BRAF Mutations Are Associated with Poor Survival Outcomes in Advanced-stage Mismatch Repair-deficient/ Microsatellite High Colorectal Cancer

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Abstract

Background: Mismatch repair-deficient (MMR-D)/microsatellite instability-high (MSI-H) metastatic colorectal cancer (mCRC) is a unique disease entity with growing interest given the rise of young-onset CRC. Given its heterogeneous behavior and potential for highly effective treatment outcomes, we sought to identify the clinical and molecular features that offer prognostic value for MMR-D CRC.

Materials/Methods: This was a retrospective cohort study of patients with metastatic CRC with MMR-D or microsatellite instability in a real-world database. Overall survival (OS) was determined by the date of metastatic disease to date of death with stratification made based on factors including BRAF and RAS mutation status, age, and MMR protein loss type.

Results: There were 1101 patients in the study. Patients with BRAF mutations had worse OS compared with patients with wild-type BRAF with a median survival of 18.9 months versus 33.2 months (hazard ratio [HR] 1.52, 95% confidence interval [CI]: 1.25-1.86, P < .001). Patients with age >50 were found to have decreased OS versus age ≤50 with a median survival of 21.4 months versus 38.7 months (HR 1.66, 95% CI: 1.33-2.07, P < .001). BRAF mutations and age >50 remained significant predictors of OS in multivariate analysis.

Conclusion: BRAF mutations and age >50 are associated with worse survival outcomes for patients with MMR-D mCRC. RAS mutations and specific MMR alterations are not associated with survival outcomes.

Key words: colorectal cancer; mismatch repair deficiency; microsatellite instability high; BRAF V600E; prognosis; KRAS; NRAS; late-onset disease.

Implications for Practice
The results of this study reveal clinically relevant markers that predict overall survival for patients with mismatch repair-deficient metastatic colorectal cancer.

Introduction

Colorectal cancer (CRC) is one of the most common malignancies with a continually rising incidence in young adults; currently, it is the fourth leading cause of cancer-related death worldwide.1 Predicted to be the second leading cause of cancer-related death for people aged 20-49 by 2040, CRC presents a concerning trend in young adults.2

More prominent in this population is the prevalence of mismatch repair deficiency (MMR-D) or microsatellite instability-high (MSI-high) disease, in up to 5% of metastatic CRC (mCRC).3 MMR-D is characterized by the loss of expression or function of any of the MMR genes including, but not limited to, MLH1, PMS2, MSH2, or MSH6. This functional protein loss in MMR genes leads to impaired repair of mismatch nucleotides that occurs during DNA replication. Repeated sequences of DNA, known as microsatellites, are particularly susceptible to errors in cases of MMR-D; accumulation of these errors in these DNA regions then leads to variable sizes of microsatellites called microsatellite instability (MSI). This subsequently leads to frameshift mutations and a high tumor mutation burden.4

MMR deficiency is classified as either being secondary to germline mutations versus sporadic mutations, which include but not limited to those with BRAF V600E mutation with MLH1 promoter hypermethylation. Making this distinction is important given the various implications: patients with
sporadic MMR-D tend to be older, have poorly differentiated disease, and have less sensitivity to chemotherapy.\(^7\)

Translation of genes with frameshift mutation alterations leads to the formation of mutation-associated neoantigens (MANAs), which is a landmark feature of MMR-D/MSI-H tumors. These MANAs are recognized by MANA-specific T cells to subsequently evoke an anti-tumor immune response orchestrated by T cells. Notably, tumor-infiltrating lymphocytes (TILs) are increased in MSI-H/MMR-D tumors, compared with microsatellite stable (MSS) tumors.\(^8\) The MANA-mediated antitumor immune response in MMR-D/MSI-H tumors observed in preclinical studies triggered clinical trials with immune checkpoint inhibitors and led to practice-changing studies in the last decade. For example, KEYNOTE-177 demonstrated a significant improvement in median progression-free survival (PFS) for patients with MMR-D/MSI-H advanced CRC treated with pembrolizumab at 16.5 months versus 8.2 months with chemotherapy (hazard ratio [HR] 0.60, 95% confidence interval [CI], 0.45-0.80, \(P = .0002\)), establishing pembrolizumab as a standard of care first-line option for this population.\(^7\)

