Microsatellite Instability and *KRAS* Mutation in Stage IV Colorectal Cancer: Prevalence, Geographic Discrepancies, and Outcomes From the National Cancer Database

Johannes Uhlig, MD, MPH[1,2]; Michael Cecchini, MD[3,4]; Amar Sheth, BS[1]; Stacey Stein, MD[3,4]; Jill Lacy, MD[3,4]; and Hyun S. Kim, MD, MHS[1,3,4]

ABSTRACT

Background: This study sought to assess microsatellite and *KRAS* status, prevalence, and impact on outcome in stage IV colorectal cancer (CRC). **Materials and Methods:** The 2010 to 2016 US National Cancer Database was queried for adult patients with stage IV CRC. Prevalence of microsatellite status (microsatellite instability–high [MSI-H] or microsatellite stable [MSS]) and *KRAS* status (*KRAS* mutation or wild-type) of the primary CRC was assessed. Overall survival (OS) was evaluated using multivariable Cox proportional hazards models in patients with complete data on both microsatellite and *KRAS* status and information on follow-up. **Results:** Information on microsatellite and *KRAS* status was available for 10,844 and 25,712 patients, respectively, and OS data were available for 5,904 patients. The overall prevalence of MSI-H status and *KRAS* mutation was 3.1% and 42.4%, respectively. Prevalence of MSI-H ranged between 1.6% (rectosigmoid junction) and 5.2% (transverse colon), and between 34.7% (sigmoid colon) and 58.2% (cecum) for *KRAS* mutation. MSI-H rates were highest in East North Central US states (4.1%), and *KRAS* mutation rates were highest in West South Central US states (44.1%). Multivariable analyses revealed longer OS for patients with *KRAS* wild-type versus mutation status (hazard ratio [HR], 0.91; 95% CI, 0.85–0.97; $P=.004$), those with MSS versus MSI-H status (HR, 0.75; 95% CI, 0.62–0.9; $P=.003$), and those with left-sided versus right-sided CRC (multivariable HR, 0.65; 95% CI, 0.6–0.7; $P<.001$). The effect of *KRAS* mutation further varied with CRC site and microsatellite status ($P=.002$ for interaction). **Conclusions:** Depending on the primary site and US geography, stage IV CRC shows distinct mutational behavior. *KRAS* mutation, MSI-H, and primary CRC sidedness independently affect OS and interact with distinct prognostic profiles. Generically classifying adenocarcinomas at different sites as CRC might deprecate this diversity.

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[1]Section of Interventional Radiology, Yale School of Medicine, New Haven, Connecticut; [2]Department of Diagnostic and Interventional Radiology, University Medical Center Goettingen, Goettingen, Germany; and [3]Section of Medical Oncology, Yale School of Medicine, and [4]Yale Cancer Center, Yale School of Medicine, New Haven, Connecticut.

Background

Colorectal cancer (CRC) is the third most common malignancy worldwide, accounting for approximately 1.9 million new cases and 880,000 deaths in 2018.[1] Especially in Western countries, CRC incidence has declined over the past decade, which might be attributable to implementation of colonoscopic screening, use of nonsteroidal anti-inflammatory drugs, and changes in lifestyle and diet.[2,3] Concurrently, major advances have been made in CRC treatment, especially regarding the combination of cytotoxic agents and introduction of targeted therapy in metastatic settings.[4,5]

Genomic instability is of crucial importance for CRC development and progression and includes several mechanisms, one of which is the loss of DNA mismatch repair (MMR) proteins, which results in microsatellite instability–high (MSI-H) tumors.[6] Mutation of the *KRAS* oncogene, which is downstream from the epidermal growth factor receptor (EGFR), is another genomic abnormality in CRC.[7]

Both MSI-H and *KRAS* mutation affect CRC treatment strategies: although immune checkpoint inhibitors have been shown to be effective in treatment of advanced solid tumors that are MSI-H,[8,9] *KRAS* mutations have been reported to mediate resistance to anti-EGFR treatment.[10,11] MSI and *KRAS* status therefore impact individualized treatment strategies for patients with CRC, particularly those with advanced or metastatic CRC (mCRC). Although *BRAF* mutations have also been described in mCRC, therapeutic targeting is challenging, which has been linked to tumoral resistance mechanisms.[12]

Furthermore, MSI-H and defects in DNA MMR genes are the mutational hallmarks in patients with Lynch


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syndrome, who have a high propensity for CRC at an early age.[13] Lynch syndrome is diagnosed in approximately 20% of patients with MSI-H CRC.[14] Given the implications for treatment and familial CRC risk, NCCN recommends MSI and MMR testing in all newly diagnosed CRC cases.[15]

Besides hereditary factors, there is increasing evidence that MSI and *KRAS* status are affected by the primary CRC location.[16,17] Still, to date, there are scarce US national data on the prevalence of MSI-H and *KRAS* mutation that account for the primary cancer sidedness. In this study, we evaluated the prevalence of microsatellite and *KRAS* status in stage IV CRC using a large-scale US national database and assessed their effect on outcomes of patients with CRC.

Materials and Methods

This study received approval from the Yale School of Medicine Institutional Review Board and was HIPAA compliant.

Study Collective

The National Cancer Database (NCDB), jointly sponsored by the American College of Surgeons and the American Cancer Society, contains approximately 34 million records from hospital cancer registries in the United States. It captures approximately 70% of newly diagnosed cancer cases annually in the United States. The NCDB was queried from 2010 to 2016 for patients who had the following diagnoses: pathologically confirmed AJCC stage IV colorectal adenocarcinoma of the cecum (C18.0), ascending colon (C18.2), hepatic flexure of the colon (C18.3), transverse colon (C18.4), splenic flexure of the colon (C18.5), descending colon (C18.6), sigmoid colon (C18.7), rectosigmoid junction (C19.9), and rectum (C20.9). Analyses were limited to cases with information on microsatellite status, *KRAS* status, and known stage IV disease. We excluded patients aged <18 years and those with overlapping lesions of the colon (C18.8); colon, not specified (C18.9); and gastrointestinal, not specified (C26.0). Patients were also excluded if their microsatellite status was obtained via immunologic testing and reported as "instability, not otherwise specified." Survival analyses were limited to patients with complete information on microsatellite and *KRAS* status, follow-up time, and survival status.

Variables

Patient comorbidities were reported using the Charlson-Deyo comorbidity index (CCI) score, stratifying patients into CCI scores of 0, 1, 2, and ≥3. Microsatellite status was assessed via PCR in tumor samples and stratified as MSI-H, MSI-low (MSI-L), and microsatellite stable (MSS). MSI-L and MSS were grouped for all analyses in this study. Patients evaluated for microsatellite status via immunohistochemistry were not considered for our analyses. *KRAS* status was stratified as *KRAS* mutation and wild-type. Radiotherapy was classified as local CRC radiotherapy, radiotherapy for CRC metastases, yttrium-90 radioembolization, and no radiotherapy.

Geographic information was provided as broader US state regions to facilitate anonymization, including the following regions: New England (Connecticut, Maine, Massachusetts, New Hampshire, Rhode Island, and Vermont), Middle Atlantic (New Jersey, New York, and Pennsylvania), East North Central (Illinois, Indiana, Michigan, Ohio, and Wisconsin), West North Central (Iowa, Kansas, Minnesota, Missouri, Nebraska, North Dakota, and South Dakota), South Atlantic (Delaware, Florida, Georgia, Maryland, North Carolina, South Carolina, Virginia, District of Columbia, and West Virginia), East South Central (Alabama, Kentucky, Mississippi, and Tennessee), West South Central (Arkansas, Louisiana, Oklahoma, and Texas), Mountain (Arizona, Colorado, Idaho, Montana, Nevada, New Mexico, Utah, and Wyoming), and Pacific (Alaska, California, Hawaii, Oregon, and Washington).

