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Gastrointestinal Cancer

Impact of Patient Age on Molecular Alterations of Left-Sided Colorectal Tumors

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Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. Colorectal cancer • Early-onset • Microsatellite instability • Tumor mutational burden • Cancer syndromes • Histone modifiers

Abstract

Background. The incidence of colorectal cancer (CRC) in younger patients is rising, mostly due to tumors in the descending colon and rectum. Therefore, we aimed to explore the molecular differences of left-sided CRC between younger ($\leq$45 years) and older patients ($\geq$65).

Subjects, Materials, and Methods. In total, 1,126 CRC tumor samples from the splenic flexure to (and including) the rectum were examined by next-generation sequencing (NGS), immunohistochemistry, and in situ hybridization. Microsatellite instability (MSI) and tumor mutational burden (TMB) were assessed by NGS.

Results. Younger patients ($n = 350$), when compared with older patients ($n = 776$), showed higher mutation rates in genes associated with cancer-predisposing syndromes (e.g., Lynch syndrome), such as *MSH6* (4.8% vs. 1.2%, $p = .005$), *MSH2* (2.7% vs. 0.0%, $p = .004$), *POLE* (1.6% vs. 0.0%, $p = .008$), *NF1* (5.9% vs. 0.5%, $p < .001$), *SMAD4* (14.3% vs. 8.3%, $p = .024$), and *BRCA2* (3.7% vs. 0.5%, $p = .002$). Genes involved in histone modification were also significantly more mutated: *KDM5C* (1.9% vs. 0%, $p = .036$), *KMT2A* (1.1% vs. 0%, $p = .033$), *KMT2C* (1.6% vs. 0%, $p = .031$), *KMT2D* (3.8% vs. 0.7%, $p = .005$), and *SETD2* (3.2% vs. 0.9%, $p = .039$). Finally, TMB-high (9.7% vs. 2.8%, $p < .001$) and MSI-high (MSI-H; 8.1% vs. 1.9%, $p = .009$) were more frequent in younger patients.

Conclusion. Our findings highlight the importance of genetic counseling and screening in younger CRC patients. MSI-H and TMB-high tumors could benefit from immune-checkpoint inhibitors, now approved for the treatment of MSI-H/deficient mismatch repair metastatic CRC patients. Finally, histone modifiers could serve as a new promising therapeutic target. With confirmatory studies, these results may influence our approach to younger adults with CRC. ***The Oncologist*** 2019;24:319–326

Implications for Practice: The increasing rate of colorectal cancers (CRC), primarily distal tumors, among young adults poses a global health issue. This study investigates the molecular differences between younger ($\leq$45 years old) and older ($\geq$65) adults with left-sided CRCs. Younger patients more frequently harbor mutations in genes associated with cancer-predisposing syndromes. Higher rates of microsatellite instability-high and tumor mutational burden-high tumors occur in younger patients, who could benefit from immune-checkpoint inhibitors. Finally, histone modifiers are more frequently mutated in younger patients and could serve as a new promising therapeutic target. This study provides new insights into mutations that may guide development of novel tailored therapy in younger CRC patients.

Introduction

Colorectal cancer (CRC) is the third most common cancer and the fourth leading cause of cancer-related death worldwide [1]. In the last 2 decades, adults aged $\geq$50 years have experienced a decrease in CRC incidence and mortality, while people aged $<$50 years show the opposite trend [2]. It has been estimated that in 2030, incidence rates of colon and rectal cancers will

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increase by 90% and 124%, respectively, in patients aged 20–34 years and by 27.7% and 46.0%, respectively, in patients aged 35–49 years [3]. The cause remains unknown, although lack of screening, obesity, physical inactivity, and Western diets may play a crucial role in early-onset CRC. Therefore, further investigations into causality are necessary to develop potential preventive strategies. Broader molecular tumor profiling should also be explored to identify biomarkers and therapeutic targets unique to younger CRC patients.

Clinicopathological differences between younger and older patients may be explained by underlying different molecular patterns [4], although data regarding the molecular features of sporadic early-onset CRC are limited and controversial [5]. On the other hand, the pathogenesis of early-onset CRC is well characterized in individuals with an inherited CRC syndrome, present in around 5%–10% of patients with CRC [6].

