TITLE: Comprehensive Genomic Landscapes in Early and Later Onset Colorectal Cancer
Christopher H. Lieu1, Erica A. Golemis2, Ilya G. Serebriiskii2,3, Justin Newberg4, Amanda Hemmerich4, Caitlin Connelly4, Wells A. Messersmith1, Cathy Eng5, S. Gail Eckhardt6, Garrett Frampton4, Matthew Cooke4, Joshua E. Meyer7

Affiliations:

1 Division of Medical Oncology, University of Colorado Cancer Center, Aurora, CO 80045; 
2 Program in Molecular Therapeutics, Fox Chase Cancer Center, Philadelphia, PA 19111; 
3 Kazan Federal University, 420000, Kazan, Russian Federation; 
4 Foundation Medicine Inc, 150 Second St., Cambridge, MA, 02141, USA; 
5 Department of Gastrointestinal Medical Oncology, University of Texas MD Anderson Cancer Center, Houston, TX 77030 
6 Department of Medical Oncology, University of Texas at Austin Dell Medical School; LIVESTRONG Cancer Institutes, Austin, TX 78712 
7 Department of Radiation Oncology, Fox Chase Cancer Center, Philadelphia, PA 19111

*Corresponding author: Christopher H. Lieu, MD christopher.lieu@ucdenver.edu

Running Title: Genomic Landscape Young-Onset CRC

Keywords: young-onset colorectal cancer, early-onset colorectal cancer, genomic analysis, colon cancer, rectal cancer, late-onset colorectal cancer

Funding: The authors were supported by NCI Core Grant P30 CA006927 (to Fox Chase Cancer Center), NIH R01 DK108195 (to EAG), CPRIT Scholar Award #RR160093 (to SGE), NIH R01 CA229259-01 (to CL), by a subsidy of the Russian Government to support the Program of Competitive Growth of Kazan Federal University (to IGS), by the Colorectal Cancer Alliance (to JEM).

Conflicts of Interest: Author C.L. has a minor conflict (consulting for Foundation Medicine)

Word Count: 2601

Figures: 5    Tables: 1    Supplementary Tables: 1    Supplementary Figures: 2
STATEMENT OF TRANSLATIONAL RELEVANCE:

The incidence of early-onset colorectal cancer continues to increase, in contrast to late-onset disease. To determine if the selective rise in incidence of this “sporadic” early-onset disease reflects a distinct profile of somatic driver mutations, we have compared the genomic landscape of early-onset colorectal cancer to that of later-onset colorectal cancer. Results of this analysis help elucidate differences in molecular carcinogenesis and may impact treatment decision-making.
ABSTRACT:

Purpose: The incidence rates of colorectal cancers (CRC) are increasing in young adults. The objective of this study was to investigate genomic differences between tumor samples collected from younger and older patients with CRC.

Experimental Design: DNA was extracted from 18,218 clinical specimens, followed by hybridization capture of 3,769 exons from 403 cancer-related genes and 47 introns of 19 genes commonly rearranged in cancer. Genomic alterations (GA) were determined, and association with patient age and microsatellite stable/microsatellite instability high (MSS/MSI-H) status established.

Results: Overall genomic alteration rates in the younger (<40) and older (≥50) cohorts were similar in the majority of the genes analyzed. Gene alteration rates in the microsatellite stable (MSS) younger and older cohorts were largely similar, with several notable differences. In particular, TP53 (FDR<0.01) and CTNNB1 (FDR=0.01) alterations were more common in younger patients with CRC, and APC (FDR<0.01), KRAS (FDR<0.01), BRAF (FDR<0.01), and FAM123B (FDR<0.01) were more commonly altered in older patients with CRC. In the MSI-H cohort, the majority of genes showed similar rate of alterations in all age group, but with significant differences seen in APC (FDR<0.01), BRAF (FDR<0.01), and KRAS (FDR<0.01).

