Reliable Detection of Mismatch Repair Deficiency in Colorectal Cancers Using Mutational Load in Next-Generation Sequencing Panels

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ABSTRACT

Purpose

Tumor screening for Lynch syndrome is recommended in all or most patients with colorectal cancer (CRC). In metastatic CRC, sequencing of *RAS/BRAF* is necessary to guide clinical management. We hypothesized that a next-generation sequencing (NGS) panel that identifies *RAS/BRAF* and other actionable mutations could also reliably identify tumors with DNA mismatch repair protein deficiency (MMR-D) on the basis of increased mutational load.

Methods

We identified all CRCs that underwent genomic mutation profiling with a custom NGS assay (MSK-IMPACT) between March 2014 and July 2015. Tumor mutational load, with exclusion of copy number changes, was determined for each case and compared with MMR status as determined by routine immunohistochemistry.

Results

Tumors from 224 patients with unique CRC analyzed for MMR status also underwent MSK-IMPACT. Thirteen percent ($n = 28$) exhibited MMR-D by immunohistochemistry. Using the 341-gene assay, 100% of the 193 tumors with < 20 mutations were MMR-proficient. Of 31 tumors with ≥ 20 mutations, 28 (90%) were MMR-D. The three remaining tumors were easily identified as being distinct from the MMR-D tumors with > 150 mutations each. Each of these tumors harbored the P286R hotspot *POLE* mutation consistent with the ultramutator phenotype. Among MMR-D tumors, the median number of mutations was 50 (range, 20 to 90) compared with six (range, 0 to 17) in MMR-proficient/*POLE* wild-type tumors ($P < .001$). With a mutational load cutoff of ≥ 20 and < 150 for MMR-D detection, sensitivity and specificity were both 1.0 (95% CI, 0.93 to 1.0).

Conclusion

A cutoff for mutational load can be identified via multigene NGS tumor profiling, which provides a highly accurate means of screening for MMR-D in the same assay that is used for tumor genotyping.

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INTRODUCTION

Approximately 15% to 20% of colorectal cancers (CRC) are deficient in one or more DNA mismatch repair (MMR) proteins, with one-quarter of these resulting from Lynch syndrome (LS), an autosomal dominant condition characterized by germline mutations in genes coding for MMR proteins.¹ Criteria for LS screening among patients with CRC have recently been expanded. Multiple guidelines recommend screening of tumors via MMR

protein expression or microsatellite instability (MSI) analysis in either all patients with CRC, in patients with CRC who were diagnosed at age younger than 70 years, or in individuals older than 70 years with a Lynch-associated family history.²⁻⁵ In addition to LS screening, in patients with early-stage (especially stage II) CRC, MMR status provides important prognostic and predictive information, with MMR-deficiency (MMR-D) being associated with both a good prognosis and a lack of efficacy from fluorouracil treatment.⁶ The recent groundbreaking success of immunotherapy with PD-1

inhibition in MMR-D CRC is likely to necessitate evaluation of MMR in all metastatic CRCs in the near future.⁷ Indeed, as of November 2015, the updated National Cancer Care Network guidelines now recommend that all metastatic CRCs be evaluated for MMR-D.

In patients with metastatic CRC, assessment of *KRAS*, *NRAS*, and *BRAF* mutational status is now accepted standard practice and determines eligibility for treatment with EGFR inhibitors.⁸ As such, at the current time, appropriate molecular profiling of a patient's CRC often requires both assessment of MMR status by immunohistochemistry (IHC) or MSI and tumor genomic profiling for *KRAS*, *NRAS*, and *BRAF*.

