original report

Microsatellite Instability Is Associated With the Presence of Lynch Syndrome Pan-Cancer

Alicia Latham, MD[1]; Preethi Srinivasan, MS[1]; Yelena Kemel, ScM[1]; Jinru Shia, MD[1]; Chaitanya Bandlamudi, PhD[1]; Diana Mandelker, MD, PhD[1]; Sumit Middha, PhD[1]; Jaclyn Hechtman, MD[1]; Ahmet Zehir, MD, PhD[1]; Marianne Dubard-Gault, MD, MS[1]; Christina Tran[1]; Carolyn Stewart[1]; Margaret Sheehan, MS[1]; Alexander Penson, PhD[1]; Deborah DeLair, MD[1]; Rona Yaeger, MD[1,2]; Joseph Vijai, PhD[1]; Semanti Mukherjee, PhD[1]; Jesse Galle[1]; Mark A. Dickson, MD[1,2]; Yelena Janjigian, MD[1,2]; Eileen M. O'Reilly, MD[1,2]; Neil Segal, MD, PhD[1,2]; Leonard B. Saltz, MD[1,2]; Diane Reidy-Lagunes, MD, MS[1,2]; Anna M. Varghese, MD[1,2]; Dean Bajorin, MD[1,2]; Maria I. Carlo, MD[1,2]; Karen Cadoo, MD[1,2]; Michael F. Walsh, MD[1,2]; Martin Weiser, MD[1,2]; Julio Garcia Aguilar, MD, PhD[1,2]; David S. Klimstra, MD[1]; Luis A. Diaz Jr, MD[1,2]; Jose Baselga, MD, PhD[1,2]; Liying Zhang, MD, PhD[1]; Marc Ladanyi, MD[1]; David M. Hyman, MD[1,2]; David B. Solit, MD[1,2]; Mark E. Robson, MD[1,2]; Barry S. Taylor, PhD[1]; Kenneth Offit, MD, MPH[1]; Michael F. Berger, PhD[1,2]; and Zsofia K. Stadler, MD[1,2]

abstract

PURPOSE Microsatellite instability (MSI) and/or mismatch repair deficiency (MMR-D) testing has traditionally been performed in patients with colorectal (CRC) and endometrial cancer (EC) to screen for Lynch syndrome (LS)–associated cancer predisposition. The recent success of immunotherapy in high-frequency MSI (MSI-H) and/or MMR-D tumors now supports testing for MSI in all advanced solid tumors. The extent to which LS accounts for MSI-H across heterogeneous tumor types is unknown. Here, we establish the prevalence of LS across solid tumors according to MSI status.

METHODS MSI status was determined using targeted next-generation sequencing, with tumors classified as MSI-H, MSI-indeterminate, or microsatellite-stable. Matched germline DNA was analyzed for mutations in LS-associated mismatch repair genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM)*. In patients with LS with MSI-H/I tumors, immunohistochemical staining for MMR-D was assessed.

RESULTS Among 15,045 unique patients (more than 50 cancer types), LS was identified in 16.3% (53 of 326), 1.9% (13 of 699), and 0.3% (37 of 14,020) of patients with MSI-H, MSI-indeterminate, and microsatellite-stable tumors, respectively ($P < .001$). Among patients with LS with MSI-H/I tumors, 50% (33 of 66) had tumors other than CRC/EC, including urothelial, prostate, pancreas, adrenocortical, small bowel, sarcoma, mesothelioma, melanoma, gastric, and germ cell tumors. In these patients with non-CRC/EC tumors, 45% (15 of 33) did not meet LS genetic testing criteria on the basis of personal/family history. Immunohistochemical staining of LS-positive MSI-H/I tumors demonstrated MMR-D in 98.2% (56 of 57) of available cases.

CONCLUSION MSI-H/MMR-D is predictive of LS across a much broader tumor spectrum than currently appreciated. Given implications for cancer surveillance and prevention measures in affected families, these data support germline genetic assessment for LS for patients with an MSI-H/MMR-D tumor, regardless of cancer type or family cancer history.

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ASSOCIATED CONTENT

See accompanying editorial on page 263

Appendix

Author affiliations and support information (if applicable) appear at the end of this article.

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INTRODUCTION

Identifying individuals appropriate for germline genetic testing for cancer susceptibility has traditionally relied on clinical criteria, such as age at cancer diagnosis and personal or family cancer history. In Lynch syndrome (LS), a cancer predisposition syndrome characterized by the presence of germline mutations in the DNA mismatch repair (MMR) genes, this has been replaced by emphasis on universal screening of all colorectal cancer (CRC) and endometrial cancer (EC) by initially screening tumors for microsatellite instability–high (MSI-H)/MMR- deficiency (MMR-D), the hallmarks of these LS-associated tumors.[1-4] At the same time, the identification of MSI-H/MMR-D as a biomarker for response to immune checkpoint blockade represents a breakthrough in the treatment of individuals with advanced solid tumors.[5,6] The US Food and Drug Administration (FDA) approval of pembrolizumab for all advanced MSI-H/MMR-D solid tumors is the first regulatory drug authorization based solely on a biomarker, agnostic of cancer type.[7] Given this, testing for MSI-H/MMR-D is now increasingly being incorporated into routine oncological care of patients with advanced solid tumors across a broad spectrum of cancers. Notably, such tumor testing does not necessarily result in downstream germline testing, and, in fact, the underlying pan-cancer prevalence of LS in these cases remains unknown.

Traditionally, identification of MSI-H/MMR-D has relied on polymerase chain reaction (PCR)–based MSI analysis or immunohistochemical (IHC) analysis for MMR protein expression.[8] Inferring MSI via next-generation sequencing (MSI-NGS) of tumors is an alternative method for MSI determination, with two FDA-authorized NGS platforms now incorporating MSI-calling algorithms.[9-12]

Although the MSI-H phenotype has been observed in a broad spectrum of tumor types and is best characterized in CRC/EC, MSI prevalence varies significantly across cancers, with recent studies observing cancer-specific MSI patterns.[12-15] Although studies have assessed germline MMR gene mutations in a limited set of MSI-H tumors[13] or in tumors agnostic of MSI status,[16] a systematic evaluation of germline MMR mutation prevalence across a heterogeneous group of solid tumors according to MSI status has not been performed. We sought to determine the prevalence of LS across multiple cancer types as a function of tumor MSI status.

METHODS

Study Population

The study comprised 15,045 patients with cancer, encompassing more than 50 cancer types, at Memorial Sloan Kettering (MSK) Cancer Center who provided written consent for an institutional review board–approved prospective protocol (ClinicalTrials.gov identifier, NCT01775072) for tumor and matched normal DNA sequencing via MSK-IMPACT (MSK–Integrated Mutation Profiling of Actionable Cancer Targets), a clinical NGS platform FDA authorized to identify genetic variants in up to 468 cancer-related genes as well as MSI status.[10,12,17,18] Patients were enrolled between January 1, 2014 and June 30, 2017.

MSI Analysis

For MSK-IMPACT–sequenced tumors, MSI status was assessed via MSIsensor, a computational algorithm that analyzes sequencing reads at designated microsatellite regions in tumor-normal pairs, reporting the percentage of unstable loci as a cumulative score.[11,12] MSIsensor scores ≥ 10 defined MSI-H status, scores ≥ 3 to < 10 an indeterminate (MSI-I) status, and scores < 3 microsatellite stable (MSS) status.[11,12] For patients with MSK-IMPACT on multiple tumors, the tumor with the highest MSIsensor score was used for analysis.

Germline Analysis

DNA from blood samples was used for germline analysis of five MMR genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM*). For individuals with non-MSS tumors (MSIsensor scores ≥ 3), germline analysis was performed in an identified manner via institutional review board approval. Baseline demographics, tumor stage and pathology, and cancer family history were obtained via electronic medical records. Family and personal cancer history analysis allowed for evaluation of the extent to which patients with LS met criteria for LS genetic testing, using the National Comprehensive Cancer Network guidelines[4] and revised Bethesda criteria.[19] For patients positive for LS, clinical confirmation of research results and disclosure was facilitated by the MSK Genomic Advisory Panel and the Clinical Genetics Service. For MSS tumors, germline analysis was performed in an anonymized fashion.

Germline variant calling was performed as previously described.[18] Germline copy number aberrations were identified by comparing sequence coverage of targeted regions to a standard diploid normal.[20] Regions with fold change of coverage < -1.5 and $P < .05$ were used to detect germline copy number losses as previously described.[20] Only samples with two or more contiguous exons deleted were included, unless single-exon deletions were confirmed by orthogonal method. Only rearrangements/deletions involving the 3′ region of *EPCAM*, implicated as causative of LS,[21,22] were included. *PMS2* deletions including exons 13 and/or 14 were excluded, given frequent alignment artifacts as a result of pseudogene presence. Identified variants were independently assessed and manually curated, applying current standards for variant classification by the American College of Medical Genetics and Genomics and compared with variant databases and publications.[23-25] Patients with germline mutations classified as likely pathogenic/pathogenic were considered as having LS.

