Recent Advances in Lynch Syndrome: Diagnosis, Treatment, and Cancer Prevention

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OVERVIEW

Identification of individuals with inherited predispositions to cancer, including Lynch syndrome, can help prevent cancer and cancer-related death by allowing for the uptake of specific cancer prevention and screening as well as the use of therapies directed toward the underlying neoplastic process for individuals with advanced cancer. In the 25 years since the discovery of microsatellite instability (MSI) and the first recognition of germline mismatch repair (MMR) gene variants as the etiologic basis of Lynch syndrome, there has been tremendous progress in the understanding of the spectrum of cancer risk associated with Lynch syndrome as well as in cancer prevention and risk-reduction strategies. The past few years, in particular, have brought transformative changes in the treatment of Lynch syndrome–associated cancers with immune checkpoint inhibitors. In parallel, advances in next-generation sequencing (NGS) technologies now allow rapid and scalable somatic and germline sequencing that promises to help identify Lynch syndrome in individuals who otherwise lack classic phenotypes. Last, real progress is being made to understand more sophisticated methods of precision cancer prevention, including chemotherapeutic prevention agents (e.g., aspirin) and strategies that leverage the immune system to facilitate primary cancer prevention in otherwise-healthy Lynch syndrome carriers.

University of Michigan pathologist Aldred Warthin, MD, PhD, is widely credited as the first person to describe the cancer predisposition syndrome now known as Lynch syndrome (formerly called hereditary nonpolyposis colorectal cancer [HNPCC]) when, in 1895, his seamstress correctly predicted that she would die as a result of cancer after she watched numerous family members succumb to cancers of the gastrointestinal or gynecologic tract. During Warthin's time and past the mid-20th century, the prevailing notion was that inherited cancer risk did not exist outside of rare conditions, such as familial adenomatous polyposis, in which there was an obvious premalignant phenotype.[1]

Now, more than 120 years later, Lynch syndrome is known as one of the most common forms of inherited cancer predisposition; the general population prevalence (estimated at 1 in 279) rivals that of germline *BRCA1*/*BRCA2* variants.[2] Although most classically associated with increased risks of colorectal and endometrial cancers, Lynch syndrome predisposes individuals to a wide array of malignancies, including ovarian, gastric, urinary tract (kidney, renal pelvis, ureter, bladder, and prostate), pancreaticobiliary, small intestinal, and brain cancers, as well as sebaceous neoplasms of the skin and possibly slightly increased risks of female breast cancer and prostate cancer (Table 1).[3-8] Lynch syndrome is caused by pathogenic germline variants in the DNA MMR genes *MLH1*, *MSH2*, *MSH6*, or *PMS2* (and, rarely, in the non-MMR gene *EPCAM*, in which deletions induce epigenetic silencing of *MSH2*).[1,7]

When the MMR genes were identified as the underlying genetic etiology of Lynch syndrome in the early 1990s, little was known about the optimal means of diagnosis of families with Lynch syndrome or prevention of Lynch-associated cancers, and the malignancies that developed were treated in exactly the same way as their sporadic counterparts.[1] With groundbreaking advances in germline and somatic sequencing, clinical risk prediction models, immuno-oncology, and precision cancer prevention, our ability to identify, prevent, and durably treat cancers associated with Lynch syndrome continues to grow in both scope and sophistication.

DIAGNOSING LYNCH SYNDROME

Currently, there are two general approaches to the diagnosis of Lynch syndrome: (1) molecular screening of colorectal and endometrial tumor specimens for evidence of defective MMR function (MMR-D) or high-level MSI (MSI-H) to identify patients with cancer who should undergo germline testing for pathogenic MMR gene variants; or (2) direct germline testing performed on patients whose personal and/or family histories of cancer are suspicious for Lynch syndrome. Molecular testing has garnered particular attention

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in recent years because of its sensitivity and specificity for identification of Lynch syndrome probands, as well as because of the ever-growing prognostic and therapeutic implications. From the standpoint of Lynch syndrome diagnosis, four main pathology tests can aid in the molecular identification of patients with cancer who are likely to have Lynch syndrome: (1) polymerase chain reaction (PCR)–based MSI testing; (2) immunohistochemical staining (or immunohistochemistry [IHC]) for the MMR proteins; (3) *MLH1* promoter methylation analysis (or somatic *BRAF* V600E mutation analysis); and (4) next-generation somatic (and/or germline) sequencing assays.

Microsatellite Instability Testing

MSI in colorectal cancers was first described in 1993[9-11] and was quickly recognized as a hallmark characteristic of Lynch syndrome–associated cancers.[12] MSI is defined as changes in the length of repetitive DNA sequences (typically mono- or dinucleotide repeat sequences) in tumors compared with the length of the same microsatellite loci in normal non-neoplastic tissue. This slippage develops as a result of defective DNA MMR machinery, which is characteristic in Lynch syndrome. Historically, five microsatellite loci are evaluated by PCR; if more than 20% are unstable, the tumor is considered to have MSI-H.[13,14] More recently, it has been shown that MSI status can be directly assessed by NGS of tumors either by direct assessment of numerous microsatellite loci[15-18] or by assessment of a tumor's overall mutational burden as a surrogate for MSI status.[19,20]

PRACTICAL APPLICATIONS

- **Universal testing of all colorectal and endometrial cancers with MMR protein IHC (or PCR-based MSI analysis) is recommended as a screen for Lynch syndrome.**
- **The use of NGS may revolutionize the diagnosis of Lynch syndrome even more, both by facilitating NGS-based assessment of tumor specimens to screen for MSI and through the growing availability of NGS-based multigene panels for direct germline testing.**
- **Treatment of advanced/metastatic Lynch-associated cancers (and non-Lynch cancers with MSI) with anti–PD-1 monoclonal antibodies (pembrolizumab or nivolumab) yields 70% or greater disease control rates, many of which are quite durable.**
- **Aspirin (600 mg/day) for 2 or more years reduces the risk of Lynch-associated colorectal cancer by greater than 50% and may reduce the risk of other Lynch-associated cancers.**
- **Individuals with Lynch syndrome may experience auto-vaccination against microsatellite instability–induced frameshift neopeptides that serve as innate immunosurveillance, which suggests that immune-based mechanisms for primary cancer prevention are promising as an avenue of future research.**