Tumor genomic profiling via next-generation sequencing (NGS) panels to define the spectrum of mutations in an individual's tumor is becoming increasingly widespread. NGS panel-based profiling may help guide treatment decisions and also determine trial eligibility for targeted agents.⁹ Given yield and efficiency, *KRAS*, *NRAS*, and *BRAF* mutational analysis in colorectal tumors at our institution is routinely performed as part of a multigene NGS panel with inclusion of numerous other cancer-related genes. Not surprisingly, given that a deficiency in the DNA MMR apparatus would be expected to result in more uncorrected mismatched bases and thus a greater mutation rate, we observed that tumors known to harbor MMR-D exhibited large numbers of mutations on our NGS assay. We therefore hypothesized that we could use the numeric mutational load of a multigene panel to accurately exclude patients whose tumors were MMR proficient (MMR-P), thereby obviating, in the majority of patients, the need for separate MMR status determination via IHC or MSI screening.

METHODS

As part of an ongoing effort to identify patients eligible for studies of targeted agents, Memorial Sloan Kettering Cancer Center offers tumor genomic mutation profiling with a custom hybridization capture-based NGS assay (MSK-IMPACT).¹⁰ MSK-IMPACT is also routinely used for *KRAS*, *NRAS*, and *BRAF* genotyping for patients with CRC. With Memorial Sloan Kettering Cancer Center institutional review board approval, electronic clinical database review identified 349 patients with CRC whose tumors had undergone MSK-IMPACT profiling between March 2014 and July 2015. Among this cohort, we identified all patients who had also had MMR protein analysis via IHC (Fig 1). Clinical characteristics, including germline genetic test results, were extracted from the medical record.

Sequencing

The MSK-IMPACT assay was conducted as previously described.¹⁰ In brief, DNA from formalin-fixed paraffin-embedded primary or metastatic colorectal tumors and patient-matched normal blood samples was extracted and sheared. Barcoded libraries from tumor and normal samples were captured, sequenced, and subjected to a custom pipeline to identify somatic mutations. Sequencing consisted of deep sequencing of all exons and selected introns of a custom 341 cancer-associated gene panel, with an updated panel containing 410 genes. All exons tested had a minimum depth of coverage of 100×. The alterations within the panel were all somatic alterations, because mutations were called against the patient's matched normal sample. To calculate mutational load, we determined the number of somatic nonsilent protein-coding mutations with exclusion of copy number gene alterations and structural rearrangements. For patients in whom the newer 410-gene panel was applied, the corresponding mutational burden was also calculated on the basis of the 341-gene panel

Fig 1. Colorectal tumor profiling via next-generation sequencing assay. CRC, colorectal cancer; IHC, immunohistochemistry; MMR, mismatch repair.

via subtraction of mutations occurring in the 69 additional genes present on the 410-gene panel.

DNA Mismatch Repair Analysis

Immunohistochemistry staining of CRCs was performed using the standard streptavidin-biotin-peroxidase procedure. Primary monoclonal antibodies against *MLH1* (clone G168-728, diluted 1:250 [BD PharMingen, San Diego, CA]), *MSH2* (clone FE11, diluted 1:50 [Oncogene Research Products, La Jolla, CA]), *MSH6* (clone GRBP.P1/2.D4, diluted 1:200 [AbD Serotec, Kidlington, UK]), and *PMS2* (clone A16-4, diluted 1:200 [BD PharMingen]) were used with external controls. Tumors showing total absence of nuclear staining, with adjacent benign tissue showing nuclear staining, were scored as negative for expression of that protein. Specialized pathologists at Memorial Sloan Kettering Cancer Center interpreted the histopathology and IHC results.

Statistical Analysis

Descriptive statistics summarize the patient populations. Student's *t* test was used to compare the mutational burden in MMR-P versus MMR-D cases. In patients for whom MSK-IMPACT was performed on more than one tumor sample, the tumor with the highest mutational burden was used for the MMR-P cases and, conversely, the sample with the lowest mutational burden was included for the MMR-D samples, thereby ensuring the most conservative statistical approach for the analysis.

