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Article

Assessing Oncologists' Adoption of Biomarker Testing in Metastatic Colorectal Cancer Using Real-World Data

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Abstract

Background: Despite national guideline recommendations for universal biomarker testing (*KRAS*, *NRAS*, *BRAF*, and mismatch repair and microsatellite instability [MMR/MSI]) in all patients with metastatic colorectal cancer (mCRC), little is known regarding adherence to these recommendations in routine practice. **Methods:** We retrospectively reviewed patients with mCRC diagnosed between January 1, 2013, and December 27, 2018, from a de-identified electronic health record–derived database. We analyzed disparities in *KRAS*, *NRAS*, *BRAF*, and MMR/MSI testing by race, age, sex, and insurance status using χ^2 tests and *t* tests. We evaluated changes in biomarker testing over time with attention to changes around dates of landmark publications and guideline updates using χ^2 tests and Cochran-Armitage tests. **Results:** A total of 20 333 patients were identified of which 66.6% had test results for any biomarker. Rates of test results for all 4 biomarkers statistically significantly increased over time ($P < .001$). However, as of June 30, 2018, the rate of test results was only 46% for *NRAS*, 56% for *KRAS*, and 46% for *BRAF*. As of December 31, 2017, the rate of MMR/MSI testing was 59%. Higher documented testing rates were associated with younger age, lower Eastern Cooperative Oncology Group performance status, and commercial insurance. There were no clinically meaningful and/or statistically significant differences in documented testing rates by tumor sidedness, race, sex, or initial stage. **Conclusions:** Increased rates of documented testing for *NRAS*, *BRAF*, and MMR/MSI in mCRC was seen between 2013 and 2018 reflecting adoption of guideline recommendations. However, the rate of documented testing remains lower than expected and warrants additional research to understand the extent to which this may represent a clinical practice quality concern.

Colorectal cancer (CRC) is the third most common cancer worldwide and the second leading cause of cancer death (1). Approximately 50%-60% of patients who are diagnosed with CRC will eventually develop metastatic CRC (mCRC) and the standard of care in the majority of these cases is systemic therapy (2,3). Recent data suggest that mCRC is a molecularly heterogenous disease, and this heterogeneity results in variable prognoses and responses to treatment. Therefore, testing for *RAS*, *BRAF* (V600E), mismatch repair (MMR) and Microsatelite Instability (MSI), and HER-2 amplification are standard of care for patients with mCRC based on landmark trials and clinical practice guideline endorsement by the National Comprehensive Cancer Network (NCCN) (3).

The *RAS* genes (*KRAS*, *NRAS*, and *HRAS*) are mutated in approximately 50% of CRCs (4,5). Post hoc analyses of large prospective trials have suggested that treatment with anti–epidermal growth factor receptor (EGFR) antibodies do not confer any benefit to patients with *RAS*-mutant mCRC (6-8). Based on this data, in 2009 the NCCN recommended testing all patients diagnosed with mCRC for *KRAS* exon 2, codons 12 and 13 (9). Guidelines have since expanded to include testing for *KRAS* exons 3 and 4 and *NRAS* exons 2, 3, and 4 (3). Further studies have suggested that even among patients with *RAS* wild-type (WT) tumors, those with right-sided tumors do not benefit from the addition of an anti-EGFR antibody in the first-line setting (6,8,10,11). Therefore, the NCCN guidelines currently recommend anti-EGFR therapy only for patients with *RAS* WT, left-sided tumors in the front-line treatment, although some providers still use anti–vascular endothelial growth factor (VEGF) inhibitors with chemotherapy in this setting (12).

BRAF is downstream of *RAS* in the mitogen-activated protein kinase pathway, and the presence of *BRAF* V600E mutation, found in approximately 10% of CRC, is associated with a poor prognosis (13,14). Accumulating evidence has suggested that