Immunohistochemistry for DNA MMR Proteins

Immunohistochemical staining for expression of the DNA MMR proteins can be used as a fast, reproducible, and inexpensive proxy for MSI status.[21-23] Reliable antibodies are available for the four main mismatch repair proteins (mutL homolog 1 [MLH1], mutS homolog 2 [MSH2], mutS homolog 6 [MSH6], and PMS1 homolog 2 [PMS2]), and the turnaround time of such testing is as fast as any standard IHC testing. Tumors that demonstrate an absent staining for any of these four proteins are considered to have underlying dysfunction in the DNA MMR machinery (MMR-D) as a result of epigenetic, somatic, and/or germline MMR gene inactivation.

MLH1 Methylation and *BRAF* Mutation Analyses

Most MMR-D and MSI-H colorectal and endometrial cancers do not develop because of Lynch syndrome but instead because of an acquired somatic MMR gene function inactivation. In the most common such situation, MLH1 function is silenced by acquired methylation of the *MLH1* promoter region.[14-26] This is more common in women and in elderly patients and accounts for 69% of all colorectal cancer occurrences that have an absence of MLH1 and PMS2 on IHC.[27] *MLH1* promoter methylation accounts for 94% of endometrial cancer occurrences that have an absence of MLH1 and PMS2.[28] Because *MLH1* promoter methylation is so common, it is typical to rule it out before germline genetic testing in patients with MSI-H tumors and/or those tumors that demonstrate an absent expression of the MLH1 and PMS2 proteins on IHC. This elimination can be done by directly assessing methylation of the *MLH1* promoter region, which is the most sensitive and specific approach at ruling out Lynch syndrome in such cases, but it requires DNA extraction and bisulfite treatment of the DNA that is not readily available at most hospitals. In colorectal cancers, promoter methylation also can be assessed indirectly by testing for the presence of somatic *BRAF* V600E mutations, as a surrogate for *MLH1* methylation status.[29] Somatic *BRAF* V600E mutations occur in a small fraction of colorectal cancers overall but are found in 69% to 78% of colorectal cancers with *MLH1* promoter methylation and are virtually never seen in Lynch syndrome–associated cancers, so *BRAF* mutation has a high negative predictive value.[30,31] Some centers have adopted a hybrid model of testing in which *BRAF* V600E mutation analysis is performed first and, if negative, is followed by *MLH1* methylation testing; other centers have switched entirely to *MLH1* methylation testing, because it is applicable for both colorectal and endometrial cancers. (*BRAF* V600E mutation analysis is not useful to determine whether MMR-D/MSI-H endometrial cancers are Lynch associated.) Regardless of the strategy, use of *MLH1* promoter methylation and/or *BRAF* mutation analyses can reduce the number of patients who need germline MMR gene testing by half.[30]

Other Forms of Biallelic Somatic MMR Gene Inactivation

Recently, it has become more widely understood that other mechanisms can induce somatic biallelic inactivation of

TABLE 1. Spectrum of Cancers Associated With Lynch Syndrome and Proven Prevention Strategies

Cancer Type	Estimated Cumulative Cancer Risk (%)[3-5]	Prevention Strategies
Colorectal cancer	10–82*	• Frequent (every 1–2 years) colonoscopy reduces incidence and mortality; typically begin at age 20–25 • Aspirin (600 mg/day) for ≥ 2 years reduces incidence** • Subtotal colectomy recommended vs. segmental resection in the setting of known Lynch-associated colorectal cancer
Endometrial cancer	15–60	• Risk-reducing hysterectomy reduces incidence, but no proven mortality benefit • Observational data suggest protective benefit of exogenous progestins • No proven benefit to endometrial cancer screening, although guidelines recommend consideration of transvaginal ultrasound and endometrial biopsy beginning at age 30–35 until hysterectomy
Ovarian cancer	1–38	• Risk-reducing salpingo-oophorectomy reduces incidence, but no proven mortality benefit • No proven benefit to ovarian cancer screening, although guidelines recommend consideration of transvaginal ultrasound beginning at age 30–35 years until salpingo-oophorectomy
Gastric cancer	< 1–13	• No proven benefit to screening, but guidelines recommend consideration of upper endoscopy to biopsy for *Helicobacter pylori*
Urinary tract cancer (kidney, renal pelvis, ureter, and bladder)	< 1–18†	• No proven benefit to screening; one study of urine cytology screening demonstrated 29% sensitivity and high rate of false-positive screens[6]
Small bowel cancer	< 1–6	• No data to suggest benefit to screening
Pancreatic cancer	1–6	• No data to suggest benefit to screening, although some guidelines recommend consideration of screening MRI and/or endoscopic ultrasound in the setting of a family history of pancreatic cancer[7]
Biliary tract cancer	< 1–4	• No data to suggest benefit to screening
Brain cancer	< 1–5	• No data to suggest benefit to screening
Female breast cancer	11–56‡	• No data to suggest benefit to increased screening compared with the general population
Prostate cancer	17–38‡	• No data to suggest benefit to increased screening compared with the general population
Sebaceous adenomas/carcinomas (skin)	1–9	• Guidelines typically recommend routine dermatologic surveillance but no data on efficacy

*Risk markedly higher for *MLH1* and *MSH2* carriers than for *MSH6* or *PMS2* carriers.

**Aspirin use was associated with reduced risk of any Lynch-associated cancer.[8]

†Risk likely highest for *MSH2* carriers.

‡Most data suggest minimal increased risk compared with the general population.