RESULTS

Patient Characteristics

A total of 224 patients with CRC were identified, whose tumors had undergone MSK-IMPACT tumor profiling and for whom IHC results for the MMR proteins were available (Fig 1). Among these patients, 87% (*n* = 196) were MMR-P, whereas 13% (*n* = 28) exhibited MMR-D. Of the MMR-D cases, 15 had *MLH1*/*PMS2* protein loss, six *MSH2*/*MSH6* loss, whereas five had only *MSH6* loss and two only *PMS2* loss. Germline genetic testing identified 12 patients with LS (*MLH1* = 3; *MSH2* = 4; *MSH6* = 4; and *PMS2* = 1) and six sporadic MMR-D CRCs (*MLH1*/*PMS2*

protein absence; presence of somatic *BRAF* V600E mutation and/or *MLH1* promoter hypermethylation). The other 10 cases included: three with presumed LS (two with a variant of uncertain significance in *MLH1* in addition to a strong personal and family history and one with a clinical diagnosis of LS), four with mutation-negative LS (absence of pathogenic germline mutation and absence of somatic *BRAF* mutation or *MLH1* hypermethylation), and three with pending/incomplete genetic evaluation.

The mean age at diagnosis in the MMR-P group (50 years; range, 17 to 78 years) was equivalent to the MMR-D group (54 years; range, 23 to 79 years). In patients with genetically proven LS, the mean age at first CRC diagnosis (46 years; range, 23 to 75 years) was significantly younger than in the non-Lynch MMR-D group (68 years; range, 57 to 70 years; $P = .009$). Among the 28 patients in the MMR-D group, a total of 32 CRCs were diagnosed, with four patients with LS or presumed LS having two separate CRC diagnoses. Whereas the majority of patients had right-sided CRC ($n = 21$), nine patients had left-sided tumors including five patients with LS. Notably, all of the confirmed sporadic MMR-D CRCs were right-sided tumors (Table 1).

Mutational Load via NGS Panel

Using the 341-gene assay, 100% of the 193 tumors with fewer than 20 mutations were MMR-P. Of the 31 tumors with ≥ 20 mutations, 28 (90%) were MMR-D. Among the 28 MMR-D tumors, the median number of mutations identified was 50 (range, 20 to 90 mutations), whereas the three remaining tumors were distinctly different with > 150 mutations each (Table 2). These three tumors each harbored the P286R hotspot mutation within the exonuclease domain of *POLE* and therefore were consistent with the previously described ultramutator phenotype.^{11,12} As such, they were easily distinguished from both the MMR-D as well as from the other MMR-P cases. The median number of mutations was 50 (range, 20 to 90 mutations) compared with six (range, 0 to 17 mutations) in the MMR-D versus the MMR-P/*POLE* wild-type tumors, respectively ($P < .001$; Table 3). Notably, among the MMR-P/*POLE* wild-type cases, the two CRCs containing the highest number of mutations (16 and 17) each were found to have a *BRAF* V600E mutation, and so would likely exclude consideration of LS even if the mutational load were felt to be of concern. Mutational load distribution among the MMR-P/*POLE* wild-type, MMR-D, and *POLE* mutant samples is depicted in Figure 2.

Within the MMR-P/*POLE* wild-type group, the multigene assay was performed on the primary tumor in 59% and on a metastatic lesion in 41% of cases; the mean and median mutational load did not differ between primary tumors and metastases. In one case, the assay was performed on both a primary and a metastatic lesion, with the same mutational load identified in both samples. Among the 28 MMR-D cases, the multigene assay was performed on the primary lesion in 86% of cases and on a metastatic lesion in 14%. In subset analyses, we observed a statistically significant difference in mutational load between MMR-P and MMR-D cases, whether the tissue analyzed was primary or metastatic tumor.

A subgroup of 95 tumors underwent testing with the newer 410-gene panel. The median number of mutations in the 20 MMR-D CRCs was 60 (range, 21 to 100 mutations) versus seven (range, 2 to

12 mutations) of 75 patients in the MMR-P group ($P < .001$), excluding one outlier with a P286R *POLE* mutation who had 348 mutations and therefore was easily discernible (Table 3). Using a mutational load cutoff of ≥ 20 and < 150 for MMR-D detection in either the 341-gene panel or the 410-gene panel, sensitivity was 1.0 (95% CI, 0.93 to 1.0) and specificity was 1.0 (95% CI, 0.93 to 1.0).

