PRECISION MEDICINE

Clinical Pan-Cancer Assessment of Mismatch Repair Deficiency Using Tumor-Only, Targeted Next-Generation Sequencing

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PURPOSE Given regulatory approval of immune checkpoint inhibitors in patients with mismatch repair–deficient (MMR-D) cancers agnostic to tumor type, it has become important to characterize occurrence of MMR-D and develop cost-effective screening approaches. Using a next-generation sequencing (NGS) panel (OncoPanel), we developed an algorithm to identify MMR-D frequency in tumor samples and applied it in a clinical setting with pathologist review.

METHODS To predict MMR-D, we adapted methods described previously for use in NGS panels, which assess patterns of single base-pair insertion or deletion events occurring in homopolymer regions. Tumors assayed with OncoPanel between July 2013 and July 2018 were included. For tumors tested after June 2017, sequencing results were presented to pathologists in real time for clinical MMR determination, in the context of tumor mutation burden, other mutational signatures, and clinical data.

RESULTS Of 20,301 tumors sequenced, 2.7% (553) were retrospectively classified as MMR-D by the algorithm. Of 4,404 samples with pathologist sign-out of MMR status, the algorithm classified 147 (3.3%) as MMR-D: in 116 cases, MMR-D was confirmed by a pathologist, five cases were overruled by the pathologist, and 26 were assessed as indeterminate. Overall, the highest frequencies of OncoPanel-inferred MMR-D were in endometrial (21%; 152/723), colorectal (9.7%; 169/1,744), and small bowel (9.3%; 9/97) cancers. When algorithm predictions were compared with historical MMR immunohistochemistry or polymerase chain reaction results in a set of 325 tumors sequenced before initiation of pathologist assessment, the overall sensitivity and specificity of the algorithm were 91.1% and 98.2%, respectively.

CONCLUSION We show that targeted, tumor-only NGS can be leveraged to determine MMR signatures across tumor types, suggesting that broader biomarker screening approaches may have clinical value.

INTRODUCTION

As regulatory approvals for immunotherapy agents expand across tumor types, efforts to identify predictive biomarkers of response or resistance to treatment are similarly expanding. Comprehensive analysis of colorectal cancers has shown that tumors with defects in mismatch repair (MMR), resulting in microsatellite instability (MSI), have elevated lymphocytic infiltration.[1] In addition, tumors with MSI demonstrate upregulated expression of inhibitory immune checkpoints, including PD-L1, on tumor-infiltrating lymphocytes and myeloid cells. These developments and others led to clinical trials evaluating the efficacy of immune checkpoint blockade on the basis of MMR status. On the basis of data from 149 patients with mismatch repair deficient (MMR-D) or MSI-high (MSI-H) tumors in five single-arm trials, the PD-1 inhibitor pembrolizumab was approved in 2017

by the US Food and Drug Administration (FDA) for patients with unresectable or metastatic MMR-D or MSI-H solid tumors of any tissue type.[2,3] This was the first tumor-agnostic, biomarker-based drug approval, with approvals for larotrectinib and entrectinib for neurotrophic tyrosine receptor kinase fusion-positive tumors following shortly thereafter.[4,5] These breakthroughs underscore the need for broadly applicable, cost-effective methods of identifying tumors with MMR-D and other potential biomarkers.

Patients with colorectal and endometrial tumors undergo routine clinical testing for MSI and/or MMR-D using polymerase chain reaction (PCR) assays by assessing loci within the Bethesda panel or related biomarkers or by assessing gene expression in the MMR pathway via immunohistochemistry (IHC).[6] However, given the lower prevalence of MMR-D outside colorectal and endometrial cancers, MSI/MMR

CONTEXT

Key Objective

To develop a bioinformatics algorithm to infer mismatch repair–deficient (MMR-D) cancers using tumor-only next-generation sequencing (NGS) data and describe the prevalence of MMR-D across cancer types.

Knowledge Generated

We characterized MMR status in a cohort of 20,301 tumor samples across a wide range of adult and pediatric malignancies, including cancer types not commonly assayed for MMR-D as part of routine clinical practice. Overall, the algorithm classified 2.7% of samples as MMR-D.

Relevance

We show the value of using a routine NGS panel to identify new, clinically relevant biomarkers that might otherwise not be assessed because of low prevalence in certain tumor types and to identify potential candidates for immunotherapy.

testing using IHC or PCR is not routinely pursued in clinical practice in other tumor types, despite National Comprehensive Cancer Network testing recommendations for the following cancers: cervical, colon, rectal, endometrial, esophageal, gastroesophageal junction, gastric carcinoma, gallbladder, intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, neuroendocrine, ovarian, pancreatic, prostate, testicular, and vulvar. Broad implementation of a targeted, tumor-only next-generation sequencing (NGS) assay (OncoPanel) for patients at Dana-Farber Cancer Institute (DFCI), Brigham and Women's Hospital (BWH), and Boston Children's Hospital (BCH) allowed us to assess whether bioinformatic analysis of OncoPanel[7] data could characterize the prevalence of MMR-D/MSI-H (hereafter MMR-D) across tumors and increase the number of patients who could be considered for immunotherapy.