Outcomes

Two primary outcomes of interest were defined a priori: (1) correlation of microsatellite and *KRAS* status with other CRC factors (cancer site, grade, stage, and metastatic status) and (2) impact of microsatellite and *KRAS* status on CRC overall survival (OS) and interaction with primary CRC sidedness.

Statistical Analyses

Continuous variables are provided as median with interquartile range and categorical variables as absolute number with percent. Continuous variables were compared using the Wilcoxon rank-sum test and categorical variables using the chi-square test. Logistic regression models were used to assess the likelihood of MSI-H and *KRAS* mutation in separate analyses. Due to concerns for reverse causation, metastatic status and cancer treatment were not considered.

OS was evaluated using univariate and multivariable Cox proportional hazards models. Variables were considered for inclusion in multivariable models based on univariate significance and were retained in the final multivariable model if $P<.05$. For multivariable modeling, tumor location was combined into right-sided CRC (CRC of the cecum and ascending and transverse colon) and left-sided CRC (CRC of the descending and sigmoid colon and the rectum). Year of CRC diagnosis was included in the final multivariable model to account for changes in therapy over time. Statistical

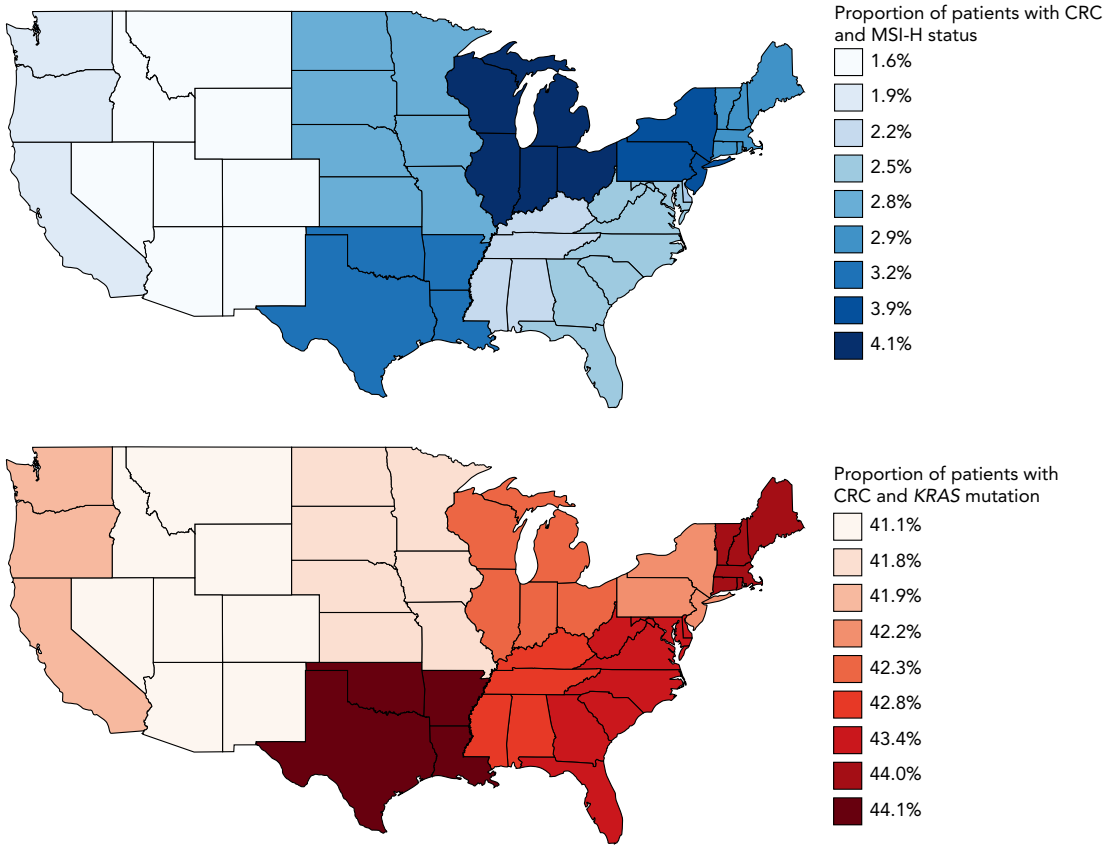

Figure 1. US geographic variation in MSI-H and *KRAS* status among patients with stage IV CRC adenocarcinoma.
Abbreviations: CRC, colorectal cancer; MSI-H, microsatellite instability–high.

interaction analyses were planned a priori between microsatellite status, *KRAS* status, and primary tumor sidedness.

All statistical analyses were performed using R version 3.4.3 (R Foundation for Statistical Computing) and RStudio version 1.1.414 (RStudio, Inc.). An α-level of .05 was chosen for statistical significance. All *P* values are 2-sided.

Results

Among 73,603 patients with mCRC and data on metastatic status, information on microsatellite status was available for 10,844 (14.7%) and on *KRAS* status for 25,712 (34.9%); these datasets were used for epidemiologic modeling. For 5,904 patients, information on both microsatellite and *KRAS* status was available (supplemental eFigure 1 and eTable 1, available with this article at JNCCN.org), which was used to assess OS and interactions between variables. The overall prevalence of MSI-H status and *KRAS* mutation was 3.1% and 42.4%, respectively. The prevalence of *KRAS* mutation was 28.9% among patients with MSI-H CRC.

Association of CRC Genomic Abnormalities With Geographic, Sociodemographic, and Cancer Factors

Multivariable logistic regression analyses (supplemental eTables 1 and 2) revealed that the likelihood of *KRAS* mutation was associated with female sex (odds ratio, 1.10; 95% CI, 1.04–1.15; $P<.001$) and African American race (odds ratio, 1.58; 95% CI, 1.40–1.72; $P<.001$). Furthermore, microsatellite and *KRAS* status independently varied according to US region (Figure 1): MSI-H prevalence was highest in East North Central states (4.1%) and prevalence of *KRAS* mutation was highest in West South Central states (44.1%). Primary CRC sidedness was another independent predictor of microsatellite and *KRAS* status (Figure 2, supplemental eTables 3 and 4): the prevalence of MSI-H ranged between 1.6% (rectosigmoid junction) and 5.2% (transverse colon), and between 58.2% (cecum) and 34.7% (sigmoid colon) for *KRAS* mutation.

Exploratory univariate analyses revealed that MSI-H was associated with fewer distant metastases than MSS (ie, 64.4% hepatic metastases in MSI-H vs 75.4% in MSS) (Tables 1 and 2). In contrast, *KRAS* mutation was associated

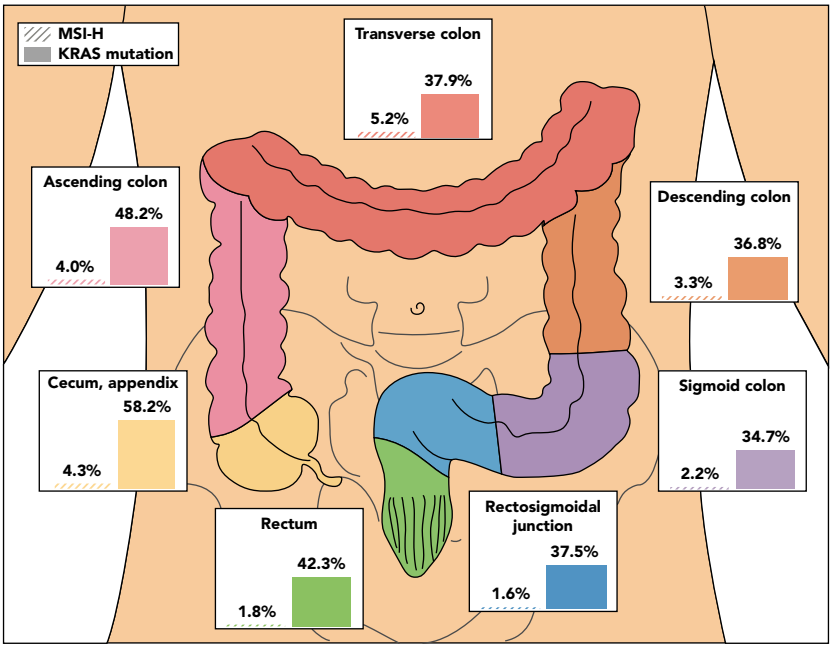

Figure 2. MSI-H and *KRAS* mutation prevalence according to primary CRC site.
Abbreviations: CRC, colorectal cancer; MSI-H, microsatellite instability–high.

with higher metastatic probability (ie, 29.8% lung metastases with *KRAS* mutation vs 20.7% with *KRAS* wild-type).