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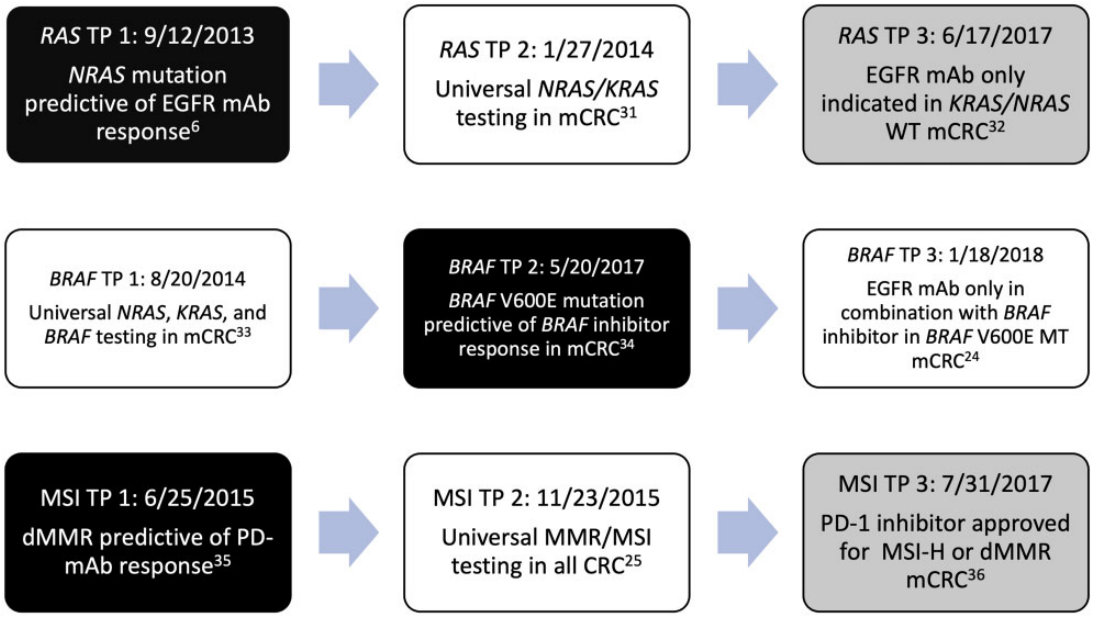

Figure 1. Landmark time points in *RAS*, *BRAF*, and MMR/MSI testing since 2012. The key time points used during the analysis are depicted in this figure for each biomarker evaluated (*RAS*, *BRAF*, and MMR/MSI). **White boxes** indicate NCCN guideline update, **black boxes** indicate landmark publications or presentations, and **gray boxes** indicated US FDA label change or approval. dMMR = deficient mismatch repair; EGFR = epidermal growth factor receptor; FDA = Food and Drug Administration; mAb = monoclonal antibody; mCRC = metastatic colorectal cancer; MMR = mismatch repair; MSI = microsatellite instability; MSI-H = microsatellite instability high; MT = mutated; NCCN = National Comprehensive Cancer Network; PD-1 = programmed death ligand 1; TP = time point; WT = wild type.

BRAF V600E mutations confer decreased response to anti-EGFR antibody therapy (15-17). Based on this data, in 2010 the NCCN initially recommended testing *BRAF* for mCRC patients who had *RAS* WT tumors, and these guidelines were expanded in 2015 to recommend testing *BRAF* in all mCRC patients (18,19). Currently, targeted agents against the mitogen-activated protein kinase pathway exist with the recent approval of the BEACON regimen combining the anti-EGFR inhibitor—cetuximab and anti BRAF inhibitor encorafenib supporting widespread testing for this biomarker (19).

Microsatellite-instability high (MSI-H) or mismatch repair deficinet (dMMR) tumors represent 4%-5% of mCRC (20). Since 2003, MMR and MSI status have been known to be prognostic and predictive of response to fluorouracil-based chemotherapy in early stage disease (21), and universal testing for MMR/MSI for the detection of Lynch syndrome was recommended for all newly diagnosed CRC by the Evaluation of Genomic Applications in Practice and Prevention working group in 2009 (22). More recently, dMMR/MSI-H mCRC tumors have demonstrated a clinically significant response to immune checkpoint inhibitor therapy in advanced stage disease (23,24). The NCCN guidelines recommended universal testing for MMR/MSI in patients with mCRC in 2015 and, in 2018, recommended the treatment of dMMR/MSI-H mCRC with pembrolizumab after progression on front-line systemic therapy (25,26). Recently, pembrolizumab was approved in the front-line setting for dMMR/MSI-H mCRC, and the NCCN guidelines were updated accordingly (24).