Methods such as mSINGS,[8] MSI-Sensor[9] and cell-free DNA[10] have previously been reported for detection of MMR-D. However, given the wide implementation and relatively low cost of tumor-only NGS, it is important to develop approaches suited for this paradigm and understand potential differences in results. It is also critical to better characterize the frequency of MMR-D across tumors to inform selective use of IHC, PCR, or NGS assays in clinical settings where NGS panels and MMR testing are used less frequently. This study expands on previous work to develop OncoPanel for clinical assessment of MMR-D using IHC and PCR for validation[11] and to characterize MMR status in 645 upper GI tract cancers[12] and 304 sarcomas.[13] Here we describe a bioinformatics algorithm that infers MMR status using an institution-wide NGS panel and report the prevalence of MMR-D across > 20,000 adult and pediatric tumor specimens.

METHODS

Patient Population

Patients treated for a cancer diagnosis at DFCI, BWH, or BCH were offered participation in the research study PROFILE (Dana-Farber/Harvard Cancer Center Institutional Review Board [IRB] protocol #11-104/17-000) as

previously described.[14] Tumor samples from patients who consented to participate in PROFILE or from patients with cancers eligible for clinical NGS were tested using a tumor-only targeted DNA sequencing panel (OncoPanel).[14] Data acquisition, bioinformatic analysis, chart review, and orthogonal MSI testing via PCR were performed under DFCI IRB protocol #17-465.

We included patients who provided written informed consent and underwent successful targeted OncoPanel sequencing of a primary or metastatic tumor between August 2013 (OncoPanel study initiation) and July 2018 to allow adequate time for patient follow-up. As of the study cutoff date, 20,301 tumors had OncoPanel results and could be assessed for MMR-D via the bioinformatics algorithm described below. Of these tumors, 4,488 were sequenced after the implementation on June 27, 2017 of a process for pathologist sign-out of NGS-based, MMR-D assessment, described in more detail below. A total of 4,404 of these 4,488 tumors met criteria for MMR assessment via the OncoPanel algorithm, which measures insertion or deletion (indel) events within homopolymeric microsatellite loci to infer MMR status, and were presented for final interpretation by a molecular pathologist. Signed-out OncoPanel reports, including MMR-D calls, were returned to physicians for clinical use.

Patients from the aforementioned study of 304 sarcomas[13] are also included in the summary data here, although with different subclassifications. Here, we used the original pathologists' diagnoses as recorded at OncoPanel sign-out, rather than more detailed subclassification of sarcomas using chart review.

Sequencing

From August 2013 to July 2014, patient samples were tested with OncoPanel Version 1, which covers 0.753334 Mb and the full coding regions of 275 genes, plus selected intronic regions of 30 genes for rearrangement detection. OncoPanel Version 2 began August 2014, covering 0.826167 Mb, with full coding regions of 300 cancer genes

plus selected intronic regions of 35 genes. OncoPanel Version 3 activated in October 2016, covering 1.315078 Mb, 447 cancer genes, and 191 regions across 60 genes for rearrangement detection. At the time of clinical pathology assessment for MMR deficiency (on or after June 27, 2017), all samples were being sequenced using OncoPanel Version 3.[14] Variants observed in public genome databases at a frequency of > 0.1% were removed, unless observed more than twice in the Catalogue of Somatic Mutations in Cancer,[15] in which case they were presented for manual review. Finally, variants were tiered and reported based on clinical significance by a molecular pathologist using methods previously described.[16] All testing was conducted in a Clinical Laboratory Improvement Amendments–certified environment.

MMR Algorithm

On the basis of methods described previously for identifying MMR-D in colorectal cancer using OncoPanel Versions 1-2,[11] an internally developed algorithm was adjusted to predict MMR status across all tumor types. For this study, a homopolymer was defined as a genomic region with more than four repeat nucleotides. Tumors harboring ≥ 1.5 single base-pair indels/Mb were called MMR-D. Only patterns of single base-pair indels in homopolymers were considered (with a minimum threshold of 1.5/Mb), and tumor mutational burden (TMB) was ignored.

Possible values resulting from the OncoPanel algorithm are proficient (MMR-P), deficient (MMR-D), and cannot assess (for tumors with < 12 variants), although a pathologist may override algorithmic calls or mark a tumor "indeterminate." For the 4,488 samples where MMR results were communicated to the ordering physician, a pathologist manually reviewed the algorithmic MMR status to assign final status. Pathologists interpreted the algorithmic MMR determination in the context of TMB, other mutational signatures validated on the platform (UV [ultraviolet], tobacco, APOBEC [apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like], temozolomide, and POLE [DNA polymerase epsilon catalytic subunit]), and existing clinical data such as MMR protein expression, when available. Recognizing that the positive and negative predictive values of the MMR algorithm are lower near the defined cutoff, and that patterns of MMR-D are not well defined for tumors outside of colon and endometrium, the pathologist could elect to override the algorithmic classification. Possible reasons for overriding the classification included homopolymer indel rates near the cutoff, low TMB for that tumor type, or no MMR gene alterations in the sequencing data. The treating clinician was also encouraged to seek confirmatory testing with IHC or PCR in this setting when clinically relevant.

TMB

TMB was calculated by including all nonsynonymous mutations in coding regions of the genome covered by OncoPanel. The sum of such mutations was divided by the size of the bait set to determine mutations per megabase (mut/Mb). TMB results were compared with other tumors sequenced with the same panel version as well as with other tumors of the same cancer type. We reported three TMB metrics for each tumor: mutations per megabase, percentile of TMB across all tumors sequenced in the current OncoPanel version, and percentile of TMB across tumors of each cancer type.