MMR gene function and result in MSI-H cancers with other patterns of MMR-D by IHC.[32-34] Among unselected colorectal cancer occurrences, this phenomenon appears to be almost as common as Lynch syndrome itself, although it has become widely recognized only in recent years.[35] Individuals with confirmed biallelic somatic MMR gene alterations do not have Lynch syndrome and should be treated according to their clinical history rather than according to Lynch syndrome surveillance guidelines. Testing for these other forms of biallelic somatic MMR gene inactivation typically occurs after abnormal tumor screening and unrevealing germline genetic testing for pathogenic MMR variants, which results in a tumor with unexplained MMR-D. As such testing becomes quickly and increasingly inexpensive, however, it may become advantageous to simply order paired tumor and germline sequencing as a single test, both to streamline workflows and to minimize confusion about non-Lynch MMR-D/MSI-H findings.

Universal Tumor Screening for Lynch Syndrome

In the past, MMR IHC was more cost effective than MSI testing for programs that universally screened all colorectal tumors for Lynch syndrome, particularly because it predicted the MMR gene in which a pathogenic germline variant was most likely.[36-38] However, with the advent of NGS-based germline testing, there is negligible incremental cost to test all MMR genes, or even to perform germline testing with a broader panel of cancer susceptibility genes beyond the five linked to Lynch syndrome.[39] Furthermore, it has become standard practice to assess all metastatic colorectal cancers for somatic alterations in *KRAS*, *NRAS*, and *BRAF* to guide therapeutic decision-making. Addition of somatic analysis of the standard microsatellite loci and/or even the MMR genes themselves into such testing is a logical next step that likely will streamline universal tumor testing programs, at least for metastatic colorectal cancers.[40]

Regardless of the test used, the underlying principle of universal tumor screening for Lynch syndrome is the same: (1) screen all patients with newly diagnosed colorectal and endometrial cancer for Lynch syndrome with one of these tumor tests; (2) follow up with *MLH1* methylation analysis (with or without *BRAF* V600E mutation analysis in colorectal cancers) for MMR-D/MSI-H tumors with absent MLH1 and PMS2 expression; (3) refer the remaining patients to genetic counseling and confirmatory germline genetic testing in a CLIA-approved laboratory. Although universal tumor screening has been recommended by multiple professional organizations,[3,7,41-45] a recent study found that only 28% of patients with colorectal cancer receive MSI or MMR IHC analyses at the time of diagnosis.[46] This screening is becoming increasingly important, not only to identify patients who are more likely to have Lynch syndrome—which can help both the patient and their mutation-positive family members receive intensive cancer surveillance to prevent future cancers—but also to identify patients who are more likely to benefit from immune checkpoint inhibitor therapy.

High-Risk Clinic-Based Assessment for Lynch Syndrome

In addition to universal tumor screening for Lynch syndrome, it is still important that any and all individuals with suspicious clinical histories for Lynch syndrome receive genetic evaluation, even if they themselves have not yet been affected by cancer. There are various clinical tools available to help identify these patients quickly on the basis of personal and family history of cancer, including a validated three-question screen[47] and the online PREMM5 prediction model.[48] Patients who answer yes to any of the questions on the three-question screen or who are predicted to have a 2.5% or greater likelihood of Lynch syndrome on PREMM5 screening warrant referral for Lynch syndrome evaluation. In the past, when germline genetic testing was more expensive and less accurate, the cancer genetics clinic would have first recommended tumor screening for a patient with a family history of colorectal or endometrial cancer, and then would have proceeded to germline genetic testing only if a tumor was MMR-D. Because NGS germline testing panels are now widely available at much lower costs, it is generally cost-effective to order multigene panel testing on patients who are evaluated for Lynch syndrome or other hereditary cancer syndromes.[39]

Data on such NGS germline panels are starting to emerge. In one study that examined a 25-gene panel of cancer susceptibility genes in individuals diagnosed with colorectal cancer before age 50, 16% of individuals harbored a pathogenic germline cancer susceptibility gene variant, only half of which were in Lynch syndrome genes.[49] In another study that examined the same 25-gene panel in more than 1,000 individuals diagnosed with colorectal cancer across all ages, 9.9% carried a pathogenic germline variant in one or more cancer susceptibility genes, only one-third of which were in Lynch syndrome genes.[50] Such studies have raised the question of whether all patients with colorectal cancer, or at least those diagnosed before age 50, should be offered germline genetic testing regardless of their tumor screening results. In addition, these studies suggest that testing should not be limited to Lynch syndrome genes but should include a broader pan-cancer panel of common hereditary cancer genes. It is always most informative to initiate genetic evaluation within a family for an individual affected by one of the cancers of concern, but such an approach often is not feasible for a wide variety of reasons. In such situations, germline testing performed on an at-risk unaffected individual with appropriate pre- and post-test counseling is a reasonable alternative.

IMMUNE-BASED THERAPIES FOR LYNCH SYNDROME–ASSOCIATED CANCERS

Although Lynch syndrome–associated colorectal cancers have superior prognoses, stage-for-stage, compared with their sporadic counterparts, some individuals with Lynch syndrome do unfortunately develop recurrent/metastatic colorectal cancer or other forms of advanced and incurable Lynch syndrome–associated cancer.[51] Recent translational and therapeutic advances that leverage the immunologic effects of MSI that are classic for Lynch syndrome–associated cancers have resulted in dramatic changes to the treatment landscape for patients with Lynch syndrome who have advanced cancers (and individuals with non–Lynch syndrome advanced MMR-D/MSI-H cancers). MSI-H, by definition, is characterized by the somatic accumulation of small insertion or deletion events at repetitive stretches of DNA termed microsatellites. When such frameshift mutations occur at hotspot microsatellite loci within coding regions of tumor suppressor genes (e.g., *TGFBR2*), they act to promote carcinogenesis.[52] However, these frameshift mutations can result in the accumulation of potentially antigenic frameshift neopeptides, which are thought to account for the tumor-infiltrating lymphocyte reactions that are classically seen in Lynch syndrome–associated (and other non-Lynch MSI-H) colorectal cancers.[53]

