主旨：新增免疫組織化學染色 MLH1、PMS2、MSH2、MSH6

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Detect MMR defect in colorectal cancer.

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Defective Mismatch Repair As a Predictive Marker for Lack of Efficacy of Fluorouracil-Based Adjuvant Therapy in Colon Cancer


See accompanying editorials on page 3207 and 3210

ABSTRACT

Purpose
Prior reports have indicated that patients with colon cancer who demonstrate high-level microsatellite instability (MSI-H) or defective DNA mismatch repair (dMMR) have improved survival and receive no benefit from fluorouracil (FU)-based adjuvant therapy compared with patients who have microsatellite-stable or proficient mismatch repair (pMMR) tumors. We examined MMR status as a predictor of adjuvant therapy benefit in patients with stages II and III colon cancer.

Methods
MSI assay or immunohistochemistry for MMR proteins were performed on 457 patients who were previously randomly assigned to FU-based therapy (either FU + levamisole or FU + leucovorin; n = 229) versus no post-surgical treatment (n = 228). Data were subsequently pooled with data from a previous analysis. The primary end point was disease-free survival (DFS).

Results
Overall, 70 (15%) of 457 patients exhibited dMMR. Adjuvant therapy significantly improved DFS (hazard ratio [HR], 0.67; 95% CI, 0.48 to 0.93; P = .02) in patients with pMMR tumors. Patients with dMMR tumors receiving FU had no improvement in DFS (HR, 1.10; 95% CI, 0.42 to 2.91; P = .88) compared with those randomly assigned to surgery alone. In the pooled data set of 1,027 patients (n = 165 with dMMR), these findings were maintained in patients with stage II disease and with dMMR tumors, treatment was associated with reduced overall survival (HR, 2.95; 95% CI, 1.02 to 8.54; P = .04).

Conclusion
Patient stratification by MMR status may provide a more tailored approach to colon cancer adjuvant therapy. These data support MMR status assessment for patients being considered for FU therapy alone and consideration of MMR status in treatment decision making.

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INTRODUCTION

Colorectal cancer (CRC) is diagnosed in approximately 145,000 individuals annually in the United States. Since the early 1990s, adjuvant therapy with fluorouracil (FU) and levamisole, and later with levorixan in treatment of stage III and selected stage II colon cancers.1-4 Adding oxaliplatin to FU-based therapy additionally improves disease-free and overall survival in patients with stage III disease,5; however, overall survival benefit in unselected patients with stage II disease is present from adding oxaliplatin.6

Pathologic tumor staging remains the key determinant of CRC prognosis and treatment. However, considerable stage-independent outcome variability is observed that likely reflects molecular heterogeneity, which underscores the need for robust prognostic and predictive markers. Although the majority of CRCs develop via a chromosomal instability pathway, approximately 15% have defective DNA mismatch repair (MMR).8 Defective MMR (dMMR) has frequently been measured by either the presence of microsatellite instability (MSI)9 or by testing for loss of the protein products for genes involved in DNA mismatch repair, most commonly MLH1, MSH2, MSH6, and PMS2. CRCs with dMMR have distinctive features that include proximal colon predominance, poor differentiation and/or mucinous histology, intra- and peritumoral...
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A Practical Approach to the Evaluation of Gastrointestinal Tract Carcinomas for Lynch Syndrome

Rish K. Pai, MD, PhD* and Reetesh K. Pai, MD†

Abstract: Lynch syndrome accounts for roughly 1 of every 35 patients with colorectal carcinoma, making it the most common hereditary form of colorectal carcinoma. Identifying patients at risk for Lynch syndrome is essential, as these patients can develop additional Lynch syndrome–related tumors, and patients and their relatives benefit from genetic counseling. The hallmark of Lynch syndrome–associated neoplasms is DNA mismatch repair protein deficiency. In most instances, the pathologist is the first to identify patients at risk for Lynch syndrome and is tasked with communicating these results to treating clinicians and genetics counselors. This review will attempt to provide the tools for pathologists to identify patients at risk for Lynch syndrome through evaluation of tumors of the gastrointestinal tract and provide up-to-date knowledge on evaluating mismatch repair protein deficiency in tumors of the gastrointestinal tract.

Key Words: Lynch syndrome, colorectal carcinoma, MSI, BRAF, mismatch repair protein, immunohistochemistry, HNPCC, Lynch-like syndrome

An estimated 130,000 individuals are diagnosed with colorectal carcinoma each year, and approximately 50,000 will die from this disease, making colorectal carcinoma the third leading cause of cancer-related death in the United States. Lynch syndrome is defined by the presence of a deleterious germline alteration in either a DNA mismatch repair (MMR) gene (MLH1, PMS2, MSH2, and MSH6) or deletions in EPCAM and is an autosomal dominant condition that predisposes to the development of tumors, most notably carcinomas of the colon, rectum, and endometrium. Roughly, 1 of every 35 patients with colorectal carcinoma has Lynch syndrome, making Lynch syndrome the most common hereditary form of colorectal carcinoma. Identifying patients with Lynch syndrome is essential as these patients and their family members may be at risk for developing Lynch syndrome–related tumors. Patients with Lynch syndrome and their affected relatives also benefit from close surveillance, which facilitates early cancer detection and intervention, which decreases disease-specific mortality.

The hallmark of Lynch syndrome–associated neoplasms is MMR protein deficiency, manifested by either high-level microsatellite instability (MSI) identified by polymerase chain reaction (PCR) techniques or by demonstration of loss of MMR protein expression within a neoplasm by immunohistochemistry. There is a continuing requirement for pathologists to play an increasing role in the identification of patients at risk for Lynch syndrome. In most instances, the pathologist is the first to identify these patients and is tasked with communicating these results to treating clinicians and genetics counselors. However, in some cases, pathologists may not feel fully proficient in explaining the implications of these findings. In addition, the terminology and definitions for conditions associated with MMR protein deficiency have undergone significant refinement over the last few years. In this review, particular emphasis will be given to evolving concepts in Lynch syndrome diagnostics (Table 1), including the growing clinical problem of individuals with Lynch-like syndrome, constitutional MMR protein deficiency, and the importance of MMR protein expression patterns in tumors in informing strategies for germline MMR and EPCAM gene–sequencing efforts. This review will also attempt to provide the tools for pathologists to identify patients at risk for Lynch syndrome and to develop optimal screening strategies and algorithms for detecting patients at risk for Lynch syndrome in the setting of colorectal carcinoma and other tumors of the gastrointestinal tract.

SELECTING A SCREENING STRATEGY FOR LYNNCH SYNDROME: FROM MORPHOLOGY TO UNIVERSAL SCREENING

The optimal strategy for screening patients with colorectal carcinoma for Lynch syndrome is a subject of continued debate in the literature. Algorithms based solely on family history are time-consuming and impractical for most physicians. The histopathologic features of colorectal carcinomas with MMR protein deficiency have been well described and include increased tumor-infiltrating...
TABLE 1. Terminology and Definitions