Mutational Spectrum via NGS Panel

Within the MMR-D tumors, 54% ($n = 15$) harbored hotspot *KRAS*, 21% ($n = 6$) *BRAF* V600E, and 32% ($n = 9$) *PIK3CA* mutations. As expected, we noted a high number of *BRAF* V600E mutations in sporadic MMR-D CRCs. Interestingly, a high frequency of *APC* mutations was present in Lynch-associated MMR-D versus sporadic MMR-D cases (83% v 17%; $P = .01$). In fact, within Lynch-associated *APC*-mutated tumors, 90% of tumors had at least two *APC* mutations, with the majority being frameshift, splice site, or nonsense deleterious somatic alterations. In the one sporadic (*BRAF* V600E-positive) MMR-D case, the identified *APC* somatic alteration was a missense mutation, and classified as a variant of uncertain significance. *TP53* mutations were present in 33% of Lynch-associated MMR-D tumors but in none of the sporadic MMR-D tumors; this observed trend did not reach statistical significance.

Among the 12 patients with LS, five had secondary somatic mutations within the gene containing the germline mutation. As such, these somatic mutations were the likely second hit in the carcinogenesis of these specific tumors. We also evaluated whether, in patients with mutation-negative LS, somatic mutations in the tumor explain the etiology of the MMR-D. Among four mutation-negative LS cases, two patients had more than one somatic mutation in the gene corresponding to the IHC abnormality. Specifically, a 53-year-old patient (with *MLH1*/*PMS2* protein loss and without a suspicious family history) had two somatic *MLH1* alterations; one was a frameshift mutation and the other a small deletion, thereby suggesting pathogenicity. Given negative germline genetic testing for *MLH1* and *PMS2*, and absence of *BRAF* V600E mutation or *MLH1* promoter hypermethylation in the tumor, the presence of the two somatic mutations may explain the etiology of this patient's MMR-D CRC. The second patient with CRC, who was 65 years old, had *MSH2*/*MSH6* protein absence; germline testing was unrevealing for mutations in *MSH2*, *MSH6*, and *EPCAM*. Somatic profiling revealed three *MSH2* mutations, including two pathogenic mutations: a deletion of nine base pairs and a nonsense mutation. As such, this suggests that the sporadic etiology of the MMR-D in this patient's CRC may be explained by these two somatic mutations.

POLE Mutations

Among MMR-P colorectal tumors, three harbored hotspot mutations in the exonuclease domain of *POLE* at codon 286, associated with an ultramutator phenotype (Table 2).^{11,12} The burden of point mutations within this small group of patients was at least three-fold higher than the mean mutation burden in MMR-D tumors, with all three tumors exhibiting > 150 somatic mutations on the 341-gene panel. Notably, all three of these tumors also had somatic mutations in *KRAS* and *TP53*.

Table 1. Mismatch Repair-Deficient Colorectal Cancer: Patient and Tumor Characteristics