Frequencies of *TP53*, *APC*, *BRAF*, and *KRAS* mutations are similar in both younger and older adults [7]. Tumors in younger adults show higher mutation rates of *FBXW7* and *POLE* [8], are more frequently. The CpG island methylator phenotype (CIMP)-low [9] and are characterized by higher frequencies of *LINE-1* hypomethylation [10, 11]. Although the majority of early-onset CRCs are microsatellite stable, microsatellite instability (MSI) is observed in 20%–40% of early-onset CRCs and is dependent on the age of onset [12]. Most MSI-high (MSI-H) tumors in younger patients are associated with Lynch syndrome, harboring a characteristic germline mutation in one of the mismatch repair (MMR) genes, and are *BRAF* wild-type. Recently, in a study of 450 patients, Pearlman et al. [13] showed a high frequency of a range of germline mutations (16%) among early-onset CRCs. Moreover, they showed that deficient-MMR (dMMR) tumors harbored a different mutational pattern from proficient-MMR (pMMR) tumors.

One compounding issue is that no global consensus exists on the age-definition of early-onset CRC [14]. Although some authors suggest using $<$50 years of age, corresponding to the CRC screening age in average-risk populations, age cutoffs vary widely in published studies, making comparisons challenging. Patients younger than 50 years of age are usually diagnosed with more advanced disease compared with those $\geq$50 years, likely due to delays in symptom recognition and diagnostic workup [15]. Moreover, younger patients tend to present with more histologically aggressive tumors (e.g., mucinous, signet ring cell, and/or poorly differentiated) [16]. However, it is uncertain whether these characteristics are related to worse outcome in young patients. Although contradictory results have been highlighted [17, 18], a range of different studies show better outcomes in terms of CRC-specific survival in younger patients when compared with their older counterparts [19].

It is important to note that for individuals aged $<$50 years, the increasing incidence rates are mostly due to tumors occurring in the descending colon and rectum [20], and tumor sidedness has emerged as an important prognostic and predictive biomarker in metastatic CRC (mCRC). Indeed, the differences between left- and right-sided colon cancer in terms of embryology, etiology, and molecular and clinical features have already been demonstrated in various studies [21–26]. Accordingly, proximal and distal colon cancer may also respond differently to first-line treatment with biologics in the metastatic setting [25].

In order to better understand the disease biology and identify molecular targets for young patients with mCRC, we explored the impact of age on the tumor biology of left-sided CRC, defined as CRC from the splenic flexure to (and including) the rectum.

To our knowledge, this is the first study to investigate the molecular differences between left-sided CRCs from younger ($\leq$45 years) versus older ($\geq$65 years) adults, aimed at broadening insights into the disease biology and potential biomarkers that might be involved in early-onset CRC.

Subjects, Materials, and Methods

Patients

This analysis included 2,413 left-sided colorectal tumors profiled at Caris Life Sciences between 2015 and 2017. Tumor tissue was formalin-fixed and paraffin-embedded (FFPE). Both specimen and tumor quality were confirmed by a board-certified pathologist using hematoxylin and eosin staining prior to multiplex testing. Immunohistochemistry (IHC) assessed protein expression; in situ hybridization determined gene amplification; and next-generation sequencing (NGS) evaluated for DNA aberrations (Illumina NextSeq; Illumina, San Diego, CA) or RNA fusions (ArcherDx FusionPlex assay; ArcherDx, Boulder, CO). All molecular techniques met Clinical Laboratory Improvement Amendments/College of American Pathology standards.

Next-Generation Sequencing

Direct sequencing was performed on genomic DNA isolated from FFPE tumor specimens using the whole-exome NextSeq platform. An Agilent SureSelect XT assay (Agilent, Santa Clara, CA) was used to enrich 592 whole-gene targets. All reported variants were detected with $>$99% confidence at an average depth of at least $\times$700. The Illumina platform calculated tumor mutational burden (TMB) by evaluating only somatic nonsynonymous missense mutations.

Microsatellite Instability by NGS and Fragment Analysis

MSI was examined using over 7,000 target microsatellite loci and compared with the reference genome hg19 from the University of California, Santa Cruz Genome Browser database. The number of microsatellite loci that were altered by somatic insertion or deletion was counted for each sample. Only insertions or deletions that increased or decreased the number of repeats were considered. Genomic variants in the microsatellite loci were detected using the same depth and frequency criteria as used for mutation detection. MSI-NGS results were compared with results from over 2,000 matching clinical cases analyzed with traditional polymerase chain reaction-based methods. The threshold to determine MSI by NGS was determined to be 46 or more loci with insertions or deletions to generate a sensitivity of $>$95% and specificity of $>$99%.