Conclusions: Tumors from younger and older patients with CRC demonstrated similar overall rates of genomic alteration. However, differences were noted in several genes relevant to biology and response to therapy. Further study will need to be conducted to determine if the differences in gene alteration rates can be leveraged to provide personalized therapies for young patients with early-onset sporadic CRC.
Introduction

Colorectal cancer (CRC) is the third most common cancer in men and the second most common in women worldwide (10.0% and 9.2% of total, respectively), and global incidence is estimated at 1.4 million cases annually, with 694,000 deaths (1). In 2019, there will be an estimated 145,600 new diagnoses of CRC and an estimated 51,020 deaths from this disease in the United States (2). Death rates from CRC have been declining in the United States since 1992, with an annual decline of 2.6% for males and 3% for females (3).

In contrast to the downturns among screening-aged individuals, CRC incidence rates in adults aged <50 years rose by 1.6% from 2000 to 2013, for an overall increase of 22% (from 5.9 to 7.2 per 100,000) (4). This increase has been driven by increasing incidence of distal colon cancer and rectal cancer, which has been increasing 3.2% annually from 1974-2013 in adults age 20-29 years (5,6). Patients younger than 50 years of age are not routinely screened for CRC and are at risk for delayed diagnosis and more advanced stage of disease at the time of diagnosis. A retrospective review found a significantly higher proportion of stage III-IV tumors in young adults (69.3%) compared to older adults (46.4%) (7,8). There is also evidence that patients diagnosed with CRC before the age of 50 have had worsened progression-free survival and overall survival compared to older patients (9,10).

Patients with early-onset CRC present with unique challenges, as younger patients may have young children, early career goals, financial toxicity, and concerns such as fertility-preservation that are not as prevalent in older patients (11). Clinically, patients with early-onset CRC may present differently than older-onset CRC with prolonged hematochezia, multiple office visits, and delayed time from onset of symptoms to diagnosis (12). These issues emphasize the importance of specifically investigating underlying biological differences in younger versus older CRC patients (9).

Although etiologies for the increase seen in young adults are yet to be fully elucidated, environmental factors may contribute including changes in lifestyle and dietary patterns. There is evidence for an increased prevalence of hereditary risk factors for CRC including familial adenomatous polyposis (FAP) and Lynch syndrome in early-onset CRC cases, but these
hereditary risk factors do not fully account for the increase seen in younger patients (12-14). Approximately 80% of patients with FAP harbor truncating germline mutations in the Adenomatous Polyposis Coli (APC) tumor suppressor gene, with identified mutations including R564X, R876X, Q1045X, 3927-3931delAAAGA, D1822V, and 2601delGA, R923X (15). Inherited mutations in four DNA mismatch repair (MMR) genes (MLH1, MSH2, MSH6, and PMS2) are the predominant cause of susceptibility to Lynch syndrome (16).

At this time, knowledge regarding the molecular features of sporadic young-onset CRC is limited, with few studies evaluating the genomic differences in this patient population. In this study, we report a large-scale study investigating comprehensive genomic differences between young-onset and later-onset CRC in hopes of elucidating further differences between the two groups.

Methods

Patients. Samples were obtained from 18,218 patients with pathologically confirmed colorectal cancer, who were referred to targeted next generation sequencing (NGS) assay by their treating physician. Samples were sent either from the original diagnostic samples or from a sample of tumor after recurrence, to identify potentially actionable genomic alterations (GAs). Multiple samples from the same patient were not allowed in the dataset. Although detailed staging information is not available, the significant majority of patients were diagnosed with advanced (unresectable) disease. This study was conducted and presented according to the most recent REporting recommendations for tumor MARKer prognostic studies (REMARK) (17). All studies were conducted in accordance with the International Ethical Guidelines for Biomedical Research Involving Human Subjects (CIOMS). All studies were performed after approval by an institutional review board (IRB). Written consent was obtained prior to data collection for this de-identified retrospective analysis.

