VOLUME 30 · NUMBER 4 · FEBRUARY 1 2012

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Association of Obesity With DNA Mismatch Repair Status and Clinical Outcome in Patients With Stage II or III Colon Carcinoma Participating in NCCTG and NSABP Adjuvant Chemotherapy Trials

Frank A. Sinicrope, Nathan R. Foster, Harry H. Yoon, Thomas C. Smyrk, George P. Kim, Carmen J. Allegra, Greg Yothers, Daniel A. Nikcevich, and Daniel J. Sargent

Frank A. Sinicrope, Nathan R. Foster, Harry H. Yoon, Thomas C. Smyrk, Daniel A. Nikcevich, and Daniel J. Sargent, North Central Cancer Treatment Group, Mayo Clinic, Rochester; Daniel A. Nikcevich, Duluth Community Clinical Oncology Program, Essentia Health, Duluth, MN; George P. Kim, Mayo Clinic, Jacksonville; Carmen J. Allegra, University of Florida, Gainesville, FL; Greg Yothers, University of Pittsburgh Graduate School of Public Health; and Carmen J. Allegra and Greg Yothers, National Surgical Adjuvant Breast and Bowel Project, Pittsburgh, PA.

Submitted September 13, 2011; accepted November 2, 2011; published online ahead of print at www.jco.org on December 27, 2011.

Supported, in part, by Grant No. K05CA-142885 (Senior Scientist Award to F.A.S.) from the National Cancer Institute, National Institutes of Health.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Clinical Trials repository link available on JCO.org.

Corresponding author: Frank A. Sinicrope, MD, FACP, Mayo Clinic and Mayo Cancer Center, 200 1st St SW, Rochester, MN 55905; e-mail: Sinicrope.frank@mayo.edu.

© 2011 by American Society of Clinical Oncology

0732-183X/12/3004-406/$20.00

DOI: 10.1200/JCO.2011.39.2563

ABSTRACT

Purpose
Although the importance of obesity in colon cancer risk and outcome is recognized, the association of body mass index (BMI) with DNA mismatch repair (MMR) status is unknown.

Patients and Methods
BMI (kg/m^2) was determined in patients with TNM stage II or III colon carcinomas (n = 2,693) who participated in randomized trials of adjuvant chemotherapy. The association of BMI with MMR status and survival was analyzed by logistic regression and Cox models, respectively.

Results
Overall, 427 (16%) tumors showed deficient MMR (dMMR), and 630 patients (23%) were obese (BMI ≥ 30 kg/m^2). Obesity was significantly associated with younger age ($P = .021$), distal tumor site ($P = .012$), and a lower rate of dMMR tumors (10% *v* 17%; $P < .001$) compared with normal weight. Obesity remained associated with lower rates of dMMR (odds ratio, 0.57; 95% CI, 0.41 to 0.79; $P < .001$) after adjusting for tumor site, stage, sex, and age. Among obese patients, rates of dMMR were lower in men compared with women (8% *v* 13%; $P = .041$). Obesity was associated with higher recurrence rates ($P = .0034$) and independently predicted worse disease-free survival (DFS; hazard ratio [HR], 1.37; 95% CI, 1.14 to 1.64; $P = .0010$) and overall survival (OS), whereas dMMR predicted better DFS (HR, 0.59; 95% CI, 0.47 to 0.74; $P < .001$) and OS. The favorable prognosis of dMMR was maintained in obese patients.

Conclusion
Colon cancers from obese patients are less likely to show dMMR, suggesting obesity-related differences in the pathogenesis of colon cancer. Although obesity was independently associated with adverse outcome, the favorable prognostic impact of dMMR was maintained among obese patients.

J Clin Oncol 30:406-412. © 2011 by American Society of Clinical Oncology

INTRODUCTION

Recent data suggest that molecular markers, including DNA mismatch repair (MMR) status, can be used to classify colorectal cancers (CRCs) into distinct subtypes, which has implications for etiology, prognosis, and treatment. MMR is involved in the etiology of CRC with approximately 10% to 20% of tumors[1] developing due to deficient function of this system, which gives rise to microsatellite instability (MSI).[2] Among tumors with deficient MMR (dMMR), approximately two thirds arise sporadically as a consequence of epigenetic inactivation of the *MLH1* MMR gene[1,3] and the other nearly one third carry a germline *MMR* gene mutation (*MLH1*, *MSH2*, *MSH6*, *PMS2*) that confers Lynch syndrome.[4] Tumors showing dMMR have a distinct phenotype that includes proximal site, poor differentiation, diploid DNA content, and abundant tumor infiltrating lymphocytes.[2,5-8] Evidence indicates that MMR status confers prognostic information in patients with colon cancer[6,9-13] and may also influence response to fluorouracil (FU) -based adjuvant chemotherapy.[9,14-16] To date, however, only limited data exist regarding the association of MMR status with body mass index (BMI)[17] or lifestyle factors[18,19] in patients with colon cancer.

Obesity is an established risk factor for developing colon cancer, with the risk being stronger in men versus women,[20-25] and may be associated with increased

mortality related to this malignancy.[26-28] Obesity is associated with increased circulating levels of estrogens in both men and women,[29] and evidence suggests that estrogen exposure may protect against the development of dMMR tumors.[30] Rates of obesity have increased two-fold in adults and three-fold in children in the past 30 years in the United States and are also rising in other parts of the developed world.[31] Data from the National Health and Nutrition Examination Survey indicate that approximately 34% of US adults—almost 73 million people—are obese, defined by the WHO[32] as having a BMI of $\geq$ 30 kg/m^2.[33] In an effort to distinguish the impact of obesity from MMR status, which have both been shown to influence patient survival, we determined their association and compared patient outcomes in a large cohort of patients with stage II or III colon cancer who participated in phase III clinical trials of adjuvant chemotherapy. Determining the association of obesity with MMR status may also provide insight into the molecular pathogenesis of colon cancer and may identify a modifiable factor associated with the MMR phenotype.