Fragment analysis (FA) included fluorescently labeled primers (Promega, Madison, WI) for co-amplification of seven biomarkers including five mononucleotide repeat markers (BAT-25, BAT-26, NR-21, NR-24, and MONO-27) and two pentanucleotide repeat markers (Penta C and D). The mononucleotide markers determined the presence of MSI, and the pentanucleotide markers were utilized for sample mix-up detection or contamination. A tumor specimen was considered dMMR if two or more mononucleotide repeats were abnormal. If one mononucleotide repeat was abnormal or repeats were identical

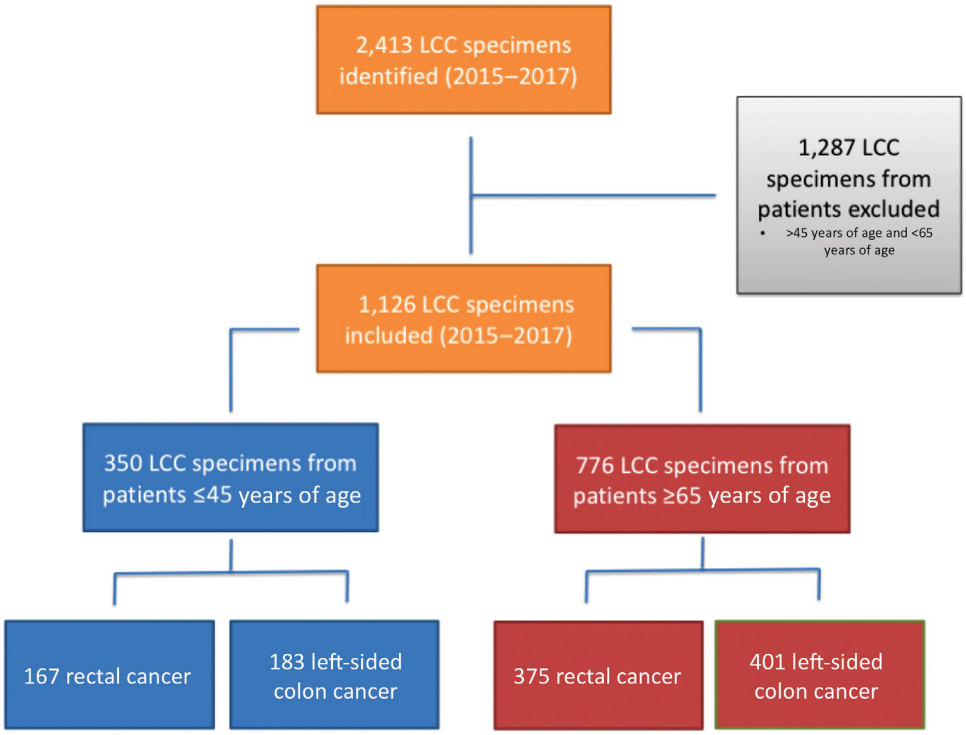

Figure 1. Consort diagram of LCC included in the study.
Abbreviation: LCC, left-sided colorectal cancer.

Table 1. Patient characteristics

LCC cohort	*n*	Age, years, median (range)	Female, %
Younger	350	40 (22–45)	52.3 (183/350)
Older	776	72 (65–89)	39.3 (305/776)

Abbreviation: LCC, left-sided colorectal cancer.

between the tumor and adjacent normal tissue, then the tumor sample was considered pMMR.

Tumor Mutational Burden by NGS

TMB was measured by counting all nonsynonymous missense mutations found per tumor that had not been previously described as germline alterations (592 genes and 1.4 megabases [MB] sequenced per tumor). The threshold to define TMB-high was greater than or equal to 17 mutations/MB and was established by comparing TMB with MSI by fragment analysis in CRC cases, based on reports of TMB having high concordance with MSI-H in CRC [26]. Values below 17 mutations/Mb were considered intermediate (7–16) or low (<7) and were grouped together for chi-square analysis.

In Situ Hybridization Methods

Using automated staining (Benchmark XT; Ventana, Tucson, AZ) and imaging (BioView, Billerca, MA) techniques, fluorescent and/or chromogenic in situ hybridization assessed *ERBB2* (human epidermal growth receptor 2 [HER2]/CEP17 [chromosome 17 centromere] probe) and *MET* (c-MET/CEP7 probe; Abbott Molecular/Vysis, Abbott Park, IL) gene copy alterations. The ratio of gene to pericentromeric regions of chromosome 7 (*MET*) and 17 (*HER2*) was utilized to determine gene amplification. Cutoffs for amplification were determined according to manufacturer's recommendation (supplemental online Appendix 1).

