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Recent Progress in Lynch Syndrome and Other Familial Colorectal Cancer Syndromes

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Abstract

Our understanding of familial colorectal cancer was limited to descriptions of affected pedigrees until the early 1990s. A series of landscape-altering discoveries revealed that there were distinct forms of familial cancer, and most were related to genes previously not known to be involved in human disease. This review largely focuses on advances in our understanding of Lynch syndrome, because of the unique relationship of this disease to defective DNA mismatch repair, and the clinical implications this has for diagnostics, prevention, and therapy. Recent advances have occurred in our understanding of the epidemiology of this disease, and the advent of broad genetic panels has altered the approach to germline and somatic diagnoses for all of the familial colorectal cancer syndromes. Important advances have been made towards a more complete mechanistic understanding of the pathogenesis of neoplasia in the setting of Lynch syndrome that has important implications for prevention. Finally, paradigm-shifting approaches to treatment of Lynch-syndrome and related tumors have occurred through the development of immune checkpoint therapies for hypermutated cancers.

Keywords

familial colorectal cancer; DNA mismatch repair; APC gene; Lynch syndrome; immune checkpoint therapy; germline mutations; genetic testing

Introduction

Familial clustering of colorectal cancer (CRC) had been noted since the beginning of the 20th century, but understanding the mechanistic basis of this problem did not begin until the 1990s.[1] Clinicians had defined the colorectal polyposis syndromes much earlier because of the distinctive clinical phenotypes, noting that familial adenomatous polyposis (FAP) and the non-adenomatous intestinal polyposis syndromes (Peutz-Jeghers Syndrome, juvenile

polyposis and others) were associated with an increased risk of CRC. However, it was less obvious what might underlie familial non-polyposis CRC. Discoveries in this research area were unexpected, and each led investigators into novel areas of cancer biology.

The first discovery seemed minor enough—the chance finding of an individual with *de novo* FAP who had multiple congenital abnormalities and a deletion in chromosome 5q that was visible cytogenetically.[2] This led to the mapping of the gene responsible for FAP in this region and the discovery that this locus tended to be lost somatically in sporadic CRCs,[3, 4] the cloning of the *APC* gene from 5q22-23 by two different laboratories[5, 6], and the first insights into the evolution of most common CRCs. The *APC* gene had an unprecedented DNA sequence, and the gene product was found to be responsible for regulating proliferation in gastrointestinal epithelium. Eventually, the genes responsible for the non-adenomatous hamartomatous polyposis syndromes were also characterized, which led investigators into previously uncharted areas of cell biology involving inactivated kinases, transcription factors and phosphatases that played key roles in regulating cell proliferation. None of this was remotely predictable before each discovery, and each new insight raised new possibilities for understanding the natural histories of these disorders and novel approaches to therapy.

Uncovering the basis of the familial non-polyposis CRC syndromes, the focus of this review, led to the discovery of a novel and unexpected pathway of carcinogenesis, with important clinical implications. It had been suspected that some clusters of CRC were not associated with polyposis and were not chance clusters of this relatively common disease.[1] In 1993, Manuel Perucho[7] discovered the microsatellite instability (MSI) phenotype in a subset of CRCs, and proposed a novel pathway for the development of CRC. Others made the same discovery in this time frame.[8, 9] Familial non-polyposis CRC was linked to a locus on chromosome 2p,[10] MSI was found in these tumors, and hereditary non-polyposis CRC was traced to mutations in four genes in the DNA mismatch repair (MMR) system. These mutations define the hereditary disease called Lynch syndrome, previously known by the somewhat confusing term “hereditary nonpolyposis colorectal cancer” or HNPCC; use of this term has fallen out of favor as it includes multiple unrelated diseases.[11] Knowing the genetic basis of Lynch syndrome led to novel concepts of tumor development, unique insights into the natural history of familial CRC, and an extraordinary new chapter in cancer therapy that involves manipulation of the immune response to cancer (Table 1).

Germline basis of familial CRC

At present, there are genes associated with nearly all of the hereditary CRC syndromes, but there are also familial clusters of polyposis and CRC for which no germline mutation can be found. It has long been recognized that having a first-degree relative (e.g., parent, sibling, or child) with CRC increases one’s own risk of developing CRC, and that having multiple such relatives with CRC further compounds this risk[12]. Roughly 15-20% of CRC diagnoses occur in individuals with at least one first-degree relative with the disease, and such families are often labeled as having “familial CRC”[12–14]. In spite of this label, however, it has generally been recognized that fewer than 5% of CRC patients harbor germline variants in CRC susceptibility genes, indicating that the majority of such familial CRC heritability remains

unexplained by identifiable monogenic germline variants. Although as yet unidentified CRC susceptibility genes may explain some of this missing heritability, polygenic, environmental, and/or behavioral factors may account for much of this unexplained risk[15–18].