CRC Mutation Status: Impact on OS and Interaction With Tumor Location

For survival analyses, we evaluated a total of 5,904 patients with data on *KRAS* mutation and microsatellite status and complete information on follow-up. Median follow-up time in this cohort was 44.5 months (interquartile range, 27.1–61.8 months). Univariate analyses revealed that patients with *KRAS* wild-type had longer OS than those with *KRAS* mutation (hazard ratio [HR], 0.71; 95% CI, 0.59–0.85; $P<.001$), and those with MSS had longer OS than those with MSI-H (HR, 0.82; 95% CI, 0.77–0.88; $P<.001$). Furthermore, CRC sidedness affected patient outcomes, with longer OS for left-sided versus righted-sided CRC (HR, 0.64; 95% CI, 0.60–0.68; $P<.001$). OS differences are summarized in Figure 3, and corresponding OS rates are summarized in supplemental eTable 4.

After adjustment for patient-, tumor-, and treatment-level variables, microsatellite status, *KRAS* status, and primary CRC site emerged as independent predictors of OS (Table 3). Longer OS was confirmed for patients with *KRAS* wild-type versus mutation (multivariable HR, 0.91; 95% CI, 0.85–0.97; $P=.004$), those with MSS versus MSI-H (multivariable HR, 0.75; 95% CI, 0.62–0.90; $P=.003$), and those with left-sided versus right-sided CRC (multivariable HR, 0.65; 95% CI, 0.60–0.70; $P<.001$).

Furthermore, a statistical interaction between microsatellite status, *KRAS* status, and primary CRC site was evident (4-*df* multivariable interaction test, $P=.002$). For example, the effect of *KRAS* wild-type on OS was stronger in left-sided CRC (*KRAS* wild-type vs mutation: HR, 0.77; 95% CI, 0.71–0.85; $P<.001$) than in right-sided CRC (*KRAS* wild-type vs mutation: HR, 1.03; 95% CI, 0.93–1.13; $P=.595$) (Figure 4).

Discussion

In the large-scale NCDB, the overall rate of MSI-H was 3.1% and that of *KRAS* mutation was 42.4% in patients with stage IV CRC. MSI rates in our study are comparable to those published for advanced CRC. For example, Fujiyoshi et al[18] reported an MSI-H prevalence of 4.1% in 401 patients with stage IV CRC. Furthermore, the *KRAS* mutation prevalence of 42.4% in our cohort is also similar to that reported elsewhere. For instance, Lowe et al[19] reported 35.9% *KRAS* mutation prevalence in a meta-analysis of patients with metastatic CRC, whereas Kafatos et al[20] showed *RAS* mutations (combined *KRAS* and *NRAS*) in 43.6% of patients with metastatic CRC pooled from 12 primary data sources.

In our study, *KRAS* mutation rates varied with patient demographics, with a higher rate of *KRAS* mutation seen in female African American individuals, which might contribute to poorer CRC outcomes observed in

Table 1. Patient and Tumor Characteristics According to Microsatellite Status

	Total n (%)	MSI-H n (%)	MSS/MSI-L n (%)	*P* Value
Total, N	10,844	334	10,510	
Mean age (SD), y	59.8 (14.0)	61.2 (15.9)	59.8 (14.0)	.049
Sex				.38
Female	5,110 (47.1)	149 (44.6)	4,961 (47.2)	
Male	5,734 (52.9)	185 (55.4)	5,549 (52.8)	
Race				.18
African American	1,501 (13.8)	36 (10.8)	1,465 (13.9)	
White	8,730 (80.5)	282 (84.4)	8,448 (80.4)	
Other	613 (5.7)	16 (4.8)	597 (5.7)	
CCI score				.43
0	8,282 (76.4)	265 (79.3)	8,017 (76.3)	
1	1,881 (17.3)	48 (14.4)	1,833 (17.4)	
2	475 (4.4)	13 (3.9)	462 (4.4)	
≥3	206 (1.9)	8 (2.4)	198 (1.9)	
Insurance status				.018
None	546 (5.0)	20 (6.0)	526 (5.0)	
Private/Managed care	5,083 (46.9)	125 (37.4)	4,958 (47.2)	
Medicaid	1,090 (10.1)	37 (11.1)	1,053 (10.0)	
Medicare	3,861 (35.6)	145 (43.4)	3,716 (35.4)	
Other government	137 (1.3)	4 (1.2)	133 (1.3)	
Unknown	127 (1.2)	3 (0.9)	124 (1.2)	
Cancer location				<.0001
Cecum	1,811 (16.7)	77 (23.1)	1,734 (16.5)	
Ascending colon	1,350 (12.4)	54 (16.2)	1,296 (12.3)	
Transverse colon	1,377 (12.7)	71 (21.3)	1,306 (12.4)	
Descending colon	581 (5.4)	19 (5.7)	562 (5.3)	
Sigmoid colon	2,757 (25.4)	61 (18.3)	2,696 (25.7)	
Rectosigmoid junction	1,072 (9.9)	17 (5.1)	1,055 (10.0)	
Rectum	1,896 (17.5)	35 (10.5)	1,861 (17.7)	
Treatment				.001
Chemotherapy alone	1,933 (17.8)	54 (16.2)	1,879 (17.9)	
No surgery or chemotherapy	448 (4.1)	16 (4.8)	432 (4.1)	
Surgery without chemotherapy	1,789 (16.5)	81 (24.3)	1,708 (16.3)	
Surgery + chemotherapy	6,674 (61.5)	183 (54.8)	6,491 (61.8)	
Radiotherapy				.033
Local radiotherapy	872 (8.0)	16 (4.8)	856 (8.1)	
No concurrent radiotherapy	9,576 (88.3)	300 (89.8)	9,276 (88.3)	
Radiotherapy for metastatic disease	356 (3.3)	15 (4.5)	341 (3.2)	
Y90 radioembolization	40 (0.4)	3 (0.9)	37 (0.4)	

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this population.[21] Race- and sex-specific differences in *KRAS* status differences are supported by earlier literature on stage III CRC and might be attributable to varying neoplasia pathways and enzyme activity.[22]

Patient age was not associated with MSI-H status in our multivariable analyses, which is counterintuitive, given that 20% of patients with MSI-H CRC are reported to have Lynch syndrome, with disease typically

Table 1. Patient and Tumor Characteristics According to Microsatellite Status (cont.)