Immunohistochemistry Analysis

Automated staining techniques (Benchmark XT; Ventana; and AutostainerLink 48; Dako, Carpinteria, CA) and commercially available detection kits were performed on FFPE tumor specimens. Positive and negative controls were included in each analysis to ensure staining efficacy and consistency across batches. Threshold values for positive expression were optimized for each antibody according to the manufacturer's recommendations, accounting for both staining intensity and percentage of tumor cells stained. Details on IHC interpretations have been described previously [27].

Statistical Analysis

Standard descriptive statistics were used for this retrospective analysis. Pearson's chi-square test was used to obtain p values (IBM SPSS Statistics for Windows, Version 24.0; Released 2016; IBM Corp., Armonk, NY). Only p values $\leq .05$ were considered statistically significant.

Ethics Statement

Human subjects were de-identified prior to analysis, with this retrospective research being exempt per Western Institutional Review Board.

Results

Patient Demographics

Of the 2,413 left-sided CRC samples, 1,126 were included in this analysis: 350 from younger patients (median age 40, range 22–45 years) and 776 from older patients (median age 72, range 65–89 years; Fig. 1). The older cohort was composed of

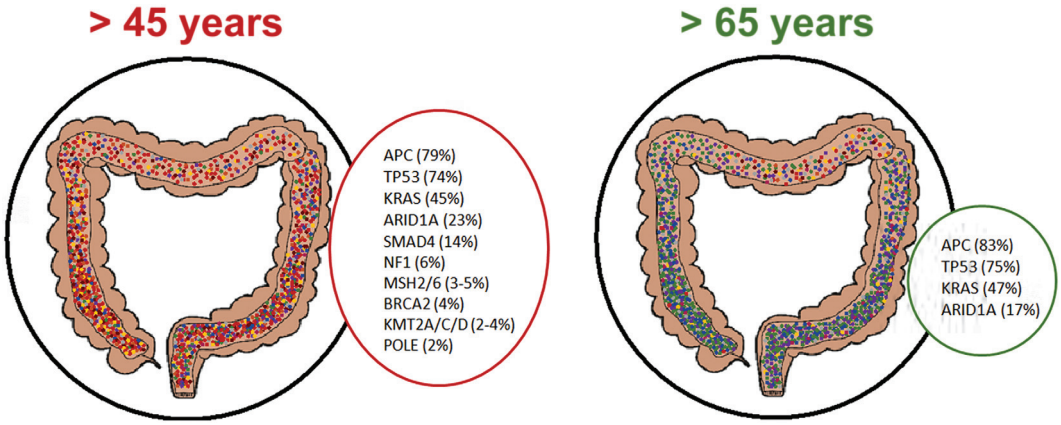

Figure 2. Molecular differences between younger and older patients with left-sided colorectal tumors via next-generation sequencing analysis.