The recent emergence of oncologic therapies such as immune checkpoint inhibitors that work through manipulation and upregulation of the patients' own immune systems have exploited this underlying biology to create game-changing progress in the treatment of Lynch syndrome–associated (and other MSI-H/MMR-D) cancers. The most notable such therapeutic examples to date are monoclonal antibodies that target PD-1. In the first study to specifically examine such agents in metastatic, refractory MMR-D/MSI-H cancers, 11 individuals with MMR-D/MSI-H colorectal cancer, 21 individuals with MMR-proficient/microsatellite-stable colorectal cancer, and nine individuals with MMR-D/MSI-H noncolorectal cancers were treated with single-agent pembrolizumab.[54] In this heavily pretreated cohort, there were markedly superior outcomes (hazard ratio for progression or death, 0.04; 95% CI, 0.01–0.21) in individuals with MMR-D/MSI-H cancers compared with those whose cancers were MMR proficient/microsatellite stable.[54] By RECIST criteria, overall response rates were 40% and 71% for MMR-D/MSI-H

colorectal cancers and noncolorectal cancers, respectively, whereas there were no responses among those with MMR-proficient/microsatellite-stable colorectal cancers. Likewise, overall disease control rates were 90% and 71% for MMR-D/MSI-H colorectal cancers and noncolorectal cancers, respectively, compared with 11% for MMR-proficient/microsatellite-stable colorectal cancers. With a median follow-up time of 36 weeks, the median progression-free survival was not reached for either cohort of patients with MMR-D/MSI-H cancers (vs. a median progression-free survival of only 2.2 months among the patients with MMR-proficient/microsatellite-stable colorectal cancer).[54] Follow-up data with pembrolizumab in 86 patients who had a wide variety of previously treated metastatic/advanced MMR-D/MSI-H cancers have shown an objective response rate of 53% (95% CI, 42%–64%) across tumor types, including a 21% complete response rate and a 77% overall disease control rate; median overall survival and progression-free survival had not been reached at a median follow-up time of 12.5 months.[55]

A complementary single-arm phase II study examined nivolumab, another anti–PD-1 monoclonal antibody, in 74 individuals with chemotherapy-refractory MMR-D/MSI-H colorectal cancer.[56] An investigator-assessed objective response rate of 31.3% (23 of 74 patients) was observed in this study, and the median duration of response was not reached during the study period (median follow-up time, 12.0 months).[56] Likewise, the median overall survival was not reached during the study period, and the median progression-free survival was 14.3 months, which indicated that the responses experienced by patients with MMR-D/MSI-H colorectal cancer in this study were quite durable.[56]

Data about the use of anti–PD-1 antibodies in advanced MMR-D/MSI-H cancers to date have not shown any significant difference in response rates or outcomes among individuals with known Lynch syndrome compared wtih those without Lynch syndrome.[54-56] Correlative translational data[55] have demonstrated marked expansion of T cells targeted toward frameshift neopeptides after treatment with anti–PD-1 antibody therapy in patients who experienced objective responses, which provides strong support to the hypothesis that these antigenic frameshift neopeptides are a fundamental factor underlying the success with immune-based therapies and which provides promise for strategies that likewise leverage immune-based mechanisms to prevent Lynch syndrome–associated cancers. Data are emerging about mechanisms underlying both primary and secondary resistance mechanisms to anti–PD-1 therapy as well, which suggests that *β-2 microglobulin* mutations that lead to downregulation of antigenic presentation mechanisms may account for a sizeable fraction of resistance to immune checkpoint blockade.[56]

These exciting successes led to the accelerated approval by the U.S. Food and Drug Administration of pembrolizumab to treat advanced, pretreated MMR-D/MSI-H cancer (regardless of primary site) and nivolumab (MMR-D/MSI-H colorectal cancer only) in 2017. Most recently, a single-arm phase II study of nivolumab with ipilimumab (a monoclonal antibody targeted against CTLA-4, another immune checkpoint protein) in 119 individuals with advanced MMR-D/MSI-H colorectal cancer demonstrated an overall response rate of 55% (with 83% of all responses lasting ≥ 6 months) and a 12-month overall survival rate of 85%.[57] Such data suggest that there may be opportunities to synergize different mechanisms of immune checkpoint blockade with one another. Other ongoing clinical trials are examining the benefit of anti–PD-1 antibodies in the adjuvant treatment of resected stage III MMR-D/MSI-H colon cancers with and without chemotherapy (NCT02912559) and in the first-line treatment of metastatic MMR-D/MSI-H colorectal cancers (NCT02563002).

CANCER PREVENTION IN LYNCH SYNDROME

Colorectal Cancer

Prospective data with long-term follow-up have demonstrated that frequent and early colonoscopic evaluation of healthy individuals with Lynch syndrome can significantly reduce colorectal cancer incidence, colorectal cancer–associated mortality, and overall mortality, thereby solidifying such screening as the core preventive intervention in Lynch syndrome.[58] Recent data from a prospective multicenter European registry,[59] however, have raised questions as to whether the preventive benefits of intensive colonoscopic surveillance with polypectomy might be overstated, in part because recent data suggest that some Lynch syndrome–associated colorectal cancers may develop as directly invasive malignancies rather than through the traditional adenoma-to-carcinoma pathway.[60] Nonetheless, guidelines from ASCO, the European Society for Medical Oncology, the National Comprehensive Cancer Network, the U.S. Multi-Society Task Force on Colorectal Cancer, the American College of Gastroenterology, and others all consistently recommend colonoscopies every 1 to 2 years for healthy individuals with Lynch syndrome.[3,7,42-44] Such guidelines mostly agree that the optimal age at which to begin colonoscopic screening is age 20 to 25, although data that demonstrate comparably lower rates of colorectal cancer for families with germline *MSH6* and *PMS2* variants (vs. those with *MLH1* and *MSH2* variants) have prompted some experts to suggest that later initiation of colonoscopies may be safe in this subset of individuals with Lynch syndrome.[4,59]

Prophylactic colectomy is not considered a standard or necessary intervention for primary colorectal cancer risk reduction in individuals with Lynch syndrome, in large part because of the efficacy of colonoscopic surveillance.[7,42,44] For individuals with Lynch syndrome who develop an early-stage colorectal cancer, however, the risk of metachronous colorectal cancer is particularly high if segmental resection is used to treat the index cancer (up to 62% at 30-year follow-up).[61] Prospective registry data suggest that the risk of metachronous colorectal cancer can be reduced substantially by performing extensive colonic resection at the time of index colon cancer diagnosis (31% reduction in risk for every 10 cm of colon resected), although there is no

proven survival benefit to more extensive surgery.[61] Thus, patient-specific factors, such as age, bowel function, comorbidities, compliance with screening, and patient preference, should all be taken into consideration to decide between segmental and more extended colonic resection for a Lynch syndrome–associated colon cancer.

