator of what would have been seen with the entire panel, because we have shown that instability in BAT-26 correlates extremely well with the complete consensus panel in the Utah subset of these colon cancers (37). It should be noted that the National Cancer Institute workshop on instability did not mandate the use of a particular panel and acknowledged that in different situations panels other than the Bethesda Consensus Panel might be more appropriate (36). There are some advantages to the approach used in this study. Determination of BAT-26 and/or *TGF β RII* instability is an extremely rapid and inexpensive way to screen large numbers of samples. In addition, the strongest relationships in this study were seen with *TGF β RII*, a coding mononucleotide repeat that is not one of the five repeats in the main Bethesda consensus panel. It is possible that the most prognostically significant microsatellite instability panel is yet to be determined.

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