PATIENTS AND METHODS

The study population consisted of patients (N = 2,693) with pathologically confirmed TNM stage II (n = 714) or III (n = 1,979) colonic adenocarcinomas who were participants in nine phase III randomized trials of adjuvant chemotherapy. Studies included those conducted by the Mayo Clinic/North Central Cancer Treatment Group (NCCTG) that evaluated FU with levamisole or leucovorin (LV) versus surgery alone (NCCTG 78-48-52[34,35] and NCCTG 84-46-52/Intergroup 0035 [INT 0035][36,37]). Other studies evaluated combinations of FU with levamisole and/or LV (NCCTG 89-46-51[38]), FU with LV and interferon-gamma with or without levamisole (NCCTG 87-46-51[39]), and FU plus LV with high versus standard dose levamisole (NCCTG 91-46-53[40]). We also included studies conducted by the National Surgical Adjuvant Breast and Bowel Project (NSABP) that randomly assigned patients to portal venous FU versus surgery alone (NSABP C-02); FU, vincristine, and semustine versus FU plus LV (NSABP C-03); FU plus LV versus FU plus LV plus levamisole (NSABP C-04[41]); and FU plus LV and oxaliplatin versus FU plus LV (NSABP C-07[42]). Within the study population, 1,759 received FU-based chemotherapy, and the remaining patients received non–FU-based treatment (n = 774) or surgery alone (n = 160).

Patient data were obtained from the cooperative group databases. Only patients with available tissue specimens were included. Tumor site was categorized as proximal when cancers were located above the splenic flexure and all other tumor sites were categorized as distal. Study patients were required to have a baseline Eastern Cooperative Oncology Group (ECOG) performance score of 0 to 2.[43] Written informed consent was obtained from all study participants, and protocols were approved by the institutional review boards at the respective study sites. This study was conducted under an active institutional review board–approved protocol.

Assessment of Patient BMI

By using patient height and weight data obtained and recorded at study entry, trained personnel calculated BMI by taking the body weight in kilograms divided by height in meters squared. These measurements were also used to calculate chemotherapy dosages. BMI was categorized as underweight (BMI, $<$ 20 kg/m^2), normal weight (BMI, 20.1 to 24.9 kg/m^2), overweight (BMI, 25 to 29.9 kg/m^2), or obese (BMI, $\geq$ 30 kg/m^2) according to WHO criteria.[32]

Assessment of Tumor MMR Status

MMR status was determined by analysis of MSI and/or MMR protein expression by immunohistochemistry (IHC). dMMR was defined as MSI high frequency (MSI-H) or loss of expression of an MMR protein by IHC. Proficient MMR (pMMR) was defined as MSI low frequency (MSI-L) or microsatellite stable (MSS) or as intact expression of all MMR proteins evaluated.

MSI testing. MSI was analyzed by polymerase chain reaction amplification of microsatellite loci in microdissected, tumor-enriched paraffin-embedded tissue. Specimens from NCCTG studies were screened by using four to 11 microsatellite markers, as previously described.[11,44] In NCCTG 91-46-53, MMR status was determined by analysis of instability at BAT26 coupled with MLH1, MSH2, and MSH6 protein expression.[40] Within NSABP studies, MSI was analyzed by using the marker panel recommended by the National Cancer Institute (BAT25, BAT26, D5S346, D2S123, and D17S250)[45] and the transforming growth factor-beta type II receptor locus.[41] Tumors were classified as MSI-H if $\geq$ 30% of the markers demonstrated instability, as MSI-L if more than 0% and less than 30% showed MSI, and as MSS if none of the markers exhibited MSI.[45]

IHC analysis of MMR proteins. Paraffin-embedded tumor sections were analyzed for MLH1 and MSH2 proteins, as previously described.[46] Staining was performed by using primary monoclonal antibodies: mouse antihuman MLH1 (clone G168-728, 1:250; BD Pharmingen, San Diego, CA) and mouse antihuman MSH2 (clone FE11, 1:50; Oncogene Research Products, Cambridge, MA). MSH6 was analyzed by using a mouse antihuman MSH6 monoclonal antibody (clone 44; Transduction Laboratories, Lexington, KY) in tumors (n = 387) from one study (NCCTG 91-46-53). Loss of an MMR protein was defined as absence of nuclear staining of tumor cells in the presence of positive nuclear staining in normal colonic epithelium and lymphocytes. Each slide was assigned a unique number that enabled blinding to patient identity and clinical characteristics.

Statistical Analysis

The χ^2 or Cochran-Armitage trend test was used to measure the association between BMI and categorical clinicopathologic variables. The Kruskal-Wallis or Wilcoxon rank sum tests were used for continuous variables. Univariate and multivariate logistic regression models were used to test for the association between obesity and MMR status after stratifying by study. Odds ratios and 95% CIs were calculated. Overall survival (OS; censored at 8 years) was calculated as the number of years from random assignment to date of death or last contact. Disease-free survival (DFS; censored at 5 years) was calculated as number of years from random assignment to first of either disease recurrence or death. The association of BMI and covariates with DFS and OS were determined by using Kaplan-Meier methodology and Cox proportional hazards models.[47] Score and likelihood ratio tests were used to evaluate the significance of each covariate in univariate and multivariate models, respectively, after stratifying by treatment and study. Statistical tests were two-sided, with $P \leq .05$ considered significant. All analyses were performed by using SAS software (SAS Institute, Cary, NC).