Targeted NGS. Analysis was performed using a clinical NGS-based assay (FoundationOne™, Foundation Medicine Inc., Cambridge, MA) as previously described (18). The sequencing method was validated on hybridization captured, adaptor ligation-based libraries using DNA extracted from ten formalin-fixed paraffin-embedded (FFPE) sections cut at 5 mm.
Comprehensive genomic profiling was performed on hybridization-captured, adaptor ligation-based libraries to a mean specimen median sequencing coverage depth of 688X for all coding exons and selected introns of up to 403 cancer-related genes. GAs (base substitutions, small indels, rearrangements and copy number alterations) were determined. Tumor mutational burden (TMB) data was available for a subset of the overall dataset.

**Data analysis.** Sequence data were evaluated for GAs including point mutations, insertions and deletions, copy number alterations, and select gene fusions/rearrangements, as described previously (18). Custom analysis was conducted as previously described (18), comparing results in younger patients (defined as < 40 years) and older patients (defined as ≥ 50 years) diagnosed with CRC. For a more in-depth analysis of individual genes, a logistic model of the type alteration status was created for each gene. Alteration status was 1 or 0 for each sample and was determined by whether there was a functional alteration in that gene for that individual. All types of alterations were included for this analysis (amplifications, deletions, truncations, point). The false discovery rate (FDR) method was utilized to correct for multiple testing for (19). Plots were created for all genes significant at FDR = 0.05.

**Statistical considerations.** Descriptive analyses were performed using frequencies and percentiles. Medians and ranges were reported for continuous variables. Fisher’s exact test was used for comparison between categorical variables. Statistical significance was defined as p < 0.05.

**Results**

DNA was extracted from 18,218 formalin-fixed, paraffin-embedded clinical specimens from patients with CRC. The age distribution for the cohort is shown in **Figure 1**, and the alteration rate across all cases is shown in **Figure 2**. There were a total of 1420 patients under the age of 40, 3248 between 40 and 49, and 13,550 age 50 and older. In accord with prior studies of CRC (20,21), the most frequent alterations were observed in *APC* (76.8%), *TP53* (75.9%), *KRAS* (50.5%), *PIK3CA* (17.8%), and *SMAD4* (14.9%). Notable fusion events which may confer
sensitivity to targeted therapy, were detected at extremely low rates (< 1%) in ALK, BRAF, FGFR1, FGFR3, RET, and ROS1, as has been reported previously (Supplemental Table 1) (22).

When comparing the < 40 cohort to the ≥ 50 cohort, gene alteration rates in the MSS younger and older cohorts were largely similar for the majority of the genes analyzed. However, several notable differences were seen between the MSS < 40 and ≥ 50 age cohorts. TP53 (p<0.01, FDR<0.01) and CTNNB1 (p<0.001, FDR<0.01) alterations were found to be more common in tumor samples from patients < 40 years old diagnosed with CRC. In contrast, APC (p < 0.01, FDR < 0.01), KRAS (p<0.001, FDR<0.01), BRAF (p<0.001, FDR<0.01), PIK3CA (p<0.001, FDR<0.01), and FAM123B (p<0.001, FDR<0.01) were more commonly altered in tumor samples from patients ≥ 50 years old with CRC (Table 1, Figure 3). In the MSI-H cohort, several notable differences between the two age cohorts were also observed, with statistically significant increases in alteration rates observed in early-onset CRC in APC (p<0.001, FDR<0.01) and KRAS (p<0.001, FDR<0.01), and statistically significant increases in alteration rates in later-onset CRC in BRAF (p<0.001, FDR<0.01) and MLH1 (p<0.001, FDR<0.01).

However, similar to the MSS cohort, other genes of interest showed very few differences.

In order to perform a more detailed analysis of gene alteration rates by patient age, alteration rates were evaluated using age as a continuous variable within the MSS set. In this analysis, several genes were noted to have a statistically significant increase in mutation rate with patient age, including ASXL1, BRAF, CEBPA, CDKN2A, DNMT3A, FAM123B, RNF43, SF3B1, SOX9, TET2 (Figure 4A). Genes showing a decreasing alteration rate with age include CTNNB1, GEN1, MYC, POLE, and TP53 (Figure 4B). Some specific common oncogenically activating mutations shown to have driver function in CRC also differed dependent on patient age. A detailed analysis of specific BRAF mutations performed in a smaller subset of patients (10,070 patients) indicated, BRAF V600E mutations increased with age, whereas other non-V600E BRAF mutations did not (Figure 5). For RNF43, the most common mutation (G659fs*41) showed a substantial increase with age, whereas other mutations in this gene did not (Figure 5).