Characterization of MMR-D	Patient	Age at Diagnosis (years)	IHC for MMR Status	Primary CRC Tumor Location	Stage	Genetic and Tumor Characteristics	Mutation Count on 341-Genes NGS Assay	Mutation Count on 410-Genes NGS Assay
Sporadic	1	67	MLH1/PMS2 absent	Right	II, with recurrence	<i>BRAF</i> V600E	62	70
	2	60	MLH1/PMS2 absent	Right	III	<i>BRAF</i> V600E	44	49
	3	76	MLH1/PMS2 absent	Right	II	<i>BRAF</i> V600E	38	N/A
	4	79	MLH1, PMS2 absent	Right	II	<i>MLH1</i> -HM positive	56	60
	5	70	MLH1/PMS2 absent	Right	IIA	<i>BRAF</i> V600E	43	47
	6	57	MLH1/PMS2 absent	Right	IV	<i>BRAF</i> V600E	50	55
Lynch syndrome	7	41	MSH2/MSH6 absent	Synchronous left and rectum	II, II	Germline <i>MSH2</i>	57 and 98	N/A
	8	33	MLH1/PMS2 absent	Right	III, with recurrence	Germline <i>MLH1</i>	59	67
	9	50	MSH6 absent	Right	II	Germline <i>MSH6</i>	40	45
	10	32	MSH2/MSH6 absent	Right	II	Germline <i>MSH2</i>	72	82
	11	64	MSH6 absent	Right	II	Germline <i>MSH6</i>	61	65
	12	31	MSH6 absent	Left	III, with recurrence	Germline <i>MSH6</i>	49	N/A
Presumed Lynch syndrome	13	23	MLH1/PMS2 absent	Right	II, with recurrence	Germline <i>MLH1</i>	23	N/A
	14	75	MSH2/MSH6 absent	Left	IV	Germline <i>MSH2</i>	70	80
	15	29, 53	MLH1/PMS2 absent	Metachronous rectum and right and rectum	II, I	Germline <i>MLH1</i>	40	44
	16	64	MSH2/MSH6 absent	Synchronous left and rectum	II, II	Germline <i>MSH2</i>	69	81
	17	59	PMS2 absent	Right	III	Germline <i>PMS2</i>	90	100
	18	53	MSH6 absent	Right	I	Germline <i>MSH6</i>	65	69
Mutation-negative Lynch syndrome*	19	45, 61	MLH1 weak/PMS2 absent	Right	III, IIB	Clinical diagnosis (strong personal and FHx)	48	60
	20	38	PMS2 absent	Rectum	III	Strong FHx, VUS <i>MLH1</i> , <i>BRAF</i> WT, and <i>MLH1</i> -HM	28	32
Mutation-negative Lynch syndrome*	21	54	MLH1/PMS2 absent	Right	III, with recurrence	Strong FHx, VUS <i>MLH1</i>	20	21
	22	53	MLH1 weak/PMS2 absent	Left	II	Strong FHx, VUS <i>MLH1</i>	50	56
	23	74	MLH1 absent/PMS2 weak	Right	IV	Lynch-like	49	N/A
Unknown	24	41	MSH2/MSH6 absent	Rectum	III	Lynch-like	45	48
	25	65	MSH2/MSH6 absent	Right	IV	Lynch-like	89	91
	26	46	MSH6 absent	Right	IV	Not tested	69	N/A
	27	63	MLH1/PMS2 absent	Right	III, with recurrence	Not tested	57	N/A
	28	63	MLH1/PMS2 absent	Left	IV	Not tested	26	N/A

Abbreviations: CRC, colorectal cancer; FHx, Family history; IHC, immunohistochemistry; MLH1-HM, *MLH1* promoter hypermethylation; MMR, mismatch repair; MMR-D, mismatch repair-deficiency; NGS, next-generation sequencing; N/A, not available; VUS, variant of unknown significance; WT, wild-type.
 *Mutation-negative Lynch syndrome refers to patients with MMR-D CRC who have no identifiable germline mutation in the corresponding *MMR* gene(s) and whose tumors also lack a *BRAF* V600E mutation or *MLH1* promoter hypermethylation.

Table 2. Mismatch Repair-Proficient Tumors With High Mutational Load on Next-Generation Sequencing Assay

Patient	Age at Diagnosis (years)	Stage	Primary CRC Location	Somatic POLE Mutation	NGS Mutation Load (341-gene panel)	Other Selected Mutations Present	Other Tumor Features
29	45	III (pT3N2a); subsequent recurrence to liver	Left (sigmoid)	P286R	250	<i>KRAS</i> <i>TP53</i> <i>PIK3CA</i> <i>KIT</i>	Minimal TILs; mucinous
30	54	III (pT4aN2b); subsequent recurrence to RP LNs	Right (cecum)	P286R	158	<i>KRAS</i> <i>TP53</i>	No TILs Moderately differentiated
31	24	II (pT3N0)	Left (rectosigmoid)	P286R	316 (410-gene panel: 348)	<i>KRAS</i> <i>TP53</i>	TILs present; poorly differentiated