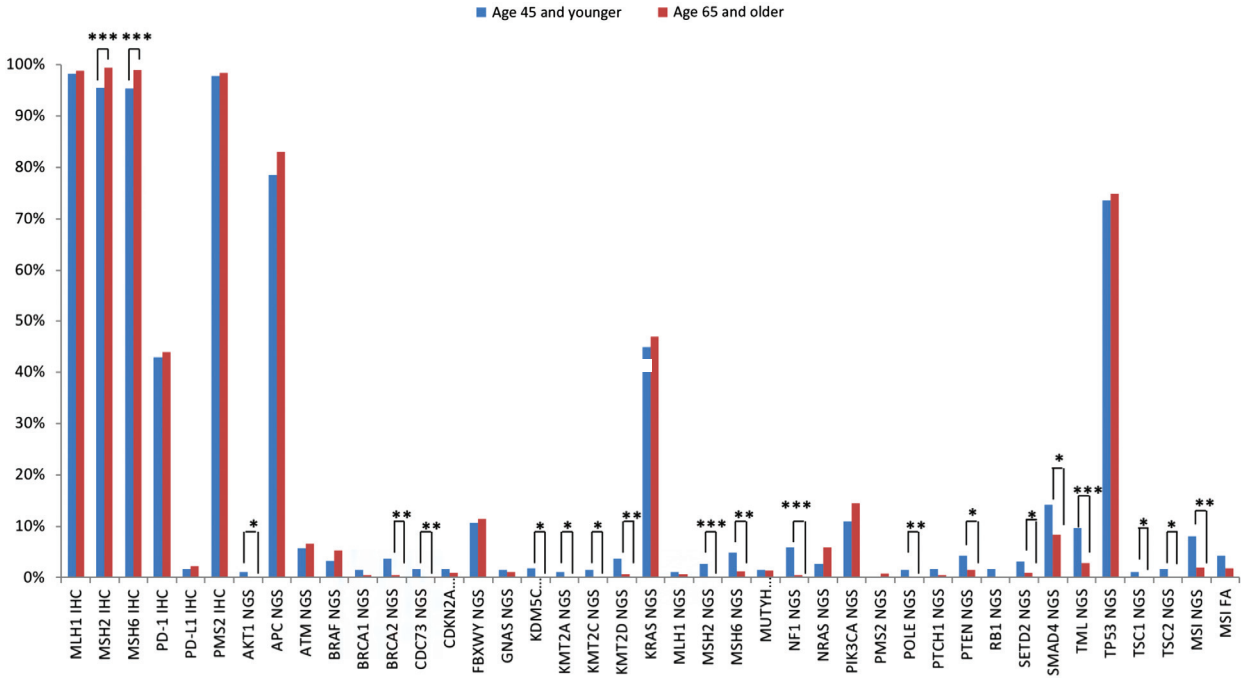

Figure 3. Mutational profile via NGS and protein expression via IHC.
Abbreviations: *, $p \leq .05$; **, $p \leq .01$; ***, $p \leq .001$; FA, fragment analysis; IHC, immunohistochemistry; MSI, microsatellite instability; NGS, next-generation sequencing.

39.3% (305/776) female patients compared with 52.3% (183/350) in the younger cohort (Table 1).

Mutational Profile via Next-Generation Sequencing

Across the two cohorts, the most frequently mutated genes included the following: *APC* (81.8%), *TP53* (74.6%), *KRAS* (46.4%), *ARID1A* (19.4%), *PIK3CA* (13.4%), *FBXW7* (11.2%), *SMAD4* (10.1%), *ATM* (6.3%), *NRAS* (4.9%), and *BRAF* (4.6%; Fig. 2). There were no significant differences in mutation frequencies of these genes between younger and older patient specimens. However, numerous genes that are associated with cancer-predisposing syndromes, such as Lynch syndrome, also known as hereditary nonpolyposis colorectal cancer (HNPCC), neurofibromatosis type 1 (NF1), *PTEN* hamartoma tumors syndrome (PHTS), tuberous sclerosis complex (TSC), and hereditary breast ovarian cancer syndrome (HBOC), were found to carry higher mutations rates in younger compared with older patients. Specifically, *MSH6* (4.8% vs. 1.2%, $p = .005$), *MSH2* (2.7% vs. 0.0%, $p = .004$), *POLE* (1.6% vs. 0.0%, $p = .008$), *NF1* (5.9% vs. 0.5%, $p < .001$), *SMAD4* (14.3% vs. 8.3%, $p = .024$), *PTEN* (4.3% vs. 1.4%, $p = .03$), *TSC1* (1.1% vs. 0.0%, $p = .031$), *TSC2* (1.6% vs. 0.2%, $p = .048$), *AKT1* (1.1% vs. 0%, $p = .031$), and *BRCA2* (3.7% vs. 0.5%, $p = .002$) showed significantly higher mutations in younger versus older patients (Fig. 3; Table 2).

Furthermore, genes responsible for histone modification were also significantly more frequently mutated in younger than in older patients: *CDC73* (1.7% vs. 0%, $p = .01$), *KDM5C* (1.9% vs. 0%, $p = .036$), *KMT2A* (1.1% vs. 0%, $p = .033$), *KMT2C* (1.6% vs. 0%, $p = .031$), *KMT2D* (3.8% vs. 0.7%, $p = .005$), and *SETD2* (3.2% vs. 0.9%, $p = .039$; Fig. 3; Table 2). Interestingly, when we excluded MSI-H and *POLE*-mutated specimens from the analysis, only *MITF* (1.9% vs. 0.0%, $p = .007$) and *NF1* (2.9% vs. 0.3%, $p = .013$) remained significant.