Endometrial, Ovarian, and Other Lynch Syndrome–Associated Cancers

For women with Lynch syndrome, endometrial cancer and ovarian cancer represent the second- and third-most common associated malignancy, respectively, after colorectal cancer. Numerous studies have attempted to research the benefit of screening for endometrial and ovarian cancer in women with Lynch syndrome with techniques that include transvaginal ultrasonography, endometrial biopsies, and cancer antigen 125 (CA-125) tumor marker testing.[62] Although such studies have shown some modest sensitivity for detection of endometrial carcinoma or endometrial hyperplasia with routine endometrial biopsies with or without ultrasonography, none of these screening techniques have consistently demonstrated high sensitivity to detect Lynch syndrome–associated endometrial or ovarian cancer, nor has such screening ever been shown to affect cancer incidence or mortality.[62]

Compelling observational data have shown that risk-reducing surgery with hysterectomy and salpingo-oophorectomy have marked efficacy for prevention of endometrial and ovarian cancer in women with Lynch syndrome, although it remains unclear whether such surgery confers any actual survival benefit.[63] Given the associated surgical risks as well as the associated psychological, cardiovascular, endocrinologic, skeletal, and sexual consequences of early-onset surgical menopause, however, it remains unclear as to how clinicians can best guide women with Lynch syndrome about the optimal timing of risk-reducing surgery. Furthermore, given the comparably lower risk of endometrial and ovarian cancers in women with *PMS2* variants (and possibly *MSH6* variants), some have questioned whether risk-reducing surgery might be overtreatment for some women with Lynch syndrome.[4] Despite these gaps in knowledge, most guidelines currently recommend consideration of risk-reducing hysterectomy and salpingo-oophorectomy at the completion of childbearing and/or in the early 40s, with consideration of annual transvaginal ultrasound and endometrial biopsy at age 30 to 35 (continued until risk-reducing surgery).[7,43,44]

Currently, there are no compelling data on effective screening for other Lynch syndrome–associated cancers, including gastric, urinary tract, pancreaticobiliary, small intestinal, or brain cancers. However, some guidelines recommend consideration of specialized screening in the setting of strong family histories of a particular cancer.[3]

Chemotherapeuticand Immune-Based Prevention

Aspirin and other cyclooxygenase-2 inhibitors have long been suspected to have modest effects at reduction of the risk of colorectal cancer and adenomas on the basis of both observational data and various randomized prevention trials.[64-66] To investigate whether such cancer-preventing benefits applied to patients with Lynch syndrome who have an inherently high risk of colorectal cancer, the international Colorectal Adenoma/Carcinoma Prevention Program 2 (CAPP2) study was launched in the late 1990s, for which individuals with Lynch syndrome were randomly assigned to receive 600 mg/day of aspirin or placebo (participants also were randomly assigned to take 30 g/day of resistant starch vs. placebo as a second intervention in this study). Although the first analysis after a mean of 29 months showed no significant difference in colorectal adenoma or carcinoma risk among those with Lynch syndrome in CAPP2 who received aspirin compared with placebo,[67] a preplanned long-term analysis ultimately demonstrated a marked reduction in colorectal cancer incidence (incidence rate ratio, 0.37; 95% CI, 0.18–0.78) among participants who took aspirin for 2 or more years compared with those randomly assigned to placebo.[8] Surprisingly, there was also a significant reduction in the incidence of any Lynch syndrome–associated cancer (incidence rate ratio, 0.59; 95% CI, 0.39–0.90) among participants who took aspirin for 2 or more years, which suggests that the preventive benefits may extend beyond the colorectum.[8] On the basis of these compelling data, daily aspirin is now considered a standard component of Lynch syndrome cancer prevention, although the ideal dose and duration of use are as yet undefined. The ongoing CAPP3 study is examining 100 mg/day, 300 mg/day, or 600 mg/day of aspirin in a prospective, randomized trial of patients with Lynch syndrome. Interestingly, a subgroup analysis of CAPP2 participants found that obesity was associated with an increased risk of colorectal cancer and also suggested that the preventive benefits of aspirin in Lynch syndrome may be limited to obese individuals.[68] Additional studies are needed to better clarify the interplay among aspirin, obesity, and dietary/lifestyle factors on cancer risk in Lynch syndrome. Various observational data have suggested potential cancer-preventing benefits from ibuprofen, calcium supplementation, and multivitamin use to reduce colorectal cancer risk in individuals with Lynch syndrome, although such interventions should not be considered standard in the absence of confirmatory prospective randomized clinical trials.[69,70]

Various studies also have examined the potential preventive benefits of exogenous hormone use to reduce the risk of endometrial cancer in women with Lynch syndrome. In one large observational study, use of hormonal contraceptives for 1 year or more was associated with a significantly reduced likelihood of endometrial cancer (HR 0.39; 95% CI, 0.23–0.64) in women with Lynch syndrome.[71] The same study also showed a mildly reduced likelihood of endometrial cancer in the setting of nulliparity and earlier onset of menarche.[71] Confirmatory prospective data about the preventive effects of hormonal factors in Lynch syndrome–associated endometrial cancer incidence are lacking, though one small prospective biomarker study demonstrated that progestin-containing oral contraceptives and

depo-medroxyprogesterone acetate use resulted in significantly reduced endometrial proliferation in pre- and postintervention biopsies.[72]