IHC Staining and Tumor Signature Analysis

In patients with LS with MSI-H/I tumors, IHC staining for MMR protein expression was performed using standard procedures.[26] Previous studies demonstrated that genomic mutational signatures can be derived from targeted-capture and sequencing data.[17] Using the patterns and nucleotide context of observed somatic synonymous and nonsynonymous substitutions in tumors from patients with LS, mutations in each tumor were assigned to a set of 30 previously described mutational signatures using a signature decomposition approach previously described.[27] To ensure robust mutational signature decomposition, we only considered samples with 20 or more somatic mutations. Because of the wide age range in our cohort and the similarity of the aging signature (signature 1) to a known MMR signature (signature 6), the dominant signature for each tumor was calculated after excluding signature 1. We considered any tumor where the dominant signature matched a known MMR-D signature (6, 15, 20, 26) as having a genomic mutational signature consistent with MMR-D.[27]

Statistical Analysis

Continuous and categorical variables were compared using a two-tailed *t* test and χ^2 statistics, respectively. *P* values $< .05$ were considered statistically significant.

RESULTS

Of 15,045 tumors assessed by MSK-IMPACT, 93.2% (n = 14,020) were MSS, 4.6% (n = 699) MSI-I, and 2.2%

(n = 326) MSI-H (Fig 1). More than 50 different cancer types were represented, with breast (n = 2,371) and lung (n = 1,952) cancer representing 28.7% of tumors. The mean age at diagnosis, sex, race, and cancer stage in the MSI-H and MSI-I groups are listed in Table 1. Although CRC and EC comprised only 9% of all tumors (n = 1,351), these canonical LS-associated tumors represented 62% (n = 201) of the MSI-H cohort ($P < .001$). The highest proportion of MSI-H tumors was noted in small bowel cancer, followed by EC and CRC (Fig 1).

In the MSI-H, MSI-I, and MSS groups, the overall prevalence of LS was 16.3% (53 of 326), 1.9% (13 of 699), and 0.3% (37 of 14,020), respectively ($P < .001$; Fig 2). Of patients with LS and an MSI-H/I tumor, 50% (33 of 66) had a primary tumor other than CRC/EC (Table 2; Fig 2). Germline MMR mutations were identified in individuals with MSI-H/I cancers not previously, or rarely, implicated in LS, including mesothelioma, melanoma, soft tissue sarcoma, adrenocortical, prostate, pancreatic, small bowel, glioma, and ovarian germ cell tumor.[28-30] A history of prior malignancy was observed in 22.7% (15 of 66) of patients with LS with MSI-H/I tumors. In the MSI-H/I groups, compared with LS-positive CRC/ECs, patients with LS with other tumors had lower MSIsensor scores and a higher prevalence of MSI-I tumors (non-CRC/EC MSI-I, 30.3% [10 of 33] *v* CRC/EC MSI-I, 9.1% [three of 33]; $P = .03$; Appendix Tables A1 and A2, online only).

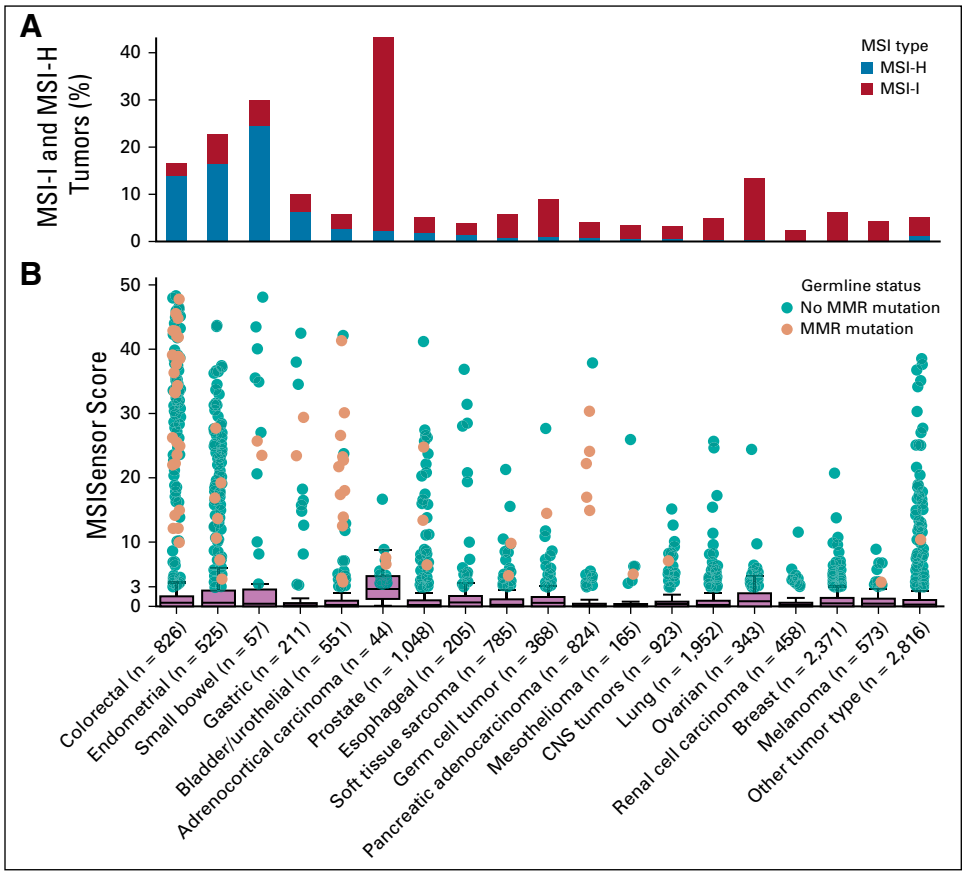

FIG 1. Distribution of microsatellite instability (MSI) status across cancer types. Tumor types are indicated on the *x*-axis. (A) Bar graphs demonstrate the percentage of tumors that are high-frequency MSI (MSI-H; blue) and indeterminate MSI (MSI-I; red) by cancer type. (B) MSIsensor score is indicated on the *y*-axis. Box and whisker plots illustrate that the majority of tumors were microsatellite stable (MSS; 93.2%), with MSIsensor score < 3, and teal dots indicate individual tumor MSIsensor scores for MSI-H (score ≥ 10) and MSI-I (score ≥ 3 to < 10) tumors. Dark orange dots indicate patients in whom germline mismatch repair (MMR) pathogenic variants were identified. The one germline MMR mutation (dark orange dot) seen in Other Tumor Type was in an adenocarcinoma of unknown primary. Bladder/urothelial category includes renal pelvis, ureter, bladder, and urethral cancers. CNS tumors category includes glioma, astrocytoma, embryonal, and miscellaneous brain tumors. Other tumor type category includes less common tumors, including ampullary carcinoma, anal carcinoma, appendiceal carcinoma, osteosarcoma, peripheral nerve sheath tumor, choriocarcinoma, cervical cancer, neuroendocrine tumor, neuroblastoma, thymic tumor, pheochromocytoma, vaginal carcinoma, Wilms tumor, cancer of unknown primary, head and neck cancer, hepatocellular carcinoma, cholangiocarcinoma, chondrosarcoma, Ewing sarcoma, non-Hodgkin lymphoma, leukemia, and retinoblastoma.

TABLE 1. Baseline Characteristics of Patients with MSI-H or MSI-I Tumors

Characteristic	MSI-H (n = 326)	MSI-I (n = 699)
Mean age at diagnosis, years	58.5	53.7
Median age at diagnosis, years	60	55
Stage at diagnosis*		
Stage 0 to III	62.6 (204)	12.4 (87)
Stage IV/recurrent	36.7 (120)	87.5 (612)
Unknown	0.6 (2)	0 (0)
Sex		
Male	44.2 (144)	37.9 (265)
Female	55.8 (182)	62 (434)
Race/ethnicity		
Non-Hispanic white	78.8 (257)	75.8 (530)
Non-Hispanic black	4.6 (15)	11 (77)
Hispanic	6.1 (20)	5.6 (39)
Asian	8 (26)	4.3 (30)
Native American	0.3 (1)	0.3 (2)
Other	0.3 (1)	1.4 (10)
Preferred not to answer	1.8 (6)	1.6 (11)

NOTE. Data presented as % (No.) unless otherwise noted.

Abbreviations: MSI-H, high-frequency microsatellite instability; MSI-I, indeterminate microsatellite instability.

*There was limited clinical and no staging information available for two patients in the MSI-H group.