Table 2. Genes with statistically significant difference in mutation rates between younger and older LCC cohorts via next-generation sequencing analysis

Gene	Overall LCC patients, %	Younger (≤45 years) LCC, %	Older (≥65 years) LCC, %	*p* value
AKT1	0.3 (2/632)	1.1 (2/190)	0.0 (0/442)	.031
BRCA2	1.4 (9/633)	3.7 (7/190)	0.5 (2/443)	.002
CDC73	0.5 (3/575)	1.7 (3/179)	0.0 (0/396)	.01
KDM5C	0.6 (2/341)	1.9 (2/107)	0.0 (0/234)	.036
KMT2A	0.3 (2/606)	1.1 (2/185)	0.0 (0/421)	.033
KMT2C	0.5 (2/420)	1.6 (2/127)	0.0 (0/293)	.031
KMT2D	1.6 (10/619)	3.8 (7/186)	0.7 (3/433)	.005
MSH2	0.8 (5/613)	2.7 (5/188)	0.0 (0/425)	.001
MSH6	2.3 (14/618)	4.8 (9/187)	1.2 (5/431)	.005
NF1	2.2 (12/550)	5.9 (10/169)	0.5 (2/381)	<.001
POLE	0.5 (3/632)	1.6 (3/190)	0.0 (0/442)	.008
PTEN	2.3 (14/603)	4.3 (8/185)	1.4 (6/418)	.03
SETD2	1.6 (10/625)	3.2 (6/189)	0.9 (4/436)	.039
SMAD4	10.1 (63/621)	14.3 (27/189)	8.3 (36/432)	.024
TSC1	0.3 (2/633)	1.1 (2/190)	0.0 (0/443)	.031
TSC2	0.6 (4/632)	1.6 (3/189)	0.2 (1/443)	.048

Abbreviation: LCC, left-sided colorectal cancer.

Table 3. Tumor mutational burden and microsatellite instability analysis between younger and older cohort

Biomarker	Overall LCC patients, %	Younger (≤45 years) LCC, %	Older (≥65 years) LCC, %	*p* value
TMB via NGS	4.9 (30/617)	9.7 (18/185)	2.8 (12/432)	<.001
MSI via NGS	3.7 (11/300)	8.1 (7/86)	1.9 (4/214)	.009
MSI via FA	2.5 (14/566)	4.2 (7/166)	1.8 (7/400)	.085

Abbreviations: FA, fragment analysis; LCC, left-sided colorectal cancer; MSI, microsatellite instability; NGS, next-generation sequencing; TMB, tumor mutational burden.

Tumor Mutational Burden and Microsatellite Instability via NGS

TMB-high (≥17 mutations/Mb) was observed more frequently in tumors of the younger patient cohort (9.7% vs. 2.8%, $p < .001$; Table 3). Moreover, when analyzed by NGS, there was a significantly higher rate of MSI-H in the cohort of younger left-sided CRCs (8.1% vs. 1.9%, $p = .009$). Interestingly, when analyzed by FA, there was no significant difference in the frequency of MSI-H between younger and older patients, although a trend toward higher rates of MSI-H was observed in younger patients (4.2% vs. 1.8%, $p = .085$; Table 3).

Protein Expression and Gene Amplification

As we observed a higher mutation rate in *MSH2* and *MSH6* via NGS in tumors of younger compared with older individuals, a lower expression of *MSH2* (4.2% vs. 0.5%, $p < .001$) and *MSH6* (4.2% vs. 1.0%, $p = .001$) in younger compared with older patients was seen by IHC.

The other proteins involved in mismatch repair (i.e., *MLH1* and *PMS2*) showed almost identical protein expression in the two groups (Table 4; supplemental online Fig. 1).

Furthermore, *ERBB2* was amplified at a higher frequency in younger patients compared with older patients (5.7% vs. 2.4%), although the difference was not statistically significant ($p = .082$). One patient in the younger cohort exhibited *MET* amplification (1.3%), whereas no patients in the older cohort showed *MET* amplification (0.0%); however, no significant difference was detected ($p = .156$; supplemental online Table 1).

Interrelationship Between MSI-H, TMB-High, *BRAF* Mutation, and PD-L1

We further investigated whether there is a difference in *BRAF* mutation rate in MSI-H tumors from younger versus older patients. Unfortunately, only a few patients in our database ($n = 40$) had data for both MSI-H and *BRAF* mutations, and in those who did, there was no significant difference in *BRAF* mutation rate (9.1% vs 20%; supplemental online Table 2). The interrelationship between MSI-H, TMB-high, *BRAF* mutations, and programmed death-ligand 1 (PD-L1) expression was studied, and there were no statistically significant differences between younger and older patient cohorts (supplemental online Fig. 2).