The commonest form of familial CRC in which specific germline variants can be identified is Lynch syndrome, which is associated with alterations in four DNA MMR genes—*MSH2* plus *EpCAM, MLH1, MSH6* and *PMS2*. This entity is recognized by the near-universal presence of MSI in the tumors. However, there are many confounders of MSI in CRC, including biallelic somatic MMR gene mutations and methylation-induced silencing of *MLH1*. The location and sequences of all of the DNA MMR genes have been known for some time, and the details can be found in an excellent review by Peltomaki.[19] Inheriting biallelic mutations in the same Lynch syndrome gene causes oligopolyposis and a pediatric cancer diathesis known as biallelic MMR deficiency (BMMRD, also called constitutional mismatch repair deficiency [CMMR-D] syndrome), as discussed in the next section.

The colonic adenomatous polyposis syndromes have undergone recent updating with the addition of the autosomal recessive forms caused by biallelic mutations in *NTLH1*[20] and *MSH3*,[21] complementing the previously known recessive form of polyposis caused by mutations in the *MutYH* gene (causing *MutYH*-associated polyposis, or MAP). Autosomal dominant oligopolyposis syndromes have been linked to germline mutations in the DNA polymerase proofreading genes *POLE* and *POLD1*.[22] Also, germline alterations in a candidate gene, *RNF43*, have been identified in at least a few patients with serrated polyposis syndrome,[23] although this entity is usually not familial. Many other putative familial CRC genes have been proposed, but most are uncommon, possibly even “private”, and limited to a single small lineage. This is discussed in detail in an excellent review by Valle.[24]

Epidemiology of Lynch syndrome

The prevalence of germline MMR gene mutations has been traditionally calculated in the context of patients diagnosed with CRC or endometrial cancer (EC), where Lynch syndrome accounts for about 3%[25–30] and 2%[31–33] of cases, respectively. When Lynch syndrome is ascertained from individuals with a personal history of cancer, studies have consistently found that germline mutations in *MLH1* and *MSH2* account for a sizeable majority (60-80%) of cases, with a relative minority of cases having germline mutations in *MSH6* and *PMS2*, and germline *EPCAM* mutations (which lead to the epigenetic inactivation of *MSH2*) being particularly rare.[25, 26, 29] Recent epidemiologic data, however, have begun to shed light on the true prevalence of Lynch syndrome in the overall population, showing it to be markedly more common and less penetrant than traditionally appreciated. Investigators recently examined clinical data from 5,744 CRC and 37,634 first-degree relatives recruited through population-based registries in the United States, Canada, and Australia via the Colon Cancer Family Registry (CCFR). Using these data in conjunction with available germline testing results, the investigators modeled the carrier frequency of pathogenic germline variants in the four MMR genes within the general population to be 0.051% (1:1946) for *MLH1* mutations, 0.035% (1:2841) for *MSH2* mutations, 0.132% (1:758) for *MSH6* mutations, and 0.140% (1:714) for *PMS2* mutations, resulting in an aggregate carrier

frequency of 0.359% (1:279) for any MMR gene mutation.[15] Interestingly, these data suggest that the genes in which pathogenic variants are most prevalent in the general population (*MSH6* and *PMS2*) have been consistently found to be comparably uncommon in Lynch syndrome patients ascertained because of a personal history of cancer. The most likely explanation for this apparent discordance is that germline variants in *MSH6* and *PMS2* confer much more modest risks of cancer than *MLH1* and *MSH2* variants.[34–39]

While Lynch syndrome appears to be common across a diversity of ethnicities, the effect of founder mutations may make it particularly prevalent in certain populations. Population-based data from Iceland, for example, have shown country's overall prevalence of Lynch syndrome to be approximately 0.442% (1:226), with the sizeable majority of cases being traced to three founder mutations: *MSH6* p.L585P (estimated carrier frequency 0.080% or 1:1250), *PMS2* p.M1? (0.092%, 1:1087), and *PMS2* p.P246Cfs*3 (0.234%, 1:427).[40] Other research has demonstrated that the *MSH6* p.Q4* founder mutation to be particularly prevalent in French Canadian individuals, with an estimated carrier frequency of 0.249% (1:402) and haplotype analysis suggests that the mutation arose in a common ancestor 430-656 years ago.[41] Although their population prevalence is not well understood, founder mutations in *MSH2* (c.1906G>C, p.A636P) and *MSH6* (c.3959_3962delCAAG and c. 3984_3987dupGTCA) appear to account for the majority of Lynch syndrome cases in individuals of Ashkenazi Jewish ancestry.[42, 43] Additional data are identifying other candidate founder mutations in various populations with undefined prevalence, including Americans of German ancestry,[44] African Americans,[45] Latinos,[46] Poles,[47] and many other groups.[48]