The highest proportions of LS among patients with MSI-H/I tumors were in urothelial (37.5%; 12 of 32), CRC (19%; 26 of 137), and gastric cancers (15.4%; two of 13; Table 2). Although only 4.1% (34 of 824) of patients with pancreatic cancer had an MSI-H/I tumor, 14.7% (five of 34) of these patients were LS-positive. When considering MSI-H pancreatic tumors alone, 83.3% (five of six) of patients were found to have LS. Of the 43.2% (19 of 44) of MSI-H/I adrenocortical tumors, 10.5% (two of 19) were LS positive. We did not identify LS among the 6.3% (150 of 2,371) of MSI-H/I breast tumors, nor did we identify LS in any ovarian tumors, including the 13.4% (43 of 343) that were found to be MSI-H/I (Table 2).

In non-LS MSI-H tumors, we assessed prevalence of somatic events at the MMR genes to determine the etiology of the MSI-H status. Double somatic events, composed of either two somatic mutations, one somatic mutation plus loss of heterozygosity, or a somatic copy number loss, were identified in 20.8% (57 of 274) of LS-negative MSI-H tumors. When selecting for non-CRC/EC, this increased to 23.3% (24 of 103). Similarly, in LS-positive MSI-H/I cases, we detected biallelic inactivation, either through loss of heterozygosity or a second somatic mutation, in 66.7% (44 of 66) of tumors.

To confirm loss of MMR protein expression in patients with germline LS mutations, IHC was performed in LS-positive MSI-H/I tumors with available tissue. IHC was concordant with the LS mutational results in 98.2% (56 of 57) of cases (Fig 3). Tumors analyzed included CRC, EC, urothelial, prostate, glioma, adrenocortical, soft tissue sarcoma, ovarian germ cell, gastric, pancreatic, and small bowel (Fig 3). The lone discordant tumor with intact MMR protein expression by IHC was a patient with CRC with an MSI-sensor score of 42 who was found to harbor the Ashkenazi Jewish germline founder mutation in *MSH2* (c.1906G>C; p.Ala636Pro), suggesting a false-negative IHC screen (Appendix Table A1). Among non-CRC/EC tumors with tissue available, IHC demonstrated concordant MMR-D in 100% (26 of 26) of cases. Among patients with LS, we identified one pediatric patient with cancer with an MSI-H ovarian germ cell tumor, harboring an *MSH2* germline mutation, with tumor demonstrating concordant absence of MSH2/MSH6 protein expression on IHC. Given a diagnosis of cancer during childhood, we considered constitutional MMR-D, with biallelic germline mutations in *MSH2*.[31] However, because the normal tissue retained MSH2/MSH6 protein expression, the diagnosis was consistent with LS rather than constitutional MMRD.

Applying the recommendation of universal tumor testing for CRC/EC tumors, 100% of patients with LS with MSI-H/I CRC/ECs met criteria for genetic testing. In comparison, 54.5% (18 of 33) of patients with LS with MSI-H/I non-CRC/EC tumors met testing criteria on the basis of personal and/or family cancer history alone.[19]

Although the majority of patients with LS exhibited MSI-H/I tumors, 36% (37 of 103) had MSS tumors. These were predominantly non-CRC/ECs (Fig 4). We considered that these patients harbored mutations in the lower-penetrance MMR genes,[32,33] they had low tumor purity that confounds the ability to detect MSI, or tumors were driven by mechanisms other than the MMR mutations. Indeed, although 71.2% (47 of 66) of germline mutations in the LS-positive MSI-H/I tumors were in *MLH1*, *MSH2*, or *EPCAM*, 78.4% (29 of 37) of germline mutations in the LS-positive MSS tumors were in the lower-penetrance *PMS2* or *MSH6* genes ($P < .001$; Fig 2B).

In addition, we assessed tumor mutational signatures among patients with LS according to MSI status. Although 87.9% (58 of 66) of MSI-H/I tumors from patients with LS exhibited MMR-D signatures, the majority (89.2%; 33 of 37) of MSS tumors from patients with LS did not have MMR-D signatures ($P < .001$). Of four LS-positive MSS tumors that manifested an MMR-D–dominant signature, three were CRC or EC, with one tumor having lower tumor purity. Three patients had *MSH6* germline mutations, associated with lower MSI levels in prior studies using MSI-PCR (Fig 4).[34] We identified LS in 0.3% (seven of 2,371) of patients with breast cancer. Among these, all corresponding tumors were MSS and lacked an MMR-D tumor signature (Fig 4). These results suggest that germline MMR mutations among the MSI-H/I groups were likely causative of the patients' cancers, compared with those patients with MSS tumor

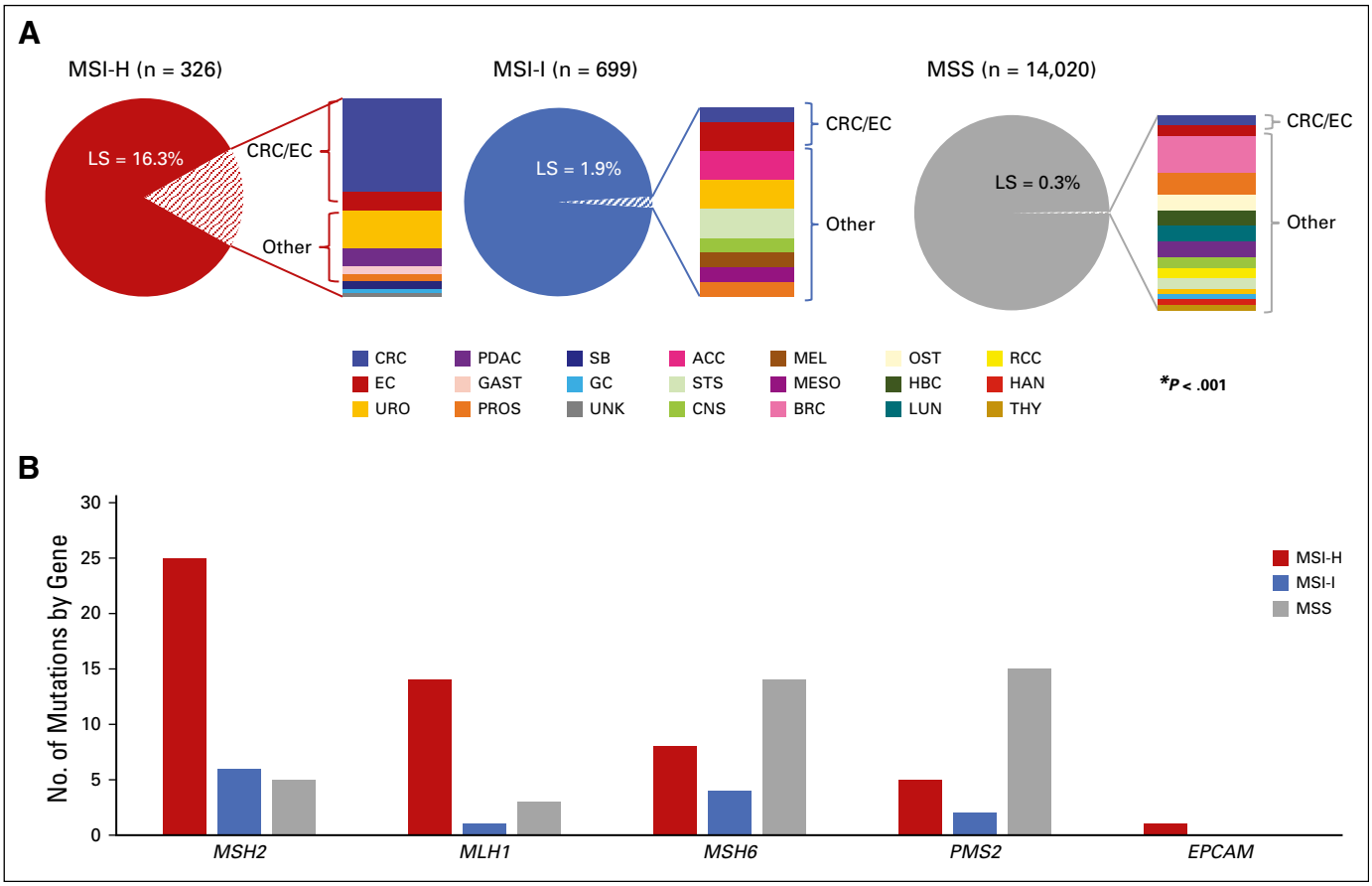

FIG 2. Distribution and prevalence of germline mismatch repair (MMR) gene mutations by microsatellite instability (MSI) status and by MMR gene type. (A) Bar graph and pie charts represent overall prevalence of pathogenic or likely pathogenic germline mutations in the DNA MMR genes, diagnostic of Lynch syndrome (LS), among high-frequency MSI (MSI-H; red), indeterminate MSI (MSI-I; blue), and microsatellite stable (MSS; gray) groups (16.3%, 1.9%, and 0.3%, respectively; $P < .001$). Tumor types are indicated in the bar within the pie graphs by individual colors, highlighting the distinct tumor types composing each group. (B) MMR genes are indicated on the *x*-axis and number of individual patients with pathogenic or likely pathogenic variants are indicated on the *y*-axis. Red, blue, and gray bars represent mutations found in MSI-H, MSI-I, and MSS cohorts across each MMR gene, respectively. ACC, adrenocortical carcinoma; BRC, breast; CRC, colorectal; EC, endometrial; GAST, gastric; GC, germ cell tumor; HAN, head and neck cancer; HBC, hepatobiliary; LUN, lung; MEL, melanoma; MESO, mesothelioma; OST, osteosarcoma; PDAC, pancreatic ductal adenocarcinoma; PROS, prostate; RCC, renal cell carcinoma; SB, small bowel; STS, soft tissue sarcoma; THY, thyroid cancer; UNK, unknown primary; URO, urothelial.

types in which the underlying germline MMR mutation was more likely an incidental finding.