RESULTS

Patient Characteristics by MMR Status and BMI Category

Of the 2,693 patients, 714 (26.5%) had stage II and 1,979 (73.5%) had stage III colon carcinomas that had been resected with curative intent. MMR status was determined in all patients, and dMMR was detected in 427 patients (16%) with cancer. A higher rate of dMMR was found in stage II (178 of 714; 25%) versus stage III (249 of 1,979; 13%) cancers ($P < .001$) and in women compared with men (18% *v* 14%; $P = .0037$). When age was dichotomized at 50 years as an indicator of menopausal status, a higher rate of dMMR was found in older versus younger women (20% *v* 12%; $P = .0025$).

Among all patients, 630 (23%) were obese (BMI $\geq$ 30 kg/m^2), 1,042 (39%) were overweight (BMI 25 to 29.9 kg/m^2), 879 (33%) were of normal weight (BMI 20 to 24.9 kg/m^2), and 142 (5%) were underweight (BMI $<$ 20 kg/m^2). Demographic and clinicopathologic features of the study population were stratified by BMI category

© 2011 by American Society of Clinical Oncology

Table 1. Clinicopathologic Features Stratified by BMI Category in Patients With Stage II or III Colon Carcinomas From Randomized Trials of FU-Based Adjuvant Chemotherapy

Variable	Total (N = 2,693)		Underweight (n = 142; 5.3%)		Normal (n = 879; 32.6%)		Overweight (n = 1,042; 38.7%)		Obese (n = 630; 23.4%)		Obese *v* Normal *P*	Overall *P*
	No.	%	No.	%	No.	%	No.	%	No.	%		
Stage											.2428*	.4651†
II	714	26.5	40	28.2	226	25.7	269	25.8	179	28.4		
III	1,979	73.5	102	71.8	653	74.3	773	74.2	451	71.6		
Site‡											.0117*	.0492*
Distal	1,449	54.4	83	59.3	444	51.2	560	54.5	362	57.7		
Proximal	1,214	45.6	57	40.7	424	48.8	468	45.5	265	42.3		
Sex											.1146*	< .001†
Female	1,240	46	113	79.6	445	50.6	389	37.3	293	46.5		
Male	1,453	54	29	20.4	434	49.4	653	62.7	337	53.5		
Grade§											.0418*	.0048†
1 to 2 (low)	1,768	77.8	70	68.6	550	76.4	697	77.9	451	81.1		
3 to 4 (high)	505	22.2	32	31.4	170	23.6	198	22.1	105	18.9		
PS§											.6412*	.5249*
0	2,030	81.8	103	83.7	652	81.3	802	83.4	473	79.5		
1	438	17.6	20	16.3	145	18.1	154	16	119	20		
2	14	0.6	0	0	5	.6	6	.6	3	.5		
Lymph nodes§											.3143*	.7966*
Negative	714	26.6	40	28.2	226	25.8	269	25.9	179	28.4		
1-3 positive	1,293	48.1	69	48.6	432	49.3	506	48.7	286	45.4		
> 3 positive	681	25.3	33	23.2	219	25	264	25.4	165	26.2		
T stage§											.4030*	.7150†
T1-2	299	11.1	8	5.7	103	11.7	123	11.8	65	10.4		
T3-4	2,383	88.9	132	94.3	774	88.3	915	88.2	562	89.6		
Age, years											.0206¶	< .001‖
Mean	59.4		56.1		59.5		60.4		58.4			
SD	11.20		12.64		11.74		10.68		10.69			
Median	61.0		58.0		61.0		62.0		59.5			
Range	21.0-86.2		25.0-76.0		24.0-86.2		21.0-85.0		22.0-81.0			
MMR status											< .001*	< .001†
pMMR	2,266	84.1	111	78.2	726	82.6	864	82.9	565	89.7		
dMMR	427	15.9	31	21.8	153	17.4	178	17.1	65	10.3		

Abbreviations: BMI, body mass index; dMMR, deficient mismatch repair; FU, fluorouracil; MMR, mismatch repair; pMMR, proficient MMR; PS, performance status; SD, standard deviation.
*χ-square test.
†Cochran-Armitage trend test.
‡Nonsignificant trend test *P* value = .1069.
§Missing cases.
¶Wilcoxon rank sum test.
‖Kruskal-Wallis exact test.

(Table 1). Across all BMI categories, statistically significant differences were observed for patient age, sex, tumor site, histologic grade, and MMR status (Table 1). Compared with those of normal weight, obese patients were significantly more likely to be younger and to have tumors located in the distal (*v* proximal) colon with low (*v* high) histologic grade. Obese patients had a dMMR rate of 10.3% that was significantly lower than the dMMR rates of 17.1%, 17.4%, and 21.8% in overweight, normal weight (Fig 1A), and underweight BMI categories, respectively ($P < .001$; Table 1). Moreover, obesity remained significantly associated with lower rates of dMMR (odds ratio, 0.57; 95% CI, 0.41 to 0.79; $P < .001$), after adjusting for factors known to be associated with MMR status (ie, tumor site, stage, sex, and age; Table 2).

When analyzed by patient sex, rates of dMMR tumors were lower in obese versus normal-weight men (8% *v* 17%; $P < .001$) and women (13% *v* 18; $P = .0817$; Fig 1B). Furthermore, obese men were significantly less likely to have dMMR tumors compared with obese women (8% *v* 13%; $P = .0413$; Fig 1B).