Orthogonal Testing

Twelve of the 26 tumor samples with discrepant calls between algorithm and pathologist, specifically those with algorithm-predicted MMR-D, a pathologist call of indeterminate, and sufficient leftover DNA, underwent orthogonal, tumor-only PCR testing. MSI PCR was performed using a five-marker microsatellite panel and capillary electrophoresis as previously described.[11] Results were interpreted by the pathologist according to revised Bethesda guidelines.[17]

RESULTS

Frequencies of MMR-D

We measured MMR-D frequencies detected using the OncoPanel algorithm in 20,301 samples across tumor types (Tables 1 and 2) and compared them to historical reports of MMR-D prevalence (Tables 1 and 3, Fig 1).[18-39] The OncoPanel cohort comprised 13,542 primary biopsies, 4,734 metastatic recurrences, 655 local recurrences, and 1,370 tumors with unspecified biopsy site.

One of the main goals of this work was to characterize prevalence of MMR-D outside the tumor types where it is commonly assayed in routine clinical practice. Overall, among 3,779 noncolorectal, nonendometrial samples for which OncoPanel results were returned to clinicians, 43 (1.14%) tumors were detected as MSI-H/MMR-D, and results were reported to the patients' treating clinicians. Without another indication for clinical MSI/MMR testing, these 43 patients are unlikely to have been tested outside of the OncoPanel program.

Across the 816 pediatric tumors profiled, eight were predicted as MMR-D algorithmically. Of these 816, 208 were also assessed for MMR status by a pathologist for the clinical OncoPanel report. One case of pediatric small bowel cancer was reported as MMR-D algorithmically, with concordant pathologist sign-out. One pediatric embryonal tumor was predicted algorithmically as MMR-D but ruled indeterminate by the pathologist. PCR-based orthogonal validation was performed on the small bowel case to confirm MSI-H status, and MSI was detected for three of four microsatellite markers.

We identified two adult appendiceal tumors with predicted MMR-D: one predicted by the algorithm in the historical cohort and the other identified by a pathologist during routine clinical sign-out. To the best of our knowledge,

TABLE 1. MMR-D Prevalence Across Cancer Types Using OncoPanel Bioinformatics Algorithm

Tumor Type	17-465 All			17-465 Pathologist Assessed		
	Total	MMR-D	Frequency (%)	Total	MMR-D	Frequency (%)
Adrenocortical carcinoma	47	0	0	9	0	0
Ampullary carcinoma	22	1	4.55	2	0	0
Anal cancer	46	0	0	6	0	0
Appendiceal cancer	80	2	2.50	19	1	5.27
Biliary cancer	198	3	1.52	42	0	0
Bladder cancer	605	15	2.48	117	3	2.57
Blastic plasmacytoid dendritic cell neoplasm	7	0	0			N/A
Breast carcinoma	1,920	17	0.89	355	0	0
Breast sarcoma	7	0	0	2	0	0
Cancer of unknown primary	682	16	2.35	213	6	2.82
Cervical cancer	103	2	1.95	19	0	0
Chondroblastoma	1	0	0			N/A
Chondrosarcoma	26	0	0	1	0	0
Chordoma	7	0	0	1	0	0
Choroid plexus tumor	6	0	0	5	0	0
Colorectal cancer	1,744	169	9.69	481	43	8.94
Embryonal tumor	154	1	0.65	37	0	0
Endometrial cancer	723	152	21.03	144	36	25
Ependymomal tumor	49	0	0	10	0	0
Esophagogastric carcinoma	757	28	3.69	198	8	4.05
Ewing sarcoma	31	0	0	6	0	0
GI neuroendocrine tumor	143	0	0	29	0	0
GI stromal tumor	195	0	0	54	0	0
Germ cell tumor	115	0	0	13	0	0
Gestational trophoblastic disease	5	0	0			N/A
Giant cell tumor	3	0	0	2	0	0
Glioma	1,475	22	1.49	288	5	1.74
Head and neck carcinoma	652	9	1.39	115	2	1.74
Hepatocellular carcinoma	77	0	0	25	0	0
Histiocytosis	25	0	0	4	0	0
Hodgkin lymphoma	9	0	0	1	0	0
Leukemia	675	5	0.75	78	0	0
Melanoma	511	2	0.39	195	0	0
Meningothelial tumor	249	0	0	51	0	0
Mesothelioma	242	2	0.83	47	1	2.13
Miscellaneous brain tumor	52	1	1.93	5	0	0
Miscellaneous neuroepithelial tumor	35	1	2.86	9	0	0
Multiple myeloma	110	1	0.91	35	0	0
Myelodysplasia	115	1	0.87	6	0	0
Myeloproliferative neoplasm	44	1	2.28	3	0	0
Nerve sheath tumor	77	1	1.29	18	0	0
Non-Hodgkin lymphoma	474	8	1.69	144	0	0
Non–small-cell lung cancer	3,008	36	1.19	604	4	0.67

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TABLE 1. MMR-D Prevalence Across Cancer Types Using OncoPanel Bioinformatics Algorithm (Continued)

	17-465 All			17-465 Pathologist Assessed		
Tumor Type	Total	MMR-D	Frequency (%)	Total	MMR-D	Frequency (%)
Osteosarcoma	37	0	0	10	0	0
Ovarian cancer	908	7	0.78	134	1	0.75
Pancreatic cancer	612	8	1.31	177	0	0
Penile cancer	7	0	0			N/A
Pheochromocytoma	13	0	0	1	0	0
Pineal tumor	10	0	0	1	0	0
Prostate cancer	578	7	1.22	144	3	2.09
Renal cell carcinoma	518	4	0.78	92	0	0
Retinoblastoma	4	0	0	2	0	0
Sellar tumor	191	1	0.53	23	0	0
Sex cord stromal tumor	42	0	0	5	0	0
Skin cancer, nonmelanoma	204	4	1.97	47	2	4.26
Small bowel cancer	97	9	9.28	42	2	4.77
Small-cell lung cancer	122	1	0.82	20	0	0
Soft tissue sarcoma	722	8	1.11	217	3	1.39
Thymic tumor	51	2	3.93	5	1	20
Thyroid cancer	445	3	0.68	67	1	1.49
Uterine sarcoma	155	2	1.3	18	0	0
Vaginal cancer	41	1	2.44	3	0	0
Wilms tumor	38	0	0	3	0	0
Total	20,301	553	2.72	4,404	122	2.78

NOTE. 17-465 All represents the overall OncoPanel cohort using algorithm prediction, and 17-465 Pathologist Assessed represents the subset with pathology sign-out.