DISCUSSION

Our comprehensive assessment of MSI, spanning more than 50 cancer types and 15,045 tumors, demonstrates that the presence of MSI-H is predictive of LS, with 16% of patients with MSI-H tumors harboring germline mutations in the MMR genes. In our study, 50% of patients with LS with MSI-H/I tumors had cancers rarely or not previously associated with LS, with 45% not meeting clinical criteria for LS genetic testing on the basis of personal or family cancer history. The FDA's recent tissue site–agnostic approval of pembrolizumab for advanced MSI-H/MMR-D solid tumors[7] and the increasing availability of tumor NGS platforms that simultaneously report MSI status[9] are expected to increase routine MSI testing across a broad spectrum of cancers. Our results support that all MSI-H/MMR-D tumors should undergo germline assessment for LS, with cascade testing of at-risk relatives, given clinical implications for increased cancer surveillance and potential risk-reducing surgeries that may be warranted. Although we used an NGS-based platform for MSI detection, concordance between MSI-H/I and IHC staining for MMR-D was 98.2% in our patients with LS, suggesting that IHC for MMR-D may also be a suitable screen in noncanonical tumors.

Among our LS-positive MSI-H/I cohort, we observed that noncanonical tumor types had a more modest MSI phenotype and were therefore more likely to be categorized as MSI-I than patients with LS with CRC/EC. Nonetheless, despite the MSI-I status, IHC staining demonstrated MMR-D in 10 of 10 of tested cases, including rare tumors such as soft tissue sarcoma, adrenocortical carcinoma, and glioma.

TABLE 2. Prevalence of Lynch Syndrome by Tumor Type and MSI Status

Tumor Type	Total Count	MSI-H/I	% MSI-H/I Lynch	95% CI
Colorectal	826	137	19 (26/137)	12.8 to 26.6
Endometrial	525	119	5.9 (7/119)	2.4 to 11.7
Small bowel	57	17	11.8 (2/17)	1.5 to 36.4
Gastric	211	13	15.4 (2/13)	1.9 to 45.5
Esophageal	205	16	0 (0/16)	0.0 to 20.6
Bladder/urothelial	551	32	37.5 (12/32)	21.1 to 56.3
Adrenocortical	44	19	10.5 (2/19)	1.3 to 33.1
Prostate	1,048	54	5.6 (3/54)	1.2 to 15.4
Germ cell	368	33	3 (1/33)	0.1 to 15.8
Soft tissue sarcoma	785	45	4.4 (2/45)	0.5 to 15.1
Pancreatic	824	34	14.7 (5/34)	5.0 to 31.1
Mesothelioma	165	6	1.7 (1/6)	0.4 to 64.1
CNS tumors	923	30	3.3 (1/30)	0.1 to 17.2
Ovarian	343	46	0 (0/46)	0.0 to 7.7
Lung	1,952	94	0 (0/94)	0.0 to 3.8
Renal	458	11	0 (0/11)	0.0 to 28.5
Breast	2,371	150	0 (0/150)	0.0 to 2.4
Melanoma	573	25	4 (1/25)	0.1 to 20.4
Other tumor type*	2,816	144	0 (1/144)*	0.0 to 3.8

NOTE. Bladder/urothelial tumors include renal pelvis, ureter, bladder, urethral cancer. Other tumor type includes less common tumors, the majority of which were ampullary carcinoma, anal carcinoma, appendiceal carcinoma, osteosarcoma, peripheral nerve sheath tumor, choriocarcinoma, cervical cancer, neuroendocrine tumor, neuroblastoma, thymic tumor, pheochromocytoma, vaginal carcinoma, Wilms tumor, cancer of unknown primary, head and neck cancer, hepatocellular carcinoma, cholangiocarcinoma, chondrosarcoma, Ewing sarcoma, non-Hodgkin lymphoma, leukemia, and retinoblastoma.

Abbreviation: MSI-H/I, high-frequency or indeterminate microsatellite instability.

*Lynch syndrome was identified in an adenocarcinoma of unknown primary.

In these LS-positive MSI-I tumors, the lower MSIsensor scores were not a result of low tumor purity. This is consistent with prior assessments of LS-associated tumors, suggesting that the extent of MSI, as detected by PCR, varies according to tumor type, with MSI-H more consistently found in ureter, gastric, and CRC cancers and lower MSI levels in EC and brain tumors.[30] Our data suggest that the MSI level needed to predict presence of LS may be different for different tumor types, with additional studies needed to address the optimal method of assessing MSI/MMR-D in noncanonical tumors. Because prior studies implicated double somatic events in the MMR genes leading to a sporadic form of MSI/MMR-D in colorectal and endometrial cancers,[35,36] we also assessed this phenomenon, finding that 20.8% (57 of 274) of LS-negative MSI-H tumors could be explained by double somatic events in the tumor.

By assessing LS prevalence in all patients regardless of MSI status, we determined the distribution of MMR gene mutations in MSS versus MSI-H/I tumors and observed an enrichment of *MSH6* and *PMS2* mutations in MSS tumors. Prior evidence has demonstrated that *MSH6* and *PMS2* mutations are more prevalent in the general population and are associated with lower cancer penetrance than other MMR genes.[32,33] Although the majority of LS-positive MSI-H/I tumors had concordant MMR-D on IHC and 87.9% had MMR-D–dominant mutational tumor signatures, among LS-positive MSS tumors, 89.2% did not exhibit an MMR-D mutational signature. This suggests that the germline MMR mutations in patients with MSS tumors were likely to be incidental findings rather than causative of the MSS tumor. In fact, our observed 0.3% LS prevalence among the 14,020 MSS tumors is identical to the estimated one in 300 prevalence of LS in the general population.[37]

We assessed the prevalence of LS in breast cancer, an area of current controversy, where some studies suggest, and others refute, an increased risk of breast cancer in *MSH6* and *PMS2* carriers.[38-40] In our cohort of patients with breast cancer, we did not identify a higher incidence of LS over the general population, and our tumor analysis did not support that breast tumors diagnosed in LS-positive patients were a result of MMR-D. Our analysis, along with other recent publications,[16,25] demonstrates the strength of integrating somatic and germline data to help elucidate tumor etiology with relevance for cancer treatment and accurate cancer penetrance estimation. We did not identify LS in any of the

A

MSI Status	IHC Concordant	IHC Discordant	IHC Not Available
MSI-H (n = 53)	46	1 (CRC)	6
MSI-I (n = 13)	10	0	3