Although guidelines recommend testing for *RAS*, *BRAF*, and MMR/MSI, rates of testing are thought to be low. One retrospective review of patients between 2010 and 2012 found that only 28% of patients had MMR/MSI testing, although testing was not universally recommended during that time period (27). More recently, a retrospective review of 1497 patients by Gutierrez et al. (28) found that guideline-aligned biomarker testing was completed in only 40% of patients between 2013 and 2017. With important treatment decisions hinging on the results of biomarker testing, the widespread adoption of testing has substantial implications for public health (29).

The purpose of our study was to analyze changes in the rates and adoption of biomarker testing over time and to evaluate adherence to clinical guideline recommendations in mCRC. We assessed the rates of testing for MMR/MSI, *KRAS*, *NRAS*, and *BRAF* mutations by key historical time points including presentation of landmark data at national meetings, key journal publications, and updates to NCCN guidelines (see Figure 1). In addition, we evaluated the therapeutic implications of biomarker testing by analyzing the use of anti-EGFR therapy over time.

Methods

Database

The patient data in this retrospective cohort study originated from the nationwide Flatiron Health database—a longitudinal, de-identified database derived from electronic health records (EHR). As of the time of this study, data originated from approximately 280 US cancer clinics (approximately 800 sites of care) and contains patient-level structured and unstructured data curated via technology-enabled chart abstraction (30,31). Institutional review board approval of the study protocol was obtained prior to study conduct and included a waiver of informed consent.

Study Design and Patient Population

We selected patients who were diagnosed with mCRC between January 1, 2013, and December 31, 2018. Eligibility criteria included individuals aged 18 years or older and with either de novo metastatic disease or a metastatic recurrence on or after

January 1, 2013. Diagnosis of mCRC was determined by chart-documented *International Classification of Disease* (ICD) codes (ICD-9 153.x or 154.x or ICD-10 C18x, C19x, C20x, or C21x).

Patient data were collected from the date of metastatic diagnosis until the end of the study period. We allowed 6 months from the date of metastatic disease diagnosis for the patient to undergo testing to be counted as "tested" to avoid biases from differential length of follow-up. We then grouped patients into 6-month intervals according to date of metastatic diagnosis and compared rates of testing by each 6-month cohort. The end of our diagnosis cutoff was June 31, 2018, and the end of our analysis was December 31, 2018, to allow for patients diagnosed close to June 2018 to have 6 months of lead time for testing. Finally, we analyzed the rate of front-line anti-EGFR treatment in the cohort of patients with documented *RAS* WT disease.

Study Measures

Baseline data collected from the Flatiron Health Database included demographic information and initial Eastern Cooperative Oncology Group performance status (ECOG PS) at time of metastatic diagnosis. We recorded every insurance type a patient held during the study period. Tumor sidedness was determined through chart extraction and characterized as either right-sided (cecum, ascending colon, hepatic flexure) or left-sided (descending colon and rectum) based on diagnosis code (C18x). Those who were not categorized as left- or right-sided as above were classified as unspecified or unknown. Transverse colon tumors were excluded from this analysis, because of challenges in defining them as left- or right-sided within the available data.

Testing data for *KRAS*, *NRAS*, *BRAF*, and MMR/MSI with either immunohistochemistry, polymerase chain reaction, or next-generation sequencing were derived from chart documentation. Chart abstraction evaluated testing of specific biomarkers following the Flatiron Health Database protocol. Technology was used to assist in surfacing documents that could contain biomarker testing information, and all identified data were confirmed by database abstractors. Biomarkers were marked as missing or undetermined if results were not available. The patients could have been tested at any laboratory and could have received full panel testing or partial testing. Information regarding the testing methods used was not available for all patients and therefore not included in the analysis. Data were gathered in de novo metastatic and metastatic recurrence. Data for MMR/MSI testing were only analyzed in de novo mCRC patients as those with recurrent disease may have been tested earlier in their diagnosis.

We aimed to examine changes in rates of testing for *NRAS*, *BRAF*, and MMR/MSI by landmark time points, which are outlined in Figure 1 (6,32-37). We did not analyze change in rates of testing by landmark time periods for *KRAS* as there were no major guideline changes for *KRAS* testing during the time periods we examined.

