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Lynch Syndrome: From Screening to Diagnosis to Treatment in the Era of Modern Molecular Oncology

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Abstract

Lynch syndrome is a hereditary cancer predisposition syndrome caused by germline alterations in the mismatch repair genes and is the most common etiology of hereditary colorectal cancer. While Lynch syndrome was initially defined by the clinical Amsterdam criteria, these criteria lack the sensitivity needed for clinical utility. This review covers the evolution of screening for Lynch syndrome from the use of tumor microsatellite instability and/or somatic alterations in mismatch repair protein expression by immunohistochemistry to the newest methods using next-generation sequencing. Additionally, it discusses the clinical implications of the diagnosis of Lynch syndrome as it affects cancer therapeutics and the role of screening in non-colorectal Lynch-associated cancers. As molecular oncology continues to evolve, it is crucial to remain current on the increasing complexity of Lynch syndrome diagnostics and treatment options.

INTRODUCTION

Up to one-third of colorectal cancer (CRC) is familial, with approximately 5–10% attributed to a germline gene alteration (21, 49, 51). There are a variety of hereditary CRC syndromes, roughly grouped into polyposis and nonpolyposis syndromes. This review focuses on the most common cause of hereditary CRC: Lynch syndrome, formerly referred to as hereditary nonpolyposis colorectal cancer, which accounts for up to 5% of CRCs (51). Lynch syndrome is caused by one or more germline pathogenic variants in one of the mismatch repair (MMR) genes (*MLH1*, *MSH2*, *MSH6*, and *PMS2*) and/or *EPCAM*. In-depth understanding of the detection, diagnosis, and clinical management of Lynch-associated malignancies is crucial to delivering optimal care in the increasingly complex field of modern molecular oncology.

CLINICAL FEATURES OF LYNCH SYNDROME

The hallmark malignancies of Lynch syndrome are CRC and endometrial cancers, with lifetime risks of up to 90% and 40%, respectively (51, 82). In addition, there is an increased risk of genitourinary (renal pelvis, ureter, bladder, or prostate), pancreatic, ovarian, stomach, small bowel, and biliary tract cancers (3, 35, 82). These risks may vary by gene; for example, individuals with *MSH2* pathogenic variants have more genitourinary cancers (103). In addition to the malignancies, there may be other benign findings, such as sebaceous lesions and keratoacanthomas (the presence of which is indicative of the variant Muir–Torre syndrome) (53, 89).

While it is commonly accepted that breast cancer is not part of Lynch syndrome, a growing number of studies have suggested an epidemiologic association (15, 36, 74, 91, 102). There have been limited molecular studies to test this hypothesis. In the international Colon Cancer Family Registry, of 35 cases of breast cancer in individuals with Lynch syndrome, 18 (51%) had immunohistochemistry (IHC) consistent with loss of MMR gene function in the breast tumor tissue (100). Only one case had a germline *MSH6* pathogenic variant, which has been suggested to have the highest breast cancer risk association (18, 74). Thus, further molecular studies are needed to evaluate breast cancer as a Lynch-associated malignancy.

CRC associated with Lynch syndrome has clinical features that are distinct from those of sporadic CRC. Lynch-associated CRC usually presents at an earlier age of diagnosis, prompting colonoscopic screening to begin at age 20–25 (95). Additionally, the tumors in Lynch-associated CRC occur more often in the proximal/right-sided colon than they do in sporadic CRC. Histologically, poor differentiation with mucinous features, Crohn's-like lymphoid reaction, and tumor-infiltrating lymphocytes are all suggestive of a Lynch-associated CRC (6, 40) and are referred to as microsatellite instability high (MSI-H) histology features.

CLINICAL CRITERIA

The Amsterdam Criteria

Consensus clinical criteria for the diagnosis of Lynch syndrome were first described in 1991 by Vasen et al. (96) as the Amsterdam criteria. These so-called 3–2–1 criteria require a minimum of three members of a family diagnosed with CRC across two successive generations (with one case being a first-degree relative of the other two), at least one case diagnosed before age 50, and the exclusion of familial adenomatous polyposis. In recognition of the spectrum of cancers within Lynch syndrome, the Amsterdam criteria were revised in 1999 (referred to as the Amsterdam II criteria) to include other noncolorectal cancers (endometrial, small bowel, ureter, and renal pelvis) now known to be associated with Lynch syndrome (97).

The Bethesda Guidelines

Once the etiology of Lynch syndrome was understood to be due to germline MMR gene alterations, the question became how to approach patient screening. The consensus Bethesda guidelines were first published in 1997 and were designed to provide recommendations on who should undergo further tumor testing for Lynch syndrome (76, 92). The consensus from this group was that individuals are at sufficiently high risk to warrant genetic testing if they have (*a*) CRC when less than 50 years old, (*b*) synchronous or metachronous Lynch-associated tumors, (*c*) CRC when less than 60 years old along with MSI-H-like histologic features, (*d*) at least one first-degree relative with a Lynch-related tumor (including colorectal, endometrial, genitourinary, stomach, small bowel, biliary, ovarian, or brain cancer) diagnosed when the relative is less than 50 years old, and/or (*e*) CRC and two or more first- or second-degree relatives with Lynch-related tumors.

Clinical Guidelines in Modern Practice

We now understand that the Amsterdam criteria and Bethesda guidelines fail to serve as good screening tests for Lynch syndrome (2). Notably, half of germline-confirmed Lynch syndrome patients fail to meet the Amsterdam II criteria (51). The Bethesda guidelines are highly sensitive but have too low a specificity to make them clinically useful. Even computer-based models [such as MMRpro (12), PREMM (43, 44), and MMRpredict (28)] have failed to make a clinically meaningful impact on current practice, likely because these models aim to detect the highest-risk individuals rather than the full spectrum of Lynch syndrome patients (42). Thus, based on the suboptimal utility of these clinical strategies and improvements in our molecular understanding of Lynch syndrome, the detection of Lynch syndrome has evolved from a clinical diagnosis using the Amsterdam criteria to tumor-based screening with germline confirmation (**Figure 1**).

TUMOR SCREENING FOR LYNCH SYNDROME

Two standard laboratory tests are available for Lynch syndrome screening of tumor tissue: DNA MSI and MMR protein IHC. These tests can be used independently or together (84, 105); however, the combination of MSI and IHC maximizes the specificity and sensitivity for Lynch syndrome screening, albeit at higher individual cost. If either test is positive, germline DNA testing is recommended to make the diagnosis of Lynch syndrome.