Association of BMI and MMR Status With Recurrence and Prognosis

Among obese versus normal-weight patients, the 5-year recurrence rates were 32% versus 25.3%, respectively ($P = .0034$). Time-to-recurrence (TTR) was also shorter in obese versus normal-weight patients (hazard ratio [HR], 1.34; 95% CI, 1.10 to 1.63; $P = .0034$). Obese patients had significantly worse DFS (HR, 1.35; 95% CI, 1.13 to 1.62; $P = .0011$) and OS (HR, 1.32; 95% CI, 1.10 to 1.58; $P = .0025$) rates compared with normal-weight patients (Fig 2; Appendix Table A1, online only). The adverse prognostic impact of obesity was similar in men and in women (DFS: $P_{interaction} = .9377$) but was stronger in proximal versus distal tumors (DFS: $P_{interaction} = .0483$) compared

 © 2011 by American Society of Clinical Oncology

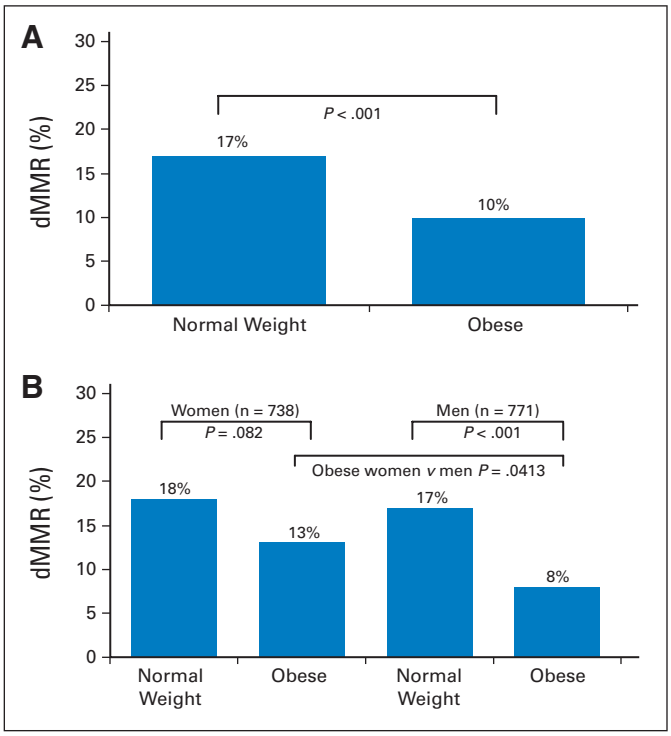

Fig 1. Percentage of resected colon carcinomas (n = 2,693) showing deficient DNA mismatch repair (dMMR; n = 427) in patients treated in randomized trials of fluorouracil-based adjuvant chemotherapy. Data are shown for (A) percentage of dMMR tumors in normal-weight (153 of 879; 17%) and obese (65 of 630; 10%) patients and (B) stratified by sex within these body mass index categories.

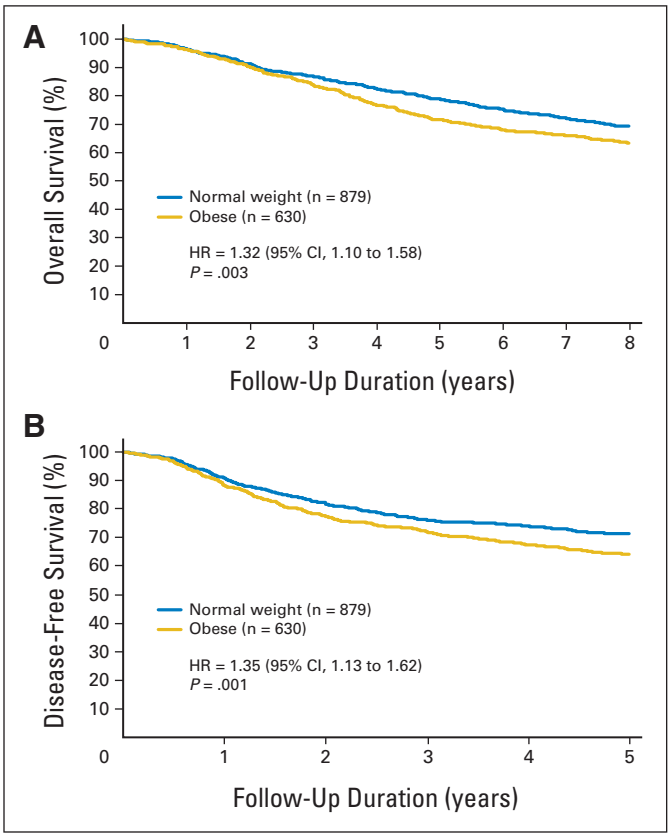

Fig 2. Prognostic impact of obesity versus normal weight status on (A) overall survival and (B) disease-free survival rates in patients with stage II and III colon carcinoma who participated in fluorouracil-based adjuvant chemotherapy trials. HR, hazard ratio.

with normal-weight patients. Specifically, obese patients (*v* normal-weight patients) with proximal cancers had significantly worse DFS (HR, 1.63; 95% CI, 1.25 to 2.14; $P < .001$), but an adverse impact was not evident in distal tumors (HR, 1.12; 95% CI, 0.87 to 1.44; $P = .3860$). Similar results were found within the obese subgroup in which proximal (*v* distal) tumors had worse outcome for DFS (HR, 1.31; 95% CI, 1.00 to 1.71; $P = .0458$) and OS (HR, 1.33; 95% CI, 1.02 to 1.74; $P = .0334$).

Survival rates among overweight and underweight patients did not differ significantly from those of normal-weight patients. Among the underweight, there was a trend toward worse DFS and OS that did not reach statistical significance, possibly because of the limited sample size (n = 142). Tumor stage (III *v* II) was associated with significantly worse DFS and OS; male sex and older age were associated with significantly worse OS (Appendix Table A1). Patients with dMMR tumors showed significantly better DFS (HR, 0.59; 95% CI, 0.47 to 0.74; $P < .001$) and OS (HR, 0.63; 95% CI, 0.51 to 0.78; $P < .001$) rates compared with patients with pMMR tumors (Appendix Table A1). The favorable prognostic impact of dMMR seen in the overall study population was maintained in obese and normal-weight subgroups ($P_{interaction} = .6560$; Fig 3).