	Total n (%)	MSI-H n (%)	MSS/MSI-L n (%)	*P* Value
Metastases				<.0001
Distant, not specified	730 (6.7)	40 (12.0)	690 (6.6)	
To 1 distant organ	7,224 (66.6)	192 (57.5)	7,032 (66.9)	
To ≥2 distant organs	1,646 (15.2)	38 (11.4)	1,608 (15.3)	
No distant organ metastases	446 (4.1)	30 (9.0)	416 (4.0)	
Peritoneal metastases	798 (7.4)	34 (10.2)	764 (7.3)	
Liver metastases				<.0001
Yes	8,138 (75.0)	215 (64.4)	7,923 (75.4)	
No	2,706 (25.0)	119 (35.6)	2,587 (24.6)	
Lung metastases				.0004
Yes	2,056 (19.0)	38 (11.4)	2,018 (19.2)	
No	8,788 (81.0)	296 (88.6)	8,492 (80.8)	
Bone metastases				.033
Yes	374 (3.4)	19 (5.7)	355 (3.4)	
No	10,470 (96.6)	315 (94.3)	10,155 (96.6)	
Brain metastases				.37
Yes	98 (0.9)	1 (0.3)	97 (0.9)	
No	10,746 (99.1)	333 (99.7)	10,413 (99.1)	
Treatment facility type				.80
Academic center	3,688 (34.0)	107 (32.0)	3,581 (34.1)	
Nonacademic center	6,376 (58.8)	192 (57.5)	6,184 (58.8)	
Suppressed for age <39 y	780 (7.2)	35 (10.5)	745 (7.1)	
Treatment facility location				.0004
East North Central	1,791 (16.5)	73 (21.9)	1,718 (16.3)	
East South Central	547 (5.0)	12 (3.6)	535 (5.1)	
Suppressed for age <39 y	780 (7.2)	35 (10.5)	745 (7.1)	
Middle Atlantic	1,697 (15.6)	67 (20.1)	1,630 (15.5)	
Mountain	568 (5.2)	9 (2.7)	559 (5.3)	
New England	654 (6.0)	19 (5.7)	635 (6.0)	
Pacific	1,343 (12.4)	25 (7.5)	1,318 (12.5)	
South Atlantic	2,059 (19.0)	52 (15.6)	2,007 (19.1)	
West North Central	709 (6.5)	20 (6.0)	689 (6.6)	
West South Central	696 (6.4)	22 (6.6)	674 (6.4)	

Continuous variables were compared between subgroups using the Wilcoxon rank-sum test, and categorical variables were compared using the chi-square test. Abbreviations: CCI, Charlson-Deyo comorbidity index; MSI-H, microsatellite instability–high; MSI-L microsatellite instability–low, MSS, microsatellite status–stable; Y90, yttrium-90.

manifesting at a younger age than CRC in other patients.[13,14] This discrepancy might be attributable to a low proportion of patients with Lynch syndrome in our cohort and to the anonymization process of the NCDB with suppressed facility location for patients aged <39 years.

Our findings further reveal that CRC genomic abnormalities independently vary with US geography in the NCDB population, with the highest MSI-H prevalence in East North Central states (Illinois, Indiana, Michigan, Ohio, and Wisconsin) and the highest *KRAS* mutation prevalence in West South Central states (Arkansas, Louisiana, Oklahoma, and Texas). Although this variability could be attributable to the patients' mutational susceptibility, differences in environmental and socioeconomic factors, dietary behavior, and CRC screening compliance might also contribute. CRC has been described as an environmentally

Table 2. Patient and Tumor Characteristics According to *KRAS* Status

	Total n (%)	*KRAS* Mutation n (%)	*KRAS* Wild-Type n (%)	*P* Value
Total, N	25,712	10,907	14,805	
Mean age (SD), y	60.8 (13.1)	61.1 (13.0)	60.5 (13.1)	.14
Sex				<.0001
Female	11,467 (44.6)	5,079 (46.6)	6,388 (43.1)	
Male	14,245 (55.4)	5,828 (53.4)	8,417 (56.9)	
Race				<.0001
African American	3,658 (14.2)	1,913 (17.5)	1,745 (11.8)	
White	20,695 (80.5)	8,460 (77.6)	12,235 (82.6)	
Other	1,359 (5.3)	534 (4.9)	825 (5.6)	
CCI score				.77
0	19,730 (76.7)	8,364 (76.7)	11,366 (76.8)	
1	4,546 (17.7)	1,934 (17.7)	2,612 (17.6)	
2	1,026 (4.0)	444 (4.1)	582 (3.9)	
≥3	410 (1.6)	165 (1.5)	245 (1.7)	
Insurance status				.68
None	1,434 (5.6)	611 (5.6)	823 (5.6)	
Private/Managed care	11,392 (44.3)	4,777 (43.8)	6,615 (44.7)	
Medicaid	2,603 (10.1)	1,107 (10.1)	1,496 (10.1)	
Medicare	9,695 (37.7)	4,150 (38.0)	5,545 (37.5)	
Other government	301 (1.2)	131 (1.2)	170 (1.1)	
Unknown	287 (1.1)	131 (1.2)	156 (1.1)	
Cancer location				<.0001
Cecum	4,148 (16.1)	2,415 (22.1)	1,733 (11.7)	
Ascending colon	3,135 (12.2)	1,512 (13.9)	1,623 (11.0)	
Transverse colon	3,082 (12.0)	1,167 (10.7)	1,915 (12.9)	
Descending colon	1,268 (4.9)	466 (4.3)	802 (5.4)	
Sigmoid colon	6,434 (25.0)	2,235 (20.5)	4,199 (28.4)	
Rectosigmoid junction	2,557 (9.9)	959 (8.8)	1,598 (10.8)	
Rectum	5,088 (19.8)	2,153 (19.7)	2,935 (19.8)	
Treatment				.0002
Chemotherapy alone	9,725 (37.8)	4,261 (39.1)	5,464 (36.9)	
No surgery or chemotherapy	1,342 (5.2)	603 (5.5)	739 (5.0)	
Surgery without chemotherapy	2,201 (8.6)	922 (8.5)	1,279 (8.6)	
Surgery + chemotherapy	12,444 (48.4)	5,121 (47.0)	7,323 (49.5)	
Radiotherapy				.96
Local radiotherapy	1,884 (7.3)	796 (7.3)	1,088 (7.3)	
No concurrent radiotherapy	22,730 (88.4)	9,640 (88.4)	13,090 (88.4)	
Radiotherapy for metastatic disease	1,012 (3.9)	436 (4.0)	576 (3.9)	
Y90 radioembolization	86 (0.3)	35 (0.3)	51 (0.3)	

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mediated entity. For example, it has been shown that immigrants assume the CRC risk of their new environment within one generation.[23] Diets rich in processed and red meat have been linked to a higher CRC risk,[24] as have smoking and excessive alcohol consumption.[25,26] Still, to date, there are only sparse data on geographic variability of CRC mutations. Turpin et al[27] evaluated patients with CRC from northern France and showed spatial heterogeneity that might

Table 2. Patient and Tumor Characteristics According to *KRAS* Status (cont.)