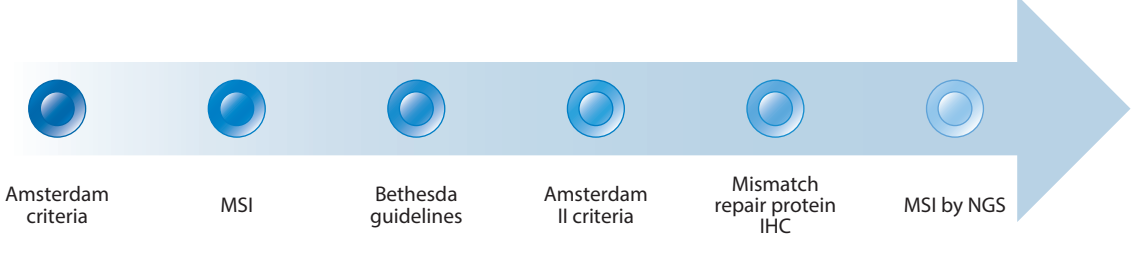

Figure 1

Evolution of Lynch syndrome screening over time. Abbreviations: IHC, immunohistochemistry; MSI, microsatellite instability; NGS, next-generation sequencing.

Microsatellite Instability

MSI is a PCR-based assay that compares variations in short repeated sequences at multiple loci. The most commonly used test interrogates five loci recommended by the National Cancer Institute. At least 30% instability (e.g., two or more of the five loci) is the generally accepted threshold for determining the presence of MSI, also referred to as MSI-H cancer (38). Less than 30% (e.g., one of the five loci) is MSI low (MSI-L), and no detected instability means microsatellite stability (MSS), although clinically MSI-L and MSS behave similarly and are often grouped together. MSI testing has a sensitivity of 55–91% for Lynch syndrome (19).

While 15% of colorectal tumors are MSI-H (29), the vast majority of these MSI-H CRCs are actually sporadic. This is often due to somatic hypermethylation of the *MLH1* promoter and associated with the *BRAF* V600E mutation (7, 52). Thus, when MSI-H alone is detected in a CRC, follow-up testing for a *BRAF* pathogenic mutation and/or *MLH1* promoter hypermethylation is often pursued to determine whether a heritable cause is likely (56). If neither is found, germline testing is recommended for confirmation.

Immunohistochemistry

IHC for the loss of expression of the MMR proteins can also be performed on CRC tumor tissue to screen for Lynch syndrome. The specific MMR IHC assays that are run vary among institutions. At a minimum, MLH1 and MSH2 expression is evaluated, as pathogenic variants in the *MLH1* and *MSH2* genes account for approximately 70% of identified Lynch syndrome cases (68). Recognizing that MLH1/PMS2 and MSH2/MSH6 make heterodimers, some centers additionally test for MSH6 and PMS2 expression, either initially as a set of four stains or secondarily if MLH1 and MSH2 are intact. To reduce costs, some centers take advantage of predictable cellular interactions with a two-stain IHC approach of testing initially for MSH6 and PMS2 expression and then testing for either MLH1 or MSH2 expression only if one of the initial proteins is absent (31, 70). For completeness of screening, if IHC is used, we recommend testing for all four MMR proteins. IHC has a sensitivity of 83% and specificity of 89% for detecting MSI CRC (19).

Due to cellular interactions, a single germline gene alteration may lead to the loss of one more of the proteins. For example, inactivation of *MSH6* leads to loss of MSH6 with or without loss of MSH2. PMS2 loss can secondarily result from either a germline *MLH1* pathogenic variant or sporadic *MLH1* hypermethylation (45). As with MSI, if MLH1 alone is absent, *BRAF* V600E and/or *MLH1* promoter hypermethylation testing may be recommended to rule out sporadic CRC prior to germline testing. Algorithms are available for interpreting different MMR deficiency patterns with or without MSI testing (62). One purported advantage of MMR IHC was that it may provide a clue for how to narrow confirmatory germline testing. However, in the era of falling costs for high-quality next-generation sequencing (NGS), this is less relevant. In contrast to the poor performance of the clinical Amsterdam criteria, MSI and/or MMR IHC detect more than 90% of Lynch syndrome patients (51).

Discordant Results

There is a common misconception that discordance between MSI and MMR IHC represents a false positive result. Discordance is often a correct result representing the differences in cellular functions detected by the two testing strategies. As abnormal IHC reflects loss of protein, and one reason that a patient with true Lynch syndrome would have a normal IHC is that the protein is expressed but is not functioning normally due to a missense pathogenic variant. This tumor

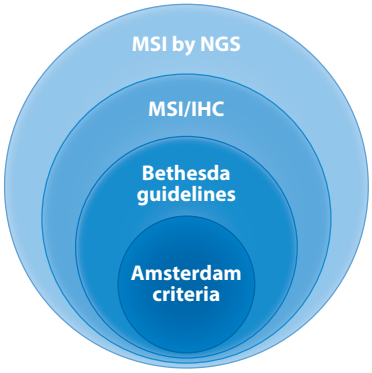

Figure 2

Relative sensitivity of Lynch syndrome screening methods. Abbreviations: MSI/IHC, microsatellite instability by PCR and/or mismatch repair protein immunohistochemistry; NGS, next-generation sequencing.

would demonstrate MSI-H and normal IHC. Conversely, some pathogenic variants, particularly in *MSH6* and *PMS2*, can result in protein loss by IHC without causing MSI-H (25). In this scenario, there will often be some evidence of MSI even if fewer than 30% of loci are unstable (i.e., MSI-L) (99, 104).

NEXT-GENERATION SEQUENCING PANEL TESTING

Next-Generation Sequencing for Tumor Screening

While MSI and MMR IHC are an improvement over the clinical criteria for Lynch syndrome, true Lynch syndrome cases can still be missed, and the multistep nature of testing can make this process quite cumbersome and sometimes incomplete. This is especially true if follow-up *BRAF* or *MLH1* testing is required. MSI by NGS [e.g., mSINGS (78) and MSIsensor (62)] is a further improvement on prior tumor screening methods with high validity and a comprehensive methodology and may become a clinical standard in the coming years (**Figure 2**). Furthermore, NGS results highlight not only deficiency in MMR but also specific pathogenic variants, which is helpful for targeted germline confirmatory sequencing.

Methods to perform MSI by NGS are well established and improve Lynch syndrome detection beyond standard MSI/IHC (64, 78). This may be because these methods use a greater number of loci in a quantifiable manner, which also allows for a lower threshold of 20% unstable loci to determine MSI-H status. The sensitivity of mSINGS for Lynch syndrome screening is 96–100%, and the specificity is 97–100% (78). In clinical practice, somatic tumor NGS panel testing has a higher Lynch syndrome detection rate than either MSI or MMR IHC, even when these are combined with *BRAF* testing (33). In addition, the single-panel test provides the ability to simultaneously evaluate other genetic biomarkers of interest (e.g., *KRAS*, *NRAS*, and *BRAF*) and potentially clinically meaningful genes of interest (e.g., *DPYD* and *TS* for predicted chemotherapy toxicity) in an efficient and cost-effective approach (22).