Abbreviations: MMR-D, mismatch repair deficiency; N/A, tumor type not assessed or specified.

MMR-D has not been reported previously in appendiceal cancer. In addition, we found one thymic MMR-D tumor among five cases (20%), indicating that thymic cancers too can be MMR-D, corroborating a recent report.[20]

The Cancer Genome Atlas (TCGA) and Therapeutically Applicable Research to Generate Effective Treatments (TARGET) projects[20] reported MSI-H status in 3.8% of tumors, using paired tumor-normal sequencing across cancer types and the algorithm MANTIS,[40] and identified MSI in 27 cancer types (see Discussion). This rate is consistent with our algorithmic predictions of MMR-D in clinically signed-out tumors (3.33%), although only 2.78% were pathologist confirmed.

We also measured algorithm sensitivity and specificity using a historical set of 325 tumors tested with IHC or PCR. This yielded a sensitivity and specificity of 91% and 98%, respectively (Appendix Table A1).

Concordance of OncoPanel MMR Algorithm With Pathologist Assessment

Concordance was assessed between the purely algorithmic MMR/OncoPanel calls and pathologist calls. For the 4,404 clinical cases with MMR status signed out by pathologists, there was substantial agreement (Cohen's κ coefficient = 0.75) between algorithmic and pathologist calls (Table 4). To better understand discordant cases, we selected 14 tumors for orthogonal MSI validation via PCR that were algorithmically predicted to be MMR-D and also had sufficient sample material. One of the samples was confirmed to be MMR-D: a pediatric embryonal tumor. None of the other samples were MSI-H by PCR, supporting the additional value of manual pathologist review. One factor among many that could play a role in discordance between algorithmic prediction and pathologist assessment (Table 5) is TMB, which was relatively high in concordant MMR-D cases (mean TMB, 55.8 mut/Mb). Concordant MMR-P cases had a mean TMB of 7.59 mut/Mb, and indeterminate cases yielded a mean TMB of 20.6 mut/Mb.

DISCUSSION

We found 2.72% of 20,301 tumor samples to be MMR-D across a wide range of adult and pediatric malignancies using a bioinformatics algorithm to process tumor-only NGS data. In a 4,404 sample subset, we report substantial

TABLE 2. Mapping of Tumor Types From Sources

Original Tumor Type	Mapped Tumor Type
Colon adenocarcinoma	Colorectal cancer
Rectal adenocarcinoma	Colorectal cancer
Colorectal adenocarcinoma	Colorectal cancer
Barret esophagus adenocarcinoma	Esophagogastric carcinoma
Esophageal carcinoma	Esophagogastric carcinoma
Gastric adenocarcinoma	Esophagogastric carcinoma
Stomach or gastric adenocarcinoma	Esophagogastric carcinoma
Esophagogastric junction carcinoma	Esophagogastric carcinoma
Small intestinal malignancies	Small bowel cancer
Extrahepatic bile duct adenocarcinoma	Biliary cancer
Pancreatic adenocarcinoma	Pancreatic cancer
Uterine carcinosarcoma	Uterine sarcoma
Kidney chromophobe	Renal cell carcinoma
Kidney renal clear cell carcinoma	Renal cell carcinoma
Kidney renal papillary cell carcinoma	Renal cell carcinoma
Kidney cancer	Renal cell carcinoma
Sarcoma	Soft tissue sarcoma
Glioblastoma multiforme	Glioma
Lower-grade or low-grade glioma	Glioma
Merkel cell carcinoma	Skin cancer, nonmelanoma
Malignant solitary fibrous tumor of the pleura	Soft tissue sarcoma
Ovarian surface epithelial carcinoma	Ovarian cancer
Nonepithelial ovarian cancer	Ovarian cancer
Cervical squamous cell carcinoma/endocervical adenocarcinoma	Cervical cancer
Pediatric acute myeloid leukemia	Acute myeloid leukemia
Pediatric high-risk Wilms tumor	Wilms tumor
Poorly differentiated carcinoma, NOS	Cancer of unknown primary
Combined small-cell lung carcinoma	Small-cell lung cancer
Lung bronchioloalveolar carcinoma	Non–small-cell lung cancer
Thyroid carcinoma	Thyroid cancer
Thymic carcinoma	Thymic tumor
Head and neck squamous carcinoma	Head and neck carcinoma
NUT midline carcinoma of the head and neck	Head and neck carcinoma
Prostatic adenocarcinoma	Prostate cancer

Abbreviations: NOS, not otherwise specified; NUT, nuclear protein in testis.

agreement between purely algorithmic calls and formal pathologist classification. It should be noted that these two measures are not independent, because the pathologists included the algorithmic calls as a part of their assessment, along with TMB, results of other mutational signatures, and existing clinical data. Orthogonal laboratory validation of tumors predicted to be MMR-D but signed out as indeterminate demonstrated the value of manual pathologist review as a final step in the MMR-D classification process and suggests that inclusion of other data types could improve future algorithms.