Founder mutations may account for a sizeable fraction of individuals with biallelic MMR deficiency (BMMRD) syndrome, caused by biallelic inheritance of pathogenic germline variants in the same MMR gene.[49] BMMRD is a rare and often devastating syndrome, characterized by pediatric-onset brain tumors (including gliomas and medulloblastomas), small bowel and large bowel adenomas and adenocarcinomas, lymphomas, leukemias, endometrial carcinomas, and café-au-lait macules (which can lead to a misdiagnosis of neurofibromatosis type 1).[50–54] Patients with BMMRD have abnormal MMR IHC in both neoplastic and normal tissue, but curiously, the cancers often lack MSI by PCR.[50] Of the small number of BMMRD cases reported in the literature to date, a surprisingly large fraction harbor biallelic germline mutations in *MSH6* or *PMS2*, presumably due to the aforementioned founder effects and the higher prevalence of these mutations in the general population.[51, 52] Another curious feature of BMMRD has been a relative paucity of Lynch syndrome-associated cancers in family members, especially considering that both parents of a BMMRD proband have Lynch syndrome, by definition.[50] This, too, may be partially explained by the relative overrepresentation of the germline mutations in the low-penetrance MMR genes (*MSH6* and *PMS2*) among BMMRD probands.

Current status of germline and somatic DNA testing

The past decade has seen a dramatic shift in the clinical evaluation of individuals with suspected hereditary cancer risk due to the widespread commercial availability of next-generation sequencing (NGS) multigene panels for germline testing as an alternative to

traditional syndrome-specific genetic testing (Table 2). Such panels typically include dozens of cancer susceptibility genes with diverse spectra of cancer risks, many of which are poorly understood. Data examining multigene panels in a variety of clinical situations[25, 55–59] consistently demonstrate that many individuals found to harbor pathogenic or likely pathogenic germline cancer susceptibility gene variants have clinical histories that fail to fulfill traditional syndrome-specific guidelines (e.g. Lynch syndrome probands who do not fulfill Amsterdam Criteria or Bethesda Guidelines) and have atypical clinical phenotypes for their specific syndrome (e.g., CRC patients with pathogenic germline *BRCA1* or *BRCA2* variants; breast cancer patients with germline MMR gene variants), including some rare individuals with pathogenic germline variants in both Lynch and non-Lynch genes.

Multigene panel testing in one large single-center study[25] of 1058 CRC patients who were not pre-selected by MMR IHC/MSI status, age, or personal/family history of cancer revealed that 9.9% of individuals carried at least one pathogenic germline variant in a 25-gene panel, including 3.1% with Lynch syndrome, nearly all of whom had MSI/MMR-deficient tumors. Beyond Lynch syndrome, however, the study found 7.0% of CRC patients with at least one non-Lynch germline mutation, including 0.8% with adenomatous polyposis (*APC* or biallelic *MutYH* mutations), 3.2% with variants in genes linked to modestly increased risks of CRC (the Ashkenazi founder *APC* p.I1307K allele, monoallelic *MutYH* variants, or *CHEK2* variants), 1.0% with deleterious *BRCA1* or *BRCA2* variants, and 1.8% with deleterious variants in other genes not known to be linked to CRC risk (*ATM*, *CDKN2A*, *PALB2*, and others).[25] Intriguingly, a large proportion of individuals with non-Lynch mutations in this study lacked clinical phenotypes suggestive of their underlying syndrome (e.g., 8 of the 11 *BRCA1/2* probands had personal/family histories that failed to fulfill National Comprehensive Cancer Network [NCCN] criteria for *BRCA1/2* testing), suggesting that such mutations would have gone undiagnosed by standard clinical care.[25] A complementary multicenter study[55] examining multigene panel testing among 450 individuals diagnosed with CRC prior to age 50 found deleterious germline variants in 16% of individuals, including 8.2% with Lynch syndrome, 2% with inherited polyposis (*APC*, biallelic *MutYH*, *SMAD4*), 2.2% with mutations in low- or moderate-penetrance CRC susceptibility genes (*APC* p.I1307K allele, monoallelic *MutYH* variants), and 1.3% with *BRCA1/2* mutations. Thus, the curation of familial cancer pedigrees facilitated the discovery of the relevant syndrome-producing genes, but the use of diagnostic genetic panels revealed that the penetrance and expressivity related to mutant genes can be quite heterogeneous.

With data consistently demonstrating a wide diversity of germline mutations in CRC patients, many of whom lack obvious clinical features of hereditary risk, the question has arisen whether all individuals diagnosed with CRC, or perhaps, all individuals diagnosed before age 50, should undergo multigene panel testing, regardless of other clinical features.[25, 55, 60] Counterarguments to this provocative suggestion include the fact the penetrance and appropriate management of many “unexpected” germline mutations remain unknown, especially for germline variants linked to only modestly increased risks of cancer, which appear to be quite common.[25] Monoallelic *MutYH* mutations, for instance, have been linked to a 1.5-2-fold increased risk of CRC[61–63] though not polyposis (as occurs in MutYH-associated polyposis, or MAP, with biallelic germline *MutYH* mutations). Such data have prompted NCCN guidelines to recommend earlier and more frequent colonoscopic

screening for monoallelic *MutYH* carriers, even in the absence of family history, though it remains unknown if this actually translates into improved outcomes.