Statistical Analysis

Patient demographic and tumor characteristics were summarized and tabulated based on mutation testing (*KRAS*, *NRAS*, or *BRAF* for the full cohort; MMR/MSI testing for those with metastatic disease at diagnosis). χ^2 and *t* tests were used to assess whether there were relationships between molecular testing at any point and patient and/or tumor characteristics. Cochran-Armitage tests for trend were used to assess whether the rates of testing change over time. We fit multivariable logistic regression models to simultaneously assess the effect of covariates of interest on testing. Models investigated the association between patient demographic/tumor characteristics and mutation testing. Additional models included an interaction between age and ECOG performance status score. Rates of front-line use of anti-EGFR targeted therapy for left- or right-side tumor were also assessed by 6-month intervals (based on first-line therapy start date) for *RAS* WT patients, with Cochran-Armitage test for trend to assess changes over time. Statistical significance was identified with a 2-sided *P* value less than .05. All analyses were done using SAS software (version 9.4).

Results

Patient Characteristics

A total of 20 333 patients were included in our analysis; their demographics are outlined in Table 1. The median age of the patients was 65 years. The majority (93.1%) of patients was treated at community centers, and 6.9% of patients were treated at academic centers. The majority of patients was diagnosed with stage IV disease (57.7%) at initial presentation. The most common type of insurance was commercial insurance (38.5%).

Rates of Testing by Demographic

KRAS, NRAS, or BRAF

Over the study period, 66.6% of patients were tested for either *KRAS*, *NRAS*, or *BRAF*, and 33.4% of patients were not tested for any these biomarkers. Of the patients, 30.5% were tested for all of the biomarkers, and 23.7% of patients were tested for *KRAS* but not *NRAS* or *BRAF*. Patients aged younger than 40 years had higher rates of testing (75%), and those aged older than 75 years had lower rates of testing (52%; $P < .001$). Rate of testing was higher among patients with lower ECOG PS (0 or 1) ($P < .001$). Patients with commercial insurance had highest rates of testing (70%), and patients with Medicaid had lowest rates of testing (61%) ($P < .001$). There were no statistically significant differences in testing by sex, race, or tumor-sidedness.

Microsatellite Instability

Demographic associations with MMR/MSI testing were similar to those found with *KRAS*, *NRAS*, and *BRAF* as patients with younger age ($P < .001$), lower ECOG PS (<.001), and commercial insurance ($P < .001$) had highest rates of testing (Table 1).

Multivariate Analysis

We found similar results on multivariable analysis as reported in the univariable analyses (Table 1). Regression results are presented in Supplementary Figures (available online). Our analysis demonstrated a statistically significant interaction between age and ECOG PS 2 or higher for *RAS* and *BRAF* testing, so that the negative effect of age (ie, older patients are less likely to be tested) was even more pronounced among those who were ECOG PS 2 or higher (results not shown).

The Adoption of Biomarker Testing Over Time

Rates of testing for *KRAS* ($P < .001$), *NRAS* ($P < .001$), *BRAF* ($P < .001$), and MMR/MSI ($P < .001$) increased statistically significantly over time for patients diagnosed with mCRC (Figure 2).

Table 1. Patient demographics and rate of mCRC recommended molecular marker testing by patients' characteristics