	Total n (%)	*KRAS* Mutation n (%)	*KRAS* Wild-Type n (%)	*P* Value
Metastases				<.0001
Distant, not specified	1,260 (4.9)	467 (4.3)	793 (5.4)	
To 1 distant organ	16,633 (64.7)	6,805 (62.4)	9,828 (66.4)	
To ≥2 distant organs	5,497 (21.4)	2,779 (25.5)	2,718 (18.4)	
No distant organ metastases	798 (3.1)	283 (2.6)	515 (3.5)	
Peritoneal metastases	1,524 (5.9)	573 (5.3)	951 (6.4)	
Liver metastases				.039
Yes	20,423 (79.4)	8,730 (80.0)	11,693 (79.0)	
No	5,289 (20.6)	2,177 (20.0)	3,112 (21.0)	
Lung metastases				<.0001
Yes	6,314 (24.6)	3,252 (29.8)	3,062 (20.7)	
No	19,398 (75.4)	7,655 (70.2)	11,743 (79.3)	
Bone metastases				.01
Yes	1,194 (4.6)	550 (5.0)	644 (4.3)	
No	24,518 (95.4)	10,357 (95.0)	14,161 (95.7)	
Brain metastases				<.0001
Yes	322 (1.3)	182 (1.7)	140 (0.9)	
No	25,390 (98.7)	10,725 (98.3)	14,665 (99.1)	
Treatment facility type				.22
Academic center	8,526 (33.2)	3,682 (33.8)	4,844 (32.7)	
Nonacademic center	15,780 (61.4)	6,685 (61.3)	9,095 (61.4)	
Suppressed for age <39 y	1,406 (5.5)	540 (5.0)	866 (5.8)	
Treatment facility location				.050
East North Central	4,316 (16.8)	1,825 (16.7)	2,491 (16.8)	
East South Central	1,435 (5.6)	614 (5.6)	821 (5.5)	
Suppressed for age <39 y	1,406 (5.5)	540 (5.0)	866 (5.8)	
Middle Atlantic	4,019 (15.6)	1,698 (15.6)	2,321 (15.7)	
Mountain	1,179 (4.6)	484 (4.4)	695 (4.7)	
New England	1,360 (5.3)	599 (5.5)	761 (5.1)	
Pacific	2,827 (11.0)	1,184 (10.9)	1,643 (11.1)	
South Atlantic	5,129 (19.9)	2,228 (20.4)	2,901 (19.6)	
West North Central	2,078 (8.1)	869 (8.0)	1,209 (8.2)	
West South Central	1,963 (7.6)	866 (7.9)	1,097 (7.4)	

Continuous variables were compared between subgroups using the Wilcoxon rank-sum test, and categorical variables were compared using the chi-square test.
Abbreviations: CCI, Charlson-Deyo comorbidity index; Y90, yttrium-90.

correlate with environmental factors, such as proximity to major highways and large cities. The association of *KRAS* mutation with African American race, female sex, and West South Central US state residence further raises the question of whether the geographic differences of *KRAS* status are affected by racial distribution differences.

Our data further showed that microsatellite and *KRAS* status varied with the primary tumor side. In tendency, both MSI-H and *KRAS* mutation were more common in right-sided versus left-sided CRC, whereas specific primary CRC locations showed distinct mutational profiles. These results are supported by Sinicrope et al,[16] who described site-specific *KRAS* mutation and DNA MMR defects in 3,018 patients with stage III disease undergoing adjuvant chemotherapy, reporting higher *KRAS* mutation and MSI-H rates in right-sided CRC. Yamauchi et al[17] described higher MSI-H incidence for CRC of stage I–IV in the proximal colon. Higher rates of *KRAS* mutation for right-sided stage II and III CRC are

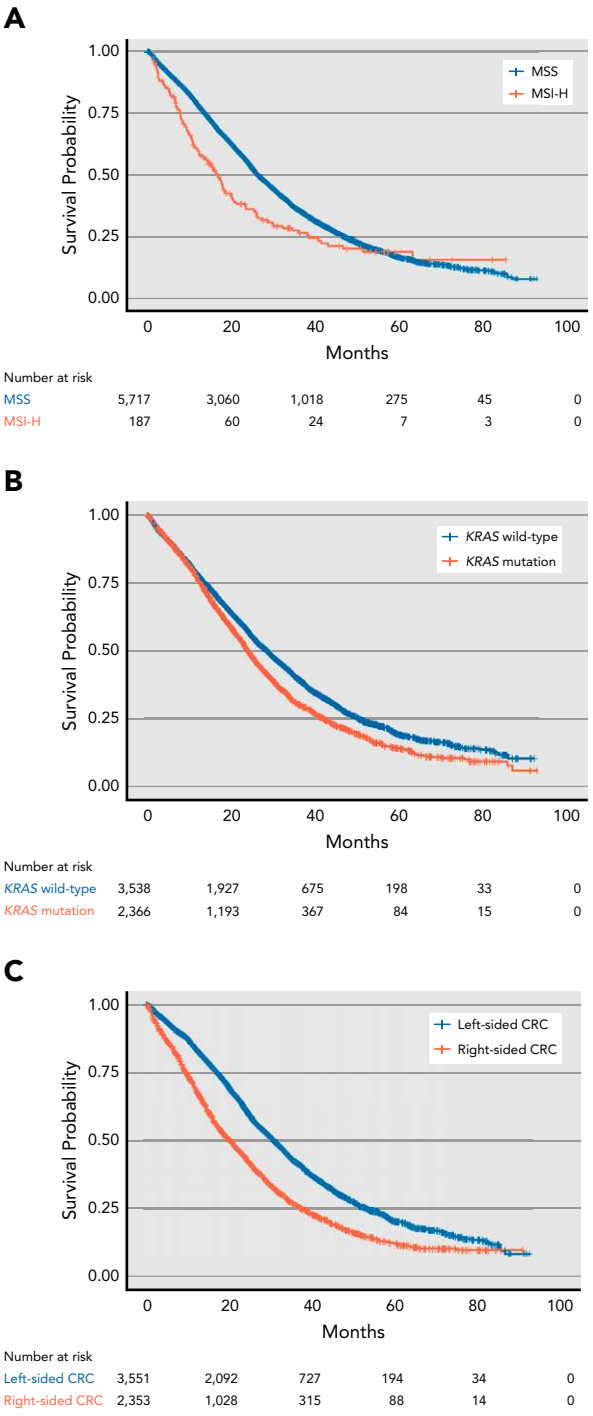

Figure 3. Kaplan-Meier plots showing impact on OS of (A) microsatellite status, (B) *KRAS* status, and (C) primary CRC site.
Abbreviations: CRC, colorectal cancer; MSI, microsatellite instability–high; MSS, microsatellite status–stable; OS, overall survival.

also noted in analyses derived from The Cancer Genome Atlas (TCGA).[28]

The underlying mechanisms of these site-specific mutation rates are not fully understood. Differences in the embryologic origin might contribute, given that the

Table 3. Multivariable Cox Proportional Hazards Model Evaluating Overall Survival Based on CRC Mutation Status

Variable	HR (95% CI)	*P* Value
CRC location		
Right-sided	Ref	
Left-sided	0.65 (0.60–0.70)	<.001
KRAS status		
KRAS mutation	Ref	
KRAS wild-type	0.91 (0.85–0.97)	.004
Microsatellite status		
MSI-H	Ref	
MSS	0.75 (0.62–0.90)	.003
Age (per 1-y increment)	1.01 (1.01–1.02)	<.001
Sex		
Female	Ref	
Male	1.04 (0.97–1.11)	.264
CCI score		
0	Ref	
≥1	1.1 (1.01–1.18)	.022
Race		
African American	Ref	
Other	0.97 (0.88–1.07)	.537
Metastases		
To 1 distant organ	Ref	
To ≥2 distant organs	1.45 (1.33–1.59)	<.001
Other or unspecified metastatic status	0.91 (0.83–1.00)	.063
Treatment facility type		
Academic center	Ref	
Nonacademic center	1.25 (1.16–1.34)	<.001
Treatment		
Chemotherapy alone	Ref	
No surgery or chemotherapy	3.5 (2.89–4.23)	<.001
Surgery without chemotherapy	1.05 (0.93–1.19)	.426
Surgery + chemotherapy	0.51 (0.47–0.55)	<.001
Radiotherapy		
No concurrent radiotherapy	Ref	
Local radiotherapy	0.82 (0.71–0.95)	.009
Radiotherapy for metastatic disease	1.1 (0.93–1.31)	.272
Y90 radioembolization	1.16 (0.70–1.94)	.56
Year of CRC diagnosis		
2010	Ref	
2011	0.83 (0.72–0.95)	.009
2012	0.97 (0.85–1.10)	.632
2013	0.99 (0.87–1.13)	.88
2014	1.02 (0.90–1.17)	.722
2015	1.14 (0.99–1.31)	.063

Abbreviations: CCI, Charlson-Deyo comorbidity index; CRC, colorectal cancer; HR, hazard ratio; MSI-H, microsatellite instability–high; MSS, microsatellite status–stable; Y90, yttrium-90.