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<td>Lynch syndrome (MIM No. 120435)</td>
<td>Presence of deleterious monoallelic germline defect in MMR genes including: 1. Germline mutations in MLH1, PMS2, MSH2, and MSH6 2. Inactivation of MSH2 due to deletion of 3’ exons of the EPCAM (TACSTD1) gene 3. Germline methylation of the MLH1 promoter (also called constitutional MLH1 inactivation) The genetic definition of Lynch syndrome encompasses Muir-Torre syndrome (MIM No. 158320) defined by the association of sebaceous gland tumors with a Lynch syndrome-associated internal malignancy alterations. The genetic basis for this condition is unknown 1. Germline mutations in MMR genes 2. Failure to detect germline MMR gene alteration using currently available testing methods 3. Incorrect interpretation of MMR immunohistochemistry results 4. Other germline gene defects, such as biallelic MUTYH mutation</td>
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<td>HNPCC</td>
<td>Clinical term for patients with carcinoma that fulfill the following Amsterdam I or II clinical criteria: 1. Three or more family members (one of whom is a first-degree relative of the other 2) with colorectal carcinoma (Amsterdam I) or HNPCC-related cancers (Amsterdam II), which include colorectal, endometrial, stomach, small intestinal, hepatobiliary, renal pelvis, or ureteral cancers 2. At least 2 successive affected generations 3. At least one of the HNPCC-related cancers diagnosed before the age of 50 4. Familial adenomatous polyposis (FAP, MIM No. 175100) should be excluded</td>
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<td>Lynch-like syndrome</td>
<td>Patients who have tumors demonstrating deficient MMR protein expression but have no deleterious germline alteration in MMR genes or EPCAM, and if the tumor is MLH1-deficient no evidence of a BRAF V600E mutation or MLH1 promoter hypermethylation within the tumor. Potential explanations for Lynch-like syndrome include: 1. Biallelic somatic (tumor) mutations in the affected MMR gene 2. Failure to detect germline MMR gene alteration using currently available testing methods 3. Incorrect interpretation of MMR immunohistochemistry results 4. Other germline gene defects, such as biallelic MUTYH mutation</td>
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<td>Familial colorectal carcinoma (type X)</td>
<td>Clinical term for patients with carcinoma that fulfill the following Amsterdam I clinical criteria but have tumors that do not harbor MMR protein deficiency and whose germline lacks MMR gene or EPCAM alterations. The genetic basis for this condition is unknown</td>
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<td>Constitutional mismatch repair deficiency (MIM No. 276300)</td>
<td>Presence of biallelic germline mutations in MMR genes. This disease is very rare and is associated with the selective approach of screening for the detection of Lynch syndrome–associated colorectal carcinomas. Moreira and colleagues also analyzed 2 additional screening approaches: (1) screening all patients with colorectal carcinoma diagnosed in patients younger than 70 years based on recommendations from a 2008 workshop in Jerusalem and (2) a selective approach of screening all patients with colorectal carcinoma diagnosed in patients younger than 70 years and in those above 70 years fulfilling the revised Bethesda guidelines. The Jerusalem recommendations had a sensitivity of only 85.4%, and the selective approach had a sensitivity of 95.1% in the detection of patients with Lynch syndrome. Finally, histology-based models, such as MsPath and PREDICT, have been proposed to predict MMR protein deficiency in colorectal carcinoma. Although the MsPath and PREDICT models are an improvement over the revised Bethesda guidelines, they were not specifically developed to detect Lynch syndrome–associated colorectal carcinomas. Both place heavy emphasis on tumor location, and a recent study demonstrated that a substantial percentage of Lynch syndrome–associated colorectal carcinomas are located in the distal colon or rectum. Thus, although histology can be helpful in identifying colorectal carcinomas with MMR protein deficiency, models that rely solely on histology may be less sensitive in detecting Lynch syndrome–associated colorectal carcinoma. Major professional medical organizations have issued Lynch syndrome–screening guidelines for patients with colorectal carcinoma (Table 3). Universal screening of all patients with colorectal carcinoma is recommended by the Evaluation of Genomic Applications in Practice and Prevention (a working group sponsored by the Centers for Disease Control), the US Multi-Society Task Force for colorectal cancer, and the American College of Gastroenterology. The National Comprehensive Cancer Network, American Society of Clinical Oncology, and European Society of Medical Oncology guidelines also endorsed universal screening of all patients with colorectal carcinoma; however, the basis of the findings of Moreira et al, these organizations also state that an alternate strategy of screening all patients with colorectal carcinoma under the age of 70 years and those over the age of 70 years who meet the revised Bethesda guidelines is acceptable. Although somewhat cost-intensive, modeling studies have shown that universal screening is cost-effective with a favorable cost-effectiveness ratio per life-year saved.</td>
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SCREENING ALGORITHM FOR LYNCH SYNDROME IN COLORECTAL CARCINOMA

Figure 2 details a screening algorithm for Lynch syndrome in colorectal carcinoma using MMR protein immunohistochemistry as the initial screening method. The details of each individual test are described below.

MMR Protein Immunohistochemistry and its Pitfalls

Early reports initially suggested that MMR protein immunohistochemistry was inferior to MSI PCR as a screening tool for Lynch syndrome. However, these early studies primarily assessed MLH1 and MSH2 immunohistochemistry without using antibodies directed against MSH6 and PMS2. More recent literature has demonstrated that the use of all 4 antibodies (MLH1, MSH2, MSH6, and PMS2) has a high sensitivity (ranging from 93% to 100%) for detecting high-level MSI and for predicting MMR gene mutation. Knowledge of the basic biology of MMR proteins helps to better understand the immunohistochemical staining patterns often observed in tumors with MMR protein deficiency. In their functional state within a cell, MLH1 dimerizes with PMS2, and MSH2 dimerizes with MSH6. MLH1 and MSH2 are the obligatory partners for their respective heterodimers. In general, mutations in MLH1 and MSH2 result in proteolytic degradation of their binding partners, PMS2 and MSH6, respectively. In contrast, mutations in MSH6 and PMS2 do not result in proteolytic degradation of their obligatory partners, as other MMR proteins have overlapping function with those of PMS2 and MSH6. Thus, a cancer from a patient with a germline mutation in MSH2 typically exhibits loss of expression of both MSH2 and MSH6, whereas a cancer from a patient with a germline MSH6 mutation exhibits isolated loss of MSH6 expression.

Given the biology of MMR proteins, a number of studies have analyzed an initial screening approach using a 2-antibody panel including only PMS2 and MSH6. These studies have demonstrated that this 2-antibody
approach is as effective as using the 4-antibody panel for detecting patients with colorectal carcinoma at risk for Lynch syndrome.22–24 MMR protein immunohistochemistry using either a 2-antibody or a 4-antibody screening approach is acceptable. Most institutions still use the 4-antibody MMR protein immunohistochemistry panel for Lynch syndrome screening in colorectal carcinoma, as PMS2 and MSH6 immunohistochemistry can be technically challenging to optimize and can at times be difficult to interpret.

There are 3 common patterns of MMR protein immunohistochemical expression: diffuse preserved (intact) expression; complete loss of expression; and heterogeneous expression (Fig. 3). When reporting MMR protein immunohistochemistry results, it is important to avoid terms such as “positive” and “negative,” as they are potentially confusing. Instead, terminology such as preserved (or intact) or loss of expression is preferred. Most colorectal

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**TABLE 2.** The Revised Bethesda Guidelines for Testing Colorectal Carcinomas for Mismatch Repair Protein Deficiency* 

| Tumor from individuals should be tested for mismatch repair protein deficiency in the following situations: |
| 1. Colorectal carcinoma diagnosed in a patient who is below 50 y of age |
| 2. Presence of synchronous, metachronous colorectal, or other Lynch syndrome–associated tumors†, regardless of age |
| 3. Colorectal carcinoma with MSI-H histology‡ diagnosed in a patient who is below 60 y of age§ |
| 4. Colorectal carcinoma diagnosed in one or more first-degree relatives with a Lynch syndrome–related tumor, with one of the cancers being diagnosed under age 50 y |
| 5. Colorectal carcinoma diagnosed in 2 or more first-degree or second-degree relatives with Lynch syndrome–related tumors, regardless of age |

