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Prognostic Role of Detailed Colorectal Location and Tumor Molecular Features: Analyses of 13,101 Colorectal Cancer Patients including 2,994 Early-onset Cases

Tomotaka Ugai, MD, PhD[1,2], **Naohiko Akimoto, MD, PhD**[1], **Koichiro Haruki, MD, PhD**[1], **Tabitha A. Harrison, MPH**[3], **Yin Cao, MPH, ScD**[4,5,6], **Conghui Qu, MS**[3], **Andrew T. Chan, MD, MPH**[7,8,9,10], **Peter T. Campbell, PhD**[11], **Sonja I. Berndt, PhD**[12], **Daniel D. Buchanan, PhD**[13,14,15], **Amanda J. Cross, PhD**[16], **Brenda Diergaarde, PhD**[17], **Steven J. Gallinger, PhD**[18], **Marc J. Gunter, PhD**[19], **Sophia Harlid, PhD**[20], **Akihisa Hidaka, MD, PhD**[3], **Michael Hoffmeister, MD, MSc**[21], **Hermann Brenner, MD, MPH**[21,22,23], **Jenny Chang-Claude, PhD**[24,25], **Li Hsu, PhD**[3], **Mark A. Jenkins, PhD**[26], **Yi Lin, MS**[3], **Roger L. Milne, PhD**[26,27,28], **Victor Moreno, PhD**[29,30,31,32], **Polly A. Newcomb, MPH, PhD**[3,33], **Reiko Nishihara, PhD**[1,34], **Mireia Obon-Santacana, MSc, PhD**[29,30,31], **Rish K. Pai, PhD**[35], **Lori C. Sakoda, MPH, PhD**[3,36], **Robert E. Schoen, MD**[37], **Martha L. Slattery, MD, MPH**[38], **Wei Sun, PhD**[3], **Efrat L. Amitay, MPH, PhD**[21], **Elizabeth Alwers, MD, PhD**[21], **Stephen N. Thibodeau, PhD**[39], **Amanda E. Toland, PhD**[40], **Bethany Van Guelpen, PhD**[41,42], **Syed H. Zaidi, PhD**[43], **John D. Potter, PhD**[3,33,44], **Jeffrey A. Meyerhardt, MD, MPH**[45], **Marios Giannakis, MD, PhD**[45,46], **Mingyang Song, MD, ScD**[2,7,8,34], **Jonathan A. Nowak, MD, PhD**[1], **Ulrike Peters, MPH, PhD**[3,33], **Amanda I. Phipps, MPH, PhD**[3,33], **Shuji Ogino, MD, PhD, MS**[1,2,46,47]

[1]Program in MPE Molecular Pathological Epidemiology, Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA;

[2]Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA;

[3]Public Health Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA;

[4]Division of Public Health Sciences, Department of Surgery, Washington University School of Medicine, St Louis, MO;

[5]Division of Gastroenterology, Department of Medicine, Washington University School of Medicine, St. Louis, MO;

[6]Alvin J. Siteman Cancer Center, St Louis, MO;

[7]Clinical and Translational Epidemiology Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA;

Co-corresponding Authors: Tomotaka Ugai, MD, PhD, Program in MPE Molecular Pathological Epidemiology, Department of Pathology, Brigham and Women's Hospital, 221 Longwood Ave., EBRC Room 404, Boston, MA, 02215, USA, Tel: +1-617-525-4304; Fax: +1-617-264-5149, tugai@bwh.harvard.edu, Shuji Ogino, MD, PhD, MS, Program in Molecular Pathological Epidemiology, Department of Pathology, Brigham and Women's Hospital, 221 Longwood Ave., Room EBRC404, Boston, MA, 02215 USA, Tel: +1-617-732-5500; Fax: +1-617-264-5149, sogino@bwh.harvard.edu.
The first four authors contributed equally.
The last four authors contributed equally.

Use of Standardized Official Symbols: We use HUGO (Human Genome Organisation)-approved official symbols (or root symbols) for genes and gene products, including BRAF, ERBB2, KRAS, HRAS, MLH1, MSH2, MSH6, NRAS, and PMS2; all of which are described at www.genenames.org. Gene symbols are italicized whereas symbols for gene products are not italicized.

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[8]Division of Gastroenterology, Massachusetts General Hospital, Boston, MA;

[9]Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA;

[10]Department of Immunology and Infectious Diseases, Harvard T.H. Chan School of Public Health, Boston, MA;

[11]Department of Epidemiology and Population Health, Albert Einstein College of Medicine, Bronx, NY;

[12]Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, MD;

[13]Colorectal Oncogenomics Group, Department of Clinical Pathology, The University of Melbourne, Parkville, Victoria, Australia;

[14]University of Melbourne Centre for Cancer Research, Victorian Comprehensive Cancer Centre, Parkville, Victoria, Australia;

[15]Genetic Medicine and Family Cancer Clinic, The Royal Melbourne Hospital, Parkville, Victoria, Australia;

[16]Department of Epidemiology & Biostatistics, Imperial College London, Norfolk Place, London, UK;

[17]Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, and UPMC Hillman Cancer Center, Pittsburgh, PA;

[18]Lunenfeld Tanenbaum Research Institute, Mount Sinai Hospital, University of Toronto, Toronto, Ontario, Canada;

[19]Nutrition and Metabolism Section, International Agency for Research on Cancer, World Health Organization, Lyon, France;

[20]Department of Radiation Sciences, Oncology Unit, Umeå University, Umeå, Sweden;

[21]Division of Clinical Epidemiology and Aging Research, German Cancer Research Center (DKFZ), Heidelberg, Germany;

[22]Division of Preventive Oncology, German Cancer Research Center (DKFZ), Heidelberg, Germany;

[23]German Cancer Consortium (Deutschen Konsortium für Translationale Krebsforschung), German Cancer Research Center (Deutsches Krebsforschungszentrum), Heidelberg, Germany;

[24]Division of Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany;

[25]Cancer Epidemiology Group, University Medical Center Hamburg-Eppendorf, Hamburg, Germany;

[26]Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Melbourne, Victoria, Australia;

[27]Cancer Epidemiology Division, Cancer Council Victoria, Melbourne, Victoria, Australia;

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[28]Precision Medicine, School of Clinical Sciences at Monash Health, Monash University, Clayton, Victoria, Australia;

[29]Unit of Biomarkers and Susceptibility, Oncology Data Analytics Program, Catalan Institute of Oncology (ICO), L'Hospitalet de Llobregat, Barcelona, Spain;

[30]Colorectal Cancer Group, ONCOBELL Program, Bellvitge Biomedical Research Institute (IDIBELL), L'Hospitalet de Llobregat, Barcelona, Spain;

[31]Consortium for Biomedical Research in Epidemiology and Public Health (CIBERESP), 28029, Madrid, Spain;

[32]Department of Clinical Sciences, Faculty of Medicine, University of Barcelona, Barcelona, Spain;

[33]Department of Epidemiology, University of Washington, Seattle, WA;

[34]Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA;

[35]Department of Laboratory Medicine and Pathology, Mayo Clinic Arizona, Scottsdale, Arizona;

[36]Division of Research, Kaiser Permanente Northern California, Oakland, CA;

[37]Department of Medicine and Epidemiology, University of Pittsburgh Medical Center, Pittsburgh, PA, USA;

[38]Department of Internal Medicine, University of Utah, Salt Lake City, UT;

[39]Division of Laboratory Genetics, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN;

[40]Departments of Cancer Biology and Genetics and Internal Medicine, Comprehensive Cancer Center, The Ohio State University, Columbus, OH;

[41]Department of Radiation Sciences, Oncology Unit, Umeå University, Umeå, Sweden;

[42]Wallenberg Centre for Molecular Medicine, Umeå University, Umeå, Sweden;

[43]Ontario Institute for Cancer Research, Toronto, Ontario, Canada;

[44]Research Centre for Hauora and Health, Massey University, Wellington, New Zealand;

[45]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA;

[46]Broad Institute of MIT and Harvard, Cambridge, MA;

[47]Cancer Immunology and Cancer Epidemiology Programs, Dana-Farber Harvard Cancer Center, Boston, MA.