B

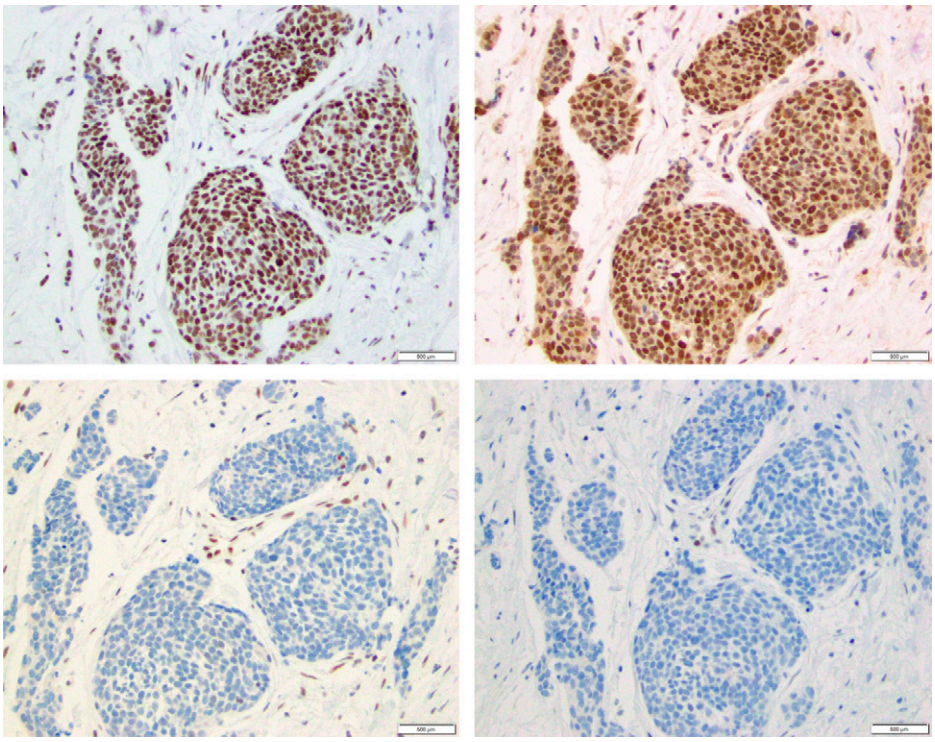

FIG 3. Concordance of immunohistochemical staining (IHC) for the mismatch repair proteins with high-frequency microsatellite instability (MSI-H) or indeterminate MSI (MSI-I) status in patients with Lynch syndrome (LS). (A) Of 53 MSI-H tumors in patients with LS, IHC was performed on 89% (47 of 53) of tumors, with 98% concordance. One MSI-H colorectal tumor (MSIsensor score, 42) had intact expression of mismatch repair proteins. Among MSI-I tumors of patients with LS, IHC was performed on 77% (10 of 13), with 100% concordance. (B) IHC on a urothelial tumor of a patient with LS with an *MSH2* germline mutation. Top panels demonstrate intact protein expression of *MLH1* (left) and *PMS2* (right), and bottom panels demonstrate absence of protein expression of *MSH2* (left) and *MSH6* (right). CRC, colorectal cancer.

343 ovarian tumors, including the 13.4% (43 of 343) that were found to be MSI-H/I. The LS prevalence in ovarian cancer is estimated to be 0.9% to 2.7%.[41] The lack of LS in our cohort may be a reflection of the relatively small sample size for this particular tumor. Moreover, although in LS nonserous adenocarcinomas of various histologic types are more common, the majority (67%) of our ovarian tumors were high-grade serous adenocarcinomas.[42,43]

There are several limitations to our study. First, our patient cohort reflects that of a large referral center primarily composed of non-Hispanic white patients. Second, in patients with LS with MSS tumors, because of patient anonymization, clinical annotation is limited, and we were also unable to perform IHC for MMR protein expression, raising the possibility of false-negative MSI screening tests in some cases. To address this, we used tumor mutational signatures, which confirmed that only four patients with LS with MSS tumors exhibited an MMR-D tumor signature, with three of these patients harboring *MSH6* germline mutations, known to be associated with a more modest MSI phenotype[34] and a possible false-negative MSI screen. Third, although MSI-I tumor status clearly predicts for a higher prevalence of LS than MSS status, this group reflects a heterogeneous population, including some patients with LS or somatic MMR mutations resulting in an MMR-D tumor as well as patients with MMR-proficient tumors.[12] This amorphous categorization is similar to the controversial significance of the MSI-low designation via MSI-PCR analysis.[44] As is often done in MSI-low tumors on the basis of MSI-PCR, a reasonable undertaking in the MSI-I category, for both predicting presence of LS as well as potential response to immunotherapy, is to perform a second level of tumor screening via IHC. Although

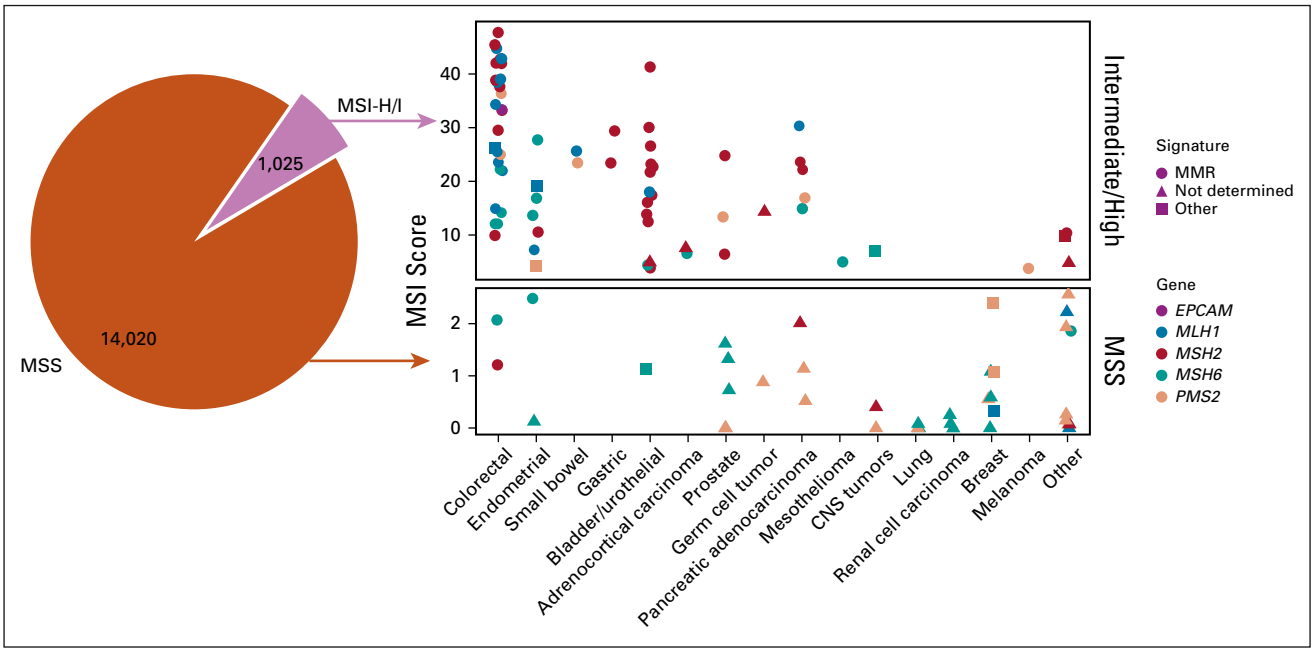

FIG 4. Tumor signatures of patients with Lynch syndrome by microsatellite instability (MSI) and mismatch repair (MMR) gene mutation status. Pie chart indicates the overall amount of all tumor types among microsatellite stable (MSS) group (blue; n = 14,020) and high-frequency microsatellite instability (MSI-H) and indeterminate MSI (MSI-I) group (gray; n = 1,025). Tumor type indicated on the *x*-axis. MSIsensor score indicated on the *y*-axis. Each point corresponds to a patient tumor in which germline analysis revealed an underlying MMR gene pathogenic or likely pathogenic variant. Color of each point indicates the specific MMR gene in which the variant was found, and shape of each point indicates the dominant tumor mutational signature (circle, MMR-deficiency [MMR-D] signature; triangle, could not be determined; square, other non–MMR-D signature). Among patients with Lynch syndrome in the MSI-H/I group (top panel), the majority (87.9%) of tumors had MMR-D dominant tumor mutational signatures, whereas the majority of patients with Lynch syndrome in the MSS group (89.2%; bottom panel) did not have MMR-D tumor signatures.

community centers may not have access to MSI-NGS algorithms, limiting the applicability of this specific method of analysis, alternative screening modalities (MSI-PCR, IHC) are widely available, and commercially available NGS assays incorporating MSI are increasingly being used by community oncologists. Last, we recognize that 36% of patients with LS had MSS tumors. Although this percentage seems high, the absolute number of LS cases incrementally detected, if universal germline screening of all patients with cancer is undertaken, is more modest, as we screened 14,020 MSS tumors to identify 37 LS cases. This 0.3% prevalence is equivalent to the LS prevalence in the general population. As such, to capture all patients with LS, universal germline screening of the population at large would need to be used, if warranted, by future studies assessing cost efficiency or decision tree analysis.

This study establishes the prevalence of LS in a pan-cancer analysis on the basis of MSI status and demonstrates that once an MSI-H/MMR-D tumor phenotype is established, germline genetic assessment for LS is necessary, regardless of tumor type and family history. Our data suggest that the spectrum of LS-associated tumors is more heterogeneous than currently deduced from classic studies. The identification of LS in a patient with an MSI-H tumor, even in the metastatic cancer setting, may have significant clinical implications, as some patients now have long-term and even complete clinical responses to immunotherapy. With these rapid advances in the treatment of patients with MSI-H/MMR-D cancer, there also exists the opportunity for phenotype-agnostic, genomic diagnosis of LS, with important implications for cancer surveillance and prevention strategies for LS families.

AFFILIATIONS

[1]Memorial Sloan Kettering Cancer Center, New York, NY
[2]Weill Cornell Medical College, New York, NY

CORRESPONDING AUTHOR

Zsofia K. Stadler, MD, Memorial Sloan Kettering Cancer Center, 300 E66th St, 10th floor, New York, NY 10065; e-mail: stadlerz@mskcc.org.

EQUAL CONTRIBUTION

A.L. and P.S. contributed to this work equally.