Tumor mutational burden (TMB) data were available for 10,070 patients within the overall dataset. Of those with TMB data available, there were a total of 9,615 patients with
microsatellite stable (MSS) CRC and 455 patients with microsatellite instability high (MSI-H) CRC. Interestingly, in the MSI-H cohort, TMB increases with increasing age, with higher TMB scores seen in older patients, and lower TMB scores seen in younger patients (Supplemental Figure 2). In the MSS subset, there was no difference in TMB by age.

Discussion
The genomic landscape of metastatic CRC has been described in great detail previously, showing that colon and rectal cancers had similar patterns of genomic alterations (20). However, a comprehensive analysis of genomic alteration rates by age has not been performed and is an area of research interest given the steady increase in young-onset CRC seen in the United States. This comprehensive analysis of younger and older onset CRC provides a number of insights into potential genomic differences between the two groups.

Overall, the genomic landscape between younger and older onset CRC appears similar, with expected rates of alterations in several genes that are critical to the selection of an optimal treatment paradigm for individual patients, including \textit{KRAS}, \textit{NRAS}, and \textit{BRAF} (23). However, several notable differences were seen between the MSS $< 40$-year-old and the $\geq 50$-year-old cohorts, with \textit{TP53} and \textit{CTNNB1} alterations more frequently seen in younger patients with CRC, and \textit{APC}, \textit{KRAS}, \textit{BRAF}, \textit{PIK3CA}, and \textit{FAM123B} more commonly altered in older patients with CRC. CRC tumors with \textit{BRAF} V600E mutations have been previously described to be more likely observed in patients aged 70 years or older (24,25). Another study has suggested that young-onset CRC have a lower rate of \textit{BRAF} alterations (26). Interestingly, non-V600E \textit{BRAF} mutations showed no differences across age groups, and recently reported data suggest that patients that harbor non-V600E \textit{BRAF} mutations have a distinct and improved prognosis (27). In a previously published study, we performed a detailed characterization of RAS mutational profiles in colon and rectal cancer in young and older patients (28), finding an association of specific mismatch substitutions with age and tumor site. Age- and site-related differences in alteration rates for specific mutations in the other genes reported here have not been described in great detail, and clearly bear greater scrutiny.
The difference in APC alteration rates is of significant interest, as mutation of APC has been previously described to have an effect on prognosis (29). CRC tumors lacking any APC mutation carry a worse prognosis than tumors with single APC mutations, and given the decreased rates of APC alterations in younger patients, this may help explain a potential difference in response to therapy in younger patients with metastatic CRC as previously reported (10). The lower incidence of APC mutations in younger patients is also of interest, given the higher expected incidence of FAP (associated with APC alterations) in the younger population. Differences in WNT pathway alteration by age is also supported by differences in RNF43 G659fs*41 alteration rates, with a clear increase seen with increasing age. The RNF43 tumor suppressor gene encodes a transmembrane ubiquitin ligase that ubiquitinates Frizzled receptors and thereby downregulates the surface expression of the WNT receptor; the truncating G659fs*41 mutation eliminates RNF43 function, elevating activity of WNT effectors (30).

When looking at age as a continuous variable, several genes showed modest decreases in alteration rates with increasing age, including the WNT effector CTNNB1, the DNA damage repair genes GEN1 and POLE, and TP53, though the alteration rates for these genes, with the exception of TP53, are quite low. The increased alteration rates in CTNNB1 in younger patients support a prior study that implicated the WNT/β-catenin pathway in sporadic early-onset CRC (31). There has been increasing interest in genes involved in DNA replication or repair (such as POLE, POLD1, MSH2) in colorectal cancer, suggesting that loss of normal DNA repair fidelity may have contributed to increased mutational burden and immune checkpoint blockade response in these tumors. To date, a role for GEN1 in colorectal cancer has not been reported, although its roles in maintaining genome integrity by promoting repair of Holliday junctions and intensifying the phenotypes associated with early onset cancers arising from Bloom’s syndrome are well established (32,33); our data suggests further investigation would be of interest. However, the overall alteration rates for these genes is extremely low, limiting their predictive value for therapeutic decision-making (34).