As outlined in this article, the phenomenon of MSI-H, which is a hallmark of Lynch-associated cancers, results in the accumulation of frameshift mutations at known microsatellite loci scattered throughout the coding and noncoding regions of the tumor genome.[52] The predictable nature of such frameshift mutations and their associated neopeptides has led to great interest in the notion of leveraging immune-based methods, such as vaccines, for primary prevention of Lynch syndrome–associated cancers.[73] Curiously, data have shown that healthy, cancer-free individuals with Lynch syndrome harbor circulating T cells that are reactive to such MSI-induced frameshift neopeptides, although they have never had a detectable cancer; this strongly suggests that innate immunosurveillance mechanisms already play a role in suppressing MSI-induced carcinogenesis in such individuals.[74] Nonneoplastic colonic crypts from healthy Lynch syndrome carriers have been shown to demonstrate MMR-D by IHC and MSI-H by PCR, which leads to the intriguing hypothesis that the healthy colon of patients with Lynch syndrome is itself a key source of immunogenic frameshift neopeptides that serve to autovaccinate such patients and suppress MSI-induced carcinogenesis.[75] A more precise understanding of the mechanisms by which Lynch syndrome–associated carcinogenesis escapes immune surveillance will be key to help leverage such discoveries into immune-based cancer prevention.[52,76]

CONCLUSION

The identification and management of individuals and families with Lynch syndrome has evolved rapidly during the past decade or so. Advances in molecular testing and NGS technologies now allow all patients with colorectal and endometrial cancers to reliably receive screening for underlying Lynch syndrome, whereas innovations in immuno-oncology promise to continue revolutionizing the treatment of Lynch-associated cancers. To continue moving the needle forward, expanded efforts to diagnose Lynch syndrome in healthy, cancer-free individuals are needed, rather than relying on the identification of Lynch syndrome through a new cancer diagnosis. Identification of Lynch syndrome offers the potential to prevent cancer-related morbidity and mortality, and continued progress in understanding the immune system's ability to recognize, eradicate, and intercept Lynch-associated neoplasia offers many intriguing possibilities for immune-based primary cancer prevention.

References

1. Boland CR, Lynch HT. The history of Lynch syndrome. *Fam Cancer*. 2013;12:145-157.
2. Win AK, Jenkins MA, Dowty JG, et al. Prevalence and penetrance of major genes and polygenes for colorectal cancer. *Cancer Epidemiol Biomarkers Prev*. 2017;26:404-412.
3. NCCN Clinical Practice Guidelines in Oncology. Genetic/Familial High-Risk Assessment: Colorectal, Version 3.2017. http://www.nccn.org/professionals/physician_gls/pdf/genetics_colon.pdf. Accessed March 15, 2018.
4. Moller P, Seppala TT, Bernstein I, et al. Cancer risk and survival in path_MMR carriers by gene and gender up to 75 years of age: a report from the Prospective Lynch Syndrome Database. *Gut*. Epub 2017 July 28.
5. Moreira L, Balaguer F, Lindor N, et al. Identification of Lynch syndrome among patients with colorectal cancer. *JAMA*. 2012;308:1555-1565.
6. Myrhoj T, Andersen MB, Bernstein I. Screening for urinary tract cancer with urine cytology in Lynch syndrome and familial colorectal cancer. *Fam Cancer*. 2008;7:303-307.
7. Syngal S, Brand RE, Church JM, et al. ACG clinical guideline: genetic testing and management of hereditary gastrointestinal cancer syndromes. *Am J Gastroenterol*. 2015;110:223-262.
8. Burn J, Gerdes AM, Macrae F, et al. Long-term effect of aspirin on cancer risk in carriers of hereditary colorectal cancer: an analysis from the CAPP2 randomised controlled trial. *Lancet*. 2011;378:2081-2087.
9. Risinger JI, Berchuck A, Kohler MF, et al. Genetic instability of microsatellites in endometrial carcinoma. *Cancer Res*. 1993;53:5100-5103.
10. Thibodeau SN, Bren G, Schaid D. Microsatellite instability in cancer of the proximal colon. *Science*. 1993;260:816-819.
11. Ionov Y, Peinado MA, Malkhosyan S, et al. Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. *Nature*. 1993;363:558-561.
12. Peltomaki P, Lothe RA, Aaltonen LA, et al. Microsatellite instability is associated with tumors that characterize the hereditary non-polyposis colorectal carcinoma syndrome. *Cancer Res*. 1993;53:5853-5855.
13. Rodriguez-Bigas M, Boland C, Hamilton S, et al. National Cancer Institute Workshop on Hereditary Nonpolyposis Colorectal Cancer Syndrome: meeting highlights and Bethesda guidelines. *J Natl Cancer Inst*. 1997;89:1758-1762.
14. Umar A, Boland CR, Terdiman JP, et al. Revised Bethesda guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. *J Natl Cancer Inst*. 2004;96:261-268.
15. Kautto EA, Bonneville R, Miya J, et al. Performance evaluation for rapid detection of pan-cancer microsatellite instability with MANTIS. *Oncotarget*. 2017;8:7452-7463.
16. Niu B, Ye K, Zhang Q, et al. MSIsensor: microsatellite instability detection using paired tumor-normal sequence data. *Bioinformatics*. 2014;30:1015-1016.
17. Salipante SJ, Scroggins SM, Hampel HL, et al. Microsatellite instability detection by next generation sequencing. *Clin Chem*. 2014;60:1192-1199.
18. Zhu L, Huang Y, Fang X, et al. A novel and reliable method to detect microsatellite instability in colorectal cancer by next-generation sequencing. *J Mol Diagn*. 2018;20:225-231.

19. Stadler ZK, Battaglin F, Middha S, et al. Reliable detection of mismatch repair deficiency in colorectal cancers using mutational load in next-generation sequencing panels. *J Clin Oncol*. 2016;34:2141-2147.