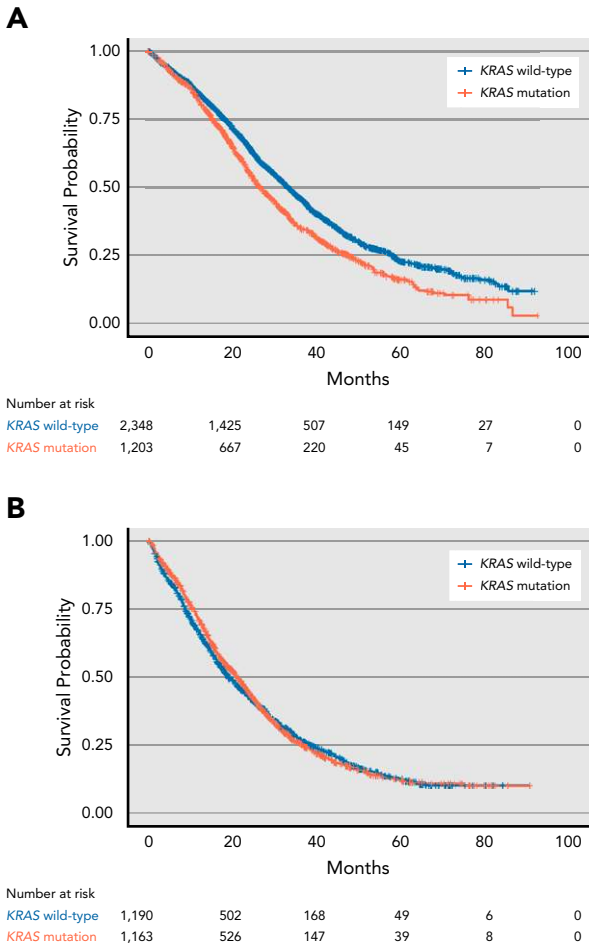

Figure 4. Kaplan-Meier plots showing differing effects of *KRAS* status according to microsatellite status and primary (A) left-sided versus (B) right-sided colorectal cancer.

right-sided colon is derived from the midgut and the left-sided colon and rectum are derived from the hindgut.[29] Furthermore, there are studies reporting locoregional differences of the microbiome in various colonic locations. For example, a higher incidence of *Escherichia coli* phylogroup B2 has been described in right-sided versus left-sided CRC.[30]

Our analyses show that MSI-H status, *KRAS* mutation, and primary CRC sidedness are independent prognosticators of OS. In general, patients with MSI-H status and *KRAS* mutation had worse OS than those with MSS status and *KRAS* wild-type. OS was also shorter in patients with right-sided versus left-sided CRC, which is supported in numerous prior reports.[31–33] Moreover, the prognostic effect of *KRAS* mutation varied with primary cancer location, as the effect size was larger in left-sided versus right-sided CRC. One explanation for site-specific differences is that proximal CRC may have an overall higher mutation rate, potentially associated with worse prognosis, as shown in the PETACC-3 study and TCGA.[28,34]

In patients with *KRAS* wild-type CRC, several studies have shown worse outcomes for right-sided versus left-sided cancers.[35,36] Sinicrope et al[16] conducted an extensive analysis of 3,018 patients with stage III CRC and described results similar to ours, with shorter OS among those with right-sided CRC and *KRAS* mutation. Furthermore, the RASCAL and RASCAL II studies associated *KRAS* mutation with earlier recurrence and death, although the smaller PETACC-3 and CALGB 89803 studies in stage II and III CRC showed contradictory results.[37–40] Discrepancies in the prognostic value of *KRAS* in CRC could possibly originate from varying patient populations and treatment regimens. Furthermore, the specific location of *KRAS* mutations (codon 12 vs 13) seems to have prognostic impact in CRC.[41]

Although MSI-H has been associated with favorable prognosis in stage II CRC, only few studies have reported on its role in stage IV CRC.[42] Price et al[43] reported on Australian registry data and showed shorter OS in patients with MSI versus MSS status, although no distinction was made between MSI-H and MSI-L. Jin et al[44] described a cohort of 1,268 patients with metastatic CRC from the Mayo Clinic and reported shorter OS in those with MSI-H versus MSS status.

Several mechanisms may contribute to inferior outcomes in patients with MSI-H CRC, including reduced efficacy of fluorouracil-based treatments[42,45] and modification of the treatment effectiveness of vascular endothelial growth factor–targeted agents.[46] Still, there are some concerns that the coincidence of *BRAF* status and MSI might confound the prognostic profile of MSI.[47]

Our study is not devoid of limitations, which are mainly inherent to the use of NCDB as the data source. First, given the stratified coding of the NCDB, no distinction between specific systemic treatment protocols is possible, thereby limiting evaluation of the predictive value of *KRAS* mutation and microsatellite status. Second, the NCDB does not provide additional details on CRC mutations, such as specific codons and microsatellite status, and *KRAS* status was not available for all patients in the NCDB, raising concerns about patient selection and the generalizability of our results. Third, there have been concerns about the generalizability of the NCDB data to the general population because it might not cover a cohort representative of the US population. Furthermore, the low proportion of patients with data on *KRAS* and MSI status among all patients with mCRC raises concerns about potential selection bias and variability in institutional policies regarding MSI testing strategies, which might impair the generalizability of our findings to a broader population.

Finally, evaluating data up to 2016, our analyses did not cover the recent use of immunotherapy for advanced CRC, with particular benefit for patients with MSI-H status.

Conclusions

In a large-scale US national cohort, we showed that MSI and *KRAS* status in stage IV CRC vary according to demography, CRC sidedness, and US geography. MSI-H, *KRAS* mutation, and primary CRC site (right- vs left-sided) independently affect OS with a worsened prognosis and distinct profiles, although recent developments might change outcomes, particularly for patients with MSI-H status. Our results corroborate that generically classifying adenocarcinomas as CRC might not appreciate the observed mutational and site-specific differences. Additional studies are warranted to evaluate whether these differences translate into site-specific treatment effectiveness for primary or metastatic disease.

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Correspondence: Hyun S. Kim, MD, MHS, University of Maryland School of Medicine, 22 South Greene Street, Suite G2K14, Baltimore, MD 21201. Email: kevin.kim@umm.edu

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Microsatellite Instability and *KRAS* Mutation in Stage IV Colorectal Cancer: Prevalence, Geographic Discrepancies, and Outcomes From the National Cancer Database

Johannes Uhlig, MD, MPH; Michael Cecchini, MD; Amar Sheth, BS; Stacey Stein, MD; Jill Lacy, MD; and Hyun S. Kim, MD, MHS