*Proposed by Umar et al.2  
†Lynch syndrome–related tumors include colorectal, endometrial, stomach, ovarian, pancreas, ureter and renal pelvis, biliary tract, brain tumors (usually glioblastoma), sebaceous tumors of the skin, keratoacanthomas, and carcinoma of the small bowel.  
‡Presence of tumor-infiltrating lymphocytes, Crohn’s-like lymphocytic reaction, mucinous/signet ring cell differentiation, or medullary growth pattern.  
§There was no consensus among the Workshop participants on whether to include the age criteria in guideline 3 above; participants voted to keep below 60 years of age in the guidelines.  
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**FIGURE 2.** A screening algorithm for Lynch syndrome in colorectal carcinoma using MMR protein immunohistochemistry as the initial screening test. A, With a universal screening approach, every patient with colorectal carcinoma will undergo MMR protein immunohistochemical analysis using either a 4-antibody (MLH1, PMS2, MSH2, and MSH6) or a 2-antibody (PMS2 and MSH6) approach. Most tumors will demonstrate preserved expression of all tested MMR proteins and will require no further testing. Patients with tumors demonstrating concurrent loss of MSH2 and MSH6, isolated loss of MSH6, or isolated loss of PMS2 expression should be referred for genetic counseling and germline MMR gene and/or EPCAM mutation testing. Patients with tumors demonstrating loss of MLH1 and PMS2 expression should have reflex BRAF mutation testing and, if BRAF wild-type, MLH1 promoter hypermethylation testing of the tumor with subsequent referral to genetic counseling if negative for MLH1 promoter hypermethylation. In the setting of positive MLH1 promoter hypermethylation studies, some authors argue for evaluation for constitutional MLH1 epimutation if the patient is below 60 years of age and/or if there is a strong personal or family history of Lynch syndrome–associated malignancy. B, If a deleterious germline MMR gene or EPCAM mutation is identified, the patient has Lynch syndrome. If no deleterious germline mutation in an MMR gene or EPCAM is identified, the patient has “Lynch-like” syndrome. Rereview of the original MMR protein immunohistochemistry should be performed to confirm loss of MMR protein expression within the patient’s tumor. Somatic MMR gene mutation may be of benefit. If biallelic somatic MMR gene mutation is identified, the patient does not have Lynch syndrome and can likely be screened as for sporadic colorectal carcinoma. If there is no evidence of biallelic somatic mutation, there is uncertainty as to whether the patient has Lynch syndrome. Most patients with Lynch-like syndrome lacking biallelic somatic mutation are counseled to follow a screening protocol as if they have confirmed Lynch syndrome given the uncertainty in the diagnosis.
carcinomas will demonstrate diffuse, strong nuclear expression of MMR proteins by immunohistochemistry and can be labeled as having preserved expression for MMR proteins. Loss of expression for MMR proteins is defined as complete absence of nuclear staining within tumor cells exhibiting nuclear expression within tumor cells. Care must be taken to not misinterpret nuclear staining within tumor-infiltrating lymphocytes as tumor cells exhibiting nuclear expression within tumor cells.

There are many pitfalls in MMR protein immunohistochemistry interpretation, particularly with regard to aberrant staining patterns and variability in

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**A)**

- **Patient with Colorectal Carcinoma**
  - MMR IHC
    - Preserved Expression of all 4 MMR proteins
      - Referral to Genetic Counseling
    - Loss of MLH1 expression
      - BRAF Mutation Testing
      - Wild-type BRAF
        - Positive for MLH1 Promoter Hypermethylation in Tumor
        - MLH1 Promoter Hypermethylation Analysis of Tumor
        - No further testing required
    - Loss of MSH2/MSH6, Isolated loss of MSH6, or Isolated loss of PMS2
      - Referral to Genetic Counseling
      - Negative for MLH1 Promoter Hypermethylation in Tumor

**B)**

- **Germline MMR Gene and/or EPCAM Mutation Testing**
  - Deleterious MMR gene or EPCAM germline mutation identified
    - Lynch Syndrome Confirmed
  - NO Deleterious MMR gene or EPCAM germline mutation identified
    - "Lynch-Like" Syndrome
      - Confirm Original MMR IHC Results
      - Screening as for sporadic carcinoma
      - Biallelic Somatic Mutation Present
      - Biallelic Somatic Mutation Absent
      - Somatic MMR Gene Mutation Testing of Tumor
staining intensity. Variation in staining patterns has resulted in a less than perfect interobserver agreement in the interpretation of MMR protein immunohistochemical stains.\textsuperscript{19,25} Aberrant staining patterns include punctate/speckled nuclear staining, nucleolar staining,\textsuperscript{26} and nuclear membrane staining (Figs. 3, 4). In most cases, these staining patterns are a result of technical issues and should not be interpreted as preserved (intact) expression of MMR proteins. Weak staining may also be a diagnostic challenge. Comparison with the internal control is often helpful to point to technical issues. However, in some cases, weak MLH1 staining may actually be a result of an MLH1 gene mutation.\textsuperscript{19}

The most common heterogenous pattern is the absence of MMR protein expression in scattered tumor cells in a background of strong, diffuse expression within the remainder of the tumor (intratumoral heterogeneity). Tissue hypoxia and fixation issues may account for this particular pattern of expression.\textsuperscript{27,28} Neoadjuvant chemoradiation may also reduce the expression of MMR

FIGURE 3. Typical patterns of expression of MMR proteins by immunohistochemistry in colorectal carcinoma. Most colorectal carcinomas will demonstrate diffuse, strong nuclear expression of MMR proteins by immunohistochemistry and can be labeled as having preserved expression for MMR proteins (A, MSH2 immunohistochemistry). Loss of expression for MMR proteins is defined as complete absence of nuclear staining within tumor cells with concurrent positive labeling in internal non-neoplastic tissues. Care must be taken to not misinterpret nuclear staining within tumor-infiltrating lymphocytes as tumor cells exhibiting nuclear expression within tumor cells. This is particularly important for colorectal carcinomas with prominent tumor-infiltrating lymphocytes, such as this case of medullary carcinoma that demonstrates loss of PMS2 expression within tumor cells with concurrent strong nuclear expression within tumor-infiltrating lymphocytes (B). Patchy, heterogenous expression of MMR proteins within colorectal carcinoma can be seen by immunohistochemistry. This is particularly common with MSH6 immunohistochemistry (C). Heterogenous staining should not be interpreted as evidence of loss of MMR protein expression. If >10% of tumor nuclei demonstrate convincing and strong nuclear expression, the tumor should be labeled as demonstrating preserved MMR protein expression. In some instances, nuclear staining that may be weak is observed in <10% of tumor nuclei (D, MSH6). In such cases, the MMR protein immunohistochemistry staining should be interpreted as equivocal. Microsatellite instability polymerase chain reaction analysis of the tumor and/or genetic counseling may be of benefit for patients with equivocal MMR expression within their tumor.
proteins. Bao et al.\textsuperscript{29} compared immunohistochemical expression of MMR proteins in pretreatment biopsies and posttreatment resections in 51 rectal carcinomas. Decreased MSH6 expression was seen in 20\% of posttreatment specimens compared with the pretreatment biopsies.\textsuperscript{29} In some cases, up to 90\% of tumor cell nuclei had loss of MSH6 expression despite demonstrating intact expression in the pretreatment biopsy. More recently, Vilkin et al.\textsuperscript{30} demonstrated decreased expression of PMS2 in 30\% of posttreatment rectal carcinomas. Decreased expression of MLH1, MSH6, and MSH2 was also seen in a small proportion of cases. Given these findings, most pathologists with experience in interpreting MMR protein immunohistochemistry regard convincingly strong expression of MMR proteins within >10\% of the tumor as indicative of preserved (intact) expression.