Another area of controversy with multigene panel testing has been deciphering the significance of high-penetrance mutations in genes not traditionally linked to CRC (e.g., *BRCA1* or *BRCA2*) when found in an individual with CRC. Some have questioned whether these unexpected findings are simply the result of detecting the background population prevalence of such alterations through what is, essentially, population-based screening.[64] Others have postulated that these findings are the result of pleiotropism (i.e., a germline variant manifesting itself with a variety of clinical phenotypes), which would suggest a (presumably weak) causative link between *BRCA1/2* alterations and some CRCs. Studies to date have observed a lack of any enrichment for *BRCA* alterations among familial CRC cohorts compared to controls, which argues against pleiotropy playing a significant role in the link between *BRCA1/2* alterations and CRC risk.[65]

One key finding from multigene panel testing data has been confirmation of the concept that tumor screening with MMR protein IHC and/or MSI testing remains a highly effective (>90% sensitivity) means of screening for Lynch syndrome among individuals with CRC or EC,[25, 55, 57] thereby supporting the ongoing use of such tumor-testing practices in CRC and EC patients ≤70 years old (or perhaps all patients), as is currently recommended by multiple guidelines (Table 3).[66–68] Smaller studies have suggested universal tumor testing with MMR IHC may also be effective in screening for Lynch syndrome among individuals with less common forms of Lynch syndrome-associated cancer, such as upper tract urothelial cancer (i.e., renal pelvis and ureter).[69] Caution should be used when interpreting MMR IHC results from sebaceous neoplasms of the skin, which appear disproportionately likely, compared to other Lynch-associated neoplasms, to demonstrate sporadic MSI-H/MMR-deficiency in the absence of underlying Lynch syndrome.[70–72] It is also important to note that for rectal cancers, when radiotherapy is administered prior to surgery, the MMR IHC assay can be inaccurate, primarily due to artefactual loss of MSH6 in the surgical specimen.[73] Thus, pre-therapy tissue or PCR-based MSI testing should be the preferred approach in this situation.

As universal tumor testing has become more widespread in clinical practice, data are now emerging that highlight the real-world challenges of capitalizing on its theoretical benefits, particularly with regards to appropriate referral for genetic counseling and testing after abnormal MMR IHC and/or MSI test results.[24, 30, 74, 75] Data also demonstrate that somatic NGS panels have the ability to assess somatic mutational burden as a highly concordant surrogate for MSI/dMMR status, suggesting that such somatic testing may be able to replace traditional MMR IHC and MSI testing, especially when universally implemented for ascertainment of other clinically relevant somatic mutations (e.g., *KRAS*, *NRAS*, and *BRAF* mutation status in CRC)[76, 77].

Another potential pitfall that has emerged as universal MMR IHC/MSI tumor testing has become widespread is the molecular diversity of sporadic forms of MSI/dMMR CRCs and ECs. Somatic hypermethylation of the *MLH1* promoter region has long been recognized[78–80] as the most common cause of MSI/dMMR in both CRC and EC, and testing for this is commonly used to determine which patients with tumors lacking MLH1/PMS2

expression by IHC can forego germline testing for Lynch syndrome[81–83]. An important caveat is that *MLH1* promoter methylation can very rarely[84, 85] arise through non-Mendelian inheritance[86, 87] of germline *MLH1* epimutations, which cause a Lynch syndrome phenotype.

Individuals with MSI/MMR-deficient CRCs or ECs who lack pathogenic germline MMR variants and whose tumors lack *MLH1* promoter hypermethylation have long been considered to have "presumed Lynch syndrome"; moreover, some publications have alternatively used the confusing term "Lynch-like syndrome" to categorize such patients. Recently, however, somatic NGS data have demonstrated that the majority of such cases have biallelic somatic inactivation of one or more MMR genes, indicating that such individuals do not have Lynch syndrome.[24, 55, 88] Unfortunately, clinical testing of tumor tissue for biallelic somatic MMR gene inactivation to rule out Lynch syndrome in such cases is not widely available and the cost may not be covered by insurance if germline Lynch syndrome testing has already been performed.