Abbreviations: CRC, colorectal cancer; MMR, mismatch repair; MMR-P, mismatch repair-proficient; NGS, next-generation sequencing; RP LNs, retroperitoneal lymph nodes; TIL, tumor-infiltrating lymphocytes.

DISCUSSION

Our results demonstrate that a multigene NGS tumor profiling panel can accurately discriminate between MMR-P versus MMR-D tumors on the basis of the numeric quantitation of the mutation load. As such, the use of a multigene tumor panel may obviate the need for parallel MMR protein assessment via either IHC or MSI analysis for initial screening of LS. Furthermore, in cases where MMR-D is suspected on the basis of mutational load, the presence/absence of a *BRAF* V600E mutation may provide simultaneous information regarding the need for further genetic work up in any given patient. Such simplification of the work up of MMR-D in CRC is especially important given recent guidelines that expand screening for LS to either all CRC or CRCs diagnosed in patients younger than 70 years. In the near future, we expect that molecular testing for CRC will continue to expand beyond that of *RAS* and *BRAF*. For example, assessment of *PIK3CA* may have implications for aspirin use in early-stage disease.¹³ As such, simplification of the molecular work-up for patients with CRC is necessary for efficiency, effective use of small tissue samples from presurgical biopsies, and cost containment.

The mutation spectrum in the MMR-D CRCs was consistent with prior studies, suggesting similar rates of *KRAS* mutations as in MMR-P CRCs and, as expected, a higher rate of *BRAF* V600E mutations, driven by the sporadic (non-Lynch) MMR-D cases.¹¹ However, an unexpected result was the significantly higher rates of *APC* (83% v 17%) and possibly *TP53* (33% v 0%) mutations in the Lynch-associated compared with the sporadic (non-Lynch: *BRAF* V600E or *MLH1* promoter hypermethylated) MMR-D cases. The Cancer Genome Atlas of CRC noted lower rates of *TP53* and *APC* mutations in hypermutated compared with nonhypermutated

cancers. The low rates of *APC* and *TP53* mutations in our sporadic (non-Lynch) MMR-D cases were in line with The Cancer Genome Atlas findings. However, in our Lynch-associated MMR-D tumors, *APC* mutations were present in 83% of cases—essentially identical to what would be expected in nonhypermutated CRCs.¹¹ Although other studies have suggested fundamental differences in the pathway to malignancy among subgroups of hypermutated CRCs,^{14,15} our study demonstrates that this difference is largely driven by the LS-associated CRCs that seem to have a clearly unique somatic mutational profile. Our data suggest that the mutation spectrum and frequency within the MMR-D group are likely to differ on the basis of the etiology of the MMR-D. Clinically, there remains substantial controversy with respect to the outcome and treatment response of Lynch-associated versus sporadic (non-Lynch) MMR-D CRCs.^{6,7,16} We anticipate that further molecular characterization may help to shed light on the inherent biologic differences among MMR-D tumors.

To our knowledge, our study is among the first to begin to characterize LS-associated CRC tumors via multigene somatic genomic profiling. Our initial assessment noted that the so-called second hit likely leading to carcinogenesis in five of the patients with LS was a somatic mutation in the gene containing the germline mutation. Notably, such somatic mutations occurred only in *MSH2* and *MSH6* carriers, suggesting the second hit in other cases, especially in *MLH1* carriers, may be loss of heterozygosity or an epigenetic event. Further evaluation of Lynch and non-Lynch MMR-D CRCs is ongoing and may help elucidate whether genomic profiling is associated with other characteristics and clinical outcome.