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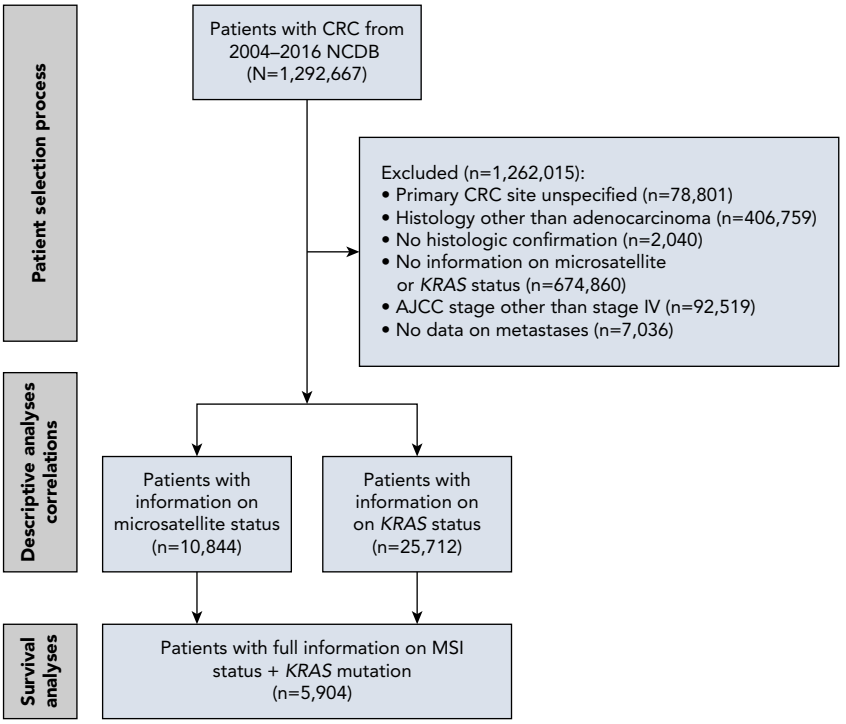

eFigure 1. Patient selection flowchart.
Abbreviations: CRC, colorectal cancer; MSI, microsatellite instability; NCDB, National Cancer Database.

eTable 1. Baseline Characteristics According to Microsatellite and *KRAS* Status

	Total n (%)	*KRAS* Mutation n (%)	*KRAS* Wild-Type n (%)	MSI-H n (%)	MSS/MSI-L n (%)
Total, N	5,904	2,366	3,538	187	5,717
Mean age (SD), y	57.8 (13.5)	58.2 (13.3)	57.5 (13.7)	58.7 (16.2)	57.7 (13.4)
Sex					
Female	2,770 (46.9)	1,190 (50.3)	1,580 (44.7)	84 (44.9)	2,686 (47.0)
Male	3,134 (53.1)	1,176 (49.7)	1,958 (55.3)	103 (55.1)	3,031 (53.0)
Race					
African American	801 (13.6)	393 (16.6)	408 (11.5)	21 (11.2)	780 (13.6)
White	4,789 (81.1)	1,857 (78.5)	2,932 (82.9)	160 (85.6)	4,629 (81.0)
Other	314 (5.3)	116 (4.9)	198 (5.6)	6 (3.2)	308 (5.4)
CCI score					
0	4,583 (77.6)	1,829 (77.3)	2,754 (77.8)	148 (79.1)	4,435 (77.6)
1	997 (16.9)	384 (16.2)	613 (17.3)	27 (14.4)	970 (17.0)
2	241 (4.1)	115 (4.9)	126 (3.6)	7 (3.7)	234 (4.1)
≥3	83 (1.4)	38 (1.6)	45 (1.3)	5 (2.7)	78 (1.4)
Insurance status					
None	282 (4.8)	108 (4.6)	174 (4.9)	8 (4.3)	274 (4.8)
Private/Managed care	3,074 (52.1)	1,254 (53.0)	1,820 (51.4)	78 (41.7)	2,996 (52.4)
Medicaid	604 (10.2)	236 (10.0)	368 (10.4)	20 (10.7)	584 (10.2)
Medicare	1,805 (30.6)	715 (30.2)	1,090 (30.8)	77 (41.2)	1,728 (30.2)
Other government	76 (1.3)	27 (1.1)	49 (1.4)	2 (1.1)	74 (1.3)
Unknown	63 (1.1)	26 (1.1)	37 (1.0)	2 (1.1)	61 (1.1)
Cancer location					
Cecum	953 (16.1)	574 (24.3)	379 (10.7)	40 (21.4)	913 (16.0)
Ascending colon	695 (11.8)	321 (13.6)	374 (10.6)	33 (17.6)	662 (11.6)
Transverse colon	705 (11.9)	268 (11.3)	437 (12.4)	40 (21.4)	665 (11.6)
Descending colon	348 (5.9)	114 (4.8)	234 (6.6)	11 (5.9)	337 (5.9)
Sigmoid colon	1,611 (27.3)	509 (21.5)	1,102 (31.1)	42 (22.5)	1,569 (27.4)
Rectosigmoid junction	584 (9.9)	198 (8.4)	386 (10.9)	7 (3.7)	577 (10.1)
Rectum	1,008 (17.1)	382 (16.1)	626 (17.7)	14 (7.5)	994 (17.4)
Treatment					
Chemotherapy alone	1,266 (21.4)	515 (21.8)	751 (21.2)	36 (19.3)	1,230 (21.5)
No surgery or chemotherapy	165 (2.8)	64 (2.7)	101 (2.9)	5 (2.7)	160 (2.8)
Surgery without chemotherapy	607 (10.3)	248 (10.5)	359 (10.1)	33 (17.6)	574 (10.0)
Surgery + chemotherapy	3,866 (65.5)	1,539 (65.0)	2,327 (65.8)	113 (60.4)	3,753 (65.6)
Radiotherapy					
No concurrent radiotherapy	5,234 (88.7)	2,107 (89.1)	3,127 (88.4)	162 (86.6)	5,072 (88.7)
Local radiotherapy	424 (7.2)	172 (7.3)	252 (7.1)	8 (4.3)	416 (7.3)
Radiotherapy for metastatic disease	222 (3.8)	77 (3.3)	145 (4.1)	14 (7.5)	208 (3.6)
Y90 radioembolization	24 (0.4)	10 (0.4)	14 (0.4)	3 (1.6)	21 (0.4)

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eTable 1. Baseline Characteristics According to Microsatellite and *KRAS* Status (cont.)

	Total n (%)	*KRAS* Mutation n (%)	*KRAS* Wild-Type n (%)	MSI-H n (%)	MSS/MSI-L n (%)
Metastases					
Distant, not specified	335 (5.7)	126 (5.3)	209 (5.9)	22 (11.8)	313 (5.5)
To 1 distant organ	3,982 (67.4)	1,558 (65.8)	2,424 (68.5)	112 (59.9)	3,870 (67.7)
To ≥2 distant organs	977 (16.5)	450 (19.0)	527 (14.9)	22 (11.8)	955 (16.7)
No distant organ metastases	192 (3.3)	62 (2.6)	130 (3.7)	15 (8.0)	177 (3.1)
Peritoneal metastases	418 (7.1)	170 (7.2)	248 (7.0)	16 (8.6)	402 (7.0)
Liver metastases					
Yes	4,582 (77.6)	1,815 (76.7)	2,767 (78.2)	130 (69.5)	4,452 (77.9)
No	1,322 (22.4)	551 (23.3)	771 (21.8)	57 (30.5)	1,265 (22.1)
Lung metastases					
Yes	1,183 (20.0)	577 (24.4)	606 (17.1)	17 (9.1)	1,166 (20.4)
No	4,721 (80.0)	1,789 (75.6)	2,932 (82.9)	170 (90.9)	4,551 (79.6)
Bone metastases					
Yes	209 (3.5)	77 (3.3)	132 (3.7)	12 (6.4)	197 (3.4)
No	5,695 (96.5)	2,289 (96.7)	3,406 (96.3)	175 (93.6)	5,520 (96.6)
Brain metastases					
Yes	54 (0.9)	26 (1.1)	28 (0.8)	1 (0.5)	53 (0.9)
No	5,850 (99.1)	2,340 (98.9)	3,510 (99.2)	186 (99.5)	5,664 (99.1)
Treatment facility type					
Academic center	1,984 (33.6)	826 (34.9)	1,158 (32.7)	52 (27.8)	1,932 (33.8)
Nonacademic center	3,403 (57.6)	1,346 (56.9)	2,057 (58.1)	108 (57.8)	3,295 (57.6)
Suppressed for age <39 y	517 (8.8)	194 (8.2)	323 (9.1)	27 (14.4)	490 (8.6)
Treatment facility location					
East North Central	855 (14.5)	360 (15.2)	495 (14.0)	36 (19.3)	819 (14.3)
East South Central	283 (4.8)	112 (4.7)	171 (4.8)	6 (3.2)	277 (4.8)
Suppressed for age <39 y	517 (8.8)	194 (8.2)	323 (9.1)	27 (14.4)	490 (8.6)
Middle Atlantic	998 (16.9)	391 (16.5)	607 (17.2)	33 (17.6)	965 (16.9)
Mountain	340 (5.8)	123 (5.2)	217 (6.1)	6 (3.2)	334 (5.8)
New England	361 (6.1)	160 (6.8)	201 (5.7)	14 (7.5)	347 (6.1)
Pacific	750 (12.7)	290 (12.3)	460 (13.0)	14 (7.5)	736 (12.9)
South Atlantic	1,074 (18.2)	439 (18.6)	635 (17.9)	26 (13.9)	1,048 (18.3)
West North Central	399 (6.8)	154 (6.5)	245 (6.9)	15 (8.0)	384 (6.7)
West South Central	327 (5.5)	143 (6.0)	184 (5.2)	10 (5.3)	317 (5.5)