		KRAS, *NRAS*, or *BRAF* testing[a]			MMR/MSI testing for de novo mCRC patients		
Patient characteristic	Total, No. (%)	Not tested, No. (%)	Tested, No. (%)	*P*	Not tested, No. (%)	Tested, No. (%)	*P*
Total	20 333	6798 (33.4)	13 535 (66.6)		5302 (45.22)	6423 (54.78)	
Median age at metastatic diagnosis, y (range)	65 (18-85)	69 (18-85)	64 (18-85)	<.001	67 (23-85)	61 (18-85)	<.001
Age group, y				<.001			<.001
40 and younger	788 (3.88)	199 (25.25)	589 (74.75)		159 (28.55)	398 (71.45)	
41-65	9422 (46.34)	2562 (27.19)	6860 (72.81)		2325 (39.61)	3544 (60.39)	
66-75	5488 (26.99)	1798 (32.76)	3690 (67.24)		1454 (48.87)	1521 (51.13)	
Older than 75	4635 (22.80)	2239 (48.31)	2396 (51.69)		1364 (58.69)	960 (41.31)	
Gender				.0487			.28
Female	11 201 (55.09)	3117 (34.15)	6011 (65.85)		2375 (44.66)	2943 (55.34)	
Male	9128 (44.89)	3678 (32.84)	7523 (67.16)		2925 (45.67)	3480 (54.33)	
Stage at initial diagnosis				<.001			
I	548 (2.70)	195 (35.58)	353 (64.42)		0	0	
II	2228 (10.96)	814 (36.54)	1414 (63.46)		0	0	
III	4902 (24.11)	1549 (31.58)	3354 (68.42)		0	0	
IV	11 725 (57.66)	3777 (32.21)	7948 (67.79)		5302	6423	
Unknown	930 (4.57)	—	—		—	—	
Race				.24			.42
African American	2123 (10.44)	656 (30.90)	1467 (69.10)		561 (44.24)	707 (55.76)	
Asian	74 (0.36)	172 (31.73)	370 (68.27)		120 (40.82)	174 (59.18)	
Hispanic or Latino	542 (2.67)	21 (30.90)	53 (69.10)		23 (50)	23 (50)	
White	13 245 (65.14)	4360 (32.92)	—8885 (67.08)		3397 (45.09)	4136 (54.91)	
Other or unknown[b]	4349 (21.39)				—	—	
ECOG performance status				<.001			<.001
0	6003 (29.52)	1640 (27.32)	4363 (72.68)		1218 (36.38)	2130 (63.62)	
1	5102 (25.09)	1469 (28.79)	3633 (71.21)		1216 (41.09)	1743 (58.91)	
2	1688 (8.30)	651 (38.57)	1037 (61.43)		495 (48.43)	527 (51.57)	
3	485 (2.39)	243 (50.10)	242 (49.90)		170 (58.22)	122 (41.78)	
4	50 (0.25)	27 (54.00)	23 (46.00		14 (48.28)	15 (51.72)	
Unknown[c]	7005 (34.45)	—	—		—	—	
Type of insurance				<.001			<.001
Commercial	7874 (38.53)	2324 (29.67)	5510 (70.33)		1883 (39.75)	2854 (60.25)	
Commercial and Medicare	3566 (17.54)	1243 (34.86)	2323 (65.14)		890 (48.85)	932 (51.15)	
Medicare	3238 (15.92)	1262 (32.22)	1976 (67.78)		905 (50.53)	886 (49.47)	
Medicaid	987 (4.85)	318 (38.97)	669 (61.03)		288 (45.28)	348 (54.72)	
Medicare and Medicaid	832 (4.09)	291 (34.98)	541 (65.02)		205 (48.46)	218 (51.54)	
Other or unknown	3876 (19.06)	1360 (35.09)	2516 (64.91)		1231 (48.95)	1285 (51.05)	
Tumor location				.10			.21
Left	8702 (42.80)	2804 (32.22)	5898 (67.78)		2025 (41.26)	2883 (58.74)	
Right	4045 (19.89)	1244 (30.75)	2801 (69.25)		1014 (42.80)	1355 (57.20)	
Unknown or unspecified	7586 (35.93)						
Practice type		N/A[c]	N/A	—	N/A	N/A	—
Academic	1403 (6.90)	—	—		—	—	
Community	18 930 (93.10)	—	—		—	—	

[a]Patients could have been tested for either *BRAF*, *KRAS*, or *NRAS*. "—" signifies no data; ECOG = Eastern Cooperative Oncology Group; mCRC = metastatic colorectal cancer; MMR = mismatch repair; MSI = microsatellite instability.
[b]Patient without documented data in this category in the database.
[c]Practice type information was only available for the full cohort for this analysis.

The rates of biomarker testing by landmark time point are outlined in Table 2. Although testing of *NRAS*, *BRAF*, and MMR/MSI increased across almost all landmark time points analyzed, rates of testing remained below 60% (below 50% for *NRAS* and *BRAF*) by the final time period analyzed.

Use of Anti-EGFR Therapy in Front-Line Over Time

Patients with left-sided, *RAS* WT tumors were increasingly treated with anti-EGFR therapy in the front-line setting ($P = .002$) though rates were only 19.12% by the final time period analyzed. Patients with right-sided, *RAS* WT tumors trended toward decreasing rates of front-line anti-EGFR therapy ($P = .21$).