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AUTHOR CONTRIBUTIONS

Conception and design: Preethi Srinivasan, Yelena Kemel, Ahmet Zehir, David S. Klimstra, Jose Baselga, David B. Solit, Mark E. Robson, Kenneth Offit, Michael F. Berger, Zsofia K. Stadler

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Administrative support: Marianne Dubard-Gault, Carolyn Stewart, David M. Hyman, David B. Solit, Zsofia K. Stadler

Provision of study material or patients: Margaret Sheehan, Mark A. Dickson, Yelena Janjigian, Eileen M. O'Reilly, Neil Segal, Anna M. Varghese, Dean Bajorin, Liying Zhang, Marc Ladanyi, David B. Solit, Kenneth Offit

Collection and assembly of data: Alicia Latham, Yelena Kemel, Jinru Shia, Chaitanya Bandlamudi, Ahmet Zehir, Marianne Dubard-Gault, Christina Tran, Carolyn Stewart, Margaret Sheehan, Rona Yaeger, Jesse Galle, Mark A. Dickson, Yelena Janjigian, Eileen M. O'Reilly, Neil Segal, Anna M. Varghese, Dean Bajorin, Marc Ladanyi, David B. Solit, Kenneth Offit, Michael F. Berger, Zsofia K. Stadler

Data analysis and interpretation: Alicia Latham, Preethi Srinivasan, Yelena Kemel, Jinru Shia, Chaitanya Bandlamudi, Diana Mandelker, Sumit Middha, Jaclyn Hechtman, Ahmet Zehir, Alexander Penson, Deborah DeLair, Rona Yaeger, Joseph Vijai, Semanti Mukherjee, Mark A. Dickson, Yelena Janjigian, Eileen M. O'Reilly, Leonard B. Saltz, Diane Reidy-Lagunes, Dean Bajorin, Maria I. Carlo, Karen Cadoo, Michael F. Walsh, Martin Weiser, Julio Garcia Aguilar, Luis A. Diaz Jr, Liying Zhang, Marc Ladanyi, David M. Hyman, David B. Solit, Mark E. Robson, Barry S. Taylor, Kenneth Offit, Michael F. Berger, Zsofia K. Stadler

Manuscript writing: All authors

Final approval of manuscript: All authors

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Jaclyn Hechtman
Honoraria: Medscape
Consulting or Advisory Role: Navigant Consulting

Rona Yaeger
Research Funding: Array BioPharma, GlaxoSmithKline, Novartis
Travel, Accommodations, Expenses: Array BioPharma

Semanti Mukherjee
Employment: Regeneron
Stock and Other Ownership Interests: Regeneron
Research Funding: Regeneron

Mark A. Dickson
Consulting or Advisory Role: Celgene
Research Funding: Eli Lilly

Yelena Janjigian
Consulting or Advisory Role: Eli Lilly, Pfizer, Merck, Bristol-Myers Squibb
Research Funding: Boehringer Ingelheim, Bayer, Eli Lilly, Amgen, Roche, Genentech

Eileen M. O'Reilly
Consulting or Advisory Role: Celgene. Astellas Pharma (I), Celsion (I), Sanofi, Silenseed, Gilead Sciences, Merrimack, Bristol-Myers Squibb (I), Bristol-Myers Squibb, Agios (I), ASLAN Pharmaceuticals (I), Bayer (I), Boston Scientific (I), CASI Pharmaceuticals (I), Delcath Systems (I), Eisai (I), Halozyme (I), Ipsen (I), Merck (I), Onxeo (I), Servier (I), SillaJen (I), Sirtex Medical (I), AstraZeneca (I), Amgen, Antengene (I), Aptus Clinical, CASI Pharmaceuticals (I), CytomX Therapeutics, Debiopharm Group (I), Daiichi Sankyo (I), Exelixis (I), Inovio Pharmaceuticals, PCI Biotech (I), Sanofi, Yakult (I)
Research Funding: Momenta Pharmaceuticals, (Inst), Incyte (Inst), OncoMed (Inst), Celgene (Inst), Bristol-Myers Squibb (Inst), AstraZeneca/MedImmune (Inst), Mabvax (Inst), Roche (Inst)

Neil Segal
Consulting or Advisory Role: Bristol-Myers Squibb, Pfizer, AstraZeneca/MedImmune, Imugene, Roche/Genentech, Amgen, Calithera Biosciences, Pieris Pharmaceuticals, Synlogic, Kyocera, Aduro Biotech, Kyn Therapeutics, Boehringer Ingelheim
Research Funding: MedImmune, Bristol-Myers Squibb, Pfizer, Genentech, Merck, Incyte

Leonard B. Saltz
Consulting or Advisory Role: McNeil PPC (I)
Research Funding: Taiho Pharmaceutical

Diane Reidy-Lagunes
Honoraria: Novartis
Consulting or Advisory Role: Ipsen, Pfizer, Novartis
Research Funding: Novartis

Anna M. Varghese
Research Funding: Eli Lilly (Inst), Verastem Oncology (Inst), BioMed Valley Discoveries (Inst), Bristol-Myers Squibb, Silenseed

Dean Bajorin
Honoraria: Merck Sharp & Dohme, Bristol-Myers Squibb, Novartis, Genentech, Merck, Roche, Eli Lilly, Fidia Farmaceutici, Eisai, UroGen Pharma, Pfizer, EMD Serono
Research Funding: Dendreon (Inst), Novartis (Inst), Amgen (Inst), Genentech (Inst), Merck (Inst), Bristol-Myers Squibb (Inst)
Travel, Accommodations, Expenses: Genentech, Merck, Bristol-Myers Squibb, Eli Lilly, UroGen Pharma

Maria I. Carlo
Consulting or Advisory Role: Pfizer

Karen Cadoo
Research Funding: AstraZeneca (Inst), Syndax Pharmaceuticals (Inst)
Travel, Accommodations, Expenses: AstraZeneca

Julio Garcia Aguilar
Consulting or Advisory Role: Medtronic, Intuitive Surgical, Johnson & Johnson

David S. Klimstra
Leadership: PAIGE
Stock and Other Ownership Interests: PAIGE
Consulting or Advisory Role: Wren Laboratories, Ipsen, Merck

Luis A. Diaz Jr
Leadership: Personal Genome Diagnostics
Stock and Other Ownership Interests: PapGene, Personal Genome Diagnostics, Jounce Therapeutics
Consulting or Advisory Role: Merck, Personal Genome Diagnostics, Genentech, Cell Design Labs
Research Funding: Merck (Inst)
Patents, Royalties, Other Intellectual Property: PapGene, Personal Genome Diagnostics, and other entities have licensed several patent applications from Johns Hopkins, where I am an inventor. These relationships are subject to certain restrictions under Johns Hopkins University policy, and the terms of these arrangements are managed by the university in accordance with its conflict-of-interest policies.
Travel, Accommodations, Expenses: Merck

Jose Baselga
Leadership: Infinity Pharmaceuticals, Varian Medical Systems, Bristol-Myers Squibb, Foghorn Therapeutics
Stock and Other Ownership Interests: PMV Pharma, Juno Therapeutics, GRAIL, Tango Therapeutics, Venthera, Northern Biologics, Apogen Biotechnologies, Aura Biosciences
Consulting or Advisory Role: Novartis, Roche/Genentech
Patents, Royalties, Other Intellectual Property: Combination therapy using PDK1 And PI3K inhibitors; Use of phosphoinositide 3-kinase inhibitors for treatment of vascular malformations; Inhibition of KMT2D for the treatment of cancer
Travel, Accommodations, Expenses: Roche, Daiichi Sankyo

Liying Zhang
Employment: Shanghai Genome Center (I)
Leadership: Shanghai Genome Center (I)
Stock and Other Ownership Interests: Shanghai Genome Center (I)
Honoraria: Future Technology Research
Travel, Accommodations, Expenses: Shanghai Genome Center (I), Roche Diagnostics Asia Pacific

David M. Hyman
Consulting or Advisory Role: Atara Biotherapeutics, Chugai Pharmaceutical, CytomX Therapeutics, Boehringer Ingelheim, AstraZeneca, Pfizer, Bayer, Debiopharm Group, ArQule, Genentech
Research Funding: AstraZeneca, Puma Biotechnology, Loxo

David B. Solit
Honoraria: Loxo Oncology, Pfizer
Consulting or Advisory Role: Pfizer, Loxo Oncology

Mark E. Robson
Honoraria: AstraZeneca, Pfizer
Consulting or Advisory Role: McKesson, AstraZeneca
Research Funding: AstraZeneca (Inst), Myriad (Inst), Invitae (Inst), Pfizer (Inst), AbbVie (Inst), Tesaro (Inst), Medivation (Inst)