Table 2. Effect of Obesity on DNA MMR Status in Stage II and III Colon Carcinomas After Adjusting for Covariates (n = 1,495)

Variable	OR	95% CI	*P*
Obese *v* normal weight	0.57	0.41 to 0.79	< .001
Proximal *v* distal	4.01	2.87 to 5.60	< .001
Stage III *v* stage II	0.39	0.28 to 0.55	< .001
Age (1-year increase)	0.98	0.971 to 0.997	.0195
Male *v* female	0.75	0.55 to 1.02	.0703

Abbreviations: MMR, mismatch repair; OR, odds ratio.

In a multivariable analysis, obesity was an independent prognostic factor for worse DFS (HR, 1.37; 95% CI, 1.14 to 1.64; $P = .0010$), OS (HR, 1.34; 95% CI, 1.12 to 1.61; $P = .0017$), and TTR (HR, 1.35; 95% CI, 1.11 to 1.64; $P = .0032$), even after adjusting for stage, tumor site, sex, age, and MMR status (Table 3). When stratified by tumor site, the adverse prognostic impact of obesity (*v* normal weight) was more evident in proximal versus distal tumors, yet the association weakened after adjustment for covariates, including MMR (DFS: $P_{interaction} = .0746$). We also found that dMMR status was independently associated with significantly better DFS and OS rates compared with pMMR status (Table 3). No interdependence between obesity and MMR status was found in the multivariable model (DFS: $P_{interaction} = .4576$; OS: $P_{interaction} = .8147$).

Within our study population, a predictive analysis for obesity and the effect of FU-based therapy was not appropriate, given that relatively few patients received surgery alone. In a prior study[26] that used participants in NCCTG and Southwest Oncology Group (SWOG) adjuvant studies, we failed to find a significant relationship between BMI category and treatment efficacy for FU in multivariable models.

DISCUSSION

We studied the association of BMI with the status of the DNA MMR system in patients with resected colon cancers who participated in

© 2011 by American Society of Clinical Oncology

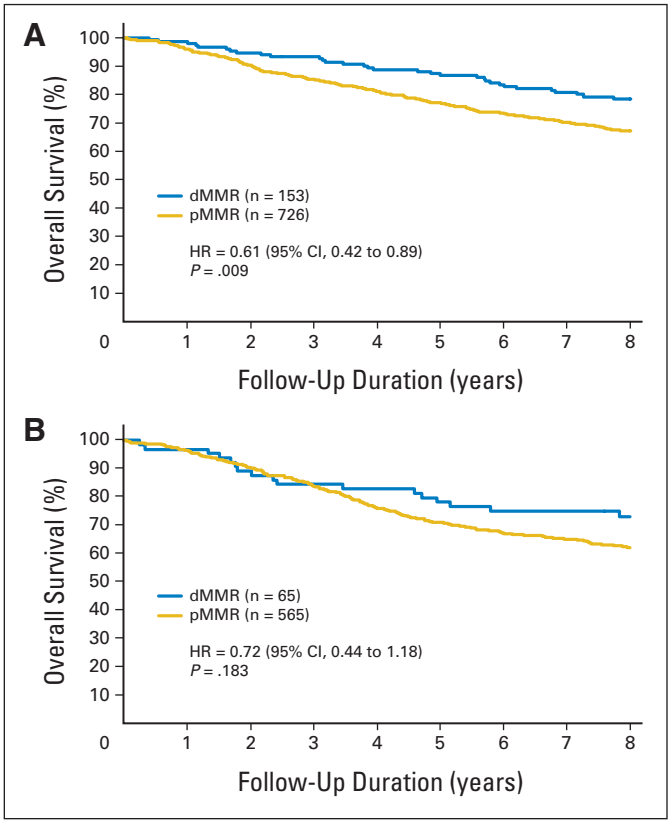

Fig 3. Prognostic impact of deficient mismatch repair (dMMR) versus proficient MMR (pMMR) status for overall survival in (A) normal-weight and (B) obese patients with stage II and III colon cancers. HR, hazard ratio.

adjuvant chemotherapy trials. Overall, obesity was associated with a significantly lower rate of dMMR compared with all other BMI categories. Specifically, obese patients had a 43% lower odds of having a dMMR tumor compared with normal-weight patients after adjusting for covariates associated with MMR status. Theses data indicate that obesity-associated colon cancers are predominantly of the pMMR molecular subtype that shows chromosomal instability[48] and exhibits more aggressive behavior compared with dMMR tumors.[6,9-13] In this regard, obesity and pMMR were each independently associated with adverse clinical outcome. Our data expand the molecular phenotype of obesity-associated colon cancers to include increased rates of pMMR. The relationship between prediagnosis BMI, MSI status, and CRC risk was examined in a case-control study in which a high BMI (self-reported) was associated with an increased risk of developing CRCs showing MSS but not MSI-H.[49] Together with our study findings, these data suggest that obesity may influence the molecular pathogenesis of colon cancer and reduce the favorable prognostic dMMR subtype.