Zonal heterogeneity is another pattern occasionally seen in colorectal carcinomas\textsuperscript{31} and more recently described in endometrial carcinomas.\textsuperscript{32} In this pattern, large areas of tumor demonstrate abrupt loss of expression of a particular MMR protein (Fig. 4). Abrupt loss of MLH1 and PMS2 expression within large areas of a tumor is typically the result of hypermethylation of the \textit{MLH1} promoter within different parts of the tumor.\textsuperscript{31} This pattern of abnormal MLH1 and PMS2 expression is not associated with Lynch syndrome. Another frequently

\textbf{FIGURE 4.} Unusual patterns of immunohistochemical expression of MMR proteins in colorectal carcinoma. Punctate/speckled nuclear staining can be seen, particularly with MLH1 immunohistochemistry (A). This pattern of staining should not be interpreted as preserved MLH1 expression and is likely a technical issue with the MLH1 staining protocol. This pattern of punctate/speckled nuclear staining with MLH1 is often associated with loss of PMS2 expression within the tumor and with \textit{MLH1} promoter hypermethylation and \textit{BRAF} V600E mutation. Prominent and diffuse nucleolar MSH6 expression may also be seen (B) and should not be interpreted as evidence of preserved MSH6 expression. In cases of MSH6 nucleolar expression, microsatellite instability polymerase chain reaction analysis of the tumor and genetic counseling should be considered. Rare colorectal carcinomas may demonstrate distinct staining of the nuclear membrane of tumor cell nuclei without diffuse nuclear staining (C, courtesy of Dr Thomas Plesec, Cleveland Clinic). Nuclear membrane staining alone should not be interpreted as preserved MMR protein expression and is likely a technical issue with the staining protocol.
encountered scenario is loss of MSH6 expression in tumors that also demonstrate concurrent loss of MLH1 and/or PMS2 expression. The loss of MSH6 expression in such cases can be abrupt and seen in only parts of the tumor and, in most cases, is due to a secondary mutation in a coding mononucleotide microsatellite within the MSH6 gene as a result of MMR protein deficiency due to loss of MLH1 and/or PMS2 expression within the tumor.33

Practically, one should be cautious in issuing a report when an unusual pattern of MMR protein expression is seen. In cases with an aberrant pattern of expression (cytoplasmic, nuclear membrane, or nucleolar expression), repeating the immunohistochemistry, preferably on another tissue block, may be helpful. If a definitive result is still not obtained, performing MSI testing by PCR may be of use. Most cases with intratumoral heterogeneity can be safely interpreted as having preserved (intact) expression. When only rare tumor cells (<10% of tumor cell nuclei) demonstrate nuclear expression for a given MMR protein, MSI testing by PCR should also be considered. The rare cases with marked zonal heterogeneity or loss of an unusual combination of MMR proteins (eg, MLH1, PMS2, and MSH6) often need additional molecular testing. Testing for BRAF mutation and/or MLH1 promoter hypermethylation studies can help to exclude Lynch syndrome in these scenarios, although recently a case of Lynch syndrome with a deleterious MSH6 mutation and MLH1 promoter hypermethylation was described.34 Close communication with the patient’s treating clinicians and genetic counselor can also help guide the subsequent steps in these unusual situations.

The pattern of abnormal MMR protein expression within a patient’s tumor helps to direct germline mutation testing efforts in a patient. However, current guidelines on germline mutation testing are not entirely clear for the scenario of isolated loss of PMS2 immunohistochemical expression. Isolated loss of PMS2 expression accounts for approximately 4% of colorectal carcinomas with abnormal MMR protein expression. Recent data indicate that approximately 25% of patients with tumors demonstrating isolated loss of PMS2 immunohistochemical expression will harbor germline MLH1 mutations.35 A subset of germline MLH1 mutations results in functionally inactive MLH1 protein, which is antigenically intact and will be detected by commonly used anti-MLH1 antibody clones (Fig. 5). Most such MLH1 mutations are nontruncating point mutations or mutations in noncoding intervening sequences that result in decreased MLH1 protein stability and/or quantity, compromised stability of the MLH1-PMS2 complex, and subsequent PMS2 degradation. For such patients, falsely preserved MLH1 protein expression will be observed in a patient’s tumor; however, diminished or weak MLH1 staining intensity may be a clue that an underlying germline MLH1 mutation is the cause of the abnormal MMR protein expression. Thus, patients with colorectal carcinoma demonstrating isolated loss of PMS2 by immunohistochemistry should undergo germline MLH1 analysis if no mutations are detected through germline PMS2 testing.

**MSI PCR**

In 1997, the National Cancer Institute recommended a panel of 5 microsatellites, referred to as the Bethesda panel, which included 2 mononucleotide repeats (BAT25 and BAT26) and 3 dinucleotide repeats (D2S123, D5S346, and D17S250). The Bethesda panel is still one of the most widely used panels of microsatellites. However, many investigators have found mononucleotide markers to be more sensitive than dinucleotide markers for the detection of MSI and commercially available panels of quasimonomorphic mononucleotide markers are being increasingly used.36 On the basis of established criteria for the Bethesda panel of microsatellites, if no markers show instability, the tumor is classified as microsatellite stable. If ≥30% of the markers show instability by PCR, the tumor is classified as high-level MSI (MSI-H). Occasionally, when using the Bethesda panel, colorectal carcinoma may demonstrate instability in <30% of microsatellite markers. In such instances, the tumor can be designated as low-level MSI (MSI-L).37 Most MSI-L colorectal carcinoma will demonstrate preserved/intact MMR protein expression by immunohistochemistry.38 However, mutations in MSH6 primarily induce instability in mononucleotide and not dinucleotide repeats. A small subset of patients with tumors harboring MSI-L with the Bethesda panel of microsatellite markers may demonstrate loss of MSH6 expression within their tumor by immunohistochemistry and harbor a germline MSH6 mutation.38 Panels of mononucleotide markers have been shown to be more sensitive in detecting MSI-H caused by MSH6 mutations.39

Detection of MSI in colorectal carcinoma by using next-generation sequencing methodology has also been recently described.40 With increasing use of next-generation sequencing for detection of molecular mutations in colorectal carcinoma to predict response to certain therapy, next-generation sequencing methods to detect MSI may replace the current method of detecting MSI by conventional PCR techniques.40

**Selecting a Screening Test: MMR Protein Immunohistochemistry Versus MSI PCR**

Both MMR protein immunohistochemistry and MSI PCR are equally valid initial screening tests for Lynch syndrome. The decision of which screening test to use primarily depends on the availability of resources and expertise. However, MMR protein immunohistochemistry has several advantages over MSI PCR. As MSI PCR typically requires normal tissue for interpretation, it has limited utility in biopsies in which normal tissue is not sampled. More importantly, MMR protein immunohistochemistry helps direct germline mutation testing efforts, whereas MSI PCR does not. In addition, MMR protein immunohistochemistry allows for the distinction between sporadic and Lynch syndrome–associated neoplasms, as loss of expression of proteins other than MLH1 raises concern for Lynch syndrome. Figure 2 details a screening algorithm using MMR protein immunohistochemistry as the screening test. If MSI PCR is used as the initial screening test, the identification of MSI-H within a tumor typically requires
further evaluation of the tumor by MMR protein immunohistochemistry to determine which MMR proteins are deficient within the patient’s tumor.

**Sporadic MLH1 Deficiency in Colorectal Carcinoma From Promoter Hypermethylation**

Sporadic MMR protein deficiency accounts for approximately 10% to 15% of colorectal carcinoma and frequently arises due to hypermethylation of CpG islands in the \textit{MLH1} promoter. The majority of colorectal carcinomas with sporadic MMR protein deficiency occur in individuals above 60 years of age with an approximately 2 to 3:1 female predominance. Acquired methylation of both copies of the \textit{MLH1} promoter leads to \textit{MLH1} gene silencing and concurrent loss of MLH1 and PMS2 protein expression. The \textit{BRAF} V600E mutation is seen in approximately 50% to 70% of colorectal carcinomas with \textit{MLH1} promoter hypermethylation and is very seldom identified in tumors with Lynch syndrome. Thus, screening tumors with loss of MLH1 and PMS2 protein expression for a \textit{BRAF} V600E mutation is a simple way to exclude Lynch syndrome in patients (Fig. 2). If the tumor harbors the \textit{BRAF} V600E mutation, further counseling and testing for MMR gene mutation is not necessary, provided there is no strong family or personal history of Lynch syndrome–associated neoplasia. Importantly, the absence of a \textit{BRAF} V600E mutation does not imply Lynch syndrome. If a colorectal carcinoma is \textit{BRAF} wild-type, further testing for \textit{MLH1} promoter hypermethylation can be performed and, if positive, helps to exclude Lynch syndrome (Fig. 2). \textit{BRAF} mutation analysis is only helpful in the Lynch syndrome–screening algorithm of colorectal carcinoma and is not useful for extracolonic cancers. Extracolonic cancers of...
the gastrointestinal tract may harbor MLH1 promoter hypermethylation that is not associated with a BRAF V600E mutation.