Next-Generation Sequencing for Germline Confirmation

Originally, germline genetic sequencing was performed on candidate genes individually, which meant that for a person with suspected Lynch syndrome, up to five genes would need to be tested.

Now that sequencing costs have come down and technologies have improved, most patients with a suspicion of hereditary CRC are evaluated with a germline genetic panel. Cost-effectiveness studies have addressed optimal panel sizes, although these findings must be reassessed as costs continue to drop (22). Use of germline panels may be recommended as follow-up to a positive MSI/IHC or MSI by NGS tumor screening or as part of a larger testing strategy for an individual with a suspicious clinical or family history. Additionally, NGS germline testing has the ability to evaluate other known CRC predisposition genes (such as *APC*, *MUTYH*, *STK11*, *BMPR1A*, and *SMAD4*) in a comprehensive fashion, again eliminating the need for stepwise testing.

In an example of the utility of NGS panel testing, 450 CRC cases from an Ohio population series diagnosed at less than 50 years old were evaluated with a 20-gene germline panel (69). Of these 450 cases, 48 (10.7%) were MMR deficient; 37 of these had Lynch syndrome, and 9 had double somatic MSI CRC. An additional 32 patients had a pathogenic germline variant in a CRC-associated gene, and 13 had a variant in a gene that is not typically associated with CRC but is associated with other cancers, such as *BRCA1* or *PALB2*. Given the not-insignificant proportion of patients overall who had a germline variant (16%) and the spectrum of these variants, germline panel testing may be appropriate for all younger individuals at diagnosis. These variants are further enriched in clinic-based and younger (<35 years) populations (60).

UNIVERSAL SCREENING

Colorectal Cancer

Several large studies have demonstrated that universal tumor screening for Lynch syndrome is both feasible and beneficial, due to an increase in the detection rate of people with Lynch syndrome (1, 32, 37, 46). For example, Hampel et al. (32) performed MSI and IHC testing for the four MMR proteins on 500 unselected CRC patients, and 18 of them (3.6%) had Lynch syndrome. Notably, only 8 of those 18 (44%) were diagnosed at less than 50 years old. For each proband diagnosed with Lynch syndrome, an average of three additional unaffected family members were found to also have Lynch syndrome, highlighting the ability to use incident cases to help prevent future cancers in family members through increased surveillance. The plethora of data supporting universal CRC screening has resulted in recommendations for universal tumor screening by several major groups, including the National Comprehensive Cancer Network (62), Evaluation of Genomic Applications in Practice and Prevention Working Group (19), and National Society of Genetic Counselors (101).

A universal tumor screening approach has been shown to be cost-effective, given the possibility of preventing additional cancers in affected individuals as well as primary cancers in unaffected at-risk relatives (11, 46, 61). However, universal Lynch syndrome screening has not been widely integrated into standard clinical practice (4, 39). One study published in 2012 noted that while 70% of academic centers had a universal screening program, only 15% of community hospitals included it as part of their standard practice (4). A more recent analysis of more than 150,000 individuals in the National Cancer Database noted that only 28% of CRC patients underwent MMR deficiency testing and only 43% of patients were diagnosed at less than 50 years old (83). Thus, continued education and dissemination of the supportive data, in the form of both guideline recommendations and implementation guides (13), will be helpful for improving screening compliance.

Endometrial Cancer

Universal Lynch syndrome screening has also been studied and is recommended for endometrial cancer (63) but is less well implemented than it is for CRC. Multiple studies have evaluated

universal tumor screening in endometrial cancer (1, 17, 26, 73). For example, among 1,002 endometrial cancers from the GOG210 clinical trial screened with MSI, 11.8% were MSI-H (26). Of the 51 MSI-H cases that were tested, 21 (41%) had Lynch syndrome, which would be approximately 5% of cases overall. Similar series have detected Lynch syndrome in approximately 2% of women with endometrial cancer (1, 17). The age of diagnosis is often earlier, but there are cases of Lynch-associated endometrial cancer diagnosed in patients over 60 years old, highlighting the need for universal screening (26). Thus, while there are similar data in endometrial cancer as there are for CRC to support universal tumor screening, and universal screening is recommended, implementation has been less common in endometrial cancer.

Other Cancers Associated with Lynch Syndrome

The Memorial Sloan Kettering–Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) study was a single-center prospective tumor-normal DNA sequencing study in more than 15,000 individuals with advanced cancer (54). In this series, 2.2% of cases were MSI-H, but only 16.3% of those cases were due to germline alterations consistent with Lynch syndrome (81). Notably, of the 1,025 tumors that were either MSI-H or MSI indeterminate, only 25% were either CRC or endometrial cancer. In the era of immunotherapy (as described in the next section), universal testing for MSI now has a role in all advanced cancers. This will also allow for greater screening, and therefore diagnosis, of Lynch syndrome.

CLINICAL IMPLICATIONS OF MICROSATELLITE INSTABILITY AND LYNCH SYNDROME

Even when adjusting for stage, MSI is recognized to be a marker of better prognosis (29, 47, 80). This is especially true for proximally located MSI CRCs (47). In stage II colon cancer, retrospective analysis notes that individuals with MSI-H tumors do not benefit from adjuvant 5-fluorouracil (71, 79, 87). This effect may be stronger in sporadic rather than germline (Lynch-associated) MSI-H CRC (86). These older chemotherapy retrospective analyses often did not account for modern CRC biomarkers, such as *BRAF* and *KRAS*, preventing greater generalization of MSI as a predictive biomarker, but its prognostic role is well established.

The role of MSI as a predictive marker with modern combination chemotherapy regimens, such as FOLFOX and FOLFIRI, is uncertain (16, 98). This uncertainty is a consequence of differences in the studies' chemotherapy regimens and assays for MSI, which make it difficult to compare studies and develop a robust and generalizable conclusion about the role of MSI as a predictive marker for modern cytotoxic chemotherapy. A retrospective analysis of the benefit from adjuvant oxaliplatin in stage II and stage III colon cancer noted no difference by MSI status (23). Thus, all stage III patients receive oxaliplatin-based chemotherapy regardless of whether the CRC is MSI-H. One trial noted that MSI may be predictive of response to adjuvant irinotecan, but this finding has not been reliable, and the current opinion based on the trials overall is that irinotecan is indicated only for metastatic CRC (5, 20, 93).