Discussion

Increasing rates of CRC among young adults poses a global health issue and an incredible burden on patients, families, and health care providers. This rise in early-onset CRC is primarily driven by distal tumors, yet both the etiology and risk factors remain unknown. Obesity and its associated behaviors, such as unhealthy dietary patterns and sedentary lifestyles, as well as gut microbiota [27] may play a crucial role in CRC risk for young

Table 4. Mismatch repair protein expression by immunohistochemistry

Biomarker	Overall LCC patients %	Younger (≤45 years) LCC, %	Older (≥65 years) LCC, %	*p* value
MLH1[a]	1.2 (11/918)	1.4 (4/285)	1.1 (7/633)	.701
MSH2[a,b]	1.6 (15/916)	4.2 (12/286)	0.5 (3/630)	<.001
MSH6[a,b]	2.0 (28/908)	4.2 (12/283)	1.0 (6/625)	.001
PMS2[a]	1.6 (15/910)	1.8 (5/282)	1.6 (10/628)	.843

[a]Percentages reflect lacking the protein.
[b]Significant differences in protein expression between younger and older LCC patients.
Abbreviation: LCC, left-sided colorectal cancer.

adults. However, from a molecular perspective, much remains unknown. In addition, the lack of a global consensus on the age cutoff to define younger patients makes any comparison a challenge.

Herein, we demonstrated that younger patients with left-sided CRCs exhibit a molecular profile that is different from that of left-sided CRCs from older patients. Accordingly, younger patients have a higher TMB-high and a greater MSI-H rate.

Patients with MSI-H or dMMR mCRC can now be given pembrolizumab (Keytruda; Merck & Co., Inc., Kenilworth, NJ) [28] and nivolumab (Opdivo; Bristol-Myers Squibb, New York City, NY) [29] owing to recent U.S. Food and Drug Administration (FDA) approval of these two immune checkpoint inhibitors in this setting. Therefore, the findings reported in this current paper may have direct impact on treatment choices for younger patients.

We found significantly higher *MSH6* and *MSH2* mutation rates by NGS and significantly lower protein expression via IHC in younger compared with older patients (Fig. 3; Tables 2 and 4). Mutations that occur in *MSH6* and *MSH2* are found to be responsible for dMMR. In sporadic CRC, promoter hypermethylation of *MLH1* is responsible for 80%–90% of MSI-H cases, and isolated loss of MSH2 or MSH6 proteins, seen by IHC, has high specificity for a germline mutation in these genes [30]. Accordingly, we found that MSI-H status was more frequent in left-sided CRC specimens from younger patients than left-sided CRCs from older patients via NGS analysis, and although FA showed the same trend, frequency differences did not reach statistical significance (Table 3). Although it is well established that dMMR can be accurately measured by directly enumerating known MSI loci using targeted deep sequencing (MSI-NGS) [31] or by TMB [32], our results warrant some caution, and further validation is needed to elucidate this aspect. However, our findings are in accordance with previous studies that showed that MSI-H occurs more frequently in younger patients, although frequencies vary widely [33, 34], and that the pattern of mutations leading to MSI-H in younger patients is different from older patients [35, 36]. In fact, currently, we are examining whether mutations in specific MMR genes have a different impact on immune-related biomarker expression (e.g., PD-L1 overexpression and TMB level).

Our study confirms previous observations suggesting a higher rate of *POLE* mutations in younger patients, although in our cohort, only 1.6% of patients aged ≤45 years harbored *POLE* mutations, whereas 9.8% were detected in a prior study [8]. Recently, in 4,500 stage II/III CRCs, Domingo et al. [37] showed that *POLE* mutations are mutually exclusive with dMMR. They occur in 1% of patients, are characterized by an excellent prognosis, and are more frequent in younger adult at diagnosis.

MSI-H status and *POLE* mutations, whether germline or somatic, lead to a hypermutated cancer phenotype [38, 39]. It is now well established that hypermutational status causes an enrichment of neoepitopes, which in turn can be recognized by the immune system and eventually lead to a potent cytotoxic T-cell response. Accordingly, we showed that younger patients displayed a TMB-high rate (defined as ≥17 mutations/Mb) threefold more frequently than older patients (9.7% vs. 2.8%, $p < .001$), probably owing to mutations in *MSH6*, *MSH2*, and *POLE*; in a prior study, we have shown a high correlation between TMB-high and MSI-H across several gastrointestinal cancers [40]. It is noteworthy that TMB-high status is an emerging biomarker for response to immunotherapy [41, 42] and prognosis [43]. Of note, the observed increased rates of MSH2 and MSH6 and increased rates of MSI and TMB could be due to the increased proportion of younger CRC patients with hereditary conditions (e.g., Lynch syndrome).