A monoclonal antibody (VE1) targeting the BRAF V600E mutant protein has become available with variable efficacy in literature reports. Most studies have demonstrated that BRAF VE1 immunohistochemistry is sensitive for detecting colorectal carcinomas harboring a BRAF V600E mutation. However, there are reports of significant variability in staining with the BRAF VE1 antibody, particularly between new antibody lots purchased from the vendor or when new reagents were used in the assay. In addition, some colorectal carcinomas can demonstrate weak staining with BRAF VE1 immunohistochemistry but lack the BRAF V600E mutation on PCR. Given the variability of staining and the issue of weak, nonspecific staining in wild-type colorectal carcinomas, BRAF VE1 immunohistochemistry is not currently commonly used in Lynch syndrome diagnostics.

**Patient Follow-up of Abnormal Test Results**

Implementation of universal screening requires close cooperation and effective communication across multiple clinical disciplines. In most instances, the pathologist is the first to identify patients at risk for Lynch syndrome and is tasked with communicating these results to treating clinicians and genetics counselors. In 1 study, communication of the results solely to colorectal surgeons resulted in only 32% of patients at risk for Lynch syndrome undergoing genetic counseling. In contrast, communication of the results directly to genetics counselors resulted in 71% of patients at risk for Lynch syndrome undergoing genetic counseling. Before implementation of universal screening for Lynch syndrome in colorectal carcinoma, pathologists are strongly encouraged to establish close cooperation and effective strategies for identifying patients at risk for Lynch syndrome and establishing appropriate plans for patient contact and patient follow-up. In addition, if a patient at risk for Lynch syndrome is identified by a pathologist during universal screening, the communication of the results to treating clinicians and/or genetics counselors responsible for patient follow-up should be explicitly documented in the pathology report.

**ISSUES IN LYNCH SYNDROME DIAGNOSTICS**

**Definition of Lynch Syndrome and its Related Conditions**

Table 1 provides a summary of definitions for the often confusing and changing terminology used for Lynch syndrome and its related conditions. Lynch syndrome is defined by the presence of a deleterious germline alteration in either a DNA MMR gene (MLH1, PMS2, MSH2, and MSH6) or deletions in EPCAM. Lynch syndrome and hereditary nonpolyposis colorectal carcinoma (HNPPC) have been historically linked; however, these 2 conditions are not synonymous. HNPCC is a clinical term for patients with carcinoma that fulfill Amsterdam clinical criteria that are based solely on family history (Table 1). Between 50% and 60% of patients with HNPCC will have tumors that are MMR protein deficient. Approximately 40% of patients with HNPCC do not harbor MMR protein deficiency within their tumor or have a germline DNA MMR gene or EPCAM alteration and have been labeled as having familial colorectal carcinoma type X (FCCTX) (Table 1). Unlike Lynch syndrome, patients with FCCTX do not have an increased risk for extracolonic cancers but have a 2-fold increased risk for colorectal carcinoma over the general population. The genetic etiology of FCCTX is largely unknown, but candidate genes are currently under investigation.

**Lynch Syndrome Versus “Lynch-like” Syndrome**

Although loss of MMR protein expression within a patient’s tumor raises concern for Lynch syndrome, it should not be considered diagnostic of Lynch syndrome. Some patients have no evidence of a germline alteration in MMR genes or EPCAM despite harboring tumors with MMR protein deficiency. Lynch-like syndrome is a provisional term that has emerged in the literature to describe those patients who have tumors demonstrating deficient MMR protein expression but have no deleterious germline alteration in MMR genes or EPCAM and, if the tumor is MLH1 deficient, no evidence of a BRAF V600E mutation or MLH1 promoter hypermethylation within the tumor. This group of patients has also been termed “suspected Lynch syndrome” by others. The terms “Lynch-like syndrome” or “suspected Lynch syndrome” are probably not the best terms to describe patients who develop tumors with MMR protein deficiency through mechanisms other than germline MMR gene or EPCAM alteration or MLH1 promoter hypermethylation. However, for the purposes of this discussion, the term Lynch-like syndrome will be used to describe these patients.

Patients with Lynch-like syndrome represent a major challenge in Lynch syndrome screening as it is uncertain whether such patients should undergo the same intensive lifelong screening protocol used for patients with confirmed Lynch syndrome. The proportion of patients with Lynch-like syndrome among patients with tumors demonstrating MMR protein deficiency concerning for Lynch syndrome varies in the literature. A recent review by Buchanan et al found that up to 59% of colorectal carcinomas with MMR protein deficiency have been identified as having Lynch-like syndrome, with the proportion ranging from 56% to 71%. In our experience with universal screening in colorectal carcinoma, 32% of patients with colorectal carcinoma harboring abnormal MMR protein expression by immunohistochemistry within their tumor raising concern for Lynch syndrome have been negative for a germline mutation in MMR genes or EPCAM and have been classified as having Lynch-like syndrome.

There are a number of potential reasons for Lynch-like syndrome (Table 4). First, and easiest to evaluate, is misinterpretation of MMR protein immunohistochemistry.
When faced with a discrepancy between the MMR protein immunohistochemistry results and germline testing, it is prudent to reevaluate the MMR protein immunohistochemical stains and possibly repeat any stains that are equivocal to confirm the presence of abnormal MMR protein expression within the tumor. In 1 analysis of patients with Lynch-like syndrome, 19% were found to be the result of incorrect interpretation of MMR protein immunohistochemistry, with infrequent loss of heterozygosity of the other MMR gene.

Some patients with Lynch-like syndrome likely still have a germline mutation that current testing methods failed to detect. An excellent example is that of the recently identified inversion of MSH2 exons 1 to 7, which have been found to explain up to 60% of previously unexplained cases of Lynch syndrome due to MSH2 germ-line mutations. Inversion of MSH2 exons 1 to 7 has not been specifically analyzed by many commercial genetic laboratories or has only recently been added to the testing protocol of other laboratories. In addition, there have been reports of somatic mosaicism in which the patient harbors a deleterious mutation in only a portion of their cells that may explain Lynch-like syndrome in rare cases.

Finally, other germline genetic defects may explain Lynch-like syndrome. In 1 recent study, approximately 3% of patients with Lynch-like syndrome had biallelic MUTYH mutations.

More recent literature reports have identified biallelic mutations in MMR genes in most tumors in patients with Lynch-like syndrome. Thus, these patients do not have Lynch syndrome but have somatic (tumor) MMR gene mutations that explain the MMR protein deficiency within their tumor. In 1 series, Haraldsdottir and colleagues analyzed tumors with deficient MMR protein immunohistochemical expression from 32 patients with no germline MMR gene mutation or alteration and performed somatic MMR gene mutation analysis. Twenty-two of 32 (69%) tumors had 2 somatic mutations in MMR genes that explained the loss of MMR protein expression by immunohistochemistry. Overall, approximately 70% of patients who lack a germline MMR gene mutation or alteration and who harbor tumors demonstrating loss of MMR protein expression can be explained by biallelic somatic MMR gene mutations in their tumor. Interestingly, on the basis of the accumulated data from the literature, different patterns of somatic mutations are evident based on which MMR genes are affected. For Lynch-like tumors with loss of MLH1 and PMS2 protein expression by immunohistochemistry, the most common scenario is a somatic MLH1 gene mutation (deletion, frameshift, insertion, or duplication) associated with loss of heterozygosity of the other MLH1 allele. In contrast, for Lynch-like tumors with loss of MSH2 and MSH6 protein expression by immunohistochemistry, the most common scenario is biallelic somatic MSH2 gene mutation with infrequent loss of heterozygosity of MSH2. Too few cases of patients harboring tumors with isolated loss of either MSH6 or PMS2 have been analyzed in the literature to comment on patterns of somatic mutation, if any. Somatic MMR gene mutations are likely sporadic events; however, the possibility that these somatic biallelic mutations are secondary to other germline gene defects, including MUTYH, still remains.