Confirming prior clinical observations, the highest frequencies of predicted MMR-D were in endometrial and colorectal cancers, but MMR-D was also observed in many different cancer types not commonly assessed for MMR-D in routine clinical practice, including appendiceal cancer and nonmelanoma skin cancer.

Further manual review of predicted MMR-D tumors signed out as indeterminate highlighted the tendency for tumors with a pattern of indels in homopolymer regions but low TMB to be called indeterminate. Orthogonal PCR validation of 14 discordant cases confirmed only one as MSI-H. It could thus be useful to incorporate TMB explicitly in future MMR-D prediction algorithms. Our results suggest confirmatory laboratory testing should continue to be used, especially when borderline NGS results are obtained or MMR-D is predicted in a tumor with low MMR-D prevalence. Correlation between high TMB and MSI-H has been reported in other NGS studies, including in prostate cancer, where 71% of 111 tumors with high TMB ($\geq$ 20 mut/Mb) were MSI-H,[41] and in adrenocortical carcinoma (n = 92) and cervical squamous cell and cervical adenocarcinoma (n = 305).[20] In a larger NGS panel study (n = 11,000), only 27% of tumors with high TMB (defined as $\geq$ 17 mut/Mb) were MSI-H ($\geq$ 46 altered microsatellite loci/Mb), whereas 70% of MSI-H cases had high TMB.[21] As exemplified in these studies, however, there is significant variability in the definitions of "high TMB" and the methodologies used to measure it.[42] Recent FDA approval of pembrolizumab for high-TMB tumors defined high TMB as $\geq$ 10 mut/Mb, with a targeted gene panel as a companion diagnostic. Although TMB and MSI are both now broadly relevant biomarkers for immunotherapy, they reflect different aspects of tumor biology. Thus, assessing MMR independently from TMB allows clinicians to evaluate these data independently when creating patient treatment plans.[43]

Certain cancers have considerable variability in reported MMR-D frequencies, including hepatocellular carcinoma (0.0%-16.2%), ampullary carcinoma (0.0%-10.4%), GI neuroendocrine tumors (0.0%-20.7%), ovarian cancer (0.0%-10.0%), and thyroid cancer (0%-63.3%). Potential reasons include variation in sample sizes and detection methods, and it also remains unknown if MMR-D differs based on the site of disease (primary *v* metastatic) or timing (treatment-naïve *v* treatment-exposed).

TABLE 3. MMR-D/MSI-H Prevalence From Pan-Cancer Studies Assessing Microsatellite Instability

Tumor Type	17-465 All			17-465 Pathologist Assessed			MSK-IMPACT Middha et al[18]			MSI Review Dudley et al[19]			TCGA/TARGET Bonneville et al[20]			Vanderwalde et al[21]		
	Total	MMR-D	Frequency (%)	Total	MMR-D	Frequency (%)	Total	MSI-H	Frequency (%)	Total	MSI-H	Frequency (%)	Total	MSI-H	Frequency (%)	Total	MSI-H	Frequency (%)
Adrenocortical carcinoma	47	0	0	9	0	0	30	0	0			N/A	92	4	4.35			N/A
Ampullary carcinoma	22	1	4.55	2	0	0	28	0	0	144	15	10.42			N/A			N/A
Anal cancer	46	0	0	6	0	0	31	0	0			N/A			N/A			N/A
Appendiceal cancer	80	2	2.50	19	1	5.27			N/A			N/A			N/A			N/A
Biliary cancer	198	3	1.52	42	0	0	236	3	1.28			N/A			N/A	29	1	3.44
Bladder cancer	605	15	2.48	117	3	2.57	355	11	3.09			N/A	412	2	0.49	143	0	0
Blastic plasmacytoid dendritic cell neoplasm	7	0	0			N/A			N/A			N/A			N/A			N/A
Breast carcinoma	1,920	17	0.89	355	0	0	1,405	0	0			N/A	1,044	16	1.54	1,024	6	0.59
Breast sarcoma	7	0	0	2	0	0	15	0	0			N/A			N/A			N/A
Cancer of unknown primary	682	16	2.35	213	6	2.82	250	5	2			N/A			N/A			N/A
Cervical cancer	103	2	1.95	19	0	0	43	0	0	344	28	8.14	305	8	2.63	168	6	3.57
Cholangiocarcinoma			N/A			N/A			N/A			N/A	74	1	1.36	177	4	2.26
Chondroblastoma	1	0	0			N/A			N/A			N/A			N/A			N/A
Chondrosarcoma	26	0	0	1	0	0	33	0	0			N/A			N/A			N/A
Chordoma	7	0	0	1	0	0	20	0	0			N/A			N/A			N/A
Choroid plexus tumor	6	0	0	5	0	0			N/A			N/A			N/A			N/A
Colorectal cancer	1,744	169	9.69	481	43	8.94	996	83	8.34	1,066	208	19.51	588	94	15.99	1,395	80	5.73
Embryonal tumor	154	1	0.65	37	0	0	81	0	0			N/A			N/A			N/A
Endometrial carcinoma	723	152	21.03	144	36	25	260	42	16.16	989	265	26.79	542	170	31.37	879	155	17.63
Ependymomal tumor	49	0	0	10	0	0	17	0	0			N/A			N/A			N/A
Esophagogastric carcinoma	757	28	3.69	198	8	4.05	282	7	2.49	76	5	6.58	624	87	13.94	373	16	4.29
Ewing sarcoma	31	0	0	6	0	0	41	0	0	55	1	1.82			N/A			N/A
GI neuroendocrine tumor	143	0	0	29	0	0			N/A	295	64	21.69			N/A			N/A
GI stromal tumor	195	0	0	54	0	0			N/A			N/A			N/A	52	0	0
Germ cell tumor	115	0	0	13	0	0	13	0	0			N/A			N/A			N/A
Gestational trophoblastic disease	5	0	0			N/A			N/A			N/A			N/A			N/A
Giant cell tumor	3	0	0	2	0	0			N/A			N/A			N/A			N/A
Glioma	1,475	22	1.49	288	5	1.74	584	0	0			N/A	909	3	0.34	522	3	0.57
Head and neck carcinoma	652	9	1.39	115	2	1.74	200	4	2	153	5	3.27	510	4	0.78	111	0	0
Hepatocellular carcinoma	77	0	0	25	0	0	114	0	0	37	6	16.22	375	3	0.80	73	2	2.74
Histiocytosis	25	0	0	4	0	0	17	0	0			N/A			N/A			N/A
Hodgkin lymphoma	9	0	0	1	0	0			N/A			N/A			N/A			N/A