Abstract

Background: The pathogenic effect of colorectal tumor molecular features may be influenced by several factors, including those related to microbiota, inflammation, metabolism, and epigenetics, which may change along colorectal segments. We hypothesized that the prognostic association of colon cancer location might differ by tumor molecular characteristics.

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Methods: Utilizing a consortium dataset of 13,101 colorectal cancer cases, including 2,994 early-onset cases, we conducted survival analyses of detailed tumor location stratified by statuses of microsatellite instability (MSI), CpG island methylator phenotype (CIMP), and *KRAS* and *BRAF* oncogenic mutation.

Results: There was a statistically significant trend for better colon cancer-specific survival in relation to tumor location from the cecum to sigmoid colon (P_{trend} = 0.002), excluding the rectum. The prognostic association of colon location differed by MSI status ($P_{interaction}$ = 0.001). Non-MSI-high tumors exhibited the cecum-to-sigmoid trend for better colon cancer-specific survival [P_{trend} <0.001; multivariable hazard ratio (HR) for the sigmoid colon (vs. cecum), 0.80; 95% confidence interval (CI), 0.70–0.92], whereas MSI-high tumors demonstrated a suggestive cecum-to-sigmoid trend for worse survival (P_{trend} = 0.020; the corresponding HR, 2.13; 95% CI, 1.15–3.92). The prognostic association of colon tumor location also differed by CIMP status ($P_{interaction}$ = 0.003) but not significantly by age, stage, and other features. Furthermore, MSI-high status was a favorable prognostic indicator in strata of stage.

Conclusions: Both detailed colonic location and tumor molecular features need to be accounted for colon cancer prognostication to advance precision medicine. Our study indicates the important role of large-scale studies to robustly examine detailed colonic subsites in molecular oncology research.

Keywords

biogeography; epigenetics; mismatch repair; molecular pathological epidemiology; young-onset cancer

BACKGROUND

Colorectal cancer consists of a heterogeneous group of tumors with different molecular features by anatomical location [1, 2]. The colon is largely divided into two segments, namely the proximal and distal anatomic segments, using the splenic flexure as a cutpoint. However, it is conceivable that biological characteristics of colorectal cancer may be influenced by a variety of factors, including those related to microbiota, inflammation, metabolism, and epigenetics, which may vary along detailed colorectal location [3–6]. Accumulating evidence indicates that the proportions of colorectal carcinomas positive for high-level microsatellite instability (MSI), CpG island methylator phenotype (CIMP), and *BRAF* mutation gradually increase along more detailed location from the rectum to ascending colon [1, 7–9]. Both MSI status and *BRAF* mutation are established prognostic and predictive biomarkers in colorectal cancer [10–12]. These facts underscore the importance of examining detailed location and molecular biomarkers of colorectal cancer.

Primary tumor location has recently attracted considerable attention as a potential prognostic feature in colon cancer. A meta-analysis has shown that distal colon cancer patients in average survive longer than proximal colon cancer patients [13]; however, a vast majority of published studies used analyses based on the dichotomy design (proximal/right-sided vs. distal/left-sided colon) but not on detailed colonic subsites. Furthermore, whether the prognostic association of detailed colon cancer location differs by clinical and molecular

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characteristics remains uncertain with limited literature data [14–18]. In our current study, we tested a hypothesis that the prognostic association of detailed tumor location might differ by key tumor molecular characteristics.

The incidence of early-onset cancers diagnosed before age 50 in many body sites including the colorectum has been increasing globally for unknown reasons [19–21]. Early-onset colorectal cancer tends to occur more frequently in the distal colon and rectum compared to later-onset colorectal cancer [22, 23]. Considering this intriguing association between colorectal cancer location and age of diagnosis, we examined whether the prognostic association of tumor location might differ by age of diagnosis.

To test our hypotheses, we leveraged a large pooled-consortium dataset of colorectal cancer cases from the Cancer Genome Atlas (TCGA), and 10 studies participating in the Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO).

METHODS

Study Sample

We used a pooled database of 13,101 colorectal cancer cases, including 2,994 early-onset cases diagnosed before age 50 years, which had available data on patient survival, tumor location, and tumor molecular characteristics. The database consisted of TCGA and the following 10 GECCO studies: the Colon Cancer Family Registry (CCFR) [24], Cancer Prevention Study II (CPS-II) [25], the German Darmkrebs: Chancen der Verhütung durch Screening Study (DACHS) [26], the Diet, Activity and Lifestyle Study (DALS) [27], the Early Detection Research Network (EDRN) [28], the European Prospective Investigation into Cancer - Sweden (EPIC_Sweden) [29], the Health Professionals Follow-up Study (HPFS) [30], the Melbourne Collaborative Cohort Study (MCCS) [31], the Nurses' Health Study (NHS) [32], and the Northern Sweden Health and Disease Study (NSHDS) [33]. Each study was approved by their relevant research ethics committee or institutional review board, and all study participants provided informed consent. All colorectal adenocarcinoma cases were identified and confirmed by review of medical records, pathological reports, and/or death certificates. Participant demographics were obtained by review of medical records. Protocols for assessing colorectal cancer-specific and overall mortality in each study have been described previously [12]. Most studies ascertained mortality status through state or national death registries, or state cancer registries, with cause of death verified by death certificates and/or medical records. NSHDS and TCGA lacked data on colorectal cancer-specific mortality. Details of the study designs and the study populations were described in previous papers [12, 34]. Participant demographics and colorectal cancer molecular characteristics according to study are shown in Supplementary Table 1.

Assessment of Tumor Location and Tumor Molecular Characteristics

Primary tumor location data (derived from medical records in each study) were documented based on the International Classification of Disease (ICD). In this analysis, cases diagnosed with synchronous carcinomas at multiple sites (N=45) were excluded. To harmonize the location data across studies, we included hepatic flexure in the transverse colon, splenic

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flexure in the descending colon, and rectosigmoid junction in the rectum. The location variable had 6 anatomical subsites, namely cecum, ascending colon, transverse colon, descending colon, sigmoid colon, and rectum.

Testing for tumor molecular markers was performed by each study and according to individual study protocols [12, 35]. Detailed analysis methods for MSI, CIMP, *BRAF* mutation, and *KRAS* mutation are described in Supplementary Methods and Supplementary Tables 2 **to** 5. We compared the results of MSI status, *BRAF* mutation, and *KRAS* mutation for participants that had both existing tumor marker data and targeted sequencing data [36]. The tumor molecular marker status by both approaches were highly concordant with more than 90% agreement for MSI status, *BRAF* mutation, and *KRAS* mutation [36].

Statistical Analyses

All statistical analyses were conducted using STATA software (version 15.1, Stata Corp., College Station, TX). All P values were two-sided and the two-sided α level of 0.005 was used as recommended by the expert statisticians [37].

We used multivariable Cox proportional hazards models to estimate hazards ratio (HR) for colorectal cancer-specific mortality (a primary outcome endpoint) and overall mortality (a secondary endpoint) according to detailed subsites. The time axis was defined as days from diagnosis, with left truncation for participating studies that enrolled cases after colorectal cancer diagnosis. Proportional hazards assumptions were assessed by Schoenfeld residuals and found to be justified. We conducted analyses in two ways. The first model (minimally adjusted model) was adjusted for a limited number of variables, including sex (female vs. male), age at diagnosis (continuous), disease stage (I vs. II-III vs. IV), and study (as a stratification variable). The second model (multivariable "fully-adjusted" model) was adjusted for the aforementioned variables, family history of colorectal cancer in any first degree relative (yes vs. no), year of diagnosis (before 1995 vs. 1995–2000 vs. after 2000), MSI status (MSI-high vs. non-MSI-high), CIMP status (high vs. low/negative), *BRAF* mutation (mutant vs. wild-type), and *KRAS* mutation (mutant vs. wild-type). We conducted tests of heterogeneity using the Q statistic and observed no statistically significant heterogeneity between studies in the prognostic association of detailed primary tumor location ($P_{heterogeneity} > 0.15$). We therefore pooled data from the studies and adjusted for each study as a stratification variable. Missing values for covariates were treated as separate indicator variables in the models. To assess differences in categorical variables across tumor subsites, the chi-square test was performed. To assess differences in continuous variables across tumor subsites, an analysis of variance assuming equal variances was performed.

