High predictability for identifying Lynch syndrome via microsatellite instability testing or immunohistochemistry in all Lynch-associated tumor types

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Lynch syndrome is an inherited cancer-prone syndrome identified in ~3% of all colorectal cancer (CRC) patients and ~2% of all endometrial cancer (EC) patients (1-3). It is defined by identification of a germline mutation in one of the DNA mismatch repair (MMR) genes (*MSH2*, *MLH1*, *MSH6*, *PMS2*, *EPCAM*) whose proteins function to maintain replication fidelity of DNA prior to mitosis, and sets it apart from other hereditary non-polyposis colorectal cancer (HNPCC) syndromes such as Familial Colorectal Cancer Type X and Polymerase Proofreading Polyposis syndrome (3). Patients with Lynch syndrome can develop synchronous or metachronous cancers at younger ages than patients with the counterpart sporadic cancers. CRC in both sexes and EC in women are the most common tumors observed, with other gastrointestinal cancers (stomach, biliary, small intestine, pancreas), female reproductive tract cancers (ovary), urinary tract cancers (renal pelvis, ureter), and brain tumors (glioblastoma) in the spectrum of Lynch tumors (1-3). The Muir-Torre variant of Lynch syndrome adds specific skin tumors such keratoacanthomas and sebaceous neoplasms to the tumor spectrum. The prevalence of Lynch syndrome in the population is estimated to be 1 in 300; yet it is underdiagnosed due to several factors including (I) little or no pre-symptomatic findings prior to cancer in patients, (II) unawareness by clinicians and patients, and (III) inadequate family history of cancer knowledge transmitted to the healthcare provider (1,3). Because surveillance of Lynch syndrome

patients extends their survival as well as quality of life, it is important to identify all patients. Universal population screening for Lynch syndrome among patients who present with colorectal and EC, the two most prevalent cancers in patients with the syndrome, has identified additional but not all Lynch patients that would have otherwise been missed and enrolled them in cancer surveillance programs, and generated the opportunity to offer genetic testing to affected and unaffected relatives (1-3).

Lynch syndrome is one of five human conditions in which tumors manifest a defect in MMR function that can be detected by microsatellite instability (MSI) testing and altered MMR protein expression (*Table 1*) (4-6). In Lynch syndrome, the patient manifests a mono-allelic MMR gene germline mutation in all cells of their body, and within developing or established tumor cells, the second MMR gene allele is inactivated somatically (two hits) thereby completely abating the expression of either allele of the MMR gene. The complete loss of the MMR protein expression partially or fully inactivates MMR function, committing daughter cells to develop point mutations and slippage mistakes at DNA microsatellite sequences, the latter detected biochemically by PCR [or by next generation sequencing (NGS)] as MSI (2,4,7-9). The presence of MSI and the loss of MMR protein expression go hand-in-hand, with over a 94% concordance rate (7). Both tests individually are used to screen CRCs and ECs for potential Lynch syndrome patients (2,3,6,9), with both a

Table 1 Human DNA mismatch repair deficiency conditions and MMR gene and protein expression

Condition	MMR inactivation mechanism	MMR protein expression
Lynch syndrome	Mono-allelic germline *MMR* gene mutation	Tumor loss of MSH2, MLH1, MSH6, or PMS2 expression
Constitutional MMR Deficiency	Bi-allelic germline *MMR* gene mutation	Tumor and potential normal tissue loss of MSH2, MLH1, MSH6, PMS2, or MSH3 expression
Lynch-like syndrome/double somatic MMR inactivation	Bi-allelic somatic *MMR* gene mutation	Tumor loss of MSH2, MLH1, MSH6, or PMS2 expression
Sporadic MSI CRC	Bi-allelic somatic *MLH1* hypermethylation	Tumor loss of MLH1 (and PMS2) expression
Inflammation-associated microsatellite alterations/EMAST	Somatic nuclear-to-cytosolic MSH3 protein shift	Reduction and heterogeneity of nuclear MSH3 expression

MMR, mismatch repair; EMAST, elevated microsatellite alterations at selected tetranucleotide repeats; CRC, colorectal cancer.

great improvement upon solely using the clinically-oriented Amsterdam criteria or Bethesda guidelines. CRCs and ECs that show MSI and/or MMR protein expression loss via immunohistochemistry (IHC) should inform the need for genetic counseling and genetic testing for a germline MMR gene mutation, the ultimate gold standard for characterizing Lynch syndrome families (1,2,6).

Can we expand screening to identify more patients with Lynch syndrome? With the study by Latham *et al.* (10), the answer is yes. Using NGS examining 15,045 unique patients with cancer spanning 50 cancer types followed by IHC in specific tumors, Latham *et al.* was able to newly identify 103 Lynch patients (overall 0.7%), with 53 of 326 (16.3%) coming from patients with MSI-high tumors, 13 of 699 (1.9%) coming from MSI-indeterminate tumors, and 37 of 14,020 (0.3%) coming from microsatellite stable (MSS) tumors (10). CRC and EC represented 201 of 326 (62%) of the MSI-high cohort while comprising only 1,351 of 15,045 (9%) of the tumors (10). Of patients with identified Lynch syndrome and an MSI-high tumor, 33 of 66 (50%) had a primary tumor other than CRC or EC, allowing the diagnosis of Lynch syndrome from the screening of other tumors rarely implicated in the syndrome, including adrenocortical cancer, mesothelioma, soft tissue sarcoma, pancreatic cancer, among others (*Table 2*) (10). IHC for MMR protein expression was concordant in 56 of 57 (98.2%) of identified Lynch syndrome patients (10). Lynch syndrome was not diagnosed via screening of all tumor types. Most notably, none of 150 MSI-high from 2,371 breast cancers, none of the 46 MSI-high from 343 ovarian cancers, none of the 94 MSI-high from 1,952 lung cancers, and none of the 11 MSI-high from 458 renal cancers belonged to the identified Lynch syndrome patients