There also appear to be significant clinicopathologic differences between patients with Lynch-like syndrome and Lynch syndrome. Colorectal carcinoma in patients with Lynch-like syndrome is even more likely to be located in the proximal colon. Certain patterns of abnormal MMR protein expression are also more likely to have Lynch syndrome confirmed by germline mutation analysis. Most patients with colorectal carcinomas demonstrating isolated loss of MSH6 expression will have Lynch syndrome confirmed by germline mutation analysis. In contrast, Lynch-like syndrome is more often identified in patients with tumors that harbor concurrent MLH1/PMS2 loss (with lack of MLH1 promoter hypermethylation) or concurrent MSH2/MSH6 loss. Synchronous or metachronous Lynch syndrome–associated carcinomas are more frequently identified in patients with confirmed Lynch syndrome compared with Lynch-like syndrome. Similar to colorectal carcinomas in patients with confirmed Lynch syndrome, carcinomas in patients

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**TABLE 4. Potential Explanations for Lynch-like Syndrome**

<table>
<thead>
<tr>
<th>Reason</th>
<th>Proportion of Cases</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biallelic (somatic) MMR gene mutation within the tumor</td>
<td>~70%</td>
<td>Biallelic somatic MMR gene mutations within the tumor occur most frequently in MLH1 and MSH2. These are likely sporadic events, and such patients can be screened as if they had sporadic colorectal carcinoma.</td>
</tr>
<tr>
<td>Incorrect interpretation of MMR protein immunohistochemistry</td>
<td>~20%</td>
<td>Reevaluation of MMR protein immunohistochemical stains and repeating any equivocal staining can identify false-“positive” results.</td>
</tr>
<tr>
<td>MMR gene mutations present but not detected by current testing methods</td>
<td>Unknown</td>
<td>One example is the inversion of MSH2 exons 1 to 7 that has recently been identified to explain some cases of Lynch syndrome. This inversion has not been routinely tested by most laboratories.</td>
</tr>
<tr>
<td>Germline mutations in other genes, such as MUTYH</td>
<td>3%</td>
<td>Germline MUTYH mutation analysis may be considered in patients with Lynch-like syndrome who lack biallelic somatic MMR gene mutation.</td>
</tr>
<tr>
<td>Somatic mosaicism</td>
<td>&lt;1%</td>
<td>Very rare. Most next-generation sequencing methodologies can detect mutations in &lt;5% of DNA, making the possibility of undetected mosaicism unlikely.</td>
</tr>
</tbody>
</table>
with Lynch-like syndrome develop through the conventional adenoma pathway and not the serrated pathway. This indicates that colorectal carcinomas in patients with Lynch-like syndrome may arise from conventional adenomas that develop biallelic somatic MMR gene mutations resulting in colorectal carcinoma with MMR protein deficiency.

**EPCAM Deletions in Lynch Syndrome**

Germline deletions affecting the **EPCAM** (TACSTD1) gene can lead to epigenetic silencing of the **MSH2** gene by hypermethylation and cause Lynch syndrome. EPCAM deletions with associated hypermethylation of the **MSH2** promoter are thought to account for up to 20% to 25% of Lynch syndrome tumors with loss of **MSH2** and **MSH6**. Patients with tumors harboring loss of **MSH2** and **MSH6** expression should be tested for germline deletions in **EPCAM**, if no **MSH2** germline mutation is identified. Immunohistochemistry using the monoclonal antibody Ber-EP4 directed at EPCAM has been proposed as helping to differentiate patients with germline EPCAM deletion from those with germline **MSH2** mutation. In 1 analysis, patients with EPCAM deletions demonstrated loss of Ber-EP4 expression within the tumor, whereas none of the patients with **MSH2** germline mutations displayed loss of Ber-EP4 expression. A subsequent study from the same group demonstrated that loss of Ber-EP4 expression occurs in many, but not all, tumors from patients with Lynch syndrome caused by EPCAM germline deletions. An acquired somatic deletion of the second **EPCAM** allele within the tumor is required for loss of Ber-EP4 expression. Those tumors that do not acquire a second somatic EPCAM deletion in addition to the germline EPCAM deletion will demonstrate preserved expression of Ber-EP4 within their tumor. Thus, Ber-EP4 immunohistochemistry is not particularly helpful in identifying patients with Lynch syndrome due to germline EPCAM deletion.

**Constitutional MLH1 Epimutation**

Constitutional epimutation refers to epigenetic hypermethylation at the promoter of a gene leading to silencing of expression from the allele within all normal somatic tissues. Constitutional epimutation of **MLH1** has helped to explain a subset of Lynch syndrome patients. In these patients, the **MLH1** promoter of the affected allele is fully methylated, resulting in silencing of the allele within all or most cells within the body. The second “hit” of the **MLH1** gene in these patients is often loss of heterozygosity or acquired sequence mutations of the second **MLH1** allele resulting in complete loss of **MLH1** protein expression within affected cells. Some constitutional **MLH1** epimutations are labile in the germline, and transmission to successive generations may not occur. Thus, constitutional **MLH1** epimutation may not follow classical Mendelian patterns of inheritance typical of other patients with Lynch syndrome with germline MMR gene sequence mutations. It also appears that cancers tend to occur at a younger age in patients with constitutional **MLH1** epimutation (mean age of onset, 39 y) compared with patients with germline **MLH1** sequence mutations (mean age of onset, 44 y).

**Constitutional MMR Deficiency**

Constitutional MMR deficiency (CMMRD) is defined by the presence of biallelic germline mutations in DNA MMR genes. This disease is very rare, with a propensity to present with malignancy early in life, including early-onset colorectal carcinoma, lymphoma, leukemia, and brain tumors. Many patients with CMMRD show features reminiscent of neurofibromatosis type 1, such as café-au-lait spots. In contrast to Lynch syndrome in which the most common germline mutations are in **MLH1** and **MSH2**, CMMRD is most often the result of biallelic mutation of **PMS2** (60% of cases) or biallelic mutation of **MSH6** (20% of cases). A possible mechanism for the relatively low frequency of **MLH1** or **MSH2** mutation in CMMRD is that highly penetrant mutations in **MLH1** or **MSH2** may be embryologically lethal when homozygous. In contrast to Lynch syndrome in which loss of expression of the affected MMR gene is only observed in neoplastic tissues, in patients with CMMRD loss of expression of the affected MMR gene will be seen in both neoplastic and non-neoplastic tissues. In young patients with malignancies, lack of MMR expression in non-neoplastic tissues should not necessarily be interpreted as a failure of the test, provided staining is demonstrated on external control tissues. In such instances, MSI PCR may be helpful to confirm the presence of MMR protein deficiency within the patient’s tumor.