The majority of clinical trials have studied patients with MMR-D/MSI-H disease collectively. However, various clinical and molecular features, other than being characterized as MMR-D/MSI-H, may lead to differences in clinical behavior and response to therapies. For example, the \(BRAF\) V600E mutation and specific MMR gene loss were found to predict response to immune checkpoint inhibitors.\(^8\)

What has not been well established is the prognostic role of individual MMR genes and \(BRAF\) mutations, as well as other molecular and clinical factors, in patients with MMR-D mCRC. The primary objective of this study is to identify prognostic factors in MMR-D/MSI-H mCRC in a large patient cohort with a real-world database.

**Methods**

Patients with stage IV colon or rectal cancer with MMR-D (defined as loss of MLH1, PMS2, MSH2, or MSH6), determined by immunohistochemistry, or MSI-H, determined by PCR of tumor samples, were selected from the nationwide de-identified, electronic health record (EHR)-derived Flatiron Health database and included in this study. The Flatiron Health database is a longitudinal database, comprising de-identified patient-level structured and unstructured data, curated via technology-enabled abstraction.\(^9,10\) During the study period, the de-identified data originated from approximately 280 US cancer clinics (~800 sites of care). The majority of patients in the database originate from community oncology settings; relative community/academic proportions vary depending on the study cohort.

\(BRAF\) and \(RAS\) mutation status, primary tumor site, and exposure to immunotherapy were noted. ECOG performance status was determined on initial evaluation. The presence of \(BRAF\) mutations was confirmed by various methods including next-generation sequencing, polymerase chain reaction, and immunohistochemistry, and was stratified by age, gender, \(RAS\) status, and tumor site. Overall survival (OS) was determined from date of metastatic disease to date of death or last visit date and stratified based on age, \(BRAF\) status, \(RAS\) status, type of MMR gene loss, tumor site, and ECOG score. The loss of MLH1 and PMS2 was classified as MLH1 loss, and loss of MSH2 and MSH6 was classified as MSH2 loss, given the functional dependence of MLH1 with PMS2 and MSH2 with MSH6. Chi-square test was used to examine the association between the \(BRAF\) mutation and clinical/molecular markers, and the Cox regression model was used for univariate and multivariate analysis. All analyses were performed utilizing SAS version 9.4 (SAS Institute Inc, Cary, NC), and R (Version 4.0.2). Institutional Review Board approval of the study protocol was obtained before study conduct and included a waiver of informed consent.

**Results**

A total of 1101 patients with MMR-D/MSI-H mCRC diagnosed from January 1, 2013 to November 25, 2020 were included from the Flatiron Health database. Most patients