Discussion

Over the last decade, international guidelines have endorsed testing recommendations for *KRAS*, *NRAS*, *BRAF*, and MMR/MSI in mCRC. Our study demonstrates an increase in testing rates over time for these biomarkers. These results suggest that

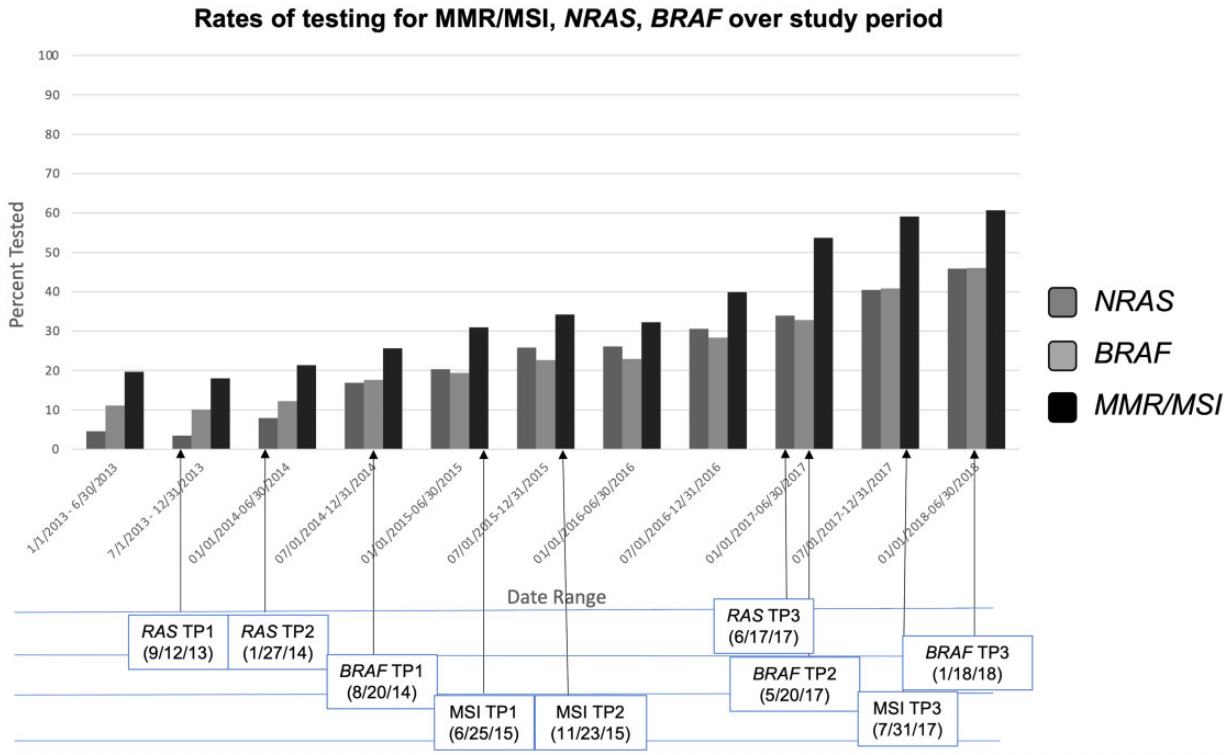

Figure 2. Rates of testing *RAS*, *BRAF*, and MMR/MSI testing over time. Changes in rates of biomarker testing are depicted in this figure for *RAS*, *BRAF*, and MSI with **arrows** depicting the relevant time point during the analysis (refer to Figure 1 for time point description). MMR = mismatch repair; MSI = microsatellite instability; TP = time point.

oncologists are receptive to landmark publications, US Food and Drug Administration labeling changes, and updates in NCCN guidelines and adapt their practice accordingly. Nevertheless, as of 2018 the rates of testing remained quite low with only 56% of patients receiving *KRAS* testing, 46% of patients receiving *NRAS* testing, and 46% of patients receiving *BRAF* testing (35). Rate of MMR/MSI testing remained high at 82% tested as of 2018, possibly because of earlier understanding of its predictive and prognostic importance, earlier testing recommendations by international guidelines, and the growing use of immunotherapy in the dMMR/MSI-H mCRC patients (3,24,38).

On average, patients who were tested tended to be younger and have a better ECOG performance status. Younger patients may receive more aggressive workup and treatment; for example, increased consideration of anti-EGFR therapy may lead to increased rates of *RAS* and *BRAF* testing (39). Conversely, patients with a poorer performance status may receive less testing as clinicians try to avoid aggressive workup in patients who may not be eligible to receive treatment.