**SCREENING FOR LYNCH SYNDROME IN SPECIAL CIRCUMSTANCES**

**Colorectal Adenomas**

In patients suspected of being at risk for Lynch syndrome and without a personal history of colorectal carcinoma, evaluation of colorectal adenomas can be helpful in establishing the diagnosis of Lynch syndrome (Fig. 6). A recent series analyzed adenomas in patients with known deleterious germline mutations in MMR genes.
and found that loss of MMR protein expression by immunohistochemistry was identified in 72% (78/109) of adenomas and correlated with the underlying germline mutation in all cases. Notably, patients with underlying germline MSH6 mutations less frequently had adenomas demonstrating loss of MSH6 protein expression by immunohistochemistry (4/11, 36%). Adenomas with a villous component or high-grade dysplasia more frequently demonstrated loss of MMR protein expression compared with adenomas without a villous component. Other studies have similarly identified a significant association between the presence of high-grade dysplasia and villous component and loss of MMR protein expression. The importance of adenoma size in selecting polyps to test for MMR protein immunohistochemistry is still unresolved. One study demonstrated that adenoma size does not seem to correlate with loss of MMR protein expression, whereas another study demonstrated that abnormal MMR protein expression correlated with adenoma size ≥ 8 mm. These results indicate that assessment of colorectal adenomas using MMR protein immunohistochemistry can be useful in patients suspected of having Lynch syndrome, and a strategy of selecting polyps with a villous component, high-grade dysplasia, and/or size ≥ 8 mm may increase the yield of MMR protein immunohistochemistry. Importantly, the presence of preserved MMR protein expression does not exclude the possibility of Lynch syndrome, as up to 30% to 50% of adenomas in patients with known Lynch syndrome have retained MMR protein expression, particularly those patients with germline MSH6 mutations. Sessile serrated adenomas can give rise to colorectal carcinoma with sporadic MLH1 deficiency that exhibits MLH1 promoter hypermethylation and the BRAF V600E mutation. Evaluation of sessile serrated adenomas by MMR protein immunohistochemistry has no role in screening for Lynch syndrome.

The presence of an advanced colorectal adenoma in a young patient can raise concern for the possibility of a hereditary cancer syndrome. A recent study analyzed MMR protein immunohistochemistry in advanced adenomas identified in patients 45 years and below, defined as adenomas with villous histology, ≥ 1 cm in diameter, or ≥ 3 polyps of any size. Only 1 of 66 polyps (1.5%) demonstrated loss of MMR protein expression, indicating the low yield of universal MMR protein immunohistochemical evaluation of advanced adenomas in young patients. A similar analysis using both MMR protein immunohistochemistry and MSI PCR found that universal screening of adenomas in patients below 40 years of age did not identify any patients with abnormal MMR protein expression or MSI-H. Thus, universal screening of colorectal adenomas in young patients is not effective in identifying patients at risk for Lynch syndrome.

Inflammatory Bowel Disease

Inflammatory bowel disease is a well-known risk factor for colorectal carcinoma, but no guidelines exist regarding testing for MMR protein deficiency in this setting. In a study of 129 colorectal carcinomas arising in inflammatory bowel disease, MSI-H was observed in 20 tumors (15%). The pattern of MMR protein expression by immunohistochemistry was quite variable with loss of MLH1 and PMS2 expression in 6 colorectal carcinomas, loss of MSH2 and MSH6 expression in 6 colorectal carcinomas, isolated loss of MSH6 expression in 6 colorectal carcinomas, and isolated loss of PMS2 expression in 2 colorectal carcinomas. MLH1 promoter hypermethylation analysis performed on the 6 cases with loss of MLH1 and PMS2 expression demonstrated hypermethylation in only 3 tumors. Germline mutational analysis was not performed. Schulmann et al also demonstrated MSI-H in 20% of ulcerative colitis–associated colorectal carcinoma.
and demonstrated that hypermethylation of CpG islands was the underlying mechanism in most MSI-H ulcerative colitis–associated colorectal carcinomas. More recently, Liu et al\(^8\(\) performed MMR protein immunohistochemistry on 55 inflammatory bowel disease–associated carcinomas and demonstrated loss of MLH1 and PMS2 expression in 4 cancers and loss of MSH2 and MSH6 expression in 1 cancer. Liu et al\(^8\(\) also demonstrated that tumors arising in the setting of inflammatory bowel disease had histologic features commonly associated with MMR protein deficiency; however, tumor-infiltrating lymphocytes were less common. These studies demonstrate that MMR protein deficiency does occur in inflammatory bowel disease–associated colorectal carcinoma in approximately 10% to 20% of cases. Although germline mutational analysis was not performed in these studies, it is unlikely that Lynch syndrome is the underlying cause of colorectal carcinomas in the setting of inflammatory bowel disease. Rather, most inflammatory bowel disease–associated colorectal carcinomas with MMR protein deficiency likely arise because of hypermethylation of CpG islands in some cases\(^8\) and chronic inflammation and reactive oxygen impairing MMR machinery in others.\(^9\) Thus, screening for Lynch syndrome in the setting of inflammatory bowel disease–associated colorectal carcinoma may not be useful.

### Synchronous Intestinal Neoplasia

Synchronous colorectal carcinomas are relatively rare and account for 1% to 4% of all surgically resected carcinomas. MMR protein deficiency is seen in approximately 35% of synchronous cancers. Importantly, in most studies there is a very high concordance of MSI status between synchronous tumors, with 87% to 98% of cases demonstrating either all microsatellite stable or MSI-H tumors. These results suggest common etiologic factors for neoplasia within a given patient.\(^1\)\(^2\) Lynch syndrome patients have a particularly high incidence of synchronous carcinomas. Recently, Hu and colleagues analyzed 58 patients with 2 synchronous colorectal carcinomas. In 21 patients, at least 1 tumor was MSI-H, with 20 of the 21 tumor pairs demonstrating MSI-H in both tumors. Of these, 8 (38%) had MMR protein immunohistochemistry concerning for Lynch syndrome, with the remainder demonstrating \(BRAF\) mutations and association with sessile serrated adenomas. Some authors have argued for testing any and all Lynch syndrome–associated tumors in a given patient.\(^9\)\(^3\) In 1 series, 31% of patients with Lynch syndrome and synchronous or metachronous LS-associated neoplasia showed discordant MMR protein expression within individual tumors.\(^9\)\(^3\) Given the possibility of discordant MMR protein expression and the potential to miss a diagnosis of Lynch syndrome if only selected tumors are screened, it seems prudent to test all Lynch syndrome–associated tumors within a given patient, especially if there are clinical features concerning for Lynch syndrome.

### Other Gastrointestinal Tract Tumors

Patients with Lynch syndrome are at increased risk for developing other tumors including within the luminal GI tract, such as adenocarcinomas of the stomach and small bowel. Patients with Lynch syndrome have up to 300-fold increased risk for developing small bowel adenocarcinoma, although the absolute lifetime risk is still relatively low at 0.6% to 1%. The frequency of deficient MMR protein expression in small bowel adenocarcinoma ranges from 5% to 35%, and Lynch syndrome is over-represented among these tumors compared with colorectal carcinoma.\(^9\) In 1 study of 61 small intestinal adenocarcinomas, 14 were MSI-H and 9 were attributable to Lynch syndrome.\(^9\) Thus, it is prudent to screen all small bowel adenocarcinomas for Lynch syndrome in patients with no other apparent risk factors for small bowel neoplasia.