Within the MSI-H cohort, several large alteration rate differences were noted in the younger and older cohorts, including differences in the alteration rates in APC, KRAS, and BRAF. Two major causes of defective MMR that can lead to MSI-H are acquired hypermethylation of the MLH1
promoter in sporadic colorectal cancer and germline MMR mutation combined with an acquired inactivation in the remaining functional allele (35). In cases of MSI, the presence of the BRAF V600E hotspot mutation practically excludes the possibility of Lynch Syndrome, and the clinical utility of the combination of these two markers is well-established (36). The difference in alteration rates in \textit{BRAF} most likely signifies the increased prevalence of sporadic MSI in the older population and the increased prevalence of inherited MSI in the younger population (37). Interestingly, the overall TMB in the MSI-H cohort appears to increase with increasing age, paralleling results seen in other tumor types (38). Prior studies investigating mutational signatures within cancer show a positive correlation between the age of cancer diagnosis and the number of mutations observed, suggesting that the mutational process may be operative at a constant rate over time leading to increased mutational burden (39,40). Other reports have suggested that the majority of young-onset CRC is MSS, and shows a higher rate of CpG island methylator phenotype (CIMP) (26). Future studies will need to investigate the response rates of patients of all ages with MSI-H metastatic CRC treated with immune checkpoint inhibitors to assess if there is an association with TMB, and if older patients receive additional benefit from these therapies.

Despite the comprehensive analysis and large sample size, there are several limitations of this current data set. There is emerging awareness that the response of metastatic CRC to targeted anti-EGFR inhibitors is impacted by the specific site of origin within the colon. Unfortunately, sidedness data is currently unavailable for this dataset, precluding an analysis into whether different mutational spectrums exist in the left versus right colon (41). Because clinical annotation data is unavailable, the predictive and prognostic effect of these gene alteration rates cannot be studied, though there are significant data on the effect of individual genes in the current literature. In addition, the dataset lacks information on factors such as hypermethylation or mRNA expression; hence, some of the genes may have significantly altered activity due to increased or decreased abundance of transcripts, modifying the estimation of their loss or gain of function as a factor of age.

In conclusion, the genomic landscapes of young onset and older onset CRC, while overall similar, display specific differences in genes that have may prognostic implications. Further
study will need to be conducted to determine if the differences in gene alteration rates, particularly within the WNT/β-catenin pathway, can be leveraged to provide personalized therapies for young patients with early-onset sporadic CRC. This study also highlights the impact of robust next-generation sequencing in the analysis of metastatic CRC, including the assessment of microsatellite instability as biomarker analysis continues to affect the personalized treatment plans for patients.

Acknowledgments.

Funding: The authors were supported by NCI Core Grant P30 CA006927 (to Fox Chase Cancer Center), NIH R01 DK108195 (to EAG), CPRIT Scholar Award #RR160093 (to SGE), NIH R01 CA229259-01 (to CL), by a subsidy of the Russian Government to support the Program of Competitive Growth of Kazan Federal University (to IGS), and by the Colorectal Cancer Alliance (to JEM, EAG). Author contributions: CC, GF, MC, VM, S Ali and JSR collected data. IGS, EH, SA, and JSR performed data analysis. EAG, IGS, SA, CL, and JEM designed the study and wrote the manuscript. Competing interests: CC, GF, MC, VM, S Ali, and JSR are employed by FM Inc, and own stock in FM Inc. Data and Materials availability: Summary level data are available by application to Foundation Medicine.
**Figure 1.** Age distribution of the entire cohort

**Figure 2.** Across all colorectal cases, the most frequent alterations were in APC (76.8%), TP53 (75.9%), KRAS (50.5%), PIK3CA (17.8%), and SMAD4 (14.9%).