Our primary hypothesis testing was to assess whether the prognostic association of an ordinal colon location variable (cecum, 1; ascending, 2; transverse, 3; descending, 4; and sigmoid, 5) might differ by clinical and key tumor molecular characteristics. We used the Wald test on an interaction term between the ordinal colon location variable and each tumor marker in the multivariable model excluding rectal cancer cases. We further examined the statistical interaction between the ordinal subsite variable and each of these variables [age (continuous), sex (female vs. male), and disease stage (I to III vs. IV)]. Considering different patient management practice for rectal cancer, we did assess the statistical trend from the

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cecum to sigmoid colon (excluding rectum). Nonetheless, the analysis model included rectal cancer patients with the rectal location variable as a separate indicator variable. In this fashion, we could include exactly the same population (i.e., all available colorectal cancer patients) as that used in our secondary analyses to assess HR for each of the colorectal subsites including the rectum. In our secondary analyses, we assessed an HR for each site (including the rectum) compared to the cecum.

We also conducted a meta-analysis with random-effects models as a sensitivity analysis. We assessed the prognostic association of colon cancer in each study separately. Then, each study-specific HR was pooled using the random-effect meta-analysis method.

RESULTS

To examine the prognostic role of colorectal tumor location, we analyzed the pooled dataset of 13,101 colorectal cancer cases. Table 1 summarizes clinical and molecular characteristics (the statuses of MSI, CIMP, *KRAS* mutation, and *BRAF* mutation) according to primary tumor location. Higher fractions of early-onset colorectal cancers were located in the rectum (38%) compared to later-onset colorectal cancers (28%). Proportions of stage IV cases were similar across primary tumor location. Molecular characteristics by disease stage and age at diagnosis are presented in Supplementary Table 6. Compared to later-onset cases, early-onset cases showed higher prevalence of MSI-high status and lower prevalence of CIMP-high status and *BRAF* mutations.

There was a statistically significant trend for better colon cancer-specific survival in relation to tumor location from the cecum to sigmoid colon (P_{trend} = 0.002; Table 2 and Figure 1). In contrast, compared to the cecum, rectal location was not significantly associated with colorectal cancer-specific survival [multivariable hazard ratio (HR), 1.10; 95% confidence interval (CI), 0.97–1.25; P = 0.12].

In our primary hypothesis testing, there was a statistically significant interaction between colonic location and MSI status for colon cancer-specific survival ($P_{interaction}$ = 0.001; Table 2). Non-MSI-high tumors exhibited the cecum-to-sigmoid trend for better colon cancer-specific survival [P_{trend} <0.001; multivariable HR for the sigmoid colon (vs. cecum), 0.80; 95% confidence interval (CI), 0.70–0.92; Figure 2], whereas MSI-high tumors demonstrated a suggestive opposite cecum-to-sigmoid trend for worse survival (P_{trend} = 0.020; the corresponding HR, 2.13; 95% CI, 1.15–3.92; Figure 3). Similar results were observed in analyses using overall survival as a secondary endpoint. A statistically significant interaction between colonic location and CIMP status was also observed for colon-cancer specific mortality ($P_{interaction}$ = 0.003). CIMP-low/negative tumors exhibited the cecum-to-sigmoid trend for better colon cancer-specific survival [P_{trend} <0.001; multivariable HR for the sigmoid colon (vs. cecum), 0.75; 95% CI, 0.64–0.88], whereas CIMP-high tumors did not demonstrate such a trend (P_{trend} = 0.10). No significant interaction was observed between tumor location and *BRAF* or *KRAS* mutation status at the stringent α level of 0.005 ($P_{interaction}$ > 0.020).

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We conducted stratified analyses by age at diagnosis (<50, 50–69, ≥70 years), sex (female vs. male) or disease stage (I to III vs. IV) (Table 3 and Supplementary Tables 7–8). The prognostic association of tumor location did not significantly differ by age of diagnosis, sex, or stage ($P_{interaction} > 0.17$).

In a sensitivity analysis, we conducted a random-effect meta-analysis that summarized multivariable HR of each individual study (Supplementary Figures 1–2, Supplementary Table 9). In general, the meta-analysis results were similar to those in the pooled analysis.

To provide additional information on the prognostic roles of the tumor markers, we conducted survival analyses of the statuses of MSI, CIMP, *KRAS* mutation, and *BRAF* mutation in overall cases as well as strata of disease stage (Table 4). MSI was consistently associated with better survival in all stages (multivariable-adjusted CRC-specific mortality HR for MSI-high vs. non-MSI-high, 0.35; 95% CI, 0.29–0.42, for overall cases).

DISCUSSION

Colorectal carcinoma represents a group of heterogenous neoplastic diseases that arise from colorectal epithelia, interacting with the local microenvironment that includes the microbiome [38, 39]. As the luminal contents of the colorectum move from the cecum to rectum, its microbial composition changes. Hence, investigations into heterogeneity of colorectal carcinomas according to detailed tumor location are of particular importance. In this study, non-MSI-high tumors exhibited the cecum-to-sigmoid trend for better colon cancer-specific survival, whereas MSI-high tumors showed a suggestive opposite cecum-to-sigmoid trend for worse survival, indicating the biological and clinical significance of both MSI status and detailed information on colorectal tumor location.

This study indicates that the prognostic role of tumor location differs by molecular features, in particular the MSI and CIMP statuses, which correlate with each other [40]. Notably, a significant trend for better survival from the cecum to sigmoid colon was observed in non-MSI-high cases and CIMP-low/negative cases. Most CIMP-high colorectal carcinomas exhibit hypermethylation of *MLH1* promoter CpG island, which is a major cause of the MSI-high phenotype [40]. A prior analysis using the CCFR, one of the participating cohorts in the current study, showed a better prognostic association of distal (vs. proximal) location in non-MSI-high colorectal cancer, while statistical power was limited in MSI-high tumors [16]. Another previous study showed that, compared to distal tumor localization, proximal localization was associated with favorable survival in stage III *RAS*-mutated colon cancer, but with unfavorable survival in stage III cancer with wild-type *RAS* and *BRAF* [41]. That study [41] used the colon dichotomy design. As tumor status (MSI status, *BRAF* and *KRAS* mutations, etc.) has become crucial for patient management [12, 41], our new knowledge on the prognostic role of detailed tumor location in strata of key molecular features can likely inform future personalized oncology practice.

The colorectal continuum model [5] is a well-recognized paradigm [42–45]. Recently, the colorectal continuum model has shown its relevance to molecular pathology of early-onset colorectal cancer [46]. However, the literature data on the prognostic relevance of the

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colorectal continuum model remain scarce. A meta-analysis has shown better prognosis associated with distal (left-sided) colon cancer compared to proximal (right-sided) colon cancers [13]. To our knowledge, only few studies have evaluated the prognostic association of more detailed colorectal tumor location with reasonable statistical power [14–18]. One study [15] showed a cecum-to-sigmoid trend for better survival, but it included only 895 stage III colon cancer cases with no tumor molecular data. Other studies did not show an apparent cecum-to-sigmoid trend [14, 16–18]. In the present study, we showed that the prognostic association of tumor location differed by MSI (and CIMP) status. Previous inconsistent results could be due to heterogenous prognostic impact of colorectal tumor location by molecular characteristics (especially MSI status); therefore, both tumor molecular features and detailed subsite data should be integrated in clinical oncology research.

In addition to the statuses of MSI, CIMP, *BRAF*, and *KRAS* mutation, several factors might influence the prognostic association of tumor location. Evidence suggests that copy number alterations of various genes play an important role in the development and progression of colorectal cancer [47]. Copy number alterations in early-onset colorectal cancer are particularly interesting future research topics. Anti-tumor immune response and the tumor microbiota have been associated with colorectal cancer survival [48–53]. Characterization of additional somatic mutations (e.g., *HRAS* and *NRAS* mutations), copy number alterations, gene amplification/expression [e.g., ERBB2 (HER2) expression], anti-tumor immune response, microbiota, and other biomarkers will allow more refined and detailed classification of colorectal cancer subtypes in future studies, which will further elucidate the prognostic association of tumor location.