20. Nowak JA, Yurgelun MB, Bruce JL, et al. Detection of mismatch repair deficiency and microsatellite instability in colorectal adenocarcinoma by targeted next-generation sequencing. *J Mol Diagn*. 2017;19:84-91.

21. Dietmaier W, Wallinger S, Bocker T, et al. Diagnostic microsatellite instability: definition and correlation with mismatch repair protein expression. *Cancer Res*. 1997;57:4749-4756.

22. Leach FS, Polyak K, Burrell M, et al. Expression of the human mismatch repair gene *hMSH2* in normal and neoplastic tissues. *Cancer Res*. 1996;56:235-240.

23. Thibodeau SN, French AJ, Roche PC, et al. Altered expression of *hMSH2* and *hMLH1* in tumors with microsatellite instability and genetic alterations in mismatch repair genes. *Cancer Res*. 1996;56:4836-4840.

24. Cunningham JM, Christensen ER, Tester DJ, et al. Hypermethylation of the hMLH1 promoter in colon cancer with microsatellite instability. *Cancer Res*. 1998;58:3455-3460.

25. Deng G, Chen A, Hong J, et al. Methylation of CpG in a small region of the hMLH1 promoter invariably correlates with the absence of gene expression. *Cancer Res*. 1999;59:2029-2033.

26. Herman JG, Umar A, Polyak K, et al. Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. *Proc Natl Acad Sci USA*. 1998;95:6870-6875.

27. Hampel H, Frankel WL, Martin E, et al. Feasibility of screening for Lynch syndrome among patients with colorectal cancer. *J Clin Oncol*. 2008;26:5783-5788.

28. Hampel H, Frankel W, Panescu J, et al. Screening for Lynch syndrome (hereditary nonpolyposis colorectal cancer) among endometrial cancer patients. *Cancer Res*. 2006;66:7810-7817.

29. Deng G, Bell I, Crawley S, et al. *BRAF* mutation is frequently present in sporadic colorectal cancer with methylated hMLH1, but not in hereditary nonpolyposis colorectal cancer. *Clin Cancer Res*. 2004;10: 191-195.

30. Adar T, Rodgers LH, Shannon KM, et al. A tailored approach to *BRAF* and *MLH1* methylation testing in a universal screening program for Lynch syndrome. *Mod Pathol*. 2017;30:440-447.

31. Palomaki GE, McClain MR, Melillo S, et al. EGAPP supplementary evidence review: DNA testing strategies aimed at reducing morbidity and mortality from Lynch syndrome. *Genet Med*. 2009;11:42-65.

32. Geurts-Giele WR, Leenen CH, Dubbink HJ, et al. Somatic aberrations of mismatch repair genes as a cause of microsatellite-unstable cancers. *J Pathol*. 2014;234:548-559.

33. Haraldsdottir S, Hampel H, Tomsic J, et al. Colon and endometrial cancers with mismatch repair deficiency can arise from somatic, rather than germline, mutations. *Gastroenterology*. 2014;147:1308-1316.e1.

34. Sourrouille I, Coulet F, Lefevre JH, et al. Somatic mosaicism and double somatic hits can lead to MSI colorectal tumors. *Fam Cancer*. 2013;12:27-33.

35. Pearlman R, Hampel H, De la Chapelle A, et al. Ohio colorectal cancer prevention initiative. *Fam Cancer*. 2017;16:1-134.

36. Grosse SD. When is genomic testing cost-effective? Testing for Lynch syndrome in patients with newly-diagnosed colorectal cancer and their relatives. *Health Care (Don Mills)*. 2015;3:860-878.

37. Grosse SD, Palomaki GE, Mvundura M, et al. The cost-effectiveness of routine testing for Lynch syndrome in newly diagnosed patients with colorectal cancer in the United States: corrected estimates. *Genet Med*. 2015;17:510-511.

38. Mvundura M, Grosse SD, Hampel H, et al. The cost-effectiveness of genetic testing strategies for Lynch syndrome among newly diagnosed patients with colorectal cancer. *Genet Med*. 2010;12:93-104.

39. Gallego CJ, Shirts BH, Bennette CS, et al. Next-generation sequencing panels for the diagnosis of colorectal cancer and polyposis syndromes: a cost-effectiveness analysis. *J Clin Oncol*. 2015;33:2084-2091.

40. Hampel H, Pearlman R, Beightol MB, et al. Assessment of tumor sequencing as a replacement for current Lynch syndrome screening tests in colorectal cancer while also identifying therapeutic targets. *JAMA Oncol*. In press.

41. Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group. 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*. 2009;11:35-41.

42. Balmana J, Balaguer F, Cervantes A, et al. Familial risk-colorectal cancer: ESMO clinical practice guidelines. *Ann Oncol*. 2013;24:vi73-vi80.

43. Giardiello FM, Allen JI, Axilbund JE, et al. Guidelines on genetic evaluation and management of Lynch syndrome: a consensus statement by the US multi-society task force on colorectal cancer. *Gastroenterology*. 2014;147:502-526.

44. Stoffel EM, Mangu PB, Gruber SB, et al. Hereditary colorectal cancer syndromes: American Society of Clinical Oncology clinical practice guideline endorsement of the familial risk-colorectal cancer: European Society for Medical Oncology clinical practice guidelines. *J Clin Oncol*. 2015;33:209-217.

45. Committee on Practice Bulletins-Gynecology; Society of Gynecology Oncology. ACOG practice bulletin No. 147: Lynch syndrome. *Obstet Gynecol*. 2014;124:1042-1054.

46. Shaikh T, Handorf EA, Meyer JE, et al. Mismatch repair deficiency testing in patients with colorectal cancer and nonadherence to testing guidelines in young adults. *JAMA Oncol*. 20184:e173580.

47. Kastrinos F, Allen JI, Stockwell DH, et al. Development and validation of a colon cancer risk assessment tool for patients undergoing colonoscopy. *Am J Gastroenterol*. 2009;104:1508-1518.