A majority of dMMR colon cancers in our series are expected to be sporadic with inactivation of *MLH1* in association with a dense pattern of DNA methylation near gene promoter regions termed the CpG island methylator phenotype (CIMP-high).[1] In a study examining lifestyle factors and CIMP status, obesity was associated with a two-fold increased risk of having a CIMP-low tumor but did not influence CIMP-high tumors.[18] Since CIMP is a pathway that inactivates *MLH1*,[3,50] this finding is consistent with our study finding of a lower rate of dMMR in colon cancers observed among obese patients. A mechanistic link may exist between obesity and DNA methylation. Sirtuin 1 (SIRT1), a histone deacetylase, is important in epigenetic gene silencing and was shown to be overexpressed in colon cancers in association with CIMP-high status and dMMR.[51] However, SIRT1 levels decrease in obesity with the potential to reactivate silenced genes that could influence MMR status in obese patients. SIRT1 increases metabolic efficiency and may provide a link between obesity, energy balance, and cancer.[52]

Overall, we found a higher frequency of dMMR in women compared with men, especially women over the age of 50 years when this age cutoff was used as a surrogate for menopausal status. These results are consistent with data demonstrating that female sex is an independent predictor of dMMR due to *MLH1* methylation with advancing age.[1] Furthermore, data suggest that estrogen production may protect against dMMR, whereas a lack of endogenous estrogen in postmenopausal women may increase the risk of dMMR tumors.[18,49] Interestingly, we found that obese men had significantly lower rates of dMMR compared with normal-weight men, but this effect did not reach statistical significance in women. Furthermore, obese men had significantly lower rates of dMMR compared with obese women. Obesity is associated with an increased production of estrogens in both sexes that is due to extragonadal aromatization of androgens and decreased plasma levels of sex hormone–binding globulin that binds estradiol.[29,53,54] Although the mechanism underlying the lower rate of dMMR tumors in obese men is unknown and likely to be multifactorial, contributing factors include the lower overall rate of dMMR in men and increased levels of estrogen that could potentially suppress dMMR.

Obese patients with colon cancer had significantly higher recurrence rates, shorter TTR, and worse DFS and OS rates compared with normal-weight patients, even after adjusting for tumor site, stage, age, sex, and MMR status. The effect of obesity on patient survival was

Table 3. Multivariable Survival Analysis in Patients With Stage II and III Colon Carcinoma

Variable	DFS HR	95% CI	*P**	OS HR	95% CI	*P**
Obese *v* normal weight	1.37	1.14 to 1.64	.0010	1.34	1.12 to 1.61	.0017
Stage III *v* II	2.37	1.82 to 3.07	< .001	2.10	1.64 to 2.69	< .001
Proximal *v* distal	1.18	0.98 to 1.42	.0894	1.20	1.00 to 1.45	.0568
Men *v* women	1.07	0.89 to 1.28	.4826	1.21	1.01 to 1.45	.0423
Age (increase of 1 year)	1.01	1.00 to 1.02	.0902	1.02	1.02 to 1.03	< .001
dMMR *v* pMMR	0.62	0.45 to 0.86	.0023	0.67	0.49 to 0.91	.0082

Abbreviations: DFS, disease-free survival; dMMR, deficient mismatch repair; HR, hazard ratio; OS, overall survival; pMMR, proficient MMR.
*Likelihood ratio *P* value from a Cox regression model after stratifying by study.

 © 2011 by American Society of Clinical Oncology

more evident in proximal compared with distal colon cancers, suggesting that obesity-associated biologic effects may influence tumor behavior in a site-dependent manner. The prognostic impact of obesity was similar in men and women. An adverse impact for obesity and colon cancer prognosis has been previously reported by our group[26] and others.[28,55] Patients with dMMR colon cancers had a statistically significant improvement in DFS and OS compared with pMMR tumors, which did not differ significantly across BMI categories. Therefore, the favorable prognostic impact of dMMR was maintained despite the presence of obesity. Although obesity is associated with an increased risk and worsened prognosis for colorectal and other types of cancer, the mechanisms underlying the obesity-cancer progression link are poorly understood. In addition to insulin resistance and hyperinsulinemia, obesity is associated with alterations in the insulin-like growth factor-1 axis, adipocyte production of adipokines including leptin,[56,57] and proinflammatory mediators that may be important contributors to tumor development and progression.[58,59]

Strengths of our study include the large number of dMMR colon cancers evaluated and the meticulous collection of long-term follow-up data within the context of clinical trials. In contrast to other studies of BMI and MMR status in which BMI was calculated from patient-reported data and recall, the recording of height and weight was performed in our study by trained medical personnel at study enrollment. The frequency of dMMR reported here is consistent with multiple clinic and population-based studies.[1,13] Since obesity is associated with comorbid illness, the strict inclusion criteria of the clinical trials requiring normal organ function and favorable performance status serve to minimize the effect of comorbidities on clinical outcome. Limitations of this study include its retrospective design and the fact that our study cohort represents a subset of the overall study populations from individual adjuvant therapy trials based on tissue availability. Furthermore, we did not have information on menopausal status or hormone replacement therapy for our study cohort.

In summary, obesity is independently associated with the molecular subtype of pMMR colon cancer that shows significantly worse survival rates compared with dMMR tumors. In fact, both obesity and MMR status were independent prognostic variables in patients with stage II or III colon cancer. Importantly, the favorable prognosis of dMMR tumors was maintained in obese patients. Together, these data indicate that colon cancers from obese patients are less likely to develop via the dMMR pathway and have a worse prognosis compared with those in normal-weight patients that is independent of other tumor variables.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Conception and design: Frank A. Sinicrope
Financial support: Frank A. Sinicrope
Provision of study materials or patients: Thomas C. Smyrk, George P. Kim, Carmen J. Allegra, Daniel A. Nikcevich
Collection and assembly of data: Frank A. Sinicrope, Nathan R. Foster, Thomas C. Smyrk, George P. Kim, Carmen J. Allegra, Greg Yothers, Daniel A. Nikcevich, Daniel J. Sargent
Data analysis and interpretation: Frank A. Sinicrope, Nathan R. Foster, Harry H. Yoon, Daniel J. Sargent
Manuscript writing: All authors
Final approval of manuscript: All authors

REFERENCES

1. Poynter JN, Siegmund KD, Weisenberger DJ, et al: Molecular characterization of MSI-H colorectal cancer by MLHI promoter methylation, immunohistochemistry, and mismatch repair germline mutation screening. Cancer Epidemiol Biomarkers Prev 17:3208-3215, 2008