Early-onset cancers that occur in over 10 different organ systems of adults before age 50 years have shown increased incidence in many parts of the world [54]. Those include early-onset cancers in not only the colorectum but also other gastrointestinal organs such as the esophagus, stomach, liver, gallbladder, extrahepatic bile duct, and pancreas [54]. Among them, early-onset colorectal cancer has become an intensive research topic. Considering the predilection of early-onset colorectal cancer to distal location, we investigated the prognostic significance of detailed tumor location by age at diagnosis. Notably, the prognostic association of tumor location did not significantly differ by age of diagnosis. Evidence indicates that there are differences in tumor characteristics (including MSI status, consensus molecular subtypes, key driver gene mutations, epigenetic features such as LINE-1 hypomethylation, and immune cell infiltrates) between age groups [19, 55–57], which might affect prognosis of early-onset colorectal cancer. Further research is warranted to clarify prognostic factors in early-onset colorectal cancer.

Our finding could be partly due to differential presence of intratumor bacteria along colonic subsites. Compelling evidence indicates that the microbiome can contribute to colorectal tumor development and progression [43, 58–60]. The composition of the intratumoral bacteria varies according to tumor locations in the colorectum [61–63]. A study has shown that the proportion of *Fusobacterium nucleatum*-high colorectal cancers gradually decreases from the cecum to rectum [64]. Evidence also indicates that intratumoral *F. nucleatum* is associated with MSI status and worse colorectal cancer survival [51, 65]. Further research is

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needed to clarify possibly interactive roles of tumor location and the microbiota in colorectal cancer prognosis.

We acknowledge limitations of the current study. First, cancer treatment information was largely unavailable. However, besides rectal cancer, it is unlikely that medical treatment strategies substantially differed by tumor location in each stage stratum during the study periods [66]. Second, it is possible that distal tumors might be detected in average earlier than proximal tumors due to higher prevalence of detectable bleeding and bowel obstruction in distal colorectal cancer patients. However, proportions of stage IV cases were similar across each subsite in this study and we adjusted for stage in our survival analyses. Third, testing for tumor molecular markers were performed using different protocols across the participating studies. However, we showed good concordance in certain molecular markers (such as MSI, *KRAS*, and *BRAF*) between the previous assays and newly-designed targeted sequencing assay [36]. Fourth, there existed measurement errors in clinical and survival data, which may be heterogeneous between cohorts. Additionally, we pooled different studies with different sampling frames (e.g., population-based or clinic-based), which might have resulted in the slightly higher proportions of MSI-high tumors and *KRAS* wild-type tumors than those reported in previous studies [11]. Nonetheless, we observed comparable prognostic associations of detailed tumor location across studies (see Supplementary Figures 1–2). Fifth, most study participants were non-Hispanic Whites. Therefore, our findings need to be validated in other populations. Lastly, findings from our study population may not be the same as those from a contemporary cohort, as most of the cases were diagnosed before 2010. Considering prolonged survival of patients with high-stage MSI-high tumors after introduction of immune checkpoint inhibitors, our findings should be replicated in more recent datasets, especially for patients with MSI-high tumors.

This study has notable strengths. First, the analysis of individual-level data with the large sample size allowed us to evaluate the prognostic significance of colorectal subsite information in strata of tumor molecular features with adequate statistical power. Second, the large sample size using the well-annotated cohorts enabled us to analyze strata of important patient subgroups, including early-onset colorectal cancer, incidence of which has been increasing worldwide for recent decades. Third, the study participants were drawn from multiple studies in several countries, which increases the generalizability of our findings.

In conclusion, the prognostic association of primary tumor location differed by MSI (and CIMP) status. There exists the cecum-to-sigmoid trend for better colon cancer-specific survival in non-MSI-high colon cancer, whereas there may be an opposite cecum-to-sigmoid trend for worse colon cancer-specific survival in MSI-high colon cancer. Both detailed colonic location and tumor molecular features need to be accounted for colon cancer prognostication to advance precision medicine. Furthermore, our study indicates the important role of large-scale studies to robustly examine detailed colonic subsites in molecular oncology research.

Supplementary Material

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Conflict of Interest:

A.T.C. previously served as a consultant for Bayer Healthcare and Pfizer Inc. A.T.C. is a Stuart and Suzanne Steele MGH Research Scholar. M.G. was on an advisory board for AstraZeneca and has received research funding from Bristol-Myers Squibb, Merck and Servier. R.N. is currently employed by Pfizer Inc. The other authors declare no potential conflicts of interest. The content is solely the responsibility of the authors and does not necessarily represent the official views of NIH. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Abbreviations:

AJCC	American Joint Committee on Cancer

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CCFR	Colon Cancer Family Registry
CI	confidence interval
CIMP	CpG island methylator phenotype
CPS-II	Cancer Prevention Study II
DACHS	Darmkrebs: Chancen der Verhütung durch Screening Study
DALS	Diet, Activity and Lifestyle Study
EDRN	Early Detection Research Network
EPIC_Sweden	European Prospective Investigation into Cancer_Sweden
GECCO	Genetics and Epidemiology of Colorectal Cancer Consortium
HPFS	Health Professionals Follow-up Study
HR	hazard ratio
ICD	International Classification of Disease
MCCS	Melbourne Collaborative Cohort Study
MSI	microsatellite instability
NHS	Nurses' Health Study
NSHDS	Northern Sweden Health and Disease Study
PCR	polymerase chain reaction
SD	standard deviation
TCGA	The Cancer Genome Atlas

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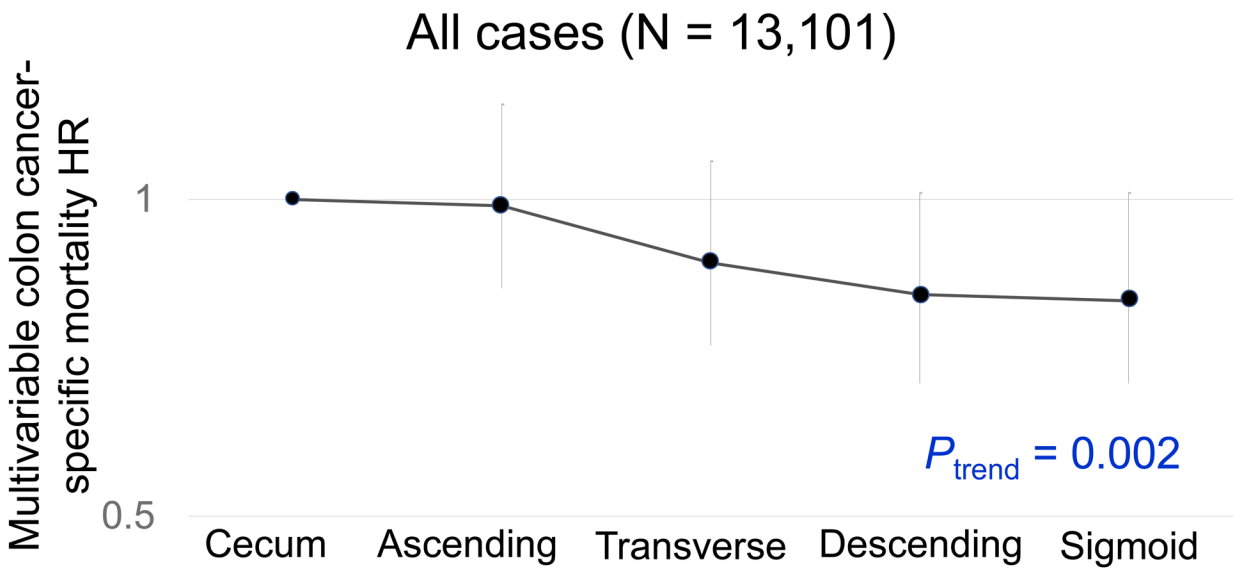

Figure 1.
Colon Cancer-Specific Mortality in Relation to Primary Tumor Location.
Abbreviations: HR, hazard ratio.