| Table 1. Baseline patient demographics and clinical/molecular characteristics. |
|-----------------------------|-----------------------------|
| Variable                    | Level                       | \(N = 1101\) % |
| Age at diagnosis            | ≤50                         | 223 20.3      |
|                             | >50                         | 878 79.7      |
| Gender                      | Female                      | 593 53.9      |
|                             | Male                        | 507 46.1      |
|                             | Missing                     | 1 -           |
| Race                        | Black or African American   | 94 9.4        |
|                             | Other Race                  | 155 15.6      |
|                             | White                       | 747 75.0      |
|                             | Missing                     | 105 -         |
| ECOG                        | 0                           | 411 46.9      |
|                             | 1                           | 320 36.5      |
|                             | 2                           | 114 13.0      |
|                             | 3                           | 29 3.3        |
|                             | 4                           | 2 0.2         |
|                             | Missing                     | 225 -         |
| MMR gene loss               | MLH1                        | 607 72.9      |
|                             | MSH2                        | 93 11.2       |
|                             | MSH6                        | 53 6.4        |
|                             | PMS2                        | 80 9.6        |
|                             | Missing                     | 268 -         |
|                             | MLH1+PMS2                   | 687 82.5      |
|                             | MSH2+MSH6                   | 146 17.5      |
|                             | Missing                     | 268 -         |
| \(BRAF\)                    | \(BRAF\) mutant             | 356 44.3      |
|                             | \(BRAF\) wild               | 447 55.7      |
|                             | Missing                     | 298 -         |
| \(RAS\)                     | \(RAS\) mutant              | 244 28.9      |
|                             | \(RAS\) wild                | 599 71.1      |
|                             | Missing                     | 258 -         |
| Tumor Site                  | Colon                       | 1009 92.5     |
|                             | Rectum                      | 82 7.5        |
|                             | Missing                     | 10 -          |
| Immunotherapy               | No                          | 750 68.1      |
|                             | Yes                         | 351 31.9      |
| Age at diagnosis            | Median                      | 67            |
|                             | Minimum                     | 18            |
|                             | Maximum                     | 85            |
|                             | Std Dev                     | 14.49         |
|                             | Missing                     | 0 -           |
were older than 50 years (79.9%), Caucasian (75%), and had ECOG 0-1 (83.4%) (Table 1). Of patients with known specific MMR gene loss \((n = 833)\), 687 (82.5%) had MLH1 and/or PMS2 loss, while 146 (17.5%) had MSH2 and/or MSH6 loss. Among patients with known BRAF status \((n = 803)\), 241 (47.2%) had a BRAF mutation and 55.7% \((n = 447)\) were BRAF wildtype (Table 1). Of patients with BRAF mutation, 301 patients had BRAF V600E mutation, 18 had a non-BRAF V600E mutation, and 27 had an unknown BRAF mutation type, and 10 had more than one BRAF mutation.

The presence of BRAF mutation was more common in age >50 versus ≤50 (52% vs 14.1%, \(P < .001\)), females versus males (54.8% vs 31.7%, \(P < .001\)), RAS WT versus RAS mut (52.8% vs 8.8%, \(P < .001\)), and colon versus rectum (46.9% vs 14.8%, \(P < .01\); Table 2). MLH1/PMS2 loss, compared with MSH2/MSH6 loss, was more common in age >50 versus ≤50 (86.9% vs 64.4%, \(P < .001\)), females (87.5% vs 76.8%, \(P < .001\)), RAS WT (86.6% vs 64.9%, \(P < .001\)), and colon (84.6% vs 52.8%, \(P < .001\); Table 2).

Patients with BRAF mutation also had worse survival outcomes compared to patients with wild-type BRAF with overall survival of 18.9 versus 33.2 months (HR 1.52, 95% CI: 1.25-1.86, \(P < .001\); Fig. 1). A subset analysis of patients with BRAF V600E mutation also showed worse OS compared with wild-type BRAF with overall survival of 17.3 versus 33.2 months (HR 1.59, 95% CI: 1.29-1.96, \(P < .001\); Supplementary Fig. 1). Patients aged >50 were found to have worse OS compared with patients age ≤50 with a median survival time of 21.4 months versus 38.7 months (HR 1.66, 95% CI: 1.33-2.07, \(P < .001\); Fig. 1B). Patients with a RAS mutation had improved OS compared with patients with wild-type RAS at 35.7 versus 22.8 months (HR 0.76, 95% CI: 0.61-0.94, \(P = .011\); Fig. 1C; Tables 3 and 4).

There was no significant difference in overall survival between individual MMR genes (\(P = .166\)); however, when MSH2/MSH6 were grouped and compared with MLH1/PMS2 mutations, a trend toward improved overall survival was noted with a median survival time of 35.2 versus 22.7 months (HR 0.79, 95% CI: 0.61-1.02, \(P = .067\); Fig. 1D).