48. Kastrinos F, Uno H, Ukaegbu C, et al. Development and validation of the PREMM5 model for comprehensive risk assessment of Lynch syndrome. *J Clin Oncol*. 2017;35:2165-2172.

49. Pearlman R, Frankel WL, Swanson B, et al. Prevalence and spectrum of germline cancer susceptibility gene mutations among patients with early-onset colorectal cancer. *JAMA Oncol*. 2017;3:464-471.

50. Yurgelun MB, Kulke MH, Fuchs CS, et al. Cancer susceptibility gene mutations in individuals with colorectal cancer. *J Clin Oncol*. 2017;35:1086-1095.

51. Sankila R, Aaltonen LA, Jarvinen HJ, et al. Better survival rates in patients with *MLH1*-associated hereditary colorectal cancer. *Gastroenterology*. 1996;110:682-687.

52. Spira A, Disis ML, Schiller JT, et al. Leveraging premalignant biology for immune-based cancer prevention. *Proc Natl Acad Sci USA*. 2016; 113:10750-10758.

53. Kloor M, von Knebel Doeberitz M. The Immune biology of microsatellite-unstable cancer. *Trends Cancer*. 2016;2:121-133.

54. Le DT, Uram JN, Wang H, et al. PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med*. 2015;372:2509-2520.

55. Le DT, Durham JN, Smith KN, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science*. 2017;357:409-413.

56. Overman MJ, McDermott R, Leach JL, et al. Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability–high colorectal cancer (CheckMate 142): an open-label, multicentre, phase 2 study. *Lancet Oncol*. 2017;18:1182-1191.

57. Overman MJ, Lonardi S, Wong KYM, et al. Durable clinical benefit with nivolumab plus ipilimumab in DNA mismatch repair-deficient/microsatellite instability-high metastatic colorectal Cancer. *J Clin Oncol*. 2018;36:773-779.

58. Jarvinen HJ, Aarnio M, Mustonen H, et al. Controlled 15-year trial on screening for colorectal cancer in families with hereditary nonpolyposis colorectal cancer. *Gastroenterology*. 2000;118:829-834.

59. Moller P, Seppala T, Bernstein I, et al. Cancer incidence and survival in Lynch syndrome patients receiving colonoscopic and gynaecological surveillance: first report from the prospective Lynch syndrome database. *Gut*. 2017;66:464-472.

60. Ahadova A, von Knebel Doeberitz M, Blaker H, et al. *CTNNB1*-mutant colorectal carcinomas with immediate invasive growth: a model of interval cancers in Lynch syndrome. *Fam Cancer*. 2016;15:579-586.

61. Parry S, Win AK, Parry B, et al. Metachronous colorectal cancer risk for mismatch repair gene mutation carriers: the advantage of more extensive colon surgery. *Gut*. 2011;60:950-957.

62. Auranen A, Joutsiniemi T. A systematic review of gynecological cancer surveillance in women belonging to hereditary nonpolyposis colorectal cancer (Lynch syndrome) families. *Acta Obstet Gynecol Scand*. 2011;90:437-444.

63. Schmeler KM, Lynch HT, Chen LM, et al. Prophylactic surgery to reduce the risk of gynecologic cancers in the Lynch syndrome. *N Engl J Med*. 2006;354:261-269.

64. Baron JA, Cole BF, Sandler RS, et al. A randomized trial of aspirin to prevent colorectal adenomas. *N Engl J Med*. 2003;348:891-899.

65. Bertagnolli MM, Eagle CJ, Zauber AG, et al. Celecoxib for the prevention of sporadic colorectal adenomas. *N Engl J Med*. 2006;355:873-884.

66. Cao Y, Nishihara R, Wu K, et al. population-wide impact of long-term use of aspirin and the risk for cancer. *JAMA Oncol*. 2016;2:762-769.

67. Burn J, Bishop DT, Mecklin JP, et al. Effect of aspirin or resistant starch on colorectal neoplasia in the Lynch syndrome. *N Engl J Med*. 2008;359:2567-2578.

68. Movahedi M, Bishop DT, Macrae F, et al. Obesity, aspirin, and risk of colorectal cancer in carriers of hereditary colorectal cancer: a prospective investigation in the CAPP2 study. *J Clin Oncol*. 2015;33:3591-3597.

69. Ait Ouakrim D, Dashti SG, Chau R, et al. Aspirin, ibuprofen, and the risk of colorectal cancer in Lynch syndrome. *J Natl Cancer Inst*. 2015;107:djv170.

70. Chau R, Dashti SG, Ait Ouakrim D, et al. Multivitamin, calcium and folic acid supplements and the risk of colorectal cancer in Lynch syndrome. *Int J Epidemiol*. 2016;45:940-953.

71. Dashti SG, Chau R, Ouakrim DA, et al. Female Hormonal factors and the risk of endometrial cancer in Lynch syndrome. *JAMA*. 2015;314:61-71.

72. Lu KH, Loose DS, Yates MS, et al. Prospective multicenter randomized intermediate biomarker study of oral contraceptive versus depo-provera for prevention of endometrial cancer in women with Lynch syndrome. *Cancer Prev Res*. 2013;6:774-781.

73. von Knebel Doeberitz M, Kloor M. Towards a vaccine to prevent cancer in Lynch syndrome patients. *Fam Cancer*. 2013;12:307-312.

74. Schwitalle Y, Kloor M, Eiermann S, et al. Immune response against frameshift-induced neopeptides in HNPCC patients and healthy HNPCC mutation carriers. *Gastroenterology*. 2008;134:988-997.

75. Kloor M, Huth C, Voigt AY, et al. Prevalence of mismatch repair-deficient crypt foci in Lynch syndrome: a pathological study. *Lancet Oncol*. 2012;13:598-606.

76. Yurgelun MB, Chenevix-Trench G, Lippman SM. Translating germline cancer risk into precision prevention. *Cell*. 2017;168:566-570.