**Figure 3.** Alteration rate in genes of interest between early-onset (< 40 years - blue) and later-onset (≥ 50 years - orange) in MSS and MSI-H CRC (patients between the ages of 40 and 49 are not included in these figures).

**Figure 4.** A. Genes of interest with increasing alteration rates by age (all p values < 0.01). The dots show the alteration rates at each age (dots are sized according to how many samples there are at that age), and the red line shows the regression fit from the logistic regression model. B. Genes of interest with decreasing alteration rates by age (all p values < 0.01)

**Figure 5:** Differing rates of specific BRAF and RNF43 mutations by age showing a clear distinction between BRAF V600E mutations versus other BRAF mutations as well as a difference in RNF43 659_660 mutations and RNF43 117 mutations.
### Table 1. Significant alterations and alterations in genes of interest between cohorts using false discovery rate (FDR) in MSS CRC and MSI-H CRC

<table>
<thead>
<tr>
<th>Gene</th>
<th>Rate observed in Under 40 group (%)</th>
<th>Rate observed in 50 and over group (%)</th>
<th>FDR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TP53</strong></td>
<td>82.3</td>
<td>76.7</td>
<td>1.56E-05</td>
</tr>
<tr>
<td><strong>APC</strong></td>
<td>65.8</td>
<td>79.7</td>
<td>4.84E-26</td>
</tr>
<tr>
<td><strong>KRAS</strong></td>
<td>45.6</td>
<td>52.4</td>
<td>1.56E-05</td>
</tr>
<tr>
<td><strong>PIK3CA</strong></td>
<td>14.1</td>
<td>17.5</td>
<td>0.002959601</td>
</tr>
<tr>
<td><strong>CTNNB1</strong></td>
<td>4</td>
<td>2.7</td>
<td>0.013488987</td>
</tr>
<tr>
<td><strong>BRAF</strong></td>
<td>5.2</td>
<td>7.7</td>
<td>0.002067048</td>
</tr>
<tr>
<td><strong>FAM123B</strong></td>
<td>2</td>
<td>6.8</td>
<td>1.35E-12</td>
</tr>
<tr>
<td><strong>NRAS</strong></td>
<td>3.7</td>
<td>4.6</td>
<td>0.171847712</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gene</th>
<th>Rate observed in Under 40 group (%)</th>
<th>Rate observed in 50 and over group (%)</th>
<th>FDR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TP53</strong></td>
<td>28.6</td>
<td>36.2</td>
<td>0.264580718</td>
</tr>
<tr>
<td><strong>APC</strong></td>
<td>70.1</td>
<td>34.4</td>
<td>1.51E-08</td>
</tr>
<tr>
<td><strong>KRAS</strong></td>
<td>49.4</td>
<td>24.1</td>
<td>2.00E-05</td>
</tr>
<tr>
<td><strong>PIK3CA</strong></td>
<td>42.9</td>
<td>30.6</td>
<td>0.066741704</td>
</tr>
<tr>
<td><strong>CTNNB1</strong></td>
<td>15.6</td>
<td>8.2</td>
<td>0.080623845</td>
</tr>
<tr>
<td><strong>BRAF</strong></td>
<td>5.2</td>
<td>48.8</td>
<td>2.05E-14</td>
</tr>
<tr>
<td><strong>FAM123B</strong></td>
<td>16.9</td>
<td>13.1</td>
<td>0.422335612</td>
</tr>
<tr>
<td><strong>NRAS</strong></td>
<td>0</td>
<td>1.4</td>
<td>0.605361263</td>
</tr>
</tbody>
</table>

**Alteration Rates in the MSS cohort**

**Alteration Rates in the MSI-H cohort**
References


41. Venook AP, Niedzwiecki D, Innocenti F, Fruth B, Greene C, O'Neil BH, et al. Impact of primary (1º) tumor location on overall survival (OS) and progression-free survival (PFS) in patients (pts) with metastatic colorectal cancer (mCRC): Analysis of CALGB/SWOG 80405 (Alliance). J Clin Oncol 2016;34(suppl; abstr 3504).
Figure 1