On the cellular level, this deficiency in MMR is associated with a higher tumor somatic mutation burden, which has important therapeutic implications for response to immunotherapy (94). Studies with pembrolizumab and nivolumab have demonstrated benefit from anti–programmed death 1 (PD-1) receptor therapy in patients with MSI cancers, including both CRC and non-CRC MSI-H tumors (48, 66). Both drugs have now received US Food and Drug Administration approval for use in metastatic MSI-H CRC. Additionally, combination immunotherapy with nivolumab and ipilimumab (a CTLA-4 inhibitor) is on track for accelerated Food and Drug

Administration approval (66). Ongoing trials are evaluating combinations of chemotherapy and immunotherapy, combinations of immunotherapy and targeted therapy, and the utility of immunotherapy in the adjuvant setting.

ADDITIONAL CLINICAL CATEGORIES DETERMINED BY MICROSATELLITE INSTABILITY STATUS

Familial Colorectal Cancer Type X

While the Amsterdam criteria can miss individuals with Lynch syndrome, there are also patients who meet the Amsterdam criteria but have negative Lynch syndrome tumor testing (i.e., MSS). Familial colorectal cancer type X (FCCTX) is the catch-all term to describe these cases (50). This first large description of FCCTX, which included 71 FCCTX families with 1,567 unaffected relatives, found an increased incidence of CRC in the cohort of FCCTX families [standardized incidence ratio (SIR) = 2.3] but to a lesser degree than in the cohort who met the Amsterdam criteria and had MSI (likely Lynch syndrome) (SIR = 6.1, $p < 0.001$) (50). The FCCTX cohort also had an increased incidence of gastric cancer (SIR = 1.4) but no increase in endometrial cancer (SIR = 0.8) or other Lynch-related cancers. Subsequent analysis demonstrated that, compared with Lynch-related CRCs, FCCTX CRCs either were less likely to be proximal or demonstrated the classic poor differentiation, mucinous features, and tumor-infiltrating lymphocytes (85). Thus, while there may be overlap in clinical history between Lynch syndrome and FCCTX, they appear to have distinct etiologies that have yet to be elucidated. Many cases with FCCTX likely result from oligogenic effects, preventing the identification of a single causative gene or pathway, as was found with Lynch syndrome and other high-penetrance CRC-associated genes, such as *APC*. Future high-quality genetic and molecular analysis is needed to explain FCCTX.

Double Somatic Microsatellite Instability Colorectal and Endometrial Cancers

Most somatic MSI CRC is due to *MLH1* hypermethylation (**Table 1**). However, some MSI CRCs remain unexplained by either *MLH1* hypermethylation or germline MMR pathogenic variants, which have historically been referred to as Lynch-like syndrome (8, 41, 55, 59, 77). Using high-quality paired tumor and germline NGS testing, Haraldsdottir et al. (34) evaluated 32 of these unexplained CRC and endometrial cancer cases and found that 22 (69%) had two or more somatic alterations in the MMR genes, which led to the hypermutated phenotype seen with MSI tumors. This report of double somatic MSI CRC solidified what has been previously observed but

Table 1 Approximate frequency of microsatellite instability (MSI) and MSI subsets, by cancer type

Cancer type	MSI	Lynch syndrome	*MLH1* hypermethylated	Double somatic MSI	References
Colorectal	15%	3–5%	10%	~2%	14, 34
Endometrial	12–25%	2–4%	10–20%	Unknown	1, 9, 17, 26, 57
Ovarian	~1%	0.5%	0.5%	Unknown	10, 27, 65, 67
Prostate[a]	~2%	<1%	Not reported	~1–2%	30, 72, 75

[a]The prevalence of MSI in prostate cancer is low overall but has been reported in up to 8% of very-high-grade localized cancers and up to 12% of metastatic disease.

found that it occurred at a higher frequency, likely due to more sensitive sequencing methods (24, 58, 88).

Clinically, these double somatic cases had a median age of diagnosis similar to those of MSS cancers but were more often right-sided with mucinous features, as would be expected for MSI or Lynch syndrome (34). Unlike Lynch syndrome, a family history of cancer was uncommon, with only 5 of 21 cases reporting a family history of CRC and 1 of 21 reporting a history of endometrial cancer. Molecular characterization of these double somatic MSI tumors revealed an increase in *PIK3CA* somatic pathogenic mutations in the double somatic MSI tumors compared with Lynch syndrome and *MLH1* hypermethylated MSI tumors (14). Similar to Lynch syndrome, *BRAF* somatic mutations were not seen in the double somatic MSI tumors. Further clinical characterization will be needed, including treatment responses and second cancer risks.

FUTURE DIRECTIONS

The ideal screening technology for Lynch syndrome will continue to evolve; however, both germline and tumor NGS are increasingly utilized. While we have focused on Lynch screening, non-MMR CRC-associated genes continue to be identified, and the detection of these may need to be considered. Germline gene panel testing for all CRCs diagnosed before the age of 50 years has been recommended due to the prevalence of positive results overall, including in those who did not meet testing criteria and those with non-Lynch germline pathogenic variants who would not be identified by tumor screening (69). Of 48 early-onset CRC cases with positive results in a gene associated with high-penetrance CRC risk, 11 (23%) were non-Lynch (69). Even in a community-based cohort of CRCs at any age without prior genetic testing, of 529 cases, 3 of the 20 positive results were in non-Lynch CRC-associated genes (A.S. Gordon, E.A. Rosenthal, D.S. Carrell, L.M. Amendola, M.O. Dorschner, et al., manuscript in review).

Lynch syndrome screening is an emerging concept in cancers outside of CRC and endometrial cancer. While there is increased uptake of MSI testing in advanced cancer where immunotherapy is a potential therapeutic option, screening in other clinical scenarios and in noncolorectal Lynch-associated cancers has made little progress (30). However, this is slowly changing in ovarian (10, 27, 65, 67) and prostate cancers (30, 72, 75). For example, emerging data show that universal screening of ovarian cancer identifies cases of Lynch-associated ovarian cancer that would not be identified by clinical criteria alone (90). Prospective evaluations to evaluate risk modification and cost-effectiveness are ongoing (ClinicalTrials.gov identifier NCT02494791).

CONCLUSIONS

The historical Amsterdam criteria have largely been replaced by tumor-based screening for MMR gene mutations using MSI, IHC, and most recently NGS-based testing. The clinical implications of the emerging double somatic MSI subgroup defined by NGS remain to be defined. This is clinically relevant given the potential for therapeutic intervention with immunotherapy and other cancer-directed therapies. Additionally, screening strategies for Lynch syndrome, which are well validated in CRC, need to be further expanded to endometrial, ovarian, prostate, and other Lynch-associated cancers.