Our study also suggests that tumor profiling may provide clinically important information in patients with an abnormal screening test for LS. With the expansion of screening for LS, a

Table 3. Mutational Load in Mismatch Repair-Deficient Versus Mismatch Repair-Proficient Colorectal Tumors as Assessed by Next-Generation Sequencing Assay

MMR Status	341-Gene Panel			410-Gene Panel		
	Sample Size	Median Mutational Load (range)	<i>P</i>	Sample Size	Median Mutational Load (range)	<i>P</i>
MMR-proficient; <i>POLE</i> wild-type	193	6 (0-17)	< .001	74	7 (2-12)	< .001
MMR-deficient	28	50 (20-90)		20	60 (21-100)	

MMR, mismatch repair.

Fig 2. Distribution of mutational load among colorectal cancer cases. (A) The number of samples by mutational load intervals for the mismatch repair proficient (MMR-P)/*POLE* wild-type (blue), mismatch repair deficient (MMR-D; gold), and *POLE* mutant (gray) colorectal cancer cases. (B) The percentage of samples from each of the three categories within a certain mutational load interval.

major clinical challenge has been the identification of an MMR-D tumor where subsequent work-up reveals absence of a germline genetic mutation and, at the same time, absence of *MLH1* hypermethylation or *BRAF* V600E mutation. Such patients, in whom LS can neither be ruled in nor ruled out, are labeled as having mutation-negative LS. The etiology of such a discordant test result may be due to a false-positive tumor IHC or MSI screen, the presence of an occult germline mutation, biallelic somatic mutations in the *MMR* genes, or somatic mosaicism for an *MMR* gene mutation. A prior study suggested that up to 70% of patients categorized as having mutation-negative LS may have acquired somatic mutations within the *MMR* genes, accounting for the MMR-D phenotype.¹⁷ Similarly, we identified four individuals with mutation-negative LS, with two patients each having two

somatic deleterious mutations in the genes implicated upon IHC analysis. We cannot prove that these mutations were in *trans* (on two different alleles) in these patients. However, this somatic analysis, in addition to the negative germline analysis and the absence of Lynch-associated cancer family history, does provide further evidence that the MMR-D is unlikely to be due to LS. Further, it may eliminate the need for more extensive cancer screening in the patient and his/her relatives.

A potential limitation of our study, regarding its applicability to other centers, is that not all institutions and laboratories routinely use NGS platforms for standard mutational analysis of CRCs. For those that do, the mutation threshold for discriminating between MMR-P and MMR-D tumors is dependent on the total number of genes assessed on any given NGS panel. Thus, each

panel would require an internal calibration to identify an appropriate cutoff. Nonetheless, for the increasing number of institutions and oncology practices that use commercially available NGS panels, such a threshold would be relatively easy to establish for each panel. For oncology practices and institutions that currently do not use NGS panels, replacement of MMR IHC with a multigene NGS panel would not be immediately possible. Although other methods for detecting an MSI-mutator phenotype have been developed via bioinformatic analyses,¹⁸ our method is straightforward, allowing clinicians to evaluate for mutational burden and thereby recognize the MMR-D phenotype with ease and without additional computational assessment. Nonetheless, the addition of mutation signatures and an assessment of microsatellite loci may help improve the discriminatory power of the analysis in cases with borderline mutational burden (eg, in our cohort, in cases with mutational ranges of 15 to 25 or 100 to 150).

In conclusion, we demonstrated that our multigene NGS assay for tumor profiling enables identification of a cutoff value for mutational load, providing an extremely sensitive and specific means of screening for MMR-D. The versatility of such a multigene assay allows for the use of a single tumor-sequencing assay to screen for both MMR-D and *RAS/BRAF* mutations in patients with CRC. The use of a single multigene sequencing assay could be highly cost effective, particularly in metastatic CRC. It could also provide additional genetic information that may be useful both for

basic research purposes, as well as for matching patients with tumors harboring putatively actionable mutations to appropriate investigational studies of agents targeting those mutations.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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