The bar chart shows the sample size distribution by age group. The age groups are categorized as follows:

- **< 40**: Sample size of approximately 1000
- **40-49**: Sample size of approximately 3000
- **≥ 50**: Sample size of approximately 13,000

The bar chart indicates a significant increase in sample size for the age group of 50 and above compared to the younger age groups.
Figure 2
Figure 3

MSS (13376 samples)

Percentage of patients

TP53  APC  KRAS  PIK3CA  CTNNB1  BRAF  AMER1  NRAS  MLH1

MSS

MSI-H (649 samples)

Percentage of patients

TP53  APC  KRAS  PIK3CA  CTNNB1  BRAF  AMER1  NRAS  MLH1
### Alteration Rates in the MSS cohort

<table>
<thead>
<tr>
<th>Gene</th>
<th>Rate observed in Under 40 group (%)</th>
<th>Rate observed in 50 and over group (%)</th>
<th>FDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53</td>
<td>82.3</td>
<td>76.7</td>
<td>1.56E-05</td>
</tr>
<tr>
<td>APC</td>
<td>65.8</td>
<td>79.7</td>
<td>4.84E-26</td>
</tr>
<tr>
<td>KRAS</td>
<td>45.6</td>
<td>52.4</td>
<td>1.56E-05</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>14.1</td>
<td>17.5</td>
<td>0.002959601</td>
</tr>
<tr>
<td>CTNNB1</td>
<td>4</td>
<td>2.7</td>
<td>0.013488987</td>
</tr>
<tr>
<td>BRAF</td>
<td>5.2</td>
<td>7.7</td>
<td>0.002067048</td>
</tr>
<tr>
<td>FAM123B</td>
<td>2</td>
<td>6.8</td>
<td>1.35E-12</td>
</tr>
<tr>
<td>NRAS</td>
<td>3.7</td>
<td>4.6</td>
<td>0.171847712</td>
</tr>
</tbody>
</table>

### Alteration Rates in the MSI-H cohort

<table>
<thead>
<tr>
<th>Gene</th>
<th>Rate observed in Under 40 group (%)</th>
<th>Rate observed in 50 and over group (%)</th>
<th>FDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53</td>
<td>28.6</td>
<td>36.2</td>
<td>0.264580718</td>
</tr>
<tr>
<td>APC</td>
<td>70.1</td>
<td>34.4</td>
<td>1.51E-08</td>
</tr>
<tr>
<td>KRAS</td>
<td>49.4</td>
<td>24.1</td>
<td>2.00E-05</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>42.9</td>
<td>30.6</td>
<td>0.066741704</td>
</tr>
<tr>
<td>CTNNB1</td>
<td>15.6</td>
<td>8.2</td>
<td>0.080623845</td>
</tr>
<tr>
<td>BRAF</td>
<td>5.2</td>
<td>48.8</td>
<td>2.05E-14</td>
</tr>
<tr>
<td>FAM123B</td>
<td>16.9</td>
<td>13.1</td>
<td>0.422335612</td>
</tr>
<tr>
<td>NRAS</td>
<td>0</td>
<td>1.4</td>
<td>0.605361263</td>
</tr>
</tbody>
</table>

**Table 1.** Significant alterations and alterations in genes of interest between cohorts using false discovery rate (FDR) in MSS CRC and MSI-H CRC
Clinical Cancer Research

Comprehensive Genomic Landscapes in Early and Later Onset Colorectal Cancer

Christopher H Lieu, Erica A. Golemis, Ilya G. Serebriiskii, et al.

Clin Cancer Res Published OnlineFirst June 26, 2019.

Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-19-0899

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2019/06/26/1078-0432.CCR-19-0899.DC1

Author Manuscript
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link http://clincancerres.aacrjournals.org/content/early/2019/06/26/1078-0432.CCR-19-0899. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.