### Table 2. Clinical characteristics based on BRAF mutation status and MMR genes.

| Covariate and level | BRAF mutation status | MLH1+PMS2 | MSH2+MSH6 |
|---------------------|----------------------|-----------|-----------|
|                     | Not present | Present | \(P\) value | Not present | Present | \(P\) value |
| Age, years          | \(n = 447\) | \(n = 356\) | \(P\) value | \(n = 687\) | \(n = 146\) | \(P\) value |
| ≤50                 | 140 (85.9%) | 23 (14.1%) | <.001 | 105 (64.4%) | 58 (35.6%) | <.001 |
| >50                 | 307 (48%) | 333 (52%) | <.001 | 582 (86.9%) | 88 (13.1%) | <.001 |
| Gender              | \(n = 146\) | \(n = 146\) | \(P\) value | \(n = 356\) | \(n = 356\) | \(P\) value |
| Female              | 200 (45.2%) | 242 (54.8%) | <.001 | 386 (87.5%) | 55 (12.5%) | <.001 |
| Male                | 246 (68.3%) | 114 (31.7%) | <.001 | 301 (76.8%) | 91 (23.2%) | <.001 |
| RAS Status          | \(n = 356\) | \(n = 356\) | \(P\) value | \(n = 146\) | \(n = 146\) | \(P\) value |
| Mutated             | 176 (91.2%) | 17 (8.8%) | <.001 | 109 (64.9%) | 59 (35.1%) | <.001 |
| Wildtype            | 241 (47.2%) | 270 (52.8%) | <.001 | 387 (86.6%) | 60 (13.4%) | <.001 |
| Tumor Site          | \(n = 687\) | \(n = 146\) | \(P\) value | \(n = 146\) | \(n = 146\) | \(P\) value |
| Colon               | 394 (53.1%) | 348 (46.9%) | <.01 | 655 (84.6%) | 119 (15.4%) | <.001 |
| Rectum              | 46 (85.2%) | 8 (14.8%) | 71 (70.7%) | 22 (29.3%) | 119 (15.4%) | <.001 |

### Discussion

Significant heterogeneity among patients with MMR-D CRC exists; therefore, to understand this disease entity better, we sought to determine the clinical and molecular features that play a prognostic role in MMR-D CRC. In our study, we identified that BRAF mutations and age >50 were associated with worse survival outcomes for patients with MMR-D mCRC. BRAF V600E mutation has been well established as a poor prognostic marker in MSS CRC. This particular point mutation maintains an active BRAF kinase, leading to constitutive activation of the MAPK pathway and upregulation of the cell cycle, propagating carcinogenesis. In MMR-D tumors, BRAF mutations are more common with an incidence of 34.6% compared with MSS tumors at 6.8%. This may be due to BRAF’s association with the high-level CpG island methylator phenotype (CIMP) and MLH1 promoter methylation.

For early-stage MMR-D CRC, BRAF mutation has been established as a poor prognostic factor, based on analysis of survival after disease recurrence from the ACCENT database. This database included 7 trials with patients with stage III CRC treated with adjuvant therapy with a total of 271 patients with MMR-D CRC. Patients with the BRAF V600E mutation with MMR-D \((n = 91)\) were noted to have worse survival outcomes compared to patients with wild-type BRAF with overall survival of 18.9 versus 33.2 months (HR 1.52, 95% CI: 1.25-1.86, \(P < .001\); Fig. 1). Treatment with immunotherapy did lead to an improved median survival time of 48.5 versus 17.2 months (HR 0.49, 95% CI: 0.38-0.64, \(P < .0001\) continued to show improved survival in multivariate analysis, while RAS mutation status lost its significance (Table 5).
compared with patients with BRAF wild-type MMR-D (n = 180) (HR 2.65, 95% CI 1.67-4.21, P < .0001).14