DISCLOSURE STATEMENT

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LITERATURE CITED

1. Adar T, Rodgers LH, Shannon KM, Yoshida M, Ma T, et al. 2018. Universal screening of both endometrial and colon cancers increases the detection of Lynch syndrome. *Cancer* 124:3145–53
2. Barnetson RA, Tenesa A, Farrington SM, Nicholl ID, Cetnarskyj R, et al. 2006. Identification and survival of carriers of mutations in DNA mismatch-repair genes in colon cancer. *N. Engl. J. Med.* 354:2751–63
3. Barrow E, Robinson L, Alduaij W, Shenton A, Clancy T, et al. 2009. Cumulative lifetime incidence of extracolonic cancers in Lynch syndrome: a report of 121 families with proven mutations. *Clin. Genet.* 75:141–49
4. Beamer LC, Grant ML, Espenschied CR, Blazer KR, Hampel HL, et al. 2012. Reflex immunohistochemistry and microsatellite instability testing of colorectal tumors for Lynch syndrome among US cancer programs and follow-up of abnormal results. *J. Clin. Oncol.* 30:1058–63
5. Bertagnolli MM, Niedzwiecki D, Compton CC, Hahn HP, Hall M, et al. 2009. Microsatellite instability predicts improved response to adjuvant therapy with irinotecan, fluorouracil, and leucovorin in stage III colon cancer: Cancer and Leukemia Group B Protocol 89803. *J. Clin. Oncol.* 27:1814–21
6. Bessa X, Alenda C, Paya A, Alvarez C, Iglesias M, et al. 2011. Validation microsatellite path score in a population-based cohort of patients with colorectal cancer. *J. Clin. Oncol.* 29:3374–80
7. Boland CR, Goel A. 2010. Microsatellite instability in colorectal cancer. *Gastroenterology* 138:2073–87.e3
8. Buchanan DD, Rosty C, Clendenning M, Spurdle AB, Win AK. 2014. Clinical problems of colorectal cancer and endometrial cancer cases with unknown cause of tumor mismatch repair deficiency (suspected Lynch syndrome). *Appl. Clin. Genet.* 7:183–93
9. Buchanan DD, Tan YY, Walsh MD, Clendenning M, Metcalf AM, et al. 2014. Tumor mismatch repair immunohistochemistry and DNA MLH1 methylation testing of patients with endometrial cancer diagnosed at age younger than 60 years optimizes triage for population-level germline mismatch repair gene mutation testing. *J. Clin. Oncol.* 32:90–100
10. Catasús L, Bussaglia E, Rodríguez I, Gallardo A, Pons C, et al. 2004. Molecular genetic alterations in endometrioid carcinomas of the ovary: similar frequency of beta-catenin abnormalities but lower rate of microsatellite instability and PTEN alterations than in uterine endometrioid carcinomas. *Hum. Pathol.* 35:1360–68
11. Cenin DR, Naber SK, Lansdorp-Vogelaar I, Jenkins MA, Buchanan DD, et al. 2018. Costs and outcomes of Lynch syndrome screening in the Australian colorectal cancer population. *J. Gastroenterol. Hepatol.* 33:1737–44
12. Chen S, Wang W, Lee S, Nafa K, Lee J, et al. 2006. Prediction of germline mutations and cancer risk in the Lynch syndrome. *JAMA* 296:1479–87
13. Cohen SA, Laurino M, Bowen DJ, Upton MP, Pritchard C, et al. 2016. Initiation of universal tumor screening for Lynch syndrome in colorectal cancer patients as a model for the implementation of genetic information into clinical oncology practice. *Cancer* 122:393–401
14. Cohen SA, Turner EH, Beightol MB, Jacobson A, Gooley TA, et al. 2016. Frequent *PIK3CA* mutations in colorectal and endometrial tumors with 2 or more somatic mutations in mismatch repair genes. *Gastroenterology* 151:440–47.e1
15. Davies H, Morganella S, Purdie CA, Jang SJ, Borgen E, et al. 2017. Whole-genome sequencing reveals breast cancers with mismatch repair deficiency. *Cancer Res.* 77:4755–62
16. Des Guetz G, Uzzan B, Nicolas P, Schischmanoff O, Perret GY, Morere JF. 2009. Microsatellite instability does not predict the efficacy of chemotherapy in metastatic colorectal cancer. A systematic review and meta-analysis. *Anticancer Res.* 29:1615–20