For individuals who have not been affected by cancer, where tumor tissue is unavailable for MMR IHC/MSI testing, or where clinical suspicion for Lynch syndrome remains high in spite of normal MMR IHC/MSI results, clinical prediction models are accurate and a cost-effective means of identifying individuals who may benefit from germline testing for Lynch syndrome[28, 89, 90]. The PREdiction Model for gene Mutations (PREMM) models, for example, analyze individuals' sex, age, and personal/family history of cancer to generate a numeric estimate of the likelihood of underlying Lynch syndrome. The newest iteration of PREMM, $PREMM_5$, (http://premm.dfci.harvard.edu), is the first clinical prediction model to provide risk assessment for all five Lynch syndrome genes (*MSH2*+*EPCAM*, *MLH1*, *MSH6*, and *PMS2*), although the model's ability to discriminate *PMS2* mutation carriers from non-carriers is suboptimal, due to the attenuated phenotype demonstrated by many *PMS2* families.[91] Current NCCN guidelines[92] recommend that individuals predicted to have a ≥5% likelihood of Lynch syndrome by $PREMM_5$ undergo genetic evaluation, although the $PREMM_5$ authors advocate for use of a more liberal ≥2.5% $PREMM_5$ score threshold for germline LS testing, due to the markedly improved sensitivity with this lower cutoff.[91] Recent implementation data have demonstrated the feasibility of incorporating Lynch syndrome risk assessment with PREMM in routine clinical gastroenterology settings,[93] giving promise to the notion that widespread Lynch syndrome risk assessment could be systematically performed in other preventive healthcare settings to detect mutation carriers prior to the development of cancer.

Optimal surveillance of the colon in Lynch syndrome

There have been several guidelines published recommending that patients with Lynch syndrome should have surveillance colonoscopy every 1-2 years[66, 68]. For non-Lynch syndrome patients at increased risk for CRC, the recommended routine surveillance intervals are usually in the range of every five years, depending upon the circumstances. This difference reflects the fact that some Lynch syndrome patients are found to have neoplastic lesions within two years after an exam that appeared to clear the colon of all adenomas and

cancers. There have been multiple possible explanations for this, but none is accepted as an explanation for all “interval cancers”.

It is generally believed that neoplastic lesions in the context of Lynch syndrome can transition from a benign adenoma to a cancer in a shorter time frame than occurs in the sporadic setting, but there is little direct evidence to prove this. One mechanism for this possibility is that adenomatous polyps could initially develop in the colons of Lynch syndrome patients by the usual, common mechanism (which is inactivation of the control of *WNT* signaling in an epithelial cell, usually through biallelic loss of *APC*), and that the MMR deficiency develops later, in a growing adenoma, accelerating the growth characteristics of the adenoma and compressing the time frame for the accumulation of additional mutations required for malignant behavior. It is known that the MMR system is involved in regulating the G2/M checkpoint and will inhibit mitosis in the presence of some critical degree of DNA damage,[94, 95] and loss of the G2/M checkpoint permits uninhibited mitotic activity and accelerated accumulation of new mutations. Experimental evidence for this mechanism can be found in the observation that dMMR activity is substantially less frequent in small adenomas in Lynch syndrome, and virtually always present in adenomas >8 mm in diameter.[96] When one does comprehensive analyses of CRC genomes, the somatic mutational spectra of hypermutated CRCs (which includes all those with MSI) is different from non-hypermutated CRCs, and the only frequently mutated gene present in both groups is *APC*,[97] which is compatible with this explanation.

That said, Sekine et al examined the somatic mutational spectrum of adenomatous polyps with either dMMR or pMMR characteristics from Lynch syndrome patients (there were examples of both), and compared this with CRCs from Lynch syndrome patients, and sporadic adenomas that were pMMR. In the dMMR tissues, they found less frequent mutations in the *APC* gene, and found frameshift mutations in the mononucleotide repeats of the *RNF43* gene, which is involved in downstream regulation of *WNT* signaling. These findings are compatible with early loss of MMR activity in adenomas (or even non-neoplastic cells), followed by signature frameshift mutations in the genes mutated in Lynch syndrome neoplasms.[98] Consequently, the timing of loss of MMR activity during Lynch syndrome tumorigenesis is not clearly resolved at this point, and there is evidence for both early and later loss of MMR.

In addition, a third possible explanation is that the CRCs in Lynch syndrome may not necessarily pass through a traditional adenoma stage. Kloor and colleagues have found isolated, non-neoplastic dMMR crypts in the colons and small intestines of patients with Lynch syndrome, and propose that some of these may grow directly into dMMR CRCs.[99] Moreover, this group had found that some Lynch syndrome-associated CRCs often do not seem to have arisen in a polyp by morphological assessment (25/40, 62.5% in Lynch versus 17/34, 50% of sporadic MSI CRCs). They propose that these tumors may have acquired the ability for “immediate invasive growth,” and have associated this with somatic mutations in the *β-catenin* gene, which was mutated in 8/46 (17.4%) of Lynch syndrome-associated CRCs, but in 0/34 of sporadic MSI CRCs.[100] Any of these mechanisms is plausible, and more study is needed to determine what is responsible for the interval CRC problem in

Lynch syndrome. One should keep an open mind here; it is possible that more than one explanation will be required to fully understand the problem.