Our findings suggest that left-sided CRCs from younger patients more frequently harbor somatic mutations in genes that are associated with different cancer syndromes. As such, younger patients may have a higher risk of developing specific types of cancer as part of a hereditary cancer syndrome if these mutations represent germline mutations, including CRC. Germline mutations in *MSH2* and *MSH6* are associated with Lynch syndrome (also known as HNPCC); *NF1* with NF1; *PTEN* with PHTS; *TSC1* and *TSC2* with TSC, *SMAD4* with juvenile polyposis syndrome, and *BRCA2* with HBOC. Unfortunately, we could not evaluate whether these mutations were germline, which is a limitation of our study. However, the strong correlation between these mutations with specific and well-known hereditary syndromes underlines the importance of genetic counseling and screening in patients with early-onset CRC.

Interestingly, in the current study, the genes that showed significantly different mutation rates between younger and older patients, although not associated with any known cancer syndrome, can be grouped together because of their function: *KDM5C*, *KMT2A*/*2C*/*2D*, *SETD2*, and *CDC73* are histone methyltransferases or demethylases. Disruption of epigenetic regulation in CRC, namely DNA and histone methylation and demethylation, have gained attention in recent years, because it is now believed that these enzymes play a crucial role in the development of CRC [44]. Indeed, epigenetic modifications provide promising new targets for anticancer therapy, and several DNA methyltransferases and histone deacetylases inhibitors are already FDA-approved anticancer drugs in hematologic malignancies (e.g., azacitidine, decitabine, vorinostat, romidepsin, and belinostat). For these reasons, numerous phase I/II trails are ongoing to test the safety and the efficacy of specific

drugs that target histone modifiers in solid tumors, including CRC [45].

Of special interest, we found higher *BRCA2* mutation rates in younger compared with older left-sided CRC patients. *BRCA2* germline mutant tumors have been shown to benefit from Poly (ADP-ribose) polymerase (PARP)-inhibitor therapy, as recently demonstrated by Mirza et al. [46]. Additionally, *ERBB2* (HER2) amplification was observed at a higher rate in younger patients (5.7%) compared with older patients (2.4%), although the difference was not significant ($p = .144$). HER2 has been shown to be a promising predictive marker of response to anti-HER2 therapy (i.e., HERACLES trial [47]). Therefore, these findings could lead to additional tailored therapies for this population of patients.

We acknowledge that our study has several limitations, such as the retrospective nature of the analysis, heterogeneous population, and the lack of clinical data that did not allow us to correlate these findings with outcomes. Therefore, future validations of these findings in prospective and larger cohorts are needed.

Conclusion

This is the first study to investigate the molecular differences between younger ($\leq$45 years) and older adults ($\geq$65 years) affected by left-sided CRC. We have shown that molecular differences between younger and older patients are predominantly due to mutations in genes that are strongly associated with cancer-predisposing syndromes, underlining the importance of genetic counseling and screening in this population of CRC patients. MSI-H and TMB-high tumors occur more frequently in younger patients, which may have a direct impact on clinical practice, as pembrolizumab and nivolumab are now FDA approved for the treatment of MSI-H or dMMR mCRC patients. In addition, younger patients harboring *ERBB2* (HER2) amplification could benefit from HER2-targeted combination therapy, whereas *BRCA2* mutant tumors may benefit from PARP-inhibitor therapy. Finally, higher mutation rates in histone methyltransferases and demethylases occur in younger individuals and could therefore present new promising therapeutic targets.

Although the differences in tumor biology observed between younger and older patients with left-sided CRCs warrant further validating studies, molecular tumor profiling in early-onset CRC should be considered in order to allow exploration of novel therapeutic options. In addition, this analysis provides new insights into mutations that may guide development of novel tailored therapy in younger CRC patients. With confirmatory and prospective studies, these initial findings may influence our approach to younger adults with CRC in the future.

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Disclosures

Heinz-Josef Lenz: Roche, Merck Serono, Bayer, Bristol-Myers Squibb, Boehringer Ingelheim, Takeda (SAB), Merck Serono, Bayer (C/A); **John L. Marshall**: Caris Life Sciences (RF, H, SAB); **David Arguello**: Caris Life Sciences (E); **Derek Raghavan**: Caris Life Sciences (C/A); **W. Michael Korn**: Caris Life Sciences (E, OI), Merck, Eli Lilly and Company (SAB); **Kelsey Poorman**: Caris Life Sciences (E); **Anthony F. Shields**: Caris Life Sciences (RF, SAB). The other authors indicated no financial relationships.

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