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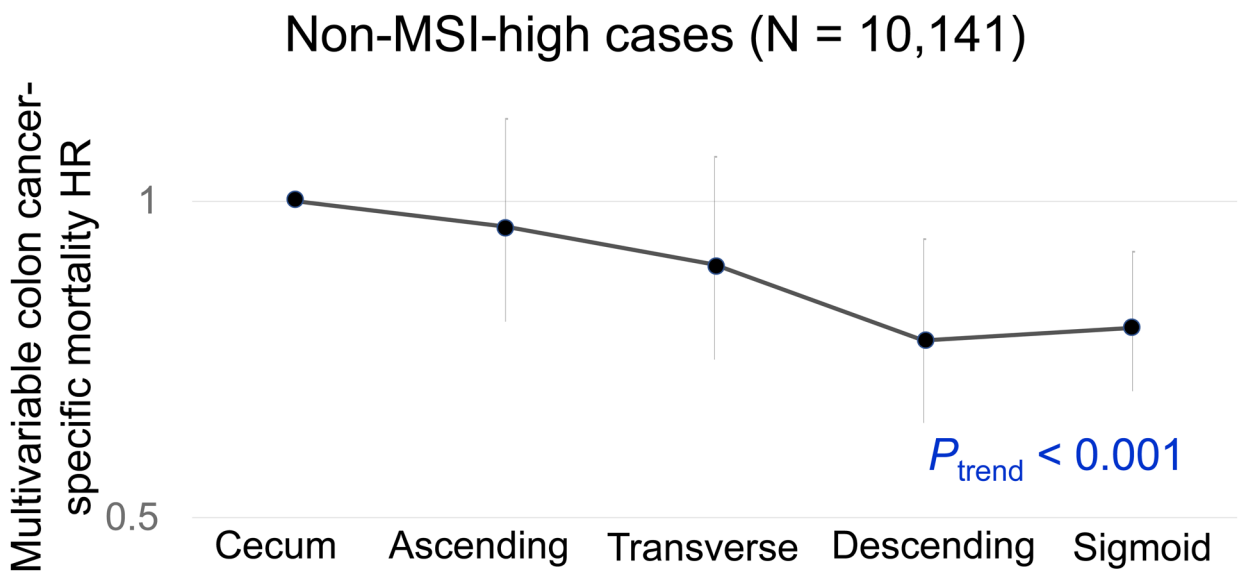

Figure 2.

Colon Cancer-Specific Mortality in Relation to Primary Tumor Location in non-MSI-high tumors.

Abbreviations: HR, hazard ratio; MSI, microsatellite instability.

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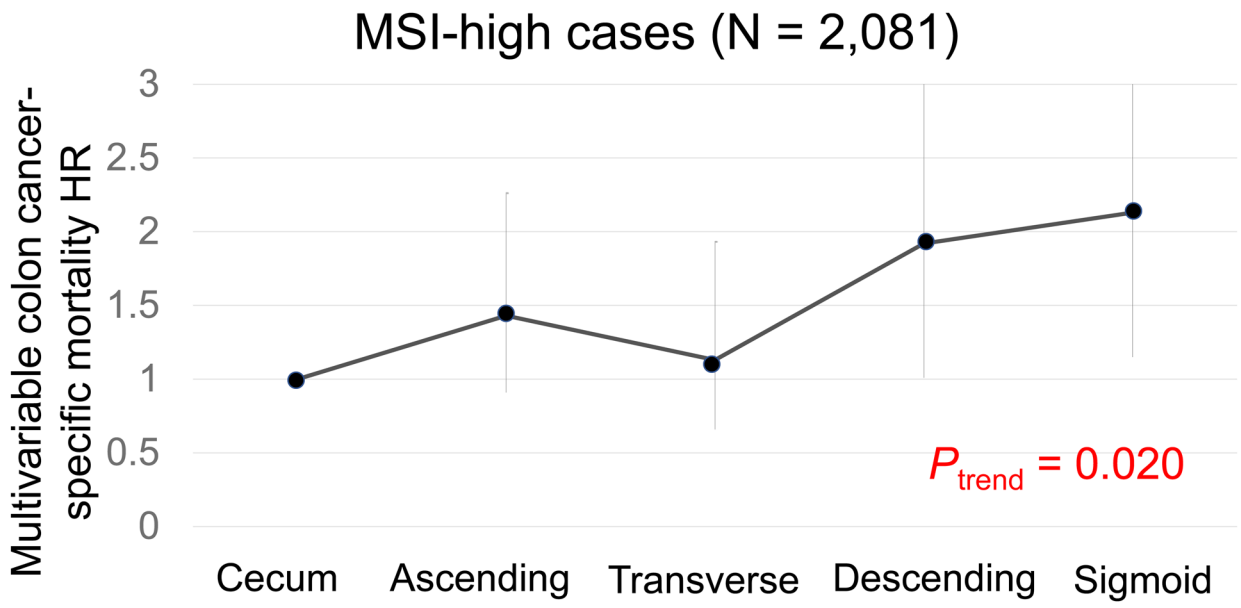

Figure 3.

Colon Cancer-Specific Mortality in Relation to Primary Tumor Location in non-MSI-high tumors.

Abbreviations: CRC, colorectal cancer; HR, hazard ratio; MSI, microsatellite instability.

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Table 1.

Participant Demographics and Colorectal Cancer Molecular Characteristics by Primary Tumor Location

Characteristics[a]	Total No.	Cecum	Ascending colon	Transverse colon	Descending colon	Sigmoid colon	Rectum	P value[b]
All cases	13,101	1,968	1,871	1,301	968	3,057	3,936	
Sex								<0.001
Female	6,339 (48%)	1,036 (53%)	1,044 (56%)	663 (51%)	481 (50%)	1,460 (48%)	1,655 (42%)	
Male	6,762 (52%)	932 (47%)	827 (44%)	638 (49%)	487 (50%)	1,597 (52%)	2,281 (58%)	
Mean age ± SD	61.3 ± 13.3	63.2 ± 13.0	64.3 ± 13.4	62.7 ± 13.8	60.1 ± 13.6	61.1 ± 12.5	58.8 ± 13.2	<0.001
<50 (Early-onset)	2,994 (23%)	360 (18%)	315 (17%)	261 (20%)	256 (26%)	662 (22%)	1,140 (29%)	
50–69	6,071 (46%)	879 (45%)	793 (42%)	561 (43%)	437 (45%)	1,512 (49%)	1,889 (48%)	
≥70	4,036 (31%)	729 (37%)	763 (41%)	479 (37%)	275 (28%)	883 (29%)	907 (23%)	
Year of diagnosis								<0.001
Before 1995	1,963 (15%)	345 (18%)	288 (16%)	231 (18%)	173 (18%)	571 (19%)	355 (9%)	
1995–2000	5,522 (43%)	915 (48%)	724 (39%)	568 (44%)	398 (42%)	1,284 (43%)	1,633 (43%)	
After 2000	5,345 (42%)	666 (35%)	824 (45%)	486 (38%)	376 (40%)	1,145 (38%)	1,848 (48%)	
Family history of colorectal cancer								<0.001
Absent	9,531 (75%)	1,404 (74%)	1,289 (72%)	898 (71%)	676 (72%)	2,303 (78%)	2,961 (78%)	
Present	3,125 (25%)	495 (26%)	510 (28%)	360 (29%)	261 (28%)	659 (22%)	840 (22%)	
AJCC disease stage								<0.001
I	3,062 (25%)	425 (23%)	415 (23%)	248 (20%)	171 (19%)	775 (28%)	1,028 (29%)	
II and III	7,677 (63%)	1,206 (65%)	1,187 (67%)	836 (69%)	630 (68%)	1,686 (60%)	2,132 (60%)	
IV	1,378 (11%)	232 (12%)	166 (8.8%)	130 (11%)	122 (13%)	333 (12%)	395 (11%)	
MSI status								<0.001
Non-MSI-high	10,141 (83%)	1,311 (71%)	1,093 (62%)	786 (67%)	764 (84%)	2,710 (94%)	3,477 (95%)	
MSI-high	2,081 (17%)	528 (29%)	669 (38%)	393 (33%)	148 (16%)	170 (5.9%)	173 (4.7%)	
CIMP status								<0.001
Low/negative	7,701 (84%)	1057 (72%)	838 (62%)	664 (71%)	589 (90%)	2,024 (94%)	2,529 (95%)	
High	1,509 (16%)	401 (28%)	520 (38%)	275 (29%)	64 (10%)	124 (5.8%)	125 (4.7%)	
BRAF								<0.001
Wild-type	10,252 (89%)	1,425 (82%)	1,236 (74%)	887 (79%)	773 (92%)	2,576 (95%)	3,355 (96%)	
Mutant	1,315 (11%)	314 (18%)	440 (26%)	234 (21%)	68 (8.1%)	137 (5.1%)	122 (3.5%)	