17. Dillon JL, Gonzalez JL, DeMars L, Bloch KJ, Tafe LJ. 2017. Universal screening for Lynch syndrome in endometrial cancers: frequency of germline mutations and identification of patients with Lynch-like syndrome. *Hum. Pathol.* 70:121–28
18. Espenschied CR, LaDuca H, Li S, McFarland R, Gau CL, Hampel H. 2017. Multigene panel testing provides a new perspective on Lynch syndrome. *J. Clin. Oncol.* 35:2568–75
19. Eval. Genom. Appl. Pract. Prev. (EGAPP) Work. Group. 2009. Recommendations from the EGAPP Working Group: genetic testing strategies in newly diagnosed individuals with colorectal cancer aimed at reducing morbidity and mortality from Lynch syndrome in relatives. *Genet. Med.* 11:35–41
20. Fallik D, Borrini F, Boige V, Viguier J, Jacob S, et al. 2003. Microsatellite instability is a predictive factor of the tumor response to irinotecan in patients with advanced colorectal cancer. *Cancer Res.* 63:5738–44
21. Fuchs CS, Giovannucci EL, Colditz GA, Hunter DJ, Speizer FE, Willett WC. 1994. A prospective study of family history and the risk of colorectal cancer. *N. Engl. J. Med.* 331:1669–74
22. Gallego CJ, Shirts BH, Bennette CS, Guzauskas G, Amendola LM, et al. 2015. Next-generation sequencing panels for the diagnosis of colorectal cancer and polyposis syndromes: a cost-effectiveness analysis. *J. Clin. Oncol.* 33:2084–91
23. Gavin PG, Colangelo LH, Fumagalli D, Tanaka N, Remillard MY, et al. 2012. Mutation profiling and microsatellite instability in stage II and III colon cancer: an assessment of their prognostic and oxaliplatin predictive value. *Clin. Cancer Res.* 18:6531–41
24. Geurts-Giele WR, Leenen CH, Dubbink HJ, Meijssen IC, Post E, et al. 2014. Somatic aberrations of mismatch repair genes as a cause of microsatellite-unstable cancers. *J. Pathol.* 234:548–59
25. Giráldez MD, Balaguer F, Bujanda L, Cuatrecasas M, Muñoz J, et al. 2010. MSH6 and MUTYH deficiency is a frequent event in early-onset colorectal cancer. *Clin. Cancer Res.* 16:5402–13
26. Goodfellow PJ, Billingsley CC, Lankes HA, Ali S, Cohn DE, et al. 2015. Combined microsatellite instability, *MLH1* methylation analysis, and immunohistochemistry for Lynch Syndrome screening in endometrial cancers from GOG210: an NRG oncology and gynecologic oncology group study. *J. Clin. Oncol.* 33:4301–8
27. Gras E, Catasus L, Argüelles R, Moreno-Bueno G, Palacios J, et al. 2001. Microsatellite instability, MLH-1 promoter hypermethylation, and frameshift mutations at coding mononucleotide repeat microsatellites in ovarian tumors. *Cancer* 92:2829–36
28. Green RC, Parfrey PS, Woods MO, Younghusband HB. 2009. Prediction of Lynch syndrome in consecutive patients with colorectal cancer. *J. Natl. Cancer Inst.* 101:331–40
29. Guastadisegni C, Colafranceschi M, Ottini L, Dogliotti E. 2010. Microsatellite instability as a marker of prognosis and response to therapy: a meta-analysis of colorectal cancer survival data. *Eur. J. Cancer* 46:2788–98
30. Guedes LB, Antonarakis ES, Schweizer MT, Mirkheshti N, Almutairi F, et al. 2017. MSH2 loss in primary prostate cancer. *Clin. Cancer Res.* 23:6863–74
31. Hall G, Clarkson A, Shi A, Langford E, Leung H, et al. 2010. Immunohistochemistry for PMS2 and MSH6 alone can replace a four antibody panel for mismatch repair deficiency screening in colorectal adenocarcinoma. *Pathology* 42:409–13
32. Hampel H, Frankel WL, Martin E, Arnold M, Khanduja K, et al. 2008. Feasibility of screening for Lynch syndrome among patients with colorectal cancer. *J. Clin. Oncol.* 26:5783–88
33. Hampel H, Pearlman R, Beightol M, Zhao W, Jones D, et al. 2018. Assessment of tumor sequencing as a replacement for Lynch syndrome screening and current molecular tests for patients with colorectal cancer. *JAMA Oncol.* 4:806–13
34. Haraldsdottir S, Hampel H, Tomsic J, Frankel WL, Pearlman R, et al. 2014. Colon and endometrial cancers with mismatch repair deficiency can arise from somatic, rather than germline, mutations. *Gastroenterology* 147:1308–16.e1
35. Haraldsdottir S, Hampel H, Wei L, Wu C, Frankel W, et al. 2014. Prostate cancer incidence in males with Lynch syndrome. *Genet. Med.* 16:553–57
36. Harkness EF, Barrow E, Newton K, Green K, Clancy T, et al. 2015. Lynch syndrome caused by *MLH1* mutations is associated with an increased risk of breast cancer: a cohort study. *J. Med. Genet.* 52:553–56

37. Heald B, Plesec T, Liu X, Pai R, Patil D, et al. 2013. Implementation of universal microsatellite instability and immunohistochemistry screening for diagnosing Lynch syndrome in a large academic medical center. *J. Clin. Oncol.* 31:1336–40
38. Hendriks YM, de Jong AE, Morreau H, Tops CM, Vasen HF, et al. 2006. Diagnostic approach and management of Lynch syndrome (hereditary nonpolyposis colorectal carcinoma): a guide for clinicians. *CA Cancer J. Clin.* 56:213–25
39. Hill AL, Sumra KK, Russell MM, Yoo J, Ko CY, et al. 2015. A single institution experience in compliance with universal screening for Lynch syndrome in colorectal cancer. *J. Gastrointest. Surg.* 19:543–50
40. Jenkins MA, Hayashi S, O'Shea AM, Burgart LJ, Smyrk TC, et al. 2007. Pathology features in Bethesda guidelines predict colorectal cancer microsatellite instability: a population-based study. *Gastroenterology* 133:48–56
41. Kang SY, Park CK, Chang DK, Kim JW, Son HJ, et al. 2015. Lynch-like syndrome: characterization and comparison with EPCAM deletion carriers. *Int. J. Cancer* 136:1568–78
42. Kastrinos F, Ojha RP, Leenen C, Alvero C, Mercado RC, et al. 2016. Comparison of prediction models for Lynch syndrome among individuals with colorectal cancer. *J. Natl. Cancer Inst.* 108:djv308
43. Kastrinos F, Steyerberg EW, Balmana J, Mercado R, Gallinger S, et al. 2013. Comparison of the clinical prediction model $PREMM_{1,2,6}$ and molecular testing for the systematic identification of Lynch syndrome in colorectal cancer. *Gut* 62:272–79
44. Kastrinos F, Steyerberg EW, Mercado R, Balmana J, Holter S, et al. 2011. The $PREMM_{1,2,6}$ model predicts risk of *MLH1*, *MSH2*, and *MSH6* germline mutations based on cancer history. *Gastroenterology* 140:73–81
45. Kato A, Sato N, Sugawara T, Takahashi K, Kito M, et al. 2016. Isolated loss of PMS2 immunohistochemical expression is frequently caused by heterogenous *MLH1* promoter hypermethylation in Lynch syndrome screening for endometrial cancer patients. *Am. J. Surg. Pathol.* 40:770–76
46. Ladabaum U, Wang G, Terdiman J, Blanco A, Kuppermann M, et al. 2011. Strategies to identify the Lynch syndrome among patients with colorectal cancer: a cost-effectiveness analysis. *Ann. Intern. Med.* 155:69–79
47. Laghi L, Malesci A. 2012. Microsatellite instability and therapeutic consequences in colorectal cancer. *Dig. Dis.* 30:304–9
48. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, et al. 2015. PD-1 blockade in tumors with mismatch-repair deficiency. *N. Engl. J. Med.* 372:2509–20
49. Lichtenstein P, Holm NV, Verkasalo PK, Iliadou A, Kaprio J, et al. 2000. Environmental and heritable factors in the causation of cancer–analyses of cohorts of twins from Sweden, Denmark, and Finland. *N. Engl. J. Med.* 343:78–85
50. Lindor NM, Rabe K, Petersen GM, Haile R, Casey G, et al. 2005. Lower cancer incidence in Amsterdam-I criteria families without mismatch repair deficiency: familial colorectal cancer type X. *JAMA* 293:1979–85
51. Lynch HT, de la Chapelle A. 2003. Hereditary colorectal cancer. *N. Engl. J. Med.* 348:919–32
52. Lynch HT, Lynch JF, Lynch PM. 2007. Toward a consensus in molecular diagnosis of hereditary nonpolyposis colorectal cancer (Lynch syndrome). *J. Natl. Cancer Inst.* 99:261–63
53. Lynch HT, Lynch PM, Pester J, Fusaro RM. 1981. The cancer family syndrome. Rare cutaneous phenotypic linkage of Torre's syndrome. *Arch. Intern. Med.* 141:607–11
54. Mandelker D, Zhang L, Kemel Y, Stadler ZK, Joseph V, et al. 2017. Mutation detection in patients with advanced cancer by universal sequencing of cancer-related genes in tumor and normal DNA versus guideline-based germline testing. *JAMA* 318:825–35
55. Mas-Moya J, Dudley B, Brand RE, Thull D, Bahary N, et al. 2015. Clinicopathological comparison of colorectal and endometrial carcinomas in patients with Lynch-like syndrome versus patients with Lynch syndrome. *Hum. Pathol.* 46:1616–25
56. McGivern A, Wynter CV, Whitehall VL, Kambara T, Spring KJ, et al. 2004. Promoter hypermethylation frequency and *BRAF* mutations distinguish hereditary non-polyposis colon cancer from sporadic MSI-H colon cancer. *Fam. Cancer* 3:101–7