2. Thibodeau SN, Bren G, Schaid D: Microsatellite instability in cancer of the proximal colon. Science 260:816-819, 1993

3. Herman JG, Umar A, Polyak K, et al: Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. Proc Natl Acad Sci U S A 95:6870-6875, 1998

4. Hampel H, Frankel WL, Martin E, et al: Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer). N Engl J Med 352: 1851-1860, 2005

5. Jass JR, Do KA, Simms LA, et al: Morphology of sporadic colorectal cancer with DNA replication errors. Gut 42:673-679, 1998

6. Samowitz WS, Curtin K, Ma KN, et al: Microsatellite instability in sporadic colon cancer is associated with an improved prognosis at the population level. Cancer Epidemiol Biomarkers Prev 10:917-923, 2001

7. Alexander J, Watanabe T, Wu TT, et al: Histopathological identification of colon cancer with microsatellite instability. Am J Pathol 158:527-535, 2001

8. Greenson JK, Bonner JD, Ben-Yzhak O, et al: Phenotype of microsatellite unstable colorectal carcinomas: Well-differentiated and focally mucinous tumors and the absence of dirty necrosis correlate with microsatellite instability. Am J Surg Pathol 27:563-570, 2003

9. Sinicrope FA, Foster NR, Thibodeau SN, et al: DNA mismatch repair status and colon cancer recurrence and survival in clinical trials of 5-fluorouracil-based adjuvant therapy. J Natl Cancer Inst 103:863-875, 2011

10. Lanza G, Gafà R, Santini A, et al: Immunohistochemical test for MLH1 and MSH2 expression predicts clinical outcome in stage II and III colorectal cancer patients. J Clin Oncol 24:2359-2367, 2006

11. Halling KC, French AJ, McDonnell SK, et al: Microsatellite instability and 8p allelic imbalance in stage B2 and C colorectal cancers. J Natl Cancer Inst 91:1295-1303, 1999

12. Gafà R, Maestri I, Matteuzzi M, et al: Sporadic colorectal adenocarcinomas with high-frequency microsatellite instability. Cancer 89:2025-2037, 2000

13. Popat S, Hubner R, Houlston RS: Systematic review of microsatellite instability and colorectal cancer prognosis. J Clin Oncol 23:609-618, 2005

14. Ribic CM, Sargent DJ, Moore MJ, et al: Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. N Engl J Med 349:247-257, 2003

15. Sargent DJ, Marsoni S, Monges G, et al: Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. J Clin Oncol 28:3219-3226, 2010

16. Jover R, Nguyen TP, Pérez-Carbonell L, et al: 5-Fluorouracil adjuvant chemotherapy does not increase survival in patients with CpG island methylator phenotype colorectal cancer. Gastroenterology 140:1174-1181, 2011

17. Campbell PT, Jacobs ET, Ulrich CM, et al: Case-control study of overweight, obesity, and colorectal cancer risk, overall and by tumor microsatellite instability status. J Natl Cancer Inst 102:391-400, 2010

18. Slattery ML, Curtin K, Anderson K, et al: Associations between cigarette smoking, lifestyle factors, and microsatellite instability in colon tumors. J Natl Cancer Inst 92:1831-1836, 2000

19. Limsui D, Vierkant RA, Tillmans LS, et al: Cigarette smoking and colorectal cancer risk by molecularly defined subtypes. J Natl Cancer Inst 102:1012-1022, 2010

20. Larsson SC, Wolk A: Obesity and colon and rectal cancer risk: A meta-analysis of prospective studies. Am J Clin Nutr 86:556-565, 2007

21. Pischon T, Lahmann PH, Boeing H, et al: Body size and risk of colon and rectal cancer in the European Prospective Investigation Into Cancer and Nutrition (EPIC). J Natl Cancer Inst 98:920-931, 2006

22. Giovannucci E, Ascherio A, Rimm EB, et al: Physical activity, obesity, and risk for colon cancer and adenoma in men. Ann Intern Med 122:327-334, 1995

23. Le Marchand L, Wilkens LR, Kolonel LN, et al: Associations of sedentary lifestyle, obesity, smoking, alcohol use, and diabetes with the risk of colorectal cancer. Cancer Res 57:4787-4794, 1997

24. Martínez ME, Giovannucci E, Spiegelman D, et al: Leisure-time physical activity, body size, and

© 2011 by American Society of Clinical Oncology

colon cancer in women: Nurses' Health Study Research Group. J Natl Cancer Inst 89:948-955, 1997

25. Ford ES: Body mass index and colon cancer in a national sample of adult US men and women. Am J Epidemiol 150:390-398, 1999

26. Sinicrope FA, Foster NR, Sargent DJ, et al: Obesity is an independent prognostic variable in colon cancer survivors. Clin Cancer Res 16:1884-1893, 2010

27. Meyerhardt JA, Catalano PJ, Haller DG, et al: Influence of body mass index on outcomes and treatment-related toxicity in patients with colon carcinoma. Cancer 98:484-495, 2003

28. Dignam JJ, Polite BN, Yothers G, et al: Body mass index and outcomes in patients who receive adjuvant chemotherapy for colon cancer. J Natl Cancer Inst 98:1647-1654, 2006

29. Kirschner MA, Schneider G, Ertel NH, et al: Obesity, androgens, estrogens, and cancer risk. Cancer Res 42:3281s-3285s, 1982

30. Slattery ML, Potter JD, Curtin K, et al: Estrogens reduce and withdrawal of estrogens increase risk of microsatellite instability-positive colon cancer. Cancer Res 61:126-130, 2001

31. Flegal KM, Carroll MD, Ogden CL, et al: Prevalence and trends in obesity among US adults, 1999-2000. JAMA 288:1723-1727, 2002

32. World Health Organization: Obesity: Preventing and Managing the Global Epidemic. Report of a WHO Consultation to Obesity (WHO Technical Report Series 894). Geneva, Switzerland, 1998