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Characteristics[a]	Total No.	Cecum	Ascending colon	Transverse colon	Descending colon	Sigmoid colon	Rectum	P value[b]
KRAS								<0.001
Wild-type	6,494 (67%)	828 (55%)	936 (67%)	674 (71%)	480 (67%)	1,641 (70%)	1,935 (68%)	
Mutant	3,269 (33%)	681 (45%)	467 (33%)	274 (29%)	241 (33%)	701 (30%)	905 (32%)	

[a]Percentage indicates the proportion of patients with a specific patient molecular characteristic among all patients or in strata of tumor location (cecum, ascending colon, transverse colon, descending colon, sigmoid colon, and rectum).

[b]To compare categorical data between subgroups classified by the tumor location, the chi-square test was performed. To compare continuous variables, an analysis of variance was performed.

Abbreviations: AJCC, American Joint Committee on Cancer; CIMP, CpG island methylator phenotype; MSI, microsatellite instability; SD, standard deviation.

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Table 2.

Colorectal Cancer-Specific and Overall Mortality in Relation to Primary Tumor Location by Tumor Molecular Features

	Cecum	Ascending colon	Transverse colon	Descending colon	Sigmoid colon	P_{trend} [c]	$P_{interaction}$ [d]	Rectum
CRC-specific mortality								
All cases								
No. of cases	1,846	1,732	1,241	925	2,866			3,685
No. of events	418	335	246	194	592			884
Minimally-adjusted HR (95% CI)[a]	1 (reference)	0.91 (0.79–1.06)	0.88 (0.75–1.03)	0.90 (0.76–1.07)	0.93 (0.82–1.05)	0.12		1.21 (1.07–1.37)
Multivariable-adjusted HR (95% CI)[b]	1 (reference)	0.99 (0.86–1.15)	0.90 (0.77–1.06)	0.85 (0.71–1.01)	0.84 (0.74–0.96)	0.002		1.10 (0.97–1.25)
MSI status							0.001	
Non-MSI-high								
No. of cases	1,224	1,000	752	726	2,532			3,240
No. of events	363	258	201	167	549			813
Minimally-adjusted HR (95% CI)[a]	1 (reference)	0.94 (0.80–1.11)	0.88 (0.74–1.05)	0.74 (0.62–0.90)	0.75 (0.66–0.86)	<0.001		0.99 (0.87–1.13)
Multivariable-adjusted HR (95% CI)[b]	1 (reference)	0.96 (0.81–1.13)	0.90 (0.75–1.07)	0.78 (0.65–0.94)	0.80 (0.70–0.92)	<0.001		1.07 (0.94–1.22)
MSI-high								
No. of cases	499	626	374	147	163			169
No. of events	33	55	27	14	17			21
Minimally-adjusted HR (95% CI)[a]	1 (reference)	1.43 (0.91–2.26)	1.13 (0.66–1.93)	1.79 (0.94–3.38)	2.06 (1.13–3.77)	0.028		2.26 (1.24–4.09)
Multivariable-adjusted HR (95% CI)[b]	1 (reference)	1.53 (0.97–2.42)	1.20 (0.70–2.06)	1.92 (1.01–3.66)	2.13 (1.15–3.92)	0.020		2.33 (1.27–4.30)
CIMP status							0.003	
CIMP-low/negative								
No. of cases	993	759	633	556	1,853			2,321
No. of events	256	167	155	109	395			592
Minimally-adjusted HR (95% CI)[a]	1 (reference)	0.82 (0.67–0.99)	0.88 (0.72–1.08)	0.69 (0.55–0.87)	0.77 (0.66–0.90)	0.002		1.10 (0.95–1.28)
Multivariable-adjusted HR (95% CI)[b]	1 (reference)	0.85 (0.70–1.03)	0.89 (0.73–1.09)	0.68 (0.54–0.86)	0.75 (0.64–0.88)	<0.001		1.07 (0.92–1.25)
CIMP-high								
No. of cases	355	470	253	59	115			115

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	Cecum	Ascending colon	Transverse colon	Descending colon	Sigmoid colon	P_{trend} [c]	$P_{interaction}$ [d]	Rectum
No. of events	63	91	49	20	30			31
Minimally-adjusted HR (95% CI)[a]	1 (reference)	1.50 (1.07–2.11)	1.48 (1.01–2.18)	1.91 (1.12–3.25)	1.53 (0.97–2.41)	0.024		2.16 (1.36–3.41)
Multivariable-adjusted HR (95% CI)[b]	1 (reference)	1.54 (1.10–2.17)	1.68 (1.13–2.48)	1.93 (1.12–3.35)	1.27 (0.80–2.02)	0.10		1.70 (0.80–2.02)
***BRAF* mutation status**							0.021	
***BRAF* wild-type**								
No. of cases	1,338	1,140	852	738	2,406			3,128
No. of events	336	230	180	154	514			750
Minimally-adjusted HR (95% CI)[a]	1 (reference)	0.89 (0.75–1.05)	0.82 (0.68–0.98)	0.78 (0.65–0.95)	0.85 (0.74–0.98)	0.030		1.14 (1.00–1.30)
Multivariable-adjusted HR (95% CI)[b]	1 (reference)	0.96 (0.81–1.13)	0.86 (0.71–1.03)	0.77 (0.63–0.93)	0.81 (0.70–0.93)	0.001		1.08 (0.95–1.24)
***BRAF* mutant**								
No. of cases	283	403	215	63	127			116
No. of events	49	85	50	16	37			32
Minimally-adjusted HR (95% CI)[a]	1 (reference)	1.16 (0.80–1.68)	1.30 (0.87–1.95)	1.33 (0.74–2.41)	1.62 (1.03–2.54)	0.034		1.68 (1.04–2.70)
Multivariable-adjusted HR (95% CI)[b]	1 (reference)	1.14 (0.79–1.66)	1.30 (0.86–1.96)	1.12 (0.61–2.05)	1.12 (0.70–1.80)	0.58		1.25 (0.76–2.05)
***KRAS* mutation status**							0.86	
***KRAS* wild-type**								
No. of cases	769	858	640	451	1,520			1,780
No. of events	170	164	137	99	337			438
Minimally-adjusted HR (95% CI)[a]	1 (reference)	0.89 (0.71–1.10)	0.93 (0.74–1.16)	0.95 (0.74–1.21)	0.90 (0.75–1.09)	0.49		1.16 (0.96–1.39)
Multivariable-adjusted HR (95% CI)[b]	1 (reference)	0.91 (0.73–1.13)	0.91 (0.72–1.14)	0.87 (0.68–1.13)	0.79 (0.65–0.96)	0.015		1.03 (0.85–1.24)
***KRAS* mutant**								
No. of cases	625	413	254	228	639			822
No. of events	187	108	70	64	145			238
Minimally-adjusted HR (95% CI)[a]	1 (reference)	1.04 (0.82–1.32)	0.88 (0.67–1.17)	0.81 (0.61–1.08)	0.83 (0.67–1.04)	0.039		1.25 (1.02–1.52)
Multivariable-adjusted HR (95% CI)[b]	1 (reference)	1.06 (0.83–1.35)	0.94 (0.71–1.24)	0.81 (0.61–1.08)	0.81 (0.65–1.01)	0.019		1.20 (0.98–1.47)
Overall mortality								
All cases								
No. of cases	1,968	1,871	1,301	968	3,057			3,936