Although patients with Lynch syndrome are at increased risk for developing gastric cancer, the lifetime risk is relatively low at 5%.\(^9\) MSI-H occurs in approximately 20% of gastric cancers, the vast majority of which arise due to hypermethylation of the \(MLH1\) promoter. These tumors are associated with older patient age, location in the antrum, well differentiation, and improved survival. Given the relative scarcity of Lynch syndrome–associated gastric carcinoma, universal screening of gastric carcinoma by MMR protein immunohistochemistry is generally not performed. Interestingly, a recent study suggested that pyloric gland adenomas may be more common in patients with Lynch syndrome.\(^9\)\(^7\) Current literature suggests that 5% to 10% of ampullary adenocarcinomas exhibit MMR protein deficiency.\(^9\)\(^8\) A large series of 170 ampullary adenocarcinomas demonstrated MSI-H by PCR in 10% of ampullary adenocarcinomas, with most cases exhibiting intestinal histology associated with tumor-infiltrating lymphocytes and mucinous differentiation.\(^9\) A subset (2%) showed deficient MMR protein expression that raised concern for Lynch syndrome. Most of the MSI-H ampullary adenocarcinomas demonstrated loss of MLH1 and PMS2 immunohistochemical expression with concomitant \(MLH1\) promoter hypermethylation, indicative of sporadic MLH1 deficiency.\(^9\)\(^8\) Agaram and colleagues identified deficient MMR protein expression in 3 of 54 cases (6%) of ampullary adenocarcinomas, with 2 cases demonstrating loss of MSH6 expression. Currently, there are no formal guidelines recommending universal screening in ampullary adenocarcinoma. However, given these data indicating that a small subset of ampullary adenocarcinoma will have MMR protein deficiency raising concern for Lynch syndrome, some institutions have opted to perform universal screening of ampullary adenocarcinoma for Lynch syndrome with MMR protein immunohistochemistry. Adenocarcinomas of the appendix infrequently demonstrate MMR protein deficiency, and most studies have demonstrated that low-grade mucinous neoplasms of the appendix do not exhibit MMR protein deficiency.\(^1\)\(^0\)\(^1\)\(^0\)\(^2\)\(^0\) Given the relative scarcity of Lynch syndrome–associated appendiceal neoplasia, universal screening of appendiceal
neoplasia by MMR protein immunohistochemistry is generally not performed.

MANAGEMENT OF PATIENTS WITH LYNCH SYNDROME

Table 5 summarizes the surveillance guidelines for patients with Lynch syndrome as recommended by the US Multi-Society Task Force for Colorectal Carcinoma.16

The guidelines for screening for colorectal carcinoma by colonoscopy are strongly recommended. However, although the level of evidence supporting the guidelines for endometrial cancer, ovarian cancer, and urinary tract cancer is low, these interventions should be offered to patients at risk for or affected by Lynch syndrome. The lifetime risk for carcinoma also varies depending on which MMR gene is affected. Patients with Lynch syndrome caused by MSH6 or PMS2 mutations have a lower cumulative lifetime risk for colorectal carcinoma of between 10% and 20% compared with patients with Lynch syndrome caused by MLH1 or MSH2 mutations who have a lifetime risk for colorectal carcinoma ranging from 30% to 74%.16 In addition, a later onset of colorectal carcinoma is seen in patients with Lynch syndrome caused by MSH6 (mean age, 54 to 63 y) or PMS2 (mean age, 47 to 66 y) compared with patients with Lynch syndrome caused by MLH1 or MSH2 mutations (mean age, 27 to 46 y).16

Given this, the initiation of screening for colorectal carcinoma in patients with Lynch syndrome caused by MSH6 or PMS2 mutation is often delayed compared with Lynch syndrome caused by MLH1 or MSH2 mutation.

Patients with Lynch-like syndrome represent a major challenge in Lynch syndrome management. Somatic MMR gene mutation analysis helps to improve Lynch syndrome diagnostics by identifying patients with biallelic somatic MMR gene mutations within their tumor that likely do not benefit from the intensive lifelong screening used for patients with confirmed Lynch syndrome. Using an updated algorithm for Lynch syndrome diagnostics (Fig. 2), if biallelic somatic mutation of an MMR gene is identified within the patient’s tumor, the patient does not have Lynch syndrome and may be screened similarly to those patients with sporadic carcinoma. However, more study is required to determine the risk of developing Lynch syndrome–associated carcinoma in patients with Lynch-like syndrome and their first-degree relatives to guide screening strategies for these patients.

EMERGING THERANOSTIC IMPLICATIONS OF MMR DEFICIENCY IN GASTROINTESTINAL TRACT CARCINOMA

Besides serving as a screening tool for Lynch syndrome, testing for MMR protein deficiency is of clinical importance as MMR protein deficiency in colorectal carcinoma is a well-known prognostic and predictive biomarker. Numerous studies have demonstrated that patients with MMR protein–deficient colorectal carcinoma have a better overall survival and reduced recurrence rate compared with patients with MMR protein–proficient colorectal carcinoma.104 This is particularly important for patients with stage II colorectal carcinoma harboring high-risk histologic features who may be eligible for adjuvant chemotherapy. If the patient has a stage II MMR protein–deficient colorectal carcinoma, adjuvant therapy is generally not given.105,106 In contrast, patients with stage III or IV colorectal carcinoma benefit from chemotherapy regardless of MMR protein status within the tumor.107 Immune checkpoint blockade using antibodies targeting the programmed cell death protein-1 (PD-1/PD-L1) pathway has emerged as a potential therapeutic option in colorectal carcinoma and other carcinomas of the gastrointestinal tract. Activation of immune checkpoints allows for tumor cells to evade antitumor immunity; PD-1

<table>
<thead>
<tr>
<th>Interventions</th>
<th>Age to Begin</th>
<th>Interval</th>
<th>Strength of Recommendation</th>
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<tbody>
<tr>
<td>Colonoscopy</td>
<td>Based on affected MMR gene as follows: MLH1 or MSH2: 20-25 y MSH6: 30 y PMS2: 35 y Or 2-5 y younger than youngest age at diagnosis of CRC in family if diagnosis before the ages listed above</td>
<td>Every 1-2 y; annual colonoscopy should be considered in confirmed mutation carriers</td>
<td>Strong recommendation</td>
</tr>
<tr>
<td>Pelvic examination with endometrial sampling</td>
<td>30-35 y</td>
<td>Annually</td>
<td>Low (expert consensus)</td>
</tr>
<tr>
<td>Transvaginal ultrasound</td>
<td>30-35 y</td>
<td>Annually</td>
<td>Low (expert consensus)</td>
</tr>
<tr>
<td>EGD with biopsy of the antrum</td>
<td>30-35 y</td>
<td>Every 2-3 y based on patient risk factors</td>
<td>Low (expert consensus)</td>
</tr>
<tr>
<td>Urinalysis</td>
<td>30-35 y</td>
<td>Annually</td>
<td>Low (expert consensus)</td>
</tr>
</tbody>
</table>

*On the basis of guidelines proposed by the US Multi-Society Task Force on Colorectal Carcinoma.16

CRC indicates colorectal carcinoma; EGD, esophagogastroduodenoscopy.
limits the activity of T cells when it interacts with its ligand PD-L1. Antibodies targeting PD-1 and PD-L1 are being evaluated in ongoing clinical trials for their efficacy in colorectal carcinoma. MMR protein–deficient colorectal carcinomas have increased expression of PD-L1 within the tumor and within tumor-infiltrating lymphocytes, allowing the tumor to evade antitumor immunity. In a recent phase 2 clinical trial, MSI-H status by PCR was predictive of response to immune checkpoint blockade with pembrolizumab, a monoclonal antibody that blocks the PD-1 pathway. The predictive effect of MSI-H and immune checkpoint blockade was identified not only in colorectal carcinoma but also in other gastrointestinal tract malignancies. Thus, MSI-H has shown early promise as a predictive biomarker for PD-L1 blockade immunotherapy.

CONCLUSIONS

Identification of individuals with a hereditary form of cancer is necessary, as these individuals benefit from genetic counseling and increased surveillance to prevent cancer development. By far, Lynch syndrome is the most common hereditary form of cancer in the gastrointestinal tract. Universal screening of all patients with colorectal carcinoma either by MMR protein immunohistochemistry or by MSI PCR is now recommended by most major professional medical organizations in the United States and Europe. Beyond the role of tumor diagnosis, pathologists are critical for the identification of patients at risk for Lynch syndrome and are tasked with establishing screening strategies for patients with carcinoma of the gastrointestinal tract using ancillary diagnostic studies, including immunohistochemistry and molecular analyses. In many instances, pathologists are the first to identify patients at risk for Lynch syndrome and must effectively communicate these findings to treating clinicians to ensure appropriate patient follow-up and genetic evaluation.

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Pai and Pai

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