Our study is the first and largest study, to our knowledge, to identify BRAF mutations’ association with worse overall survival (18.9 months vs 33.2 months, HR 1.41, P = .01) for MMR-D/MSI-H mCRC in a large patient cohort. While this mutation can be associated with other factors that can contribute to worse survival, including older age, we demonstrated that the presence of BRAF mutations is still associated with worse survival outcomes on multivariate analysis. Previously, one pooled analysis of 4 studies of MMR-D mCRC patients did not show differences in PFS or OS based on BRAF mutation status with PFS 6.1 versus 6.3 months (HR 1.07, 95% CI 0.67-1.70, P = 1.0) and OS 11.7 versus 15 months (HR 1.51, 95% CI: 0.93-2.46, P = .155).12 However, this analysis involved a sample size of just 153 patients and only 53 patients with BRAF V600E mutation. In addition to being prognostic, the BRAF mutation may be
The association of RAS mutations with poor clinical outcomes in early-stage MMR-D CRC has been established; however, studies of its role in advanced disease are limited. One study of patients with stage I-IV CRC found that KRAS mutated/MMR-D CRC had the shortest OS while KRAS WT/MMR-D CRC had the longest OS.19 Specifically, in early-stage MMR-D CRC, KRAS status has not been established to have prognostic value; this may be due to the smaller sample sizes and difficulty with reaching statistical significance.20,21 Our study evaluating over 1000 mCRC patients failed to identify this association; conversely, we discovered an association between wild-type RAS status and adverse survival outcome, although this did not persist on multivariate analysis. Notably, RAS mutations and BRAF mutations are almost mutually exclusive, and worse outcomes observed in univariate analysis for RAS wild-type patients could be related to the predominant presence of BRAF mutations in the RAS wild-type cohort. It is also important to note that, while BRAF V600E is known to be a founder mutation in MMR-D CRC, RAS mutations are not driver alterations in MMR-D CRC and occasionally occur as a result of frameshift mutations; their molecular and clinical significance remains to be seen.

We also evaluated differences in survival outcomes for MMR-D mCRC patients based on the type of protein loss leading to the MMR deficiency. Patients were grouped into MLH1/PMS2 vs. MSH2/MSH6 categories, given the functional dependence of these proteins on each other. Loss of MLH1/PMS2 protein demonstrated a trend toward worse overall survival compared with MSH2/MSH6 loss (P = .067). Of note, MLH1 loss is often seen in association with BRAF mutation, which has a poor prognosis, as mentioned previously. Also, MLH1/PMS2 loss is associated with a low tumor mutational burden, which is also a negative prognostic factor.22 Other studies evaluating the prognostic value of individual MMR proteins or MMR protein subtypes are limited; therefore, additional studies are needed to verify our findings. Of note, the type of individual MMR protein loss has been shown to confer differences in response to immunotherapy in MMR-D CRC. Patients with MSH2/MSH6 loss have had greater 1-year and 2-year PFS compared with those with MLH1/PMS2 loss (84.2% vs 57.8% and 78.2% vs 54.2%, respectively, P < .001) when treated with immunotherapy.9

Our study is limited by its retrospective nature; however, the large sample size allowed for statistically significant results that were not seen with smaller-sized studies discussed above. Also, our dataset did not include other potential biomarkers such as sidedness of cancer, tumor mutational burden, and sites of metastasis, which may also have prognostic implications. Additionally, the mutation type for those with KRAS/NRAS mutations, as well as whether or not surgery was part of patients’ treatment, were not specified in the Flatiron Health database. Furthermore, prospective studies are needed to confirm our findings.

Currently, MMR-D mCRC is viewed as one disease entity with generalized treatment guidelines. However, multiple clinical and molecular features were found in our study to confer differences in survival outcomes for patients with MMR-D mCRC, including BRAF mutation status, age, and ECOG score. A trend toward differences in overall survival was also noted based on the type of MMR protein loss. These findings highlight the heterogeneity of MMR-D CRC as well as the
importance of incorporating these factors into understanding the pathophysiology of this disease.

**Conclusion**

In conclusion, our study demonstrated that BRAF mutations and age >50 are associated with inferior survival outcomes for patients with MMR-D mCRC. RAS mutations and specific MMR alterations do not seem to be associated with survival outcomes. As we gain a better understanding of the interplay of these prognostic factors, our approach to managing MMR-D CRC can become more personalized and hopefully lead to improved survival for patients.