Prior studies have shown that older patients are less likely to be referred to a medical oncologist and to receive therapy for mCRC compared with their younger counterparts (40,41). The reasons for this disparity are likely multifactorial, including a paucity of prospective data in the elderly population because of underrepresentation in clinical trials, increased rate of medical comorbidities, and challenges with social support leading to more fragmented care (42,43). These same challenges may contribute to the decreased testing rates in the elderly. Notably, however, anti-EGFR therapy is commonly used and may be well tolerated in older adults, making biomarker testing important in this cohort (29,44). Immunotherapy may also be well tolerated by patients with a poor performance status, and therefore MMR/MSI testing remains imperative in the elderly patient population especially as data show that older patients may have higher rates of MSI-H tumors compared with younger cohorts (24,45-47).

Patients with commercial insurance had the highest rates of *RAS*, *BRAF*, and MMR/MSI testing, whereas patients with Medicaid had the lowest rates. It is well established that patients with Medicaid or lack of insurance suffer from statistically significantly worse outcomes. Large, retrospective database analyses have shown that uninsured and Medicaid-insured patients present at more advanced stages of colorectal cancer due in part to lack of early screening (48). Additionally, CRC patients with non-Medicaid insurance enjoy a statistically significantly longer survival compared with patients with Medicaid insurance (49-51). The same barriers to care that lead to reduced screening and poorer outcomes in the uninsured or Medicaid-insured population likely contribute to a reduced rate of biomarker testing in this cohort. In addition, because older individuals (or those who would be eligible for Medicare) were less likely to be tested, age and performance status may be affecting the interaction between insurance status and biomarker testing.

We found that rates of *KRAS*, *NRAS*, and *BRAF* testing increased over time, and the rates of testing appeared to increase across almost all time periods examined. Even with this increase in testing over time, however, many patients remain untested as of our final data period. In fact, only a minority of patients were tested for the 4 biomarkers demonstrating a significant need for improvement in our management of these patients. Understanding the factors that contribute to suboptimal testing rates, as well as the reasons for disparity in testing between subpopulations, is beyond the scope of this

Table 2. Analysis of biomarker testing by landmark time point (online only)

Time periods	% tested	Comparison	*P*
NRAS			
Period 1: January 1, 2013, to TP 1	4.10	Referent	
[September 12, 2013, landmark publication—NRAS MT predictive of EGFR inhibitor response (6)]			
Period 2: TP 1 to TP 2	3.82	Period 1 vs period 2	.69
[January 1, 27, 2014, NCCN guideline update recommends universal NRAS and KRAS testing in mCRC (32)]			
Period 3: TP 2 to TP 3	23.45	Period 2 vs period 3	<.001
[June 17, 2017, FDA label change—EGFR inhibitor only indicated in RAS WT mCRC (33,54)]			
Period 4: TP 3 to June 30, 2018[a]	42.78	Period 3 vs period 4	<.001
BRAF			
Period 1: January 1, 2013, to TP 1	11.54	Referent	
[August 20, 2014, NCCN guideline update recommends universal KRAS, NRAS, and BRAF testing in mCRC (34)]			
Period 2: TP 1 to TP 2	23.96	Period 1 vs period 2	<.001
[May 20, 2017, landmark publication—BRAF v600E MT predictive of BRAF inhibitor response (35)]			
Period 3: TP 2 to TP 3	40.14	Period 2 vs period 3	<.001
[January 18, 2018, NCCN guideline update recommends EGFR inhibitor only in combi nation with BRAF inhibitor in BRAF v600E mutated mCRC (25)]			
Period 4: TP 3 to June 30, 2018	45.85	Period 3 vs period 4	<.001
MMR/MSI			
Period 1: January 1, 2013, to TP 1	23.29	Referent	
[June 25, 2015, landmark publication—MMR predictive of PD-1 mAb response (36)]			
Period 2: TP 1 to TP 2	32.01	Period 1 vs period 2	<.001
[November 23, 2015, NCCN guideline update recommends MMR/MSI testing in all CRC (55)]			
Period 3: TP 2 to TP 3	42.92	Period 2 vs period 3	<.001
[July 31, 2017, FDA label change—nivolumab approved for dMMR/MSI-H mCRC (56)]			
Period 4: TP 3 to June 30, 2018	59.31	Period 3 vs period 4	<.001

[a]June 30, 2018, marks the end of available data. CRC = colorectal cancer; EGFR = epidermal growth factor receptor; FDA = Food and Drug Administration; mAb = monoclonal antibody; mCRC = metastatic colorectal cancer; MMR = mismatch repair; MSI = microsatellite instability; MSI-H = microsatellite instability high; dMMR- deficient mismatch repair; MT = mutated; NCCN = National Comprehensive Cancer Network; PD-1 = programmed death ligand 1; TP = time point; WT = wild type.

analysis and would be difficult to evaluate through this database alone. However, additional research and educational initiatives may help uncover and dismantle these barriers for testing.