57. McMeekin DS, Tritchler DL, Cohn DE, Mutch DG, Lankes HA, et al. 2016. Clinicopathologic significance of mismatch repair defects in endometrial cancer: an NRG oncology/gynecologic oncology group study. *J. Clin. Oncol.* 34:3062–68
58. Mensenkamp AR, Vogelaar IP, van Zelst-Stams WA, Goossens M, Ouchene H, et al. 2014. Somatic mutations in *MLH1* and *MSH2* are a frequent cause of mismatch-repair deficiency in Lynch syndrome-like tumors. *Gastroenterology* 146:643–46.e8
59. Mills AM, Sloan EA, Thomas M, Modesitt SC, Stoler MH, et al. 2015. Clinicopathologic comparison of Lynch syndrome-associated and "Lynch-like" endometrial carcinomas identified on universal screening using mismatch repair protein immunohistochemistry. *Am. J. Surg. Pathol.* 40:155–65
60. Mork ME, You YN, Ying J, Bannon SA, Lynch PM, et al. 2015. High prevalence of hereditary cancer syndromes in adolescents and young adults with colorectal cancer. *J. Clin. Oncol.* 33:3544–49
61. Mvundura M, Grosse SD, Hampel H, Palomaki GE. 2010. The cost-effectiveness of genetic testing strategies for Lynch syndrome among newly diagnosed patients with colorectal cancer. *Genet. Med.* 12:93–104
62. Natl. Compr. Cancer Netw. 2018. *NCCN Guidelines for colon cancer*. Version 1.2018, accessed July 24, 2018. Available at **https://www.nccn.org/professionals/physician_gls**
63. Natl. Compr. Cancer Netw. 2018. *NCCN Guidelines for genetic/familial high-risk assessment: colorectal*. Version 1.2018, accessed July 24, 2018. Available at **https://www.nccn.org/professionals/physician_gls**
64. Niu B, Ye K, Zhang Q, Lu C, Xie M, et al. 2014. MSIsensor: microsatellite instability detection using paired tumor-normal sequence data. *Bioinformatics* 30:1015–16
65. Norquist BM, Harrell MI, Brady MF, Walsh T, Lee MK, et al. 2016. Inherited mutations in women with ovarian carcinoma. *JAMA Oncol.* 2:482–90
66. Overman MJ, Lonardi S, Wong KYM, Lenz HJ, Gelsomino F, et al. 2018. Durable clinical benefit with nivolumab plus ipilimumab in DNA mismatch repair-deficient/microsatellite instability-high metastatic colorectal cancer. *J. Clin. Oncol.* 36:773–79
67. Pal T, Akbari MR, Sun P, Lee JH, Fulp J, et al. 2012. Frequency of mutations in mismatch repair genes in a population-based study of women with ovarian cancer. *Br. J. Cancer* 107:1783–90
68. Palomaki GE, McClain MR, Melillo S, Hampel HL, Thibodeau SN. 2009. EGAPP supplementary evidence review: DNA testing strategies aimed at reducing morbidity and mortality from Lynch syndrome. *Genet. Med.* 11:42–65
69. Pearlman R, Frankel WL, Swanson B, Zhao W, Yilmaz A, et al. 2017. Prevalence and spectrum of germline cancer susceptibility gene mutations among patients with early-onset colorectal cancer. *JAMA Oncol.* 3:464–71
70. Pearlman R, Markow M, Knight D, Chen W, Arnold CA, et al. 2018. Two-stain immunohistochemical screening for Lynch syndrome in colorectal cancer may fail to detect mismatch repair deficiency. *Mod. Pathol.* 31:1891–900
71. Popat S, Hubner R, Houlston RS. 2005. Systematic review of microsatellite instability and colorectal cancer prognosis. *J. Clin. Oncol.* 23:609–18
72. Pritchard CC, Morrissey C, Kumar A, Zhang X, Smith C, et al. 2014. Complex *MSH2* and *MSH6* mutations in hypermutated microsatellite unstable advanced prostate cancer. *Nat. Commun.* 5:4988
73. Ring KL, Bruegl AS, Allen BA, Elkin EP, Singh N, et al. 2016. Germline multi-gene hereditary cancer panel testing in an unselected endometrial cancer cohort. *Mod. Pathol.* 29:1381–89
74. Roberts ME, Jackson SA, Susswein LR, Zeinomar N, Ma X, et al. 2018. *MSH6* and *PMS2* germline pathogenic variants implicated in Lynch syndrome are associated with breast cancer. *Genet. Med.* 20:1167–74
75. Robinson D, Van Allen EM, Wu YM, Schultz N, Lonigro RJ, et al. 2015. Integrative clinical genomics of advanced prostate cancer. *Cell* 162:454
76. Rodriguez-Bigas MA, Boland CR, Hamilton SR, Henson DE, Jass JR, et al. 1997. A National Cancer Institute workshop on hereditary nonpolyposis colorectal cancer syndrome: meeting highlights and Bethesda guidelines. *J. Natl. Cancer Inst.* 89:1758–62
77. Rodríguez-Soler M, Pérez-Carbonell L, Guarinos C, Zapater P, Castillejo A, et al. 2013. Risk of cancer in cases of suspected Lynch syndrome without germline mutation. *Gastroenterology* 144:926–32.e1