33. Hedley AA, Ogden CL, Johnson CL, et al: Prevalence of overweight and obesity among US children, adolescents, and adults, 1999-2002. JAMA 291:2847-2850, 2004

34. Laurie JA, Moertel CG, Fleming TR, et al: Surgical adjuvant therapy of large-bowel carcinoma: An evaluation of levamisole and the combination of levamisole and fluorouracil: The North Central Cancer Treatment Group and the Mayo Clinic. J Clin Oncol 7:1447-1456, 1989

35. [No authors listed]: Efficacy of adjuvant fluorouracil and folinic acid in colon cancer: International Multicentre Pooled Analysis of Colon Cancer Trials (IMPACT) investigators. Lancet 345:939-944, 1995

36. Moertel CG, Fleming TR, Macdonald JS, et al: Levamisole and fluorouracil for adjuvant therapy of resected colon carcinoma. N Engl J Med 322:352-358, 1990

37. O'Connell MJ, Mailliard JA, Kahn MJ, et al: Controlled trial of fluorouracil and low-dose leucovorin given for 6 months as postoperative adjuvant therapy for colon cancer. J Clin Oncol 15:246-250, 1997

38. Beart RW Jr, Moertel CG, Wieand HS, et al: Adjuvant therapy for resectable colorectal carcinoma with fluorouracil administered by portal vein infusion: A study of the Mayo Clinic and the North Central Cancer Treatment Group. Arch Surg 125: 897-901, 1990

39. Wiesenfeld M, O'Connell MJ, Wieand HS, et al: Controlled clinical trial of interferon-gamma as postoperative surgical adjuvant therapy for colon cancer. J Clin Oncol 13:2324-2329, 1995

40. O'Connell MJ, Sargent DJ, Windschitl HE, et al: Randomized clinical trial of high-dose levamisole combined with 5-fluorouracil and leucovorin as surgical adjuvant therapy for high-risk colon cancer. Clin Colorectal Cancer 6:133-139, 2006

41. Kim GP, Colangelo LH, Wieand HS, et al: Prognostic and predictive roles of high-degree microsatellite instability in colon cancer: A National Cancer Institute-National Surgical Adjuvant Breast and Bowel Project Collaborative Study. J Clin Oncol 25:767-772, 2007

42. Kuebler JP, Wieand HS, O'Connell MJ, et al: Oxaliplatin combined with weekly bolus fluorouracil and leucovorin as surgical adjuvant chemotherapy for stage II and III colon cancer: Results from NSABP C-07. J Clin Oncol 25:2198-2204, 2007

43. Zubrod CG, Schneiderman MA, Frei E, et al: Appraisal of methods for the study of chemotherapy of cancer in man: Comparative therapeutic trial of nitrogen mustard and triethylene thiophosphoramide. J Chron Dis 11:7-33, 1960

44. Watanabe T, Wu TT, Catalano PJ, et al: Molecular predictors of survival after adjuvant chemotherapy for colon cancer. N Engl J Med 344:1196-1206, 2001

45. Boland CR, Thibodeau SN, Hamilton SR, et al: A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: Development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res 58:5248-5257, 1998

46. Thibodeau SN, French AJ, Roche PC, et al: Altered expression of hMSH2 and hMLH1 in tumors with microsatellite instability and genetic alterations in mismatch repair genes. Cancer Res 56:4836-4840, 1996

47. Cox DR: Regression models and life tables. J R Stat Soc B 34:187-220, 1972

48. Lengauer C, Kinzler KW, Vogelstein B: Genetic instability in colorectal cancers. Nature 386: 623-627, 1997

49. Campbell PT, Cotterchio M, Dicks E, et al: Excess body weight and colorectal cancer risk in Canada: Associations in subgroups of clinically defined familial risk of cancer. Cancer Epidemiol Biomarkers Prev 16:1735-1744, 2007

50. Toyota M, Ahuja N, Ohe-Toyota M, et al: CpG island methylator phenotype in colorectal cancer. Proc Natl Acad Sci U S A 96:8681-8686, 1999

51. Nosho K, Shima K, Irahara N, et al: SIRT1 histone deacetylase expression is associated with microsatellite instability and CpG island methylator phenotype in colorectal cancer. Mod Pathol 22:922-932, 2009

52. Hursting SD, Berger NA: Energy balance, host-related factors, and cancer progression. J Clin Oncol 28:4058-4065, 2010

53. Hautanen A: Synthesis and regulation of sex hormone-binding globulin in obesity. Int J Obes Relat Metab Disord 24:S64-S70, 2000 (suppl 2)

54. Schneider G, Kirschner MA, Berkowitz R, et al: Increased estrogen production in obese men. J Clin Endocrinol Metab 48:633-638, 1979

55. Meyerhardt JA, Niedzwiecki D, Hollis D, et al: Impact of body mass index and weight change after treatment on cancer recurrence and survival in patients with stage III colon cancer: Findings from Cancer and Leukemia Group B 89803. J Clin Oncol 26:4109-4115, 2008

56. Rondini EA, Harvey AE, Steibel JP, et al: Energy balance modulates colon tumor growth: Interactive roles of insulin and estrogen. Mol Carcinog 50:370-382, 2011

57. Yakar S, Nunez NP, Pennisi P, et al: Increased tumor growth in mice with diet-induced obesity: Impact of ovarian hormones. Endocrinology 147: 5826-5834, 2006

58. Renehan AG, Roberts DL, Dive C: Obesity and cancer: Pathophysiological and biological mechanisms. Arch Physiol Biochem 114:71-83, 2008

59. Sinicrope FA, Dannenberg AJ: Obesity and breast cancer prognosis: Weight of the evidence. J Clin Oncol 29:4-7, 2011

 © 2011 by American Society of Clinical Oncology