Abbreviations: CCI, Charlson-Deyo comorbidity index; MSI-H, microsatellite instability–high; MSI-L, microsatellite instability–low; MSS, microsatellite status–stable.

eTable 2. Univariate and Multivariable Logistic Regression Models for *KRAS* Mutation Outcome

	Univariate		Multivariate	
Variable	**OR (95% CI)**	***P* Value**	**OR (95% CI)**	***P* Value**
Age (per 1-y increase)	1.00 (1.00–1.01)	.001	—	
Sex				
Male	Ref		Ref	
Female	1.15 (1.09–1.21)	<.001	1.10 (1.04–1.15)	<.001
Race				
Other	Ref		Ref	
African American	1.59 (1.48–1.71)	<.001	1.58 (1.47–1.70)	<.001
Insurance status				
None	Ref		—	
Private/Managed care	0.97 (0.87–1.09)	.625	—	
Medicaid	1.00 (0.87–1.14)	.961	—	
Medicare	1.01 (0.90–1.13)	.888	—	
Other government	1.04 (0.81–1.33)	.771	—	
Unknown	1.13 (0.88–1.46)	.343	—	
CCI score				
0	Ref		—	
≥1	1.00 (0.95–1.07)	.871	—	
Cancer location				
Cecum	Ref		Ref	
Ascending colon	0.67 (0.61–0.73)	<.001	0.66 (0.60–0.73)	<.001
Transverse colon	0.44 (0.40–0.48)	<.001	0.43 (0.39–0.48)	<.001
Descending colon	0.42 (0.37–0.47)	<.001	0.41 (0.36–0.47)	<.001
Sigmoid colon	0.38 (0.35–0.41)	<.001	0.39 (0.36–0.42)	<.001
Rectosigmoid junction	0.43 (0.39–0.48)	<.001	0.44 (0.40–0.49)	<.001
Rectum	0.53 (0.48–0.57)	<.001	0.55 (0.50–0.60)	<.001
Treatment facility location				
Suppressed for age <39 y	Ref		Ref	
New England	1.26 (1.08–1.47)	.003	1.20 (1.03–1.40)	.019
East North Central	1.17 (1.04–1.33)	.010	1.08 (0.95–1.22)	.238
East South Central	1.20 (1.03–1.39)	.017	1.06 (0.91–1.24)	.433
Middle Atlantic	1.17 (1.04–1.33)	.012	1.10 (0.97–1.24)	.154
Mountain	1.12 (0.95–1.31)	.171	1.06 (0.90–1.25)	.459
Pacific	1.16 (1.01–1.32)	.030	1.13 (0.99–1.29)	.076
South Atlantic	1.23 (1.09–1.39)	.001	1.07 (0.95–1.21)	.275
West North Central	1.15 (1.00–1.32)	.044	1.09 (0.94–1.25)	.253
West South Central	1.27 (1.10–1.46)	.001	1.15 (0.99–1.32)	.059

Abbreviations: CCI, Charlson-Deyo comorbidity index; OR, odds ratio.

eTable 3. Univariate and Multivariable Logistic Regression Models for MSI-H Outcome

	Univariate		Multivariate	
Variable	**OR (95% CI)**	***P* Value**	**OR (95% CI)**	***P* Value**
Age (per 1-y increase)	1.01 (1.00–1.02)	.072	—	
Sex				
Female	Ref		—	
Male	1.11 (0.89–1.38)	.350	—	
Race				
African American	Ref		—	
Other	1.34 (0.96–1.93)	.101	—	
Insurance status				
None	Ref		—	
Private/Managed care	0.66 (0.42–1.10)	.094	—	
Medicaid	0.92 (0.54–1.64)	.780	—	
Medicare	1.03 (0.65–1.70)	.915	—	
Other government	0.79 (0.23–2.13)	.673	—	
Unknown	0.64 (0.15–1.89)	.471	—	
CCI score				
0	Ref		—	
≥1	0.84 (0.64–1.09)	.195	—	
Cancer location				
Cecum	Ref		Ref	
Ascending colon	0.94 (0.65–1.34)	.725	0.93 (0.65–1.33)	.696
Transverse colon	1.22 (0.88–1.70)	.230	1.19 (0.85–1.66)	.302
Descending colon	0.76 (0.44–1.24)	.296	0.72 (0.42–1.18)	.209
Sigmoid colon	0.51 (0.36–0.72)	<.001	0.49 (0.35–0.69)	<.001
Rectosigmoid junction	0.36 (0.21–0.60)	<.001	0.34 (0.19–0.57)	<.001
Rectum	0.42 (0.28–0.63)	<.001	0.40 (0.27–0.60)	<.001
Treatment facility location				
Middle Atlantic	Ref		Ref	
East North Central	1.03 (0.74–1.45)	.848	1.03 (0.73–1.45)	.871
East South Central	0.55 (0.28–0.98)	.056	0.55 (0.28–0.99)	.059
Suppressed for age <39 y	1.14 (0.75–1.72)	.531	1.33 (0.86–2.01)	.190
Mountain	0.39 (0.18–0.75)	.009	0.40 (0.18–0.76)	.010
New England	0.73 (0.42–1.20)	.229	0.72 (0.42–1.18)	.210
Pacific	0.46 (0.28–0.72)	.001	0.47 (0.29–0.73)	.001
South Atlantic	0.63 (0.43–0.91)	.014	0.61 (0.42–0.89)	.010
West North Central	0.71 (0.41–1.15)	.179	0.69 (0.41–1.13)	.160
West South Central	0.79 (0.48–1.27)	.356	0.79 (0.47–1.27)	.349

Abbreviations: CCI, Charlson-Deyo comorbidity index; MSI-H, microsatellite instability–high; OR, odds ratio.

eTable 4. Overall Survival Rates

	Overall Survival (%)			
	1-Year	2-Year	3-Year	5-Year
MSI-H	60	36.9	27.4	19.9
MSS	78.2	54.8	36.2	17.3
KRAS mutation	76.5	50	30.6	14.3
KRAS wild-type	78.4	57.2	39.6	19.5
Right-sided CRC	68.7	43.6	26.6	12.5
Left-sided CRC	83.6	61.4	42.3	20.7

Abbreviations: CRC, colorectal cancer; MSI-H, microsatellite instability–high; MSS, microsatellite status–stable.