(*Table 2*) (10). Only 15 of 66 (22.7%) of identified Lynch patients with MSI-high tumors had a prior malignancy history (10). In addition to Lynch syndrome, Latham *et al.* also detected 57 Lynch-like (double somatic MMR gene inactivation) patients (*Table 1*) out of 274 (20.8%) non-Lynch MSI-high tumors (10). Thirty-seven of the 103 identified Lynch syndrome patients came from MSS tumors, with most showing germline mutation of *MSH6* and *PMS2*, two lower penetrant MMR genes, in contrast to the majority of identified Lynch patients from MSI-high tumors showing *MLH1* and *MSH2* mutations (10). Overall, Latham *et al.* demonstrates with expansion of MSI and IHC testing to multiple cancers beyond CRCs and ECs that an additional 50% of Lynch syndrome patients can be newly identified.

There are a few other observations from Latham *et al.* First, the level of MSI varied among the various tumor types, with higher levels of MSI-high found among ureter, gastric and CRCs and lower levels found in ECs and brain tumors (10). MSI-high tumors are hypermutated due to the nature of the MMR defect, and contain hundreds of somatic mutations (9,11). Variance in the degree of hypermutation might be reflective of (I) high penetrant versus low penetrant MMR gene inactivation, (II) dominant clone and sampling from the tumor which may be heterogeneous, and/or (III) tumor cell doubling times to allow for more somatic mutation accumulation (12). Second, the race/ethnicity make-up of patients with MSI-high tumors was markedly lower for non-Hispanic blacks as compared to non-Hispanic white patients, consistent with prior population studies among sporadic CRC patients (13,14). Third, Latham *et al.* only screened patient cancers. Other non-cancers, such as sebaceous neoplasms that can be a part of Lynch syndrome,

Table 2 Lynch-associated cancers that may reveal a Lynch syndrome diagnosis (10)

Current screening for Lynch syndrome via MSI and IHC on tissues from	Expanded screening for Lynch syndrome via MSI and IHC on tissues from	Low yield tumors for screening for Lynch syndrome via MSI and IHC
• CRC	• CRC	• Ovarian cancer
• EC	• EC	• Lung cancer
	• Small intestinal cancer	• Renal cancer
	• Gastric cancer	• Breast cancer
	• Bladder/urothelial cancer	• Ampullary carcinoma
	• Adrenocortical cancer	• Anal carcinoma
	• Prostate cancer	• Appendiceal carcinoma
	• Germ cell cancer	• Osteosarcoma
	• Soft tissue sarcoma	• Peripheral nerve sheath tumors
	• Pancreatic cancer	• Choriocarcinoma
	• Mesothelioma	• Cervical cancer
	• Central nervous system tumors	• Neuroendocrine tumors
	• Melanoma	• Neuroblastoma
		• Thymic tumor
		• Pheochromocytoma
		• Vaginal carcinoma
		• Wilms tumor
		• Head and neck cancer
		• Hepatocellular carcinoma
		• Cholangiocarcinoma
		• Chondrosarcoma
		• Ewing sarcoma
		• Non-Hodgkin lymphoma
		• Leukemia
		• Retinoblastoma

MSI, microsatellite instability testing; IHC, immunohistochemistry.

have shown loss of MMR protein expression that can subsequently identify germline MMR gene mutation (15). It is possible that screening specific non-cancers in addition to cancers might also improve the yield in identifying Lynch syndrome. And fourth, Latham *et al.* noted no identified Lynch patients from MSI-high breast cancers, but did identify 7 Lynch patients from among 2,371 (0.3%) MSS breast cancers (10). Because these breast tumors lacked hypermutation and MSI, it is very likely that the breast tumor developed independently from the MMR germline

mutation. Lynch syndrome patients can develop non-MSI tumors that are thought to be sporadic in addition to their MMR-deficient driven tumors, and breast cancer has been a controversial association within Lynch syndrome. Among all MSS cancers, Lynch syndrome was identified in 0.3%, which is identical to the 1 in 300 population prevalence, strengthening the notion that the germline findings using NGS among MSS tumors is incidental and not causal.

Patients whose tumors show MSI-high, which includes all Lynch syndrome patients, may obtain a survival benefit

with immune checkpoint blockade due to the hypermutated nature of the tumors and the generation of neoantigens from transcribed and subsequently translated genes that contain microsatellite coding sequences (4,9,16). The U.S. Food and Drug Administration approved anti-PD-1 therapy for patients with MSI-high tumors, agnostic to the type of cancer. Thus, identifying Lynch syndrome patients is not only important for surveillance and prevention of future cancer, it affects the therapeutic options in identified patients that may develop a cancer. However, despite expansion to multiple tumor types to screen from and to identify Lynch syndrome, these various tumor types present in Lynch syndrome patients do not mean there is a uniform response to anti-PD-1 therapy. The extent of insertion/ deletion mutations (frameshifts at DNA microsatellites) within each tumor determines the level of PD-1 expression, and thereby the level of tumor cell response to anti-PD-1 therapy, and thereby the level of patient response to anti-PD-1 therapy (17). Thus, the level of insertion/deletion mutational burden within each tumor determines that degree of potential response. Overall, the findings from Latham *et al.* broadens the catch and identification of Lynch syndrome patients for which surveillance and therapeutic options should improve outcomes.

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Footnote

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