Michael F. Berger
Consulting or Advisory Role: Roche
Research Funding: Illumina

Zsofia K. Stadler
Consulting or Advisory Role: Allergan (I), Genentech (I), Regeneron (I), Optos (I), Adverum Biotechnologies (I), Biomarin (I), Alimera Sciences (I), Novartis (I), Spark Therapeutics (I), Fortress Biotech (I), Regenxbio (I)

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APPENDIX

TABLE A1. Baseline Patient and Tumor Characteristics for Patients With Lynch Syndrome in MSI-I/MSI-H Cohort

Patient	Age at Dx (years)	Cancer Stage	Race/Ethnicity	Tumor Type	MSIsensor Score	Gene	RefSeq Transcript	Variant	IHC Concordance	Met NCCN Testing Guidelines	Prior Malignancy
001	64	IIA	Non-Hispanic white	Colorectal cancer	47.7	*MSH2*	NM_000251	c.2169dupC; p.Thr724Hisfs*5	Y	Y	None
002	74	IIA	Non-Hispanic white	Colorectal cancer	45.45	*MSH2*	NM_000251	c.929T>G; p.Leu310Arg	Y	Y	Endometrial
003	43	III	Non-Hispanic white	Colorectal cancer	44.71	*MLH1*	NM_000249	c.866_867dupAC; p.Pro290Thrfs*8	Y	Y	None
004	36	IIB	Asian	Colorectal cancer	42.85	*MLH1*	NM_000249	c.1852_1854delAAG; p.Lys618del	Y	Y	None
005	41	IIA	Asian	Colorectal cancer	42.76	*MSH2*	NM_000251	c.2348delA; p.His783Leufs*29	Y	Y	None
006	46	IIIB	Non-Hispanic white	Colorectal cancer	42	*MSH2*	NM_000251	c.1906G>C; p.Ala636Pro	N	Y	None
007	32	IIA	Non-Hispanic white	Colorectal cancer	41.9	*MSH2*	NM_000251	c.1906G>C; p.Ala636Pro	Y	Y	None
008	43	IV	Non-Hispanic white	Urothelial	41.29	*MSH2*	NM_000251	c.1255C>T; p.Gln419*	Y	Y	None
009	50	III	Non-Hispanic white	Colorectal cancer	39.06	*MLH1*	NM_000249	c.1852_1854delAAG; p.Lys618del	Y	Y	None
010	48	IIA	Non-Hispanic white	Colorectal cancer	38.83	*MSH2*	NM_000251	c.2635C>T; p.Gln879*	Y	Y	None
011	33	IV	Non-Hispanic white	Colorectal cancer	38.44	*MLH1*	NM_000249	Whole gene deletion	Y	Y	None
012	32	IIIB	Non-Hispanic white	Colorectal cancer	37.6	*MSH2*	NM_000251	c.942+3A>T	Y	Y	None
013	62	IIIB	Non-Hispanic white	Colorectal cancer	36.28	*PMS2*	NM_000535	c.1927C>T; p.Gln643*	Y	Y	None
014	30	I	Non-Hispanic white	Colorectal cancer	34.3	*MLH1*	NM_000249	c.1582_1583insGGTT; p.His528Argfs*30	Y	Y	None
015	43	III-B	Non-Hispanic white	Colorectal cancer	33.36	*EPCAM*	NM_002354	Deletion exons 8-9	Y	Y	None
016	33	III	Non-Hispanic white	Colorectal cancer	33.2	*MSH2*	NM_000251	c.1302delA; p.Val435Phefs*3	Y	Y	None
017	45	III	Non-Hispanic white	Pancreatic cancer	30.33	*MLH1*	NM_000249	c.1731G>A; p.Ser577Ser	Not available	Y	None
018	69	II	Non-Hispanic white	Urothelial	30.05	*MSH2*	NM_000251	c.1046C>G; p.Pro349Arg	Y	Y	Colon, prostate, squamous cell carcinoma of skin
019	63	IV	Non-Hispanic white	Gastric carcinoma	29.37	*MSH2*	NM_000251	Deletion exons 1-3	Not available	N	Pancreas
020	69	IB	Non-Hispanic white	Endometrial cancer	27.69	*MSH6*	NM_000179	c.2619delG; p.Ile874Serfs*32	Y	Y	Breast
021	60	IV	Non-Hispanic white	Urothelial	26.55	*MSH2*	NM_000251	c.1906G>C; p.Ala636Pro	Y	Y	None
022	40	IV	Non-Hispanic white	Colorectal cancer	26.21	*MLH1*	NM_000249	c.793C>T;p.Arg265Cys	Y	Y	None
023	35	IV	Non-Hispanic white	Small bowel cancer	25.65	*MLH1*	NM_000249	c.1731G>A; p.Ser577Ser	Y	Y	None
024	20	IV	Non-Hispanic white	Colorectal cancer	25.44	*MLH1*	NM_000249	c.793C>A; p.Arg265Ser	Y	Y	None
025	62	IIA	Non-Hispanic white	Colorectal cancer	24.93	*PMS2*	NM_000535	Deletion exons 10-11	Y	Y	None
026	48	IV	Non-Hispanic white	Prostate cancer	24.75	*MSH2*	NM_000251	c.1228_1229delGG; p.Gly410Tyrfs*6	Not available	Y	Colon
027	75	IV	Non-Hispanic white	Pancreatic cancer	24.06	*MSH2*	NM_000251	c.1906G>C; p.Ala636Pro	Y	Y	Gastric, prostate, urothelial
028	61	IIIB	Non-Hispanic black	Colorectal cancer	23.54	*MLH1*	NM_000249	c.199G>A; p.Gly67Arg	Y	Y	Prostate
029	44	I	Non-Hispanic white	Small bowel cancer	23.44	*PMS2*	NM_000535	c.1605_1606delTC; p.Gln536Glyfs*5	Y	Y	None
030	54	IV	Non-Hispanic white	Gastric carcinoma	23.4	*MSH2*	NM_000251	c.1968C>G; p.Tyr656*	Y	N	None
031	43	II	Non-Hispanic white	Urothelial	23.26	*MSH2*	NM_000251	Deletion exons 1-8	Y	Y	None
032	31	IV	Non-Hispanic white	Urothelial	22.7	*MSH2*	NM_000251	c.1216C>T; p.Arg406*	Y	Y	None
033	64	IIIB	Non-Hispanic white	Colorectal cancer	22.23	*MSH6*	NM_000179	c.3743_3744insT; p.Tyr1249Leufs*26	Y	Y	None
034	68	Recurrent	Non-Hispanic white	Pancreatic cancer	22.18	*MSH2*	NM_000251	c.2038C>T; p.Arg680*	Not available	Y	Colon, endometrial
035	27	IV	Non-Hispanic white	Colorectal cancer	21.96	*MLH1*	NM_000249	Deletion exons 1-2	Y	Y	None
036	65	IV	Non-Hispanic white	Urothelial	21.67	*MSH2*	NM_000251	c.1906G>C; p.Ala636Pro	Y	Y	Endometrial
037	40	IV	Hispanic	Endometrial cancer	19.19	*MLH1*	NM_000249	c.1731+1G>T	Y	Y	None
038	36	IV	Non-Hispanic white	Urothelial	17.98	*MLH1*	NM_000249	c. 790+2T>C	Y	Y	Endometrial, duodenal
039	55	I	Non-Hispanic white	Urothelial	17.39	*MSH2*	NM_000251	c.1906G>C; p.Ala636Pro	Y	Y	Colon, squamous cell carcinoma of skin
040	63	II-B	Asian	Pancreatic cancer	16.9	*PMS2*	NM_000535	Deletion exon 11	Y	N	None
041	58	IA	Non-Hispanic white	Endometrial cancer	16.84	*MSH6*	NM_000179	c.3991C>T; p.Arg1331*	Not available	Y	None
042	38	III	Hispanic	Colorectal cancer	14.9	*MLH1*	NM_000249	c.790+1G>A	Not available	Y	None
043	67	IV	Asian	Pancreatic cancer	14.9	*MSH6*	NM_000179	c.3268G>T; p.Glu1090*	Y	N	None
044	9	IV	Non-Hispanic white	Germ cell tumor	14.42	*MSH2*	NM_000251	c.2089T>C; p.Cys697Arg	Y	N	None
045	63	IIA	Non-Hispanic white	Colorectal cancer	14.14	*MSH6*	NM_000179	c.3743_3744insT; p.Tyr1249Leufs*26	Y	Y	None

(continued on following page)

TABLE A1. Baseline Patient and Tumor Characteristics for Patients With Lynch Syndrome in MSI-I/MSI-H Cohort (continued)