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TABLE 3. MMR-D/MSI-H Prevalence From Pan-Cancer Studies Assessing Microsatellite Instability (Continued)

Tumor Type	17-465 All			17-465 Pathologist Assessed			MSK-IMPACT Middha et al[18]			MSI Review Dudley et al[19]			TCGA/TARGET Bonneville et al[20]			Vanderwalde et al[21]		
	Total	MMR-D	Frequency (%)	Total	MMR-D	Frequency (%)	Total	MSI-H	Frequency (%)	Total	MSI-H	Frequency (%)	Total	MSI-H	Frequency (%)	Total	MSI-H	Frequency (%)
Leukemia	675	5	0.75	78	0	0			N/A			N/A	503	1	N/A	1	0	0
Melanoma	511	2	0.39	195	0	0			N/A	56	6	10.72	470	3	0.64	345	0	0
Meningothelial tumor	249	0	0	51	0	0			N/A			N/A			N/A			N/A
Mesothelioma	242	2	0.83	47	1	2.13	122	1	0.82			N/A	83	2	2.41			N/A
Miscellaneous brain tumor	52	1	1.93	5	0	0	16	0	0			N/A			N/A			N/A
Miscellaneous neuroepithelial tumor	35	1	2.86	9	0	0	11	0	0			N/A			N/A			N/A
Multiple myeloma	110	1	0.91	35	0	0			N/A			N/A			N/A	10	0	0
Myelodysplasia	115	1	0.87	6	0	0			N/A			N/A			N/A			N/A
Myeloproliferative neoplasm	44	1	2.28	3	0	0			N/A			N/A			N/A			N/A
Nasopharyngeal carcinoma			N/A			N/A			N/A			N/A	50	0	0			N/A
Nerve sheath tumor	77	1	1.29	18	0	0	20	0	0			N/A			N/A			N/A
Non-Hodgkin lymphoma	474	8	1.69	144	0	0	44	0	0			N/A	81	0	N/A	5	0	0
Non-small-cell lung cancer	3,008	36	1.19	604	4	0.67	1,525	5	0.33			N/A	1,065	6	0.57	1,869	12	0.64
Osteosarcoma	37	0	0	10	0	0	50	0	0			N/A			N/A			N/A
Ovarian cancer	908	7	0.78	134	1	0.75	233	0	0	1,234	124	10.05	437	6	1.38	1,573	18	1.14
Pancreatic cancer	612	8	1.31	177	0	0	564	4	0.71			N/A	183	0	0	518	6	1.16
Pediatric high-risk Wilms tumor			N/A			N/A			N/A			N/A	41	1	2.44			N/A
Pediatric neuroblastoma			N/A			N/A			N/A			N/A	220	1	0.46			N/A
Penile cancer	7	0	0	1	0	0			N/A			N/A			N/A			N/A
Pheochromocytoma	13	0	0	1	0	0			N/A			N/A			N/A			N/A
Pheochromocytoma and paraganglioma			N/A			N/A			N/A			N/A	179	0	0			N/A
Pineal tumor	10	0	0	1	0	0			N/A			N/A			N/A			N/A
Prostate cancer	578	7	1.22	144	3	2.09	722	12	1.67	79	6	7.59	498	3	0.61	191	4	2.09
Renal cell carcinoma	518	4	0.78	92	0	0	349	1	0.29	152	3	1.98	627	5	0.80	155	1	0.65
Retinoblastoma	4	0	0	2	0	0			N/A			N/A			N/A			N/A
Retroperitoneal or peritoneal carcinoma or sarcoma			N/A			N/A			N/A			N/A				53	0	0
Salivary carcinoma			N/A			N/A	107	0	0			N/A			N/A			N/A
Sellar tumor	191	1	0.53	23	0	0			N/A			N/A			N/A			N/A
Sex cord stromal tumor	42	0	0	5	0	0	19	0	0			N/A			N/A			N/A
Skin (basal cell carcinoma)			N/A			N/A			N/A	53	0	0			N/A			N/A
Skin (sebaceous tumor)			N/A			N/A			N/A	45	22	48.89			N/A			N/A

(Continued on following page)

TABLE 3. MMR-D/MSI-H Prevalence From Pan-Cancer Studies Assessing Microsatellite Instability (Continued)