78. Salipante SJ, Scroggins SM, Hampel HL, Turner EH, Pritchard CC. 2014. Microsatellite instability detection by next generation sequencing. *Clin. Chem.* 60:1192–99
79. Sargent DJ, Marsoni S, Monges G, Thibodeau SN, Labianca R, et al. 2010. Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. *J. Clin. Oncol.* 28:3219–26
80. Sargent DJ, Shi Q, Yothers G, Tejpar S, Bertagnolli MM, et al. 2014. Prognostic impact of deficient mismatch repair (dMMR) in 7,803 stage II/III colon cancer (CC) patients (pts): a pooled individual pt data analysis of 17 adjuvant trials in the ACCENT database. *J. Clin. Oncol.* 32:3507
81. Schwark AL, Srinivasan P, Kernel Y, Shia J, Bandlamudi C, et al. 2018. Pan-cancer microsatellite instability to predict for presence of Lynch syndrome. *J. Clin. Oncol.* 36(Suppl.):LBA1509
82. Senter L. 2012. Genetic testing by cancer site: colon (nonpolyposis syndromes). *Cancer J.* 18:334–37
83. Shaikh T, Handorf EA, Meyer JE, Hall MJ, Esnaola NF. 2018. Mismatch repair deficiency testing in patients with colorectal cancer and nonadherence to testing guidelines in young adults. *JAMA Oncol.* 4:e173580
84. Shia J. 2008. Immunohistochemistry versus microsatellite instability testing for screening colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome. Part I. The utility of immunohistochemistry. *J. Mol. Diagnostics* 10:293–300
85. Shiovitz S, Copeland WK, Passarelli MN, Burnett-Hartman AN, Grady WM, et al. 2014. Characterisation of familial colorectal cancer Type X, Lynch syndrome, and non-familial colorectal cancer. *Br. J. Cancer* 111:598–602
86. Sinicrope FA, Foster NR, Thibodeau SN, Marsoni S, Monges G, et al. 2011. DNA mismatch repair status and colon cancer recurrence and survival in clinical trials of 5-fluorouracil-based adjuvant therapy. *J. Natl. Cancer Inst.* 103:863–75
87. Sinicrope FA, Mahoney MR, Smyrk TC, Thibodeau SN, Warren RS, et al. 2013. Prognostic impact of deficient DNA mismatch repair in patients with stage III colon cancer from a randomized trial of FOLFOX-based adjuvant chemotherapy. *J. Clin. Oncol.* 31:3664–72
88. Sourrouille I, Coulet F, Lefevre JH, Colas C, Eyries M, et al. 2013. Somatic mosaicism and double somatic hits can lead to MSI colorectal tumors. *Fam. Cancer* 12:27–33
89. South CD, Hampel H, Comeras I, Westman JA, Frankel WL, de la Chapelle A. 2008. The frequency of Muir-Torre syndrome among Lynch syndrome families. *J. Natl. Cancer Inst.* 100:277–81
90. Takeda T, Tsuji K, Banno K, Yanokura M, Kobayashi Y, et al. 2018. Screening for Lynch syndrome using risk assessment criteria in patients with ovarian cancer. *J. Gynecol. Oncol.* 29:e29
91. Therkildsen C, Ladelund S, Smith-Hansen L, Lindberg LJ, Nilbert M. 2017. Towards gene- and gender-based risk estimates in Lynch syndrome; age-specific incidences for 13 extra-colorectal cancer types. *Br. J. Cancer* 117:1702–10
92. Umar A, Boland CR, Terdiman JP, Syngal S, de la Chapelle A, et al. 2004. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. *J. Natl. Cancer Inst.* 96:261–68
93. Van Cutsem E, Labianca R, Bodoky G, Barone C, Aranda E, et al. 2009. Randomized phase III trial comparing biweekly infusional fluorouracil/leucovorin alone or with irinotecan in the adjuvant treatment of stage III colon cancer: PETACC-3. *J. Clin. Oncol.* 27:3117–25
94. Vanderwalde A, Spetzler D, Xiao N, Gatalica Z, Marshall J. 2018. Microsatellite instability status determined by next-generation sequencing and compared with PD-L1 and tumor mutational burden in 11,348 patients. *Cancer Med.* 7:746–56
95. Vasen HF. 2005. Clinical description of the Lynch syndrome [hereditary nonpolyposis colorectal cancer (HNPCC)]. *Fam. Cancer* 4:219–25
96. Vasen HF, Mecklin JP, Khan PM, Lynch HT. 1991. The International Collaborative Group on Hereditary Non-Polyposis Colorectal Cancer (ICG-HNPCC). *Dis. Colon Rectum* 34:424–25
97. Vasen HF, Watson P, Mecklin JP, Lynch HT. 1999. New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the International Collaborative Group on HNPCC. *Gastroenterology* 116:1453–56

98. Vilar E, Gruber SB. 2010. Microsatellite instability in colorectal cancer—the stable evidence. *Nat. Rev. Clin. Oncol.* 7:153–62
99. Vilar E, Mork ME, Cuddy A, Borras E, Bannon SA, et al. 2014. Role of microsatellite instability-low as a diagnostic biomarker of Lynch syndrome in colorectal cancer. *Cancer Genet.* 207:495–502
100. Walsh MD, Buchanan DD, Cummings MC, Pearson SA, Arnold ST, et al. 2010. Lynch syndrome-associated breast cancers: clinicopathologic characteristics of a case series from the colon cancer family registry. *Clin. Cancer Res.* 16:2214–24
101. Weissman SM, Burt R, Church J, Erdman S, Hampel H, et al. 2012. Identification of individuals at risk for Lynch syndrome using targeted evaluations and genetic testing: National Society of Genetic Counselors and the Collaborative Group of the Americas on Inherited Colorectal Cancer joint practice guideline. *J. Genet. Couns.* 21:484–93
102. Win AK, Lindor NM, Jenkins MA. 2013. Risk of breast cancer in Lynch syndrome: a systematic review. *Breast Cancer Res.* 15:R27
103. Win AK, Lindor NM, Young JP, Macrae FA, Young GP, et al. 2012. Risks of primary extracolonic cancers following colorectal cancer in lynch syndrome. *J. Natl. Cancer Inst.* 104:1363–72
104. Wu Y, Berends MJ, Mensink RG, Kempinga C, Sijmons RH, et al. 1999. Association of hereditary non-polyposis colorectal cancer-related tumors displaying low microsatellite instability with *MSH6* germline mutations. *Am. J. Hum. Genet.* 65:1291–98
105. Zhang L. 2008. Immunohistochemistry versus microsatellite instability testing for screening colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome. Part II. The utility of microsatellite instability testing. *J. Mol. Diagn.* 10:301–7