Patient	Age at Dx (years)	Cancer Stage	Race/Ethnicity	Tumor Type	MSIsensor Score	Gene	RefSeq Transcript	Variant	IHC Concordance	Met NCCN Testing Guidelines	Prior Malignancy
046	86	IV	Non-Hispanic white	Urothelial	13.88	*MSH2*	NM_000251	Deletion exons 9-10	Y	N	None
047	42	IIIC	Non-Hispanic white	Endometrial cancer	13.62	*MSH6*	NM_000179	c.3991C>T; p.Arg1331*	Y	Y	None
048	64	IV	Non-Hispanic white	Prostate cancer	13.35	*PMS2*	NM_000535	c.137G>T; p.Ser46Ile	Y	N	None
049	58	IV	Non-Hispanic white	Urothelial	12.46	*MSH2*	NM_000251	c.1784T>G; p.Leu595Arg	Y	N	None
050	31	IV	Non-Hispanic white	Colorectal cancer	12.1	*MSH6*	NM_000179	c.3476dupA; p.Tyr1159*	Y	Y	None
051	32	IIIC	Non-Hispanic white	Colorectal cancer	12.08	*MSH6*	NM_000179	c.3573dupT; p.Val1192Cysfs*2	Y	Y	None
052	52	IV	Non-Hispanic black	Endometrial cancer	10.54	*MSH2*	NM_000251	c.942+3A>T	Y	Y	None
053	35	IV	Non-Hispanic white	Cancer unknown primary	10.37	*MSH2*	NM_000251	c.1871T>G; p.1624Ser	Y	N	None
054	25	I	Non-Hispanic white	Colorectal cancer	9.9	*MSH2*	NM_000251	Deletion exons 1-6	Y	Y	None
055	71	II-B	Non-Hispanic white	Soft tissue sarcoma	9.78	*MSH2*	NM_000251	c.1216C>T; p.Arg406*	Y	Y	Colon, endometrial, sebaceous adenoma
056	29	IV	Non-Hispanic white	Adrenocortical carcinoma	7.55	*MSH2*	NM_000251	c.1906G>C; p.Ala636Pro	Y	Y	None
057	31	IIIC	Non-Hispanic white	Endometrial cancer	7.21	*MLH1*	NM_000249	c.2194A>T; p.Lys732*	Y	Y	Ovarian
058	35	IV	Non-Hispanic white	Glioma (glioblastoma multiforme with oligodendroglioma component)	7.04	*MSH6*	NM_000179	c.3261dupC; p.Phe1088Leufs*5	Y	N	None
059	48	IV	Non-Hispanic white	Adrenocortical carcinoma	6.53	*MSH6*	NM_000179	c.3261delC; p.Phe1088Serfs*2	Not available	N	None
060	62	IV	Non-Hispanic white	Prostate cancer	6.42	*MSH2*	NM_000251	c.1216C>T; p.Arg406*	Y	Y	Colon, squamous cell carcinoma of skin
061	73	IV	Asian	Mesothelioma	4.96	*MSH6*	NM_000179	c.2862C>G; p.Tyr954*	Not available	N	None
062	32	I	Non-Hispanic white	Soft tissue sarcoma	4.77	*MSH2*	NM_000251	c.229_230delAG; p.Ser77Cysfs*4	Y	N	None
063	84	Recurrent	Non-Hispanic white	Urothelial	4.39	*MSH6*	NM_000179	c.3261dupC; p.Phe1088Leufs*5	Y	N	None
064	66	IB	Non-Hispanic white	Endometrial cancer	4.22	*PMS2*	NM_000535	c.1A>G; p.Met1?	Y	Y	None
065	45	Recurrent	Non-Hispanic white	Urothelial	3.8	*MSH2*	NM_000251	c.942+3A>T	Y	Y	Endometrial
066	57	IV	Non-Hispanic white	Melanoma	3.76	*PMS2*	NM_000535	Deletion exons 1-8	Not available	N	None

NOTE. Table includes age at diagnosis, cancer stage, patients' race/ethnicity, tumor type, MSIsensor score, the MMR gene in which a pathogenic or likely pathogenic variant was discovered, the specific variant call, and if IHC showed concordant mismatch repair deficiency.

Abbreviations: Dx, diagnosis; IHC, immunohistochemistry; MSI-H, high-frequency microsatellite instability; MSI-I, indeterminate microsatellite instability; N, no; NCCN, National Comprehensive Cancer Network; RefSeq, National Center for Biotechnology Information reference sequence database; Y, yes.

TABLE A2. Baseline Tumor Characteristics for Patients With Lynch Syndrome in MSS Cohort

Patient	Tumor Type	MSIsensor Score	Gene	RefSeq Transcript	Variant
067	Hepatobiliary cancer	2.56	*PMS2*	NM_000535	c.943C>T; p.Arg315*
068	Endometrial cancer	2.48	*MSH6*	NM_000179	c.3959_3962delCAAG; p.Ala1320Glufs*6
069	Breast cancer	2.4	*PMS2*	NM_000535	c.1053delG; p.Leu351Phefs*5
070	Bone cancer	2.23	*MLH1*	NM_000249	c.199G>A; p.Gly67Arg
071	Colorectal cancer	2.07	*MSH6*	NM_000179	c.1458_1459delTG; p.Glu487Aspfs*10
072	Pancreatic cancer	2.01	*MSH2*	NM_000251	c.1906G>C; p.Ala636Pro
073	Soft tissue sarcoma	1.94	*PMS2*	NM_000535	del exon 8-9
074	Thyroid cancer	1.86	*MSH6*	NM_000179	c.3261delC; p.Phe1088Serfs*2
075	Prostate cancer	1.61	*MSH6*	NM_000179	c.3984_3987dupGTCA; p.Leu1330Valfs*12
076	Colorectal cancer	1.21	*MSH2*	NM_000251	c.1906G>C; p.Ala636Pro
077	Bladder cancer	1.14	*MSH6*	NM_000179	c.3463C>T; p.Gln1155*
078	Pancreatic cancer	1.14	*PMS2*	NM_000535	c.1076dupT; p.Leu359Phefs*6
079	Breast cancer	1.08	*PMS2*	NM_000535	c.736_741delinsTGTGTGTGAAG; p.Pro246Cysfs*3
080	Breast cancer	1.07	*MSH6*	NM_000179	c.3103C>T; p.Arg1035*
081	Germ cell tumor	0.88	*PMS2*	NM_000535	c.943C>T; p.Arg315*
082	Prostate cancer	0.72	*MSH6*	NM_000179	c.3959_3962delCAAG; p.Ala1320Glufs*6
083	Breast cancer	0.58	*MSH6*	NM_000179	c.3959_3962delCAAG; p.Ala1320Glufs*6
084	Breast cancer	0.55	*PMS2*	NM_000535	c.137G>T; p.Ser46Ile
085	Pancreatic cancer	0.52	*PMS2*	NM_000535	c.1831delinsTT;p.Ile611Phefs*2
086	Glioma	0.4	*MSH2*	NM_000251	c.301_306delGAAGTT; p.Glu101_Val102del
087	Breast cancer	0.33	*MLH1*	NM_000249	c.866_867dupAC; p.Pro290Thrfs*8
088	Hepatobiliary cancer	0.26	*PMS2*	NM_000535	c.137G>T;p.Ser46Ile
089	Hepatobiliary cancer	0.15	*PMS2*	NM_000535	c.137G>T;p.Ser46Ile
090	Endometrial cancer	0.13	*MSH6*	NM_000179	c.3984_3987dupGTCA; p.Leu1330Valfs*12
091	Head and neck cancer	0.08	*PMS2*	NM_000535	c.137G>T; p.Ser46Ile
092	Non–small-cell lung cancer	0.08	*MSH6*	NM_000179	c.3972_3979delGAAGATGA; p.Lys1325Sfs*13
093	Renal cell carcinoma	0.08	*MSH6*	NM_000179	c.3238_3239delCT; p.Leu1080Valfs*12
094	Soft tissue sarcoma	0.08	*MSH2*	NM_000251	c.942+3A>T
095	Bone cancer	0.07	*MSH2*	NM_000251	c.528_529delTG; p.Cys176*
096	Bone cancer	0	*MLH1*	NM_000249	c.1333C>T; p.Gln445*
097	Breast cancer	0	*MSH6*	NM_000179	c.3959_3962delCAAG; p.Ala1320Glufs*6
098	Glioma	0	*PMS2*	NM_000535	del exons 9-11
099	Non–small-cell lung cancer	0	*MSH6*	NM_000179	c.1250delA; p.Lys417Serfs*36
100	Non–small-cell lung cancer	0	*PMS2*	NM_000535	c.164-1G>C
101	Prostate cancer	0	*PMS2*	NM_000535	c.137G>T; p.Ser46Ile
102	Prostate cancer	0	*PMS2*	NM_000535	c.538-1G>C
103	Renal cell carcinoma	0	*MSH6*	NM_000179	c.2731C>T; p.Arg911*

NOTE. Table includes tumor type, MSIsensor score, the mismatch repair gene in which a pathogenic or likely pathogenic variant was discovered, and the specific variant call in the anonymized MSS group.

Abbreviations: MSI microsatellite instability; MSS, microsatellite stable; RefSeq, National Center for Biotechnology Information reference sequence database.