Tumor Type	17-465 All			17-465 Pathologist Assessed			MSK-IMPACT Middha et al[18]			MSI Review Dudley et al[19]			TCGA/TARGET Bonneville et al[20]			Vanderwalde et al[21]		
	Total	MMR-D	Frequency (%)	Total	MMR-D	Frequency (%)	Total	MSI-H	Frequency (%)	Total	MSI-H	Frequency (%)	Total	MSI-H	Frequency (%)	Total	MSI-H	Frequency (%)
Skin (squamous cell carcinoma)			N/A			N/A			N/A	30	0	0			N/A			N/A
Skin cancer, nonmelanoma	204	4	1.97	47	2	4.26			N/A			N/A			N/A	6	0	0
Small bowel cancer	97	9	9.28	42	2	4.77	32	5	15.63			N/A			N/A	72	6	8.33
Small-cell lung cancer	122	1	0.82	20	0	0	94	1	1.07			N/A			N/A	75	1	1.33
Soft tissue sarcoma	722	8	1.11	217	3	1.39	474	1	0.22	40	2	5	255	2	0.79	3	0	0
Testicular germ cell tumor			N/A			N/A			N/A			N/A	150	0	0			N/A
Thymic tumor	51	2	3.93	5	1	20	20	0	0			N/A	123	1	0.82	26	0	0
Thyroid cancer	445	3	0.68	67	1	1.49	234	1	0.43	30	19	63.34	496	0	0	42	1	2.38
Uterine sarcoma	155	2	1.3	18	0	0	97	2	2.07			N/A	57	2	3.51	128	3	2.34
Uveal melanoma			N/A			N/A			N/A			N/A	80	0	0	50	1	2.00
Vaginal cancer	41	1	2.44	3	0	0			N/A			N/A			N/A			N/A
Wilms tumor	38	0	0	3	0	0			N/A			N/A			N/A			N/A
Total	20,301	553	2.72	4,404	122	2.78	9,784	188	1.92	4,878	779	15.97	11,073	425	3.84	11,348	342	3.01

NOTE. Results from this study (17-465) are shown in the first two columns; 17-465 All represents the overall OncoPanel cohort using algorithm prediction, and 17-465 Pathologist Assessed represents the subset with pathology sign-out. Comparators are MSK-IMPACT,[18] TCGA/TARGET,[20] Vanderwalde et al,[21] and a review of MSI studies conducted by Dudley et al,[19] which included the following cancer types: ampullary,[22] cervical,[23] colorectal,[24] endometrial,[25,26] esophageal,[27] Ewing sarcoma,[28] gastric adenocarcinoma,[29] head and neck squamous cell carcinoma,[30] hepatocelluar,[31] ovarian,[32] prostate,[33] renal,[34] skin melanoma,[35] skin (sebaceous tumors),[36,37] skin (squamous cell carcinoma),[36,37] and soft tissue sarcoma.[39] Other cancer types reported in Vanderwalde et al[21] that may encompass several cancer type categories included in the table are: soft tissue tumors (1/283, 0.35%), neuroendocrine tumors (7/193, 3.63%), other female genital tract malignancies (1/57, 1.75%), lymphoma (0/27, 0.00%), male genital tract malignancy (0/15, 0.00%), and a category titled "none of these apply" (7/705, 0.99%). We reviewed the individual studies included in Dudley et al[19] to confirm and include additional details of each tumor type. As a result, values shown in the "MSI Review" section differ from those of Dudley et al in the following cases: colorectal,[24] where Dudley et al report 13%, but with 208 cases reported as MSI-H out of 1,066 from the original manuscript, we show 19.51%; endometrial,[25,26] where consolidation of two cited references from Dudley et al (118/543 in Hampel et al,[25] 147/446 in Zighelboim et al[26]) leads to a 26.79% rate of MSI-H endometrial tumors; and skin (sebaceous tumors),[36,37] where consolidation of two cited references from Dudley et al (7/20 in Cesinaro et al,[37] 15/25 in Kruse et al[36]) leads to a 48.80% rate of MSI-H sebaceous tumors.

Abbreviations: MMR-D, mismatch repair deficiency; MSI, microsatellite instability; MSI-H, microsatellite instability high; N/A, tumor type not assessed or specified.
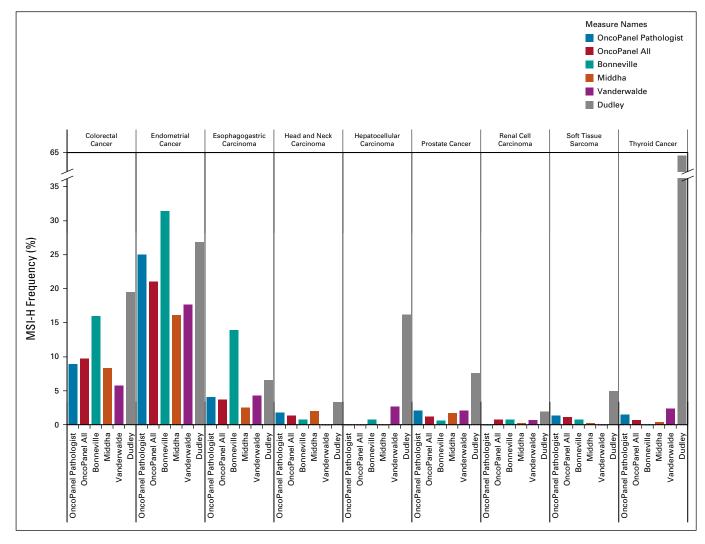
FIG 1. Comparison of mismatch repair–deficient (MMR-D)/microsatellite instability–high (MSI-H) patterns across tumor types. Across all studies described in Table 3,[18-39] the frequency of MMR-D or MSI-H was reported across all six cohorts in nine tumor types: colorectal, endometrial, esophagogastric, head and neck, hepatocellular, prostate, renal cell carcinoma, thyroid cancer, and soft tissue sarcoma. Hepatocellular carcinoma and thyroid cancer had considerably higher MSI-H rates reported in the review by Dudley et al.[19]