Chemoprevention in Lynch syndrome

The landmark Colorectal Adenoma/carcinoma Prevention Programme 2 (CAPP2) study examined the chemopreventive effects of resistant starch (30 mg/day) and high-dose (600 mg/day) aspirin in 937 individuals with Lynch syndrome in a two-by-two randomized double-blinded placebo-controlled trial. Although resistant starch was ultimately shown to have no meaningful chemopreventive benefit with respect to adenoma or carcinoma formation,[101, 102] individuals randomized to aspirin who completed at least two years' of therapy were found to have markedly lower rates of CRC (HR 0.41; 95% CI 0.19-0.86) and all Lynch syndrome-associated cancers (HR 0.45; 95% CI 0.26-0.79) after a mean follow-up of 55.7 months, compared to those randomized to placebo.[103] Importantly, there was no significant increase in adverse events in individuals randomized to aspirin, compared to placebo.[103] The ongoing CAPP3 study is examining the chemopreventive effects of different doses of aspirin (100 mg/day, 300 mg/day, and 600 mg/day) in Lynch syndrome. Subgroup analysis of the CAPP2 study revealed body mass index ≥30 kg/m^2 to be a significant risk factor for CRC in individuals taking placebo, but not aspirin, suggesting that the chemopreventive effects of aspirin in Lynch syndrome may be most pronounced in obese individuals.[104] Of note, the chemopreventive benefits of aspirin in CAPP2 did not include fewer adenomas, and the effects on cancer risk reduction were found to be delayed for nearly a decade, mirroring prior data examining aspirin for CRC prevention in the general population.[102, 103]

Observational data from the CCFR have similarly suggested a chemopreventive effect of both aspirin and ibuprofen in individuals with Lynch syndrome.[105] Among 1858 individuals with known pathogenic germline MMR variants, self-reported aspirin use was associated with a markedly reduced likelihood of developing CRC (HR 0.49, 95% 0.27-0.90 for aspirin use between 1 month and 4.9 years; HR 0.25, 95% CI 0.10-0.62 for aspirin use ≥5 years), and a comparable effect was seen with self-reported ibuprofen use (HR 0.38, 95% 0.18-0.79 for ibuprofen use between 1 month and 4.9 years; HR 0.26, 95% CI 0.10-0.69 for ibuprofen use ≥5 years). Other data from the CCFR have suggested that ≥3 years of multivitamin use (HR 0.47; 95% CI 0.32-0.69) and ≥3 years of calcium supplementation (HR 0.42; 95% CI 0.23-0.74) are associated with reduced likelihood of CRC[106] although large-scale, prospective, placebo-controlled prevention trials with these agents have failed to show reduced CRC incidence in non-Lynch syndrome populations.[107, 108] Prospective, randomized data are warranted before ibuprofen, calcium, or multivitamins should be recommended for routine chemopreventive use in Lynch syndrome.

Studies have also examined the chemopreventive effects of exogenous progestins for reducing EC risk in women with Lynch syndrome. In a recent prospective phase II biomarker study of 51 women with Lynch syndrome, participants were randomized to three months of therapy with either a single dose of depo-medroxyprogesterone acetate or daily progestin-containing oral contraceptive pills. Transvaginal ultrasonography and endometrial biopsies were taken pre- and post-treatment to examine the effects of therapy on endometrial

proliferation as a surrogate for chemopreventive activity. The study found a marked reduction in endometrial proliferative activity by endometrial biopsy in both treatment arms with concurrently reduced expression of *IGF-1*, *survivin*, and other biomarkers. The study was not designed to definitively address the chemopreventive efficacy of progestins in women with Lynch syndrome, but these data suggest that such agents warrant further investigation in this population.[109]

Treatment and Precision Medicine for CRCs with dMMR

Despite advances in diagnosis and screening, a portion of individuals with Lynch syndrome still go on to develop cancer. For early stage Lynch syndrome-associated CRCs, surgical resection represents the mainstay of therapy. The major surgical options include total abdominal colectomy or segmental resection with ongoing annual or semi-annual colonoscopic surveillance. A few factors favor more extensive surgery, particularly in younger affected individuals and those with more severe phenotypes. Importantly, retrospective data have suggested greater rates of metachronous CRCs after segmental resection as compared to subtotal colectomy—approximately 25% vs 8%.[110, 111] A post hoc pooled analysis of long term outcomes from registry data suggest that this risk grows over time, with the incidence of metachronous CRCs after 10, 20 and 30 years being 16%, 41% and 62%, respectively.[112] However, there is no clear evidence that more extensive surgery confers a survival benefit, and more extensive surgery imparts a greater risk of chronic diarrhea and/or incontinence; by modeling, quality adjusted life years (QALYs) are approximately equivalent with either strategy[113, 114]. Though subtotal or total colectomy represents a current preferred recommendation, the decision with respect to extent of surgery should consider both the patient's risk of additional cancers, surgical risk with additional resection, and patient preferences.