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	Cecum	Ascending colon	Transverse colon	Descending colon	Sigmoid colon	P_{trend} [c]	$P_{interaction}$ [d]	Rectum
No. of events	872	796	536	394	1,253			1,680
Minimally-adjusted HR (95% CI)[a]	1 (reference)	0.98 (0.89–1.08)	0.96 (0.86–1.07)	0.94 (0.83–1.06)	0.94 (0.86–1.03)	0.056		1.15 (1.05–1.25)
Multivariable-adjusted HR (95% CI)[b]	1 (reference)	1.02 (0.92–1.12)	0.97 (0.87–1.08)	0.93 (0.83–1.05)	0.91 (0.83–0.99)	0.004		1.10 (1.01–1.20)
MSI status							<0.001	
Non-MSI-high								
No. of cases	1,311	1,093	786	764	2,710			3,477
No. of events	652	489	370	325	1,130			1,511
Minimally-adjusted HR (95% CI)[a]	1 (reference)	0.92 (0.81–1.03)	0.90 (0.79–1.03)	0.84 (0.73–0.96)	0.81 (0.74–0.90)	<0.001		1.00 (0.91–1.10)
Multivariable-adjusted HR (95% CI)[b]	1 (reference)	0.93 (0.83–1.05)	0.92 (0.81–1.05)	0.86 (0.76–0.99)	0.84 (0.76–0.93)	<0.001		1.04 (0.94–1.14)
MSI-high								
No. of cases	528	669	393	148	170			173
No. of events	171	260	123	47	64			61
Minimally-adjusted HR (95% CI)[a]	1 (reference)	1.27 (1.04–1.56)	1.20 (0.95–1.53)	1.08 (0.77–1.50)	1.58 (1.18–2.13)	0.020		1.37 (1.00–1.89)
Multivariable-adjusted HR (95% CI)[b]	1 (reference)	1.32 (1.08–1.62)	1.21 (0.95–1.54)	1.14 (0.81–1.58)	1.70 (1.26–2.29)	0.006		1.48 (1.07–2.06)
CIMP status							0.019	
CIMP-low/negative								
No. of cases	1,057	838	664	589	2,024			2,529
No. of events	491	350	296	244	849			1,140
Minimally-adjusted HR (95% CI)[a]	1 (reference)	0.83 (0.73–0.96)	0.90 (0.78–1.04)	0.81 (0.70–0.95)	0.81 (0.72–0.90)	0.001		1.03 (0.93–1.15)
Multivariable-adjusted HR (95% CI)[b]	1 (reference)	0.86 (0.75–0.99)	0.92 (0.79–1.06)	0.81 (0.69–0.94)	0.79 (0.71–0.89)	<0.001		1.01 (0.90–1.13)
CIMP-high								
No. of cases	401	520	275	64	124			125
No. of events	183	268	123	38	58			54
Minimally-adjusted HR (95% CI)[a]	1 (reference)	1.42 (1.14–1.74)	1.29 (1.02–1.63)	1.33 (0.93–1.91)	1.18 (0.87–1.59)	0.17		1.65 (1.19–2.27)
Multivariable-adjusted HR (95% CI)[b]	1 (reference)	1.43 (1.17–1.75)	1.27 (1.00–1.62)	1.34 (0.93–1.94)	1.11 (0.81–1.51)	0.33		1.51 (1.09–2.10)
***BRAF* mutation status**							0.024	
***BRAF* wild-type**								
No. of cases	1,425	1,236	887	773	2,576			3,355

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	Cecum	Ascending colon	Transverse colon	Descending colon	Sigmoid colon	P_{trend} [c]	$P_{interaction}$ [d]	Rectum
No. of events	647	510	373	315	1,054			1,443
Minimally-adjusted HR (95% CI)[a]	1 (reference)	0.96 (0.86–1.08)	0.93 (0.81–1.05)	0.90 (0.78–1.03)	0.90 (0.81–0.99)	0.020		1.12 (1.02–1.23)
Multivariable-adjusted HR (95% CI)[b]	1 (reference)	1.00 (0.89–1.13)	0.95 (0.84–1.08)	0.90 (0.78–1.03)	0.88 (0.79–0.97)	0.002		1.09 (0.99–1.21)
***BRAF* mutant**								
No. of cases	314	440	234	68	137			122
No. of events	145	220	115	36	74			58
Minimally-adjusted HR (95% CI)[a]	1 (reference)	1.16 (0.93–1.45)	1.19 (0.93–1.53)	1.19 (0.82–1.74)	1.28 (0.96–1.72)	0.092		1.36 (0.99–1.87)
Multivariable-adjusted HR (95% CI)[b]	1 (reference)	1.15 (0.92–1.44)	1.15 (0.89–1.48)	1.11 (0.76–1.63)	1.14 (0.84–1.55)	0.37		1.29 (0.91–1.82)
***KRAS* mutation status**							0.99	
***KRAS* wild-type**								
No. of cases	828	936	674	480	1,641			1,935
No. of events	363	437	310	208	684			847
Minimally-adjusted HR (95% CI)[a]	1 (reference)	1.09 (0.95–1.26)	1.08 (0.93–1.26)	1.03 (0.86–1.22)	0.91 (0.80–1.04)	0.026		1.12 (0.99–1.27)
Multivariable-adjusted HR (95% CI)[b]	1 (reference)	1.08 (0.94–1.25)	1.06 (0.91–1.23)	1.01 (0.85–1.21)	0.88 (0.77–1.01)	0.013		1.07 (0.94–1.23)
***KRAS* mutant**								
No. of cases	681	467	274	241	701			905
No. of events	344	219	128	118	307			422
Minimally-adjusted HR (95% CI)[a]	1 (reference)	1.05 (0.88–1.24)	0.89 (0.73–1.10)	0.90 (0.73–1.11)	0.85 (0.73–1.00)	0.018		1.11 (0.95–1.28)
Multivariable-adjusted HR (95% CI)[b]	1 (reference)	1.06 (0.89–1.26)	0.92 (0.75–1.14)	0.92 (0.74–1.13)	0.84 (0.72–0.99)	0.012		1.10 (0.95–1.28)

[a] Adjusted for sex, age, disease stage, and study (as strata).

[b] Adjusted for sex, age, disease stage, study (as strata), family history of colorectal cancer, year of diagnosis, MSI status, CIMP status, *BRAF* mutation, and *KRAS* mutation except for stratification factors.

[c] Trend tests from cecum to sigmoid colon were performed the ordinal subsite variable (cecum: 1, ascending: 2, transverse: 3, descending: 4, and sigmoid: 5).

[d] $P_{interaction}$ was calculated using the Wald test for the cross-product of the ordinal subsite variable and molecular marker variables (binary) in the multivariable Cox regression model. Abbreviations: CI, confidence interval; CIMP, CpG island methylator phenotype; CRC, colorectal cancer; HR, hazard ratio; MSI, microsatellite instability.

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Table 3.

Colorectal Cancer-Specific and Overall Mortality in Relation to Primary Tumor Location According to Age at Diagnosis