It is encouraging, however, that MMR-D prevalence in most tumor types across independent studies was generally consistent (Fig 1), especially for other NGS-based

TABLE 4. Concordance Between Algorithmic Prediction and Final Pathology Assessment for MMR Status

Final Pathology Assessment	Clinical Algorithm Prediction		
	Predicted MMR-D	Predicted MMR-P	Total
Signed out MMR-D	116	6	122
Signed out MMR-P	5	4,213	4,218
Signed out indeterminate	26	38	64
Total	147	4,257	4,404[a]

Abbreviations: MMR-D, mismatch repair deficient; MMR-P, mismatch repair proficient.

[a]Excludes 84 samples that could not be assessed for MMR status by pathology because of insufficient variants.

assessments,[18,20,21] although more traditional MSI testing performed via PCR or IHC showed greater variability in some tumor types.[19] For the TCGA and TARGET data sets,[18] MSI-H status was observed using tumor-normal paired NGS in 27 different tumor types at an overall frequency of 3.8%.[37] Our predicted MMR-D frequency using tumor-only NGS is similar for clinically signed-out cases (3.3%), although only 2.8% were pathologist confirmed. Coupled with the high sensitivity and specificity we observed in comparison with manual review, this suggests tumor-only NGS detection of MMR-D supported by simple computational predictions may be a useful and cost-effective screening tool for research and clinical use. In this paradigm, computationally predicted MMR-D tumors should, in many cases, still undergo validation with a clinical test (eg, PCR or IHC) before being used for clinical care. PCR testing has been considered the gold standard for the

TABLE 5. Mean TMB Across 4,404 OncoPanel Tumors, Subcategorized by Algorithm-Predicted MMR Status and Pathologist-Assessed MMR Status

Pathologist Assessment	Mean TMB		
	Algorithm MMR-D	Algorithm MMR-P	All
Pathologist MMR-D	54.96 (116)	72.24 (6)	55.81 (122)
Pathologist MMR-P	17.34 (5)	7.58 (4,213)	7.59 (4,218)
Pathologist indeterminate	17.61 (26)	22.71 (38)	20.64 (64)
All	47.36 (147)	8.40 (4,257)	8.99 (4,404)

NOTE. Data are presented as mutations/Mb (No. of samples).

Abbreviations: MMR-D, mismatch repair deficient; MMR-P, mismatch repair proficient; TMB, tumor mutational burden.

determination of microsatellite stability, although the five-marker Bethesda panel[44] and updated MSI Analysis System[45] were originally optimized and validated in colorectal cancer. Across tumor types, it remains unclear which methodology to determine MMR status provides greater accuracy, and NGS computational algorithms may improve identification of these tumors compared with TMB, PCR, or IHC testing. It will also be important for future studies to measure the impact of MMR-D assessment on treatment decisions and patient outcomes.

Given the low overall frequency of MMR-D across most noncolorectal, nonendometrial tumors, we believe our algorithm provides a cost-effective initial screening tool to identify patients for PCR- or IHC-based testing. From a clinical implementation standpoint, rather than testing all patients with cancer with PCR or IHC to identify the < 3% of patients who may be candidates for immune checkpoint inhibitors on the basis of MMR-D, implementing the algorithm in the context of an existing, multipurpose NGS tumor-only panel, can provide an efficient initial screen and allow clinicians to select only those tumors most likely to be MMR-D for further testing. Biomarker studies in patients with MMR-D tumors treated with immune checkpoint inhibition will provide further insight.

In the near future, combined testing of many biomarkers across a large panel of genes as described here may be more efficient in cost, time, and sample material than sequential or more selective biomarker testing, and it also offers the ability to quickly incorporate new biomarker discoveries into clinical practice and bring effective treatments to patients who would otherwise not benefit from these discoveries. Efforts to evaluate the clinical utility of NGS-inferred MMR-D, determined by the impact of physician notification of MMR/MSI status on treatment decision making, are ongoing and may provide further support for the implementation of targeted NGS panels in routine clinical practice.

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APPENDIX

TABLE A1. Comparison of OncoPanel MMR Algorithm With Historical Clinical MSI/MMR Testing

Laboratory Assessment	Algorithm MMR-D	Algorithm MMR-P	Total
Laboratory MSI-H	41	4	45
Laboratory MSS	5	275	280
Total	46	279	325

NOTE. List of tumors tested (total number MSI-H by orthogonal testing/total number tested): appendiceal cancer (1/3), biliary cancer (1/4), bladder cancer (0/2), cancer of unknown primary (2/7), colorectal cancer (17/227), endometrial cancer (19/64), esophagogastric carcinoma (4/8), pancreatic cancer (0/4), prostate cancer (1/4), small bowel cancer (0/1), soft tissue sarcoma (0/1).

Abbreviations: MMR-D, mismatch repair deficient; MMR-P, mismatch repair proficient; MSI-H, microsatellite instability high; MSS, microsatellite stable.