Finally, we saw a statistically significant increase in the use of anti-EGFR therapy in the front-line setting for left-sided tumors and a decreased use for right-sided tumors, which was not statistically significant. This aligns with the NCCN guidelines recommendation for the use of anti-EGFR therapy plus chemotherapy for left-sided, *RAS* WT tumors in this setting, based on data demonstrating superiority over the combination of chemotherapy and anti-VEGF (3,11). However, overall use of anti-EGFR therapy remains quite low in the front-line setting with less than 20% of patients with left-sided, *RAS* WT tumors receiving such treatment by the final time point analyzed. The gradual adoption of these guidelines may be because of the short time interval between the publication of the above data in June 2017 and the end of our data collection in 2018 (52). Furthermore, the exclusion of transverse colon tumors from this analysis, because of challenges in defining them as left- or right-sided within the database may have also affected these results.

There are several limitations to our study. These data are contingent on clinical information as documented in the EHR and the manual abstraction of that information, which may introduce variability, errors, or subjectivity. The use of trained professional abstractors, following consistent abstraction guidance, policies, and procedures, may mitigate these risks (30). Missing data in the EHR are also a source of potential bias. For example, although we did not find an association with race, multiple studies have found an independent association between race and poor outcomes (48-50). This may be explained by 21% of patients being characterized as other or unknown race in our cohort. Chart documentation of patient variables may have impacted indication of biomarker testing as well. In addition, it is possible that providers used outside laboratories for biomarker testing, which was not included in the electronic medical record, or testing was performed by providers outside of the Flatiron Health network. This may have resulted in a lower rate of documented testing in our analysis. Finally, errors in recording the correct ICD code or failure to include appropriate tumor sidedness may have affected our analyses (53). However, these factors are unlikely to affect the overall observed trends over time.

The NCCN guidelines do not specify a recommended method of testing these biomarkers. Over the time period analyzed, adoption of comprehensive next-generation sequencing testing has increased, which may have led to higher rates of testing across all biomarkers (28). With this trend, we would expect to find a significant increase in the percentage of patients having their tumor samples tested. However, by the final time point analyzed, 59% of patients had MMR/MSI testing, whereas only 43% had *NRAS* testing, suggesting a large percentage of patients had either no testing or had only limited biomarker testing.

Additional research is needed to understand barriers to universal testing, and ongoing monitoring is needed to ensure testing rates are improved.

This type of big data analysis fails to capture the thought process behind the decision regarding testing of patients; for example, older patients may be tested at a lower rate because more of them may be unfit for chemotherapy, and their management may have been focused on supportive care. Moreover, some providers may have only tested for *RAS* and *BRAF* on progression after front-line therapy in mCRC, which our data may have missed given the 6-month window after diagnosis to determine compliance to testing. Data supporting the benefit of anti-EGFR therapy in the front-line setting were not available for most of the timeline of this study (2013-2018). Therefore, during first-line treatment, testing may have not been considered necessary by some providers. Finally, the large sample size of this database results in statistical significance even with minimal difference between groups. As such, the small differences in rates of testing between males and females or stages of diagnosis (although statistically significant) are unlikely to represent a clear underlying signal.

In conclusion, testing of biomarkers *KRAS*, *NRAS*, *BRAF*, and MMR/MSI have increased over time in accordance with landmark publications and guideline changes. However, the rate of documented testing remains low and can be improved on substantially. Increased testing rates could result in improved diagnoses of hereditary CRC as in Lynch syndrome, optimization of treatment, and ultimately improved patient outcomes. Additional research is warranted to confirm these findings, characterize the reasons for nontesting, and understanding barriers for adoption of testing among providers, which may ultimately lead to educational initiatives to improve testing trends.

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Data Availability

The data that support the findings of this study are openly available in electronic form at Fox Chase Cancer Center biostatistical department including the database obtained from Flatiron and the analysis conducted at our site.

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