	Cecum	Ascending colon	Transverse colon	Descending colon	Sigmoid colon	P_{trend} [c]	$P_{interaction}$ [d]	Rectum
CRC-specific mortality							0.67	
Age <50 (early-onset)								
No. of cases	350	307	254	250	649			1,106
No. of events	75	54	38	46	140			266
Minimally-adjusted HR (95% CI)[a]	1 (reference)	0.98 (0.69–1.40)	0.66 (0.44–0.97)	0.88 (0.61–1.27)	1.01 (0.76–1.35)	0.82		1.24 (0.96–1.62)
Multivariable-adjusted HR (95% CI)[b]	1 (reference)	1.06 (0.75–1.51)	0.68 (0.46–1.01)	0.82 (0.57–1.19)	0.86 (0.64–1.14)	0.39		1.09 (0.83–1.42)
Age 50–69								
No. of cases	814	736	532	411	1,400			1,740
No. of events	193	142	127	87	277			405
Minimally-adjusted HR (95% CI)[a]	1 (reference)	0.83 (0.66–1.03)	0.98 (0.78–1.23)	0.80 (0.62–1.03)	0.84 (0.70–1.01)	0.11		1.04 (0.87–1.24)
Multivariable-adjusted HR (95% CI)[b]	1 (reference)	0.87 (0.70–1.09)	1.00 (0.80–1.25)	0.73 (0.56–0.94)	0.74 (0.62–0.90)	0.002		0.93 (0.78–1.12)
Age ≥70								
No. of cases	682	689	455	264	817			839
No. of events	150	139	81	61	175			213
Minimally-adjusted HR (95% CI)[a]	1 (reference)	0.99 (0.78–1.26)	0.83 (0.63–1.10)	1.04 (0.77–1.40)	0.97 (0.78–1.21)	0.86		1.46 (1.18–1.82)
Multivariable-adjusted HR (95% CI)[b]	1 (reference)	1.05 (0.83–1.34)	0.82 (0.62–1.08)	1.04 (0.77–1.41)	0.89 (0.71–1.11)	0.28		1.32 (1.06–1.65)
Overall mortality							0.73	
Age <50 (early-onset)								
No. of cases	360	315	261	256	662			1,140
No. of events	110	82	58	68	194			375
Minimally-adjusted HR (95% CI)[a]	1 (reference)	0.96 (0.72–1.28)	0.64 (0.46–0.89)	0.86 (0.63–1.16)	0.97 (0.77–1.23)	0.91		1.17 (0.94–1.46)
Multivariable-adjusted HR (95% CI)[b]	1 (reference)	1.02 (0.77–1.36)	0.68 (0.49–0.94)	0.82 (0.60–1.12)	0.86 (0.68–1.10)	0.27		1.06 (0.85–1.32)
Age 50–69								
No. of cases	879	793	561	437	1,512			1,889
No. of events	373	302	262	180	597			810

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	Cecum	Ascending colon	Transverse colon	Descending colon	Sigmoid colon	P_{trend} [c]	$P_{interaction}$ [d]	Rectum
Minimally-adjusted HR (95% CI)[a]	1 (reference)	0.88 (0.75–1.03)	1.11 (0.95–1.30)	0.88 (0.74–1.05)	0.90 (0.79–1.03)	0.18		1.07 (0.95–1.22)
Multivariable-adjusted HR (95% CI)[b]	1 (reference)	0.90 (0.77–1.06)	1.13 (0.97–1.33)	0.85 (0.71–1.02)	0.86 (0.75–0.98)	0.039		1.03 (0.90–1.17)
Age ≥70								
No. of cases	729	763	479	275	883			907
No. of events	389	412	216	146	462			495
Minimally-adjusted HR (95% CI)[a]	1 (reference)	1.06 (0.92–1.22)	0.92 (0.77–1.08)	1.02 (0.85–1.24)	0.97 (0.85–1.11)	0.46		1.19 (1.04–1.37)
Multivariable-adjusted HR (95% CI)[b]	1 (reference)	1.11 (0.96–1.28)	0.91 (0.77–1.08)	1.05 (0.87–1.28)	0.97 (0.84–1.11)	0.25		1.18 (1.03–1.36)

[a] Adjusted for sex, disease stage, and study (as strata).

[b] Adjusted for sex, disease stage, and study (as strata), family history of colorectal cancer, year of diagnosis, MSI status, CIMP status, *BRAF* mutation, and *KRAS* mutation.

[c] Trend tests from cecum to sigmoid colon were performed using the ordinal subsite variable (cecum: 1, ascending: 2, transverse: 3, descending: 4, and sigmoid: 5).

[d] $P_{interaction}$ was calculated using the Wald test for the cross-product of the ordinal subsite variable and age (continuous) in the multivariable Cox regression model.

Abbreviations: CI, confidence interval; CIMP, CpG island methylator phenotype; CRC, colorectal cancer; HR, hazard ratio; MSI, microsatellite instability.

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Table 4.

Colorectal Cancer-Specific and Overall Mortality in Relation to Tumor Molecular Features.

	CRC-specific mortality			Overall mortality		
	No. of cases	**No. of events**	**Multivariable HR[a] (95% CI)**	**No. of cases**	**No. of events**	**Multivariable HR[a] (95% CI)**
Overall						
MSI status						
Non-MSI-high	9474	2351	1 (reference)	10141	4477	1 (reference)
MSI-high	1978	167	0.35 (0.29–0.42)	2081	726	0.72 (0.66–0.80)
CIMP status						
CIMP-low/negative	7115	1674	1 (reference)	7701	3370	1 (reference)
CIMP-high	1367	284	1.10 (0.94–1.29)	1509	724	1.14 (1.03–1.27)
BRAF mutation status						
BRAF wild-type	9602	2164	1 (reference)	10252	4324	1 (reference)
BRAF mutant	1207	269	1.37 (1.18–1.61)	1315	648	1.14 (1.03–1.26)
KRAS mutation status						
KRAS wild-type	6018	1345	1 (reference)	6494	2849	1 (reference)
KRAS mutant	2918	812	1.11 (1.01–1.22)	3269	1538	1.02 (0.96–1.09)
Stage I						
MSI status						
Non-MSI-high	2239	148	1 (reference)	2361	763	1 (reference)
MSI-high	453	11	0.53 (0.27–1.04)	473	136	1.04 (0.83–1.30)
CIMP status						
CIMP-low/negative	1693	104	1 (reference)	1792	585	1 (reference)
CIMP-high	281	11	1.31 (0.66–2.59)	310	104	1.16 (0.88–1.54)
BRAF mutation status						
BRAF wild-type	2284	145	1 (reference)	2400	783	1 (reference)
BRAF mutant	225	5	1.40 (1.13–1.73)	247	81	0.71 (0.54–0.94)
KRAS mutation status						
KRAS wild-type	1496	85	1 (reference)	1582	535	1 (reference)
KRAS mutant	659	80	1.24 (0.86–1.78)	711	255	1.01 (0.87–1.18)
Stage II and III						

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	CRC-specific mortality			Overall mortality		
	No. of cases	**No. of events**	**Multivariable HR[a] (95% CI)**	**No. of cases**	**No. of events**	**Multivariable HR[a] (95% CI)**
MSI status						
Non-MSI-high	5428	1205	1 (reference)	5826	2377	1 (reference)
MSI-high	1287	108	0.33 (0.26–0.41)	1362	472	0.70 (0.62–0.79)
CIMP status						
CIMP-low/negative	4189	891	1 (reference)	4551	1833	1 (reference)
CIMP-high	888	149	1.12 (0.89–1.39)	984	451	1.29 (1.11–1.49)
BRAF mutation status						
BRAF wild-type	5628	1130	1 (reference)	6030	2334	1 (reference)
BRAF mutant	1014	776	1.51 (1.16–1.96)	850	411	1.10 (0.95–1.26)
KRAS mutation status						
KRAS wild-type	3525	691	1 (reference)	3833	1522	1 (reference)
KRAS mutant	1762	438	1.26 (1.11–1.43)	1929	837	1.10 (1.01–1.21)
Stage IV						
MSI status						
Non-MSI-high	1106	866	1 (reference)	1218	1018	1 (reference)
MSI-high	64	31	0.44 (0.30–0.64)	71	48	0.54 (0.39–0.74)
CIMP status						
CIMP-low/negative	741	581	1 (reference)	984	451	1 (reference)
CIMP-high	130	109	1.06 (0.83–1.37)	145	129	1.02 (0.81–1.29)
BRAF mutation status						
BRAF wild-type	1014	779	1 (reference)	1116	916	1 (reference)
BRAF mutant	110	90	1.66 (0.96–2.87)	123	110	1.55 (1.22–1.97)
KRAS mutation status						
KRAS wild-type	606	482	1 (reference)	673	567	1 (reference)
KRAS mutant	381	298	1.01 (0.86–1.19)	432	352	1.00 (0.87–1.16)

[a]Adjusted for sex, age, primary tumor location, disease stage, study (as strata), family history of colorectal cancer, year of diagnosis, MSI status, CIMP status, *BRAF* mutation, and *KRAS* mutation except for the predictor.

Abbreviations: CI, confidence interval; CIMP, CpG island methylator phenotype; CRC, colorectal cancer; HR, hazard ratio; MSI, microsatellite instability.