The role of additional therapy in early Lynch-associated CRCs is stage-dependent, as with non-Lynch associated cancers. Analyses have demonstrated improved prognoses for individuals with MSI/dMMR CRCs (compared with MMR-proficient [pMMR] CRCs), particularly in early stage disease.[115] Importantly, data has suggested that 5-FU-based adjuvant therapy does not provide a survival benefit when administered after surgery with curative intent[115, 116]. A pooled analysis of patients with stage II or III colon cancer treated in randomized trials of adjuvant 5-FU based chemotherapy confirmed this lack of benefit.[117] In this study, while treatment benefit was observed in pMMR patients, a trend toward worse outcomes was observed in Stage II dMMR patients receiving chemotherapy. Thus, at this point, published guidelines advise against the use adjuvant chemotherapy for MSI stage II colon cancers.[118]

For stage III colon cancers, in general, adjuvant chemotherapy is widely accepted, with a fluoropyrimidine/oxaliplatin chemotherapy doublet such as FOLFOX (5-FU, leucovorin, oxaliplatin) or CAPOX (capecitabine, oxaliplatin) being the standard of care. However, the evidence for benefit in the specific case of CRCs with MSI is mixed. Given that ~10% of patients with stage III CRCs have dMMR tumors, only a minority of which are a result of Lynch syndrome, and the confounding issue of improved survival of patients whose CRCs have dMMR activity, individual studies have been underpowered to delineate the true benefit

of adjuvant chemotherapy in Lynch syndrome. Moreover, it is likely that Lynch syndrome CRCs differ biologically from those dMMR CRCs associated with methylated *MLH1* promoters; the latter group emerges from a background of the CpG island methylator phenotype (CIMP) and has a higher frequency of *BRAF* mutations, which are not found in Lynch syndrome and confer a more deadly phenotype.[119] The MOSAIC trial, which contributed to the establishment of adjuvant FOLFOX as a standard of care, suggested a trend toward benefit for oxaliplatin in the subset of dMMR patients (n=95). The addition of oxaliplatin to 5-FU provided an absolute improvement in median survival of 2% in the pMMR population, with a 16.9% improvement in those with dMMR tumors.[120] A retrospective, non-randomized analysis of 433 consecutive patients with stage II or III dMMR colon cancers treated at 11 French centers represents the largest relevant published series. Patients with stage II or III tumors underwent surgical resection alone or were treated with resection followed by adjuvant therapy—a fluoropyrimidine with or without oxaliplatin. Compared with surgery alone, adjuvant oxaliplatin-based therapy improved disease-free survival in multivariate analysis (HR 0.35, p<.001), in contrast to 5-FU (HR 0.73, p =.38).[121] The number of patients, events, and nature of these analyses significantly raise the risk of spurious findings and make firm conclusions problematic. However, based upon the totality of the data, for eligible patients, a fluoropyrimidine doublet (FOLFOX or CAPOX) represents the standard of care in stage III CRCs, including dMMR/MSI tumors. Based upon the aforementioned data, for stage III colon cancer patients who are not candidates for oxaliplatin therapy (e.g. due to the existence of peripheral neuropathy), there may be no benefit to adjuvant chemotherapy with a single agent fluoropyrimidine (5-FU or capecitabine).

Unfortunately, even though conferred a better prognosis, a significant minority of patients with MSI CRCs will experience distant spread or recurrence of disease. MSI tumors constitute just 3-5% of all stage IV CRCs, probably reflecting the underlying differences in biology of dMMR vs pMMR CRCs.[122, 123] Compared with patients harboring microsatellite stable (MSS) tumors, those with MSI tumors are more likely to experience local recurrence and peritoneal metastases, with a lower frequency of lung or liver metastases.[124] *BRAF* mutations, typically associated with poor prognosis and virtually never seen in Lynch Syndrome, are much more common in MSI tumors than in MSS CRCs at 35% vs 7%.[123] A metastatic MSI CRC confers a comparable or somewhat worse prognosis than a metastatic MSS tumor, though the existence of *BRAF* mutations appears to be the major driver of this effect[123, 125]. The advent of therapies that manipulate the immune response to MSI CRCs is likely to completely alter our approach to this problem.

While chemotherapy remains the mainstay in advanced CRC, the introduction of effective immunotherapeutic agents stands as the major landscape-changing discovery of recent years. Two Programed Death-1 (PD-1) targeting drugs—the monoclonal antibodies pembrolizumab (Keytruda) and nivolumab (Opdivo)—are now FDA-approved for the treatment of advanced MSI CRC after failure of cytotoxic chemotherapy. PD-1 is a 228-amino acid membrane receptor protein that is induced on activated T-cells through the T-cell antigen and cytokine receptors.[126] PD-1 has two primary ligands with distinct expression patterns. PD-L1 (B7-H1, CD274) is expressed constitutively at low levels on antigen presenting cells and also expressed on non-hematopoietic cells, including vascular

endothelial cells. PD-L2 is expressed on activated dendritic cells and macrophages. Both ligands are upregulated by pro-inflammatory cytokines, most potently by interferon-γ, but also by TNF-α, VEGF and additional members of the interferon family. The binding of PD-1 on an activated T-cell by PD-L1 or PD-L2 induces the T-cell into a quiescent state through multiple potential mechanisms, including down-regulated signaling through T-cell receptor proximal kinases and altered T-cell metabolism.[127] Serving as a physiological counter-regulatory process to prevent unfettered inflammation, PD-L1 or PD-L2 upregulation represents an escape mechanism by which many tumors can avoid immune-mediated elimination.