**Conflict of Interest**

**Iman Imanirad:** Natera Inc (C/A); **Jessica Frakes:** Boston Scientific (C/A); **Christine Walko:** Mission Healthcare (E); **Jackson Laboratory for Genomic Medicine and Intermountain Precision Genomics (C/A);** **Richard Kim:** Bristol-Myers Squibb, Lilly, Bayer, and Taiho Oncology (C/A); Lilly and Incyte (Other--speaker's bureau); Bayer, Eisai, and Bristol-Myers Squibb (RF); **Jason B. Fleming:** Johnson & Johnson, GlycosBio, Moleculin Biotech, Perthera (C/A). The other authors indicated no financial relationships.

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**Table 4. Univariate Cox regression analysis with reported HRs.**

| Covariate                      | Level | N  | HR (95% CI) | Log-rank P-value | P-value |
|--------------------------------|-------|----|-------------|------------------|---------|
| **Age at diagnosis**           | >50   | 869| 1.66 (1.33-2.07) | <.001            | <.001   |
|                                | ≤50   | 223| -           | -                | -       |
| **Gender**                     | Male  | 502| 1.13 (0.96-1.34) | .146             | .147    |
|                                | Female| 589| -           | -                | -       |
| **Race**                       | Black or African American | 92 | 0.98 (0.73-1.31) | .874             | .787    |
|                                | Other Race | 154 | 1.09 (0.85-1.39) | .519             | -       |
|                                | White | 742| -           | -                | -       |
| **ECOG performance status**    | 2-4   | 142| 2.23 (1.76-2.83) | <.001            | <.001   |
|                                | 0-1   | 728| -           | -                | -       |
| **MMR gene loss**              | MSH2  | 93 | 0.72 (0.52-1.00) | .047             | .166    |
|                                | MSH6  | 53 | 0.95 (0.65-1.40) | .812             | -       |
|                                | PMS2  | 79 | 1.13 (0.83-1.54) | .451             | -       |
|                                | MLH1  | 601| -           | -                | -       |
| **MMR gene loss**              | MSH2+MSH6 | 146 | 0.79 (0.61-1.02) | .068             | .067    |
|                                | MLH1+PMS2 | 680 | -           | -                | -       |
| **BRAF**                       | BRAF mutant | 355 | 1.52 (1.25-1.86) | <.001            | <.001   |
|                                | BRAF wild | 442 | -           | -                | -       |
| **RAS**                        | RAS mutant | 243 | 0.76 (0.61-0.94) | .011             | .011    |
|                                | RAS wild | 596 | -           | -                | -       |
| **Tumor Site**                 | Rectum | 81 | 1.08 (0.80-1.45) | .615             | .614    |
|                                | Colon   | 1001| -           | -                | -       |
| **Immunotherapy**              | Yes    | 351| 0.47 (0.39-0.57) | <.001            | <.001   |
|                                | No     | 741| -           | -                | -       |
| **Age at diagnosis**           | 1092  | 1.02 (1.02-1.02) | <.001            | <.001   |
| **Duration on immunotherapy (months)** | 351 | 0.89 (0.87-0.92) | <.001            | -       |

Bold values are statistically significant.

Abbreviations: CI, confidence interval; HR, hazard ratio; OS, overall survival.

| Variable                   | Level | HR (95% CI) | P-value |
|----------------------------|-------|-------------|---------|
| **Age at diagnosis**       | 1.01 (1, 1.02) | .0254 |         |
| **ECOG performance status**| 1.87 (1.38, 2.54) | <.0001 |         |
| **BRAF**                   | 1.41 (1.08, 1.85) | .0121 |         |
| **Immunotherapy**          | 0.49 (0.38, 0.64) | <.0001 |         |

Abbreviations: CI, confidence interval; HR, hazard ratio.

importance of incorporating these factors into understanding the pathophysiology of this disease.
Author Contributions
Conception/design: I.H.S. Provision of study material or patients: I.H.S., E.T. Collection and/or assembly of data: I.H.S., E.T., J.W. Data analysis and interpretation: I.H.S., E.T., J.W. Manuscript writing: All authors. Final approval of manuscript: All authors.

Data Availability
The data underlying this article will be shared on reasonable request to the corresponding author.

Supplementary Material
Supplementary material is available at The Oncologist online.

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