Early in the development of PD-1 and PD-L1 targeting compounds there was a clinical anecdote of a single CRC patient achieving a complete response that was durable off therapy for greater than three years after treatment with nivolumab. The tumor was an MSI CRC, was notable for the presence of membranous PD-L1 expression on infiltrating macrophages and lymphocytes, and associated with infiltrating PD-1+ CD3+ T cells.[128] Of relevance, MSI CRCs have higher densities of tumor infiltrating lymphocytes (TILs) as well as more frequent inflammatory Crohn's-like reactions than MSS tumors.[129] The TCGA clearly demonstrated that MSI CRCs—regardless of whether Lynch syndrome, hypermethylation-induced *MLH1* silencing or biallelic somatic MMR alterations—carry significantly higher mutation loads than non-MSI cancers.[97] Tumors carrying germline or somatic mutations in the catalytic domain of *POLE*, which are distinct from MSI tumors, possess an ultra-hypermutated phenotype, making them similarly of interest in the context of immune checkpoint therapy. It has been long postulated that elevated levels of neoantigens, produced by novel frameshift-associated peptides are responsible for the increased T-cell infiltrate seen in MSI tumors.[130] Recent investigations have firmly supported this hypothesis, directly linking the number of frameshift mutations to the density of TILs.[131, 132] Thus, preclinical evidence supported MSI tumors as a subset experiencing active immune surveillance in an immunologically primed microenvironment; further signs suggested that clinical grade drugs might be capable of reactivating otherwise quiescent T-cells. Moreover, this phenomenon underlies the prediction that immunization of patients with Lynch syndrome might be possible using these neoantigenic peptides.[133]

Based upon these observations, clinical studies commenced to test PD-1 targeting therapies in MSI CRCs. The first landmark study enrolled patients with MSI CRCs as well as MSI non-CRCs, the majority of whom were afflicted by Lynch syndrome, and treated them with pembrolizumab monotherapy.[134] Remarkably, overwhelming benefit was demonstrated as 52% of patients exhibited significant regression and 21% achieved a complete response, many remaining highly durable and maintained off therapy.[135] Additional data has also demonstrated clear benefit with nivolumab in advanced dMMR/MSI CRC that had failed prior cytotoxic chemotherapy, which produced a response in 31% of cases in a recent phase 2 study and an overall disease control rate of 69%.[136] Both responses and stable disease have been highly durable with nivolumab, as only 3/23 (13%) responders have exhibited subsequent disease progression and the median duration of response has not yet been established.[136] As with pembrolizumab, a clinical or known history of Lynch syndrome appeared to have no bearing on benefit with nivolumab. In addition, neither tumor PD-L1 positivity, immune cell PD-L1 expression, nor *BRAF/KRAS* mutational status impacted the

likelihood of achieving clinical benefit in this study.[136] Based upon these data, both pembrolizumab and nivolumab have been added to the NCCN guidelines for use as second-line or later therapy of MSI metastatic CRCs.[118] Immune checkpoint therapy is now approved as the first cancer therapy in which a biomarker—MSI—defines the indication for its use.[137]

The benefit of PD-1 targeting therapy is clear, but reliable predictors of benefit (beyond MSI status) remain lacking. PD-L1 expression, in spite of early signs of predictive capacity, has not been validated as a predictive biomarker for response to this therapy. Tumor mutational burden appears to be a potential predictor across cancers on the whole, though the predictive power of mutational load calculation is not clear within the confines of the hypermutated subset of cancers (MSI and DNA polymerase-proofreading mutated).[138, 139] Early data suggests that disparities in mutational load cannot predict the differential effects of PD-1 inhibition among MSI CRCs.[135]

Equally problematic, a sizeable portion of patients with MSI tumors do not receive any benefit (i.e. show primary resistance) or exhibit progression after an initial effect (acquired resistance) from immune checkpoint therapy. Data drawn from four melanoma patients with progressive tumors due to acquired resistance to anti-PD-1 drugs demonstrated the presence of mutations in immune-response genes—the interferon receptor-associated *Janus kinase 1* (*JAK1*) or *Janus kinase 2* (*JAK2*)—or in a gene required for antigen presentation, i.e., β_2-*microglobulin* (*B2M*).[140] Interestingly, follow-up of pembrolizumab-treated MSI CRC patients shows a similar trend. An analysis examining the primary tumors and metastases in patients with acquired resistance revealed recurrent mutations in *B2M* in a majority of cases. On the other hand, in this small series, *B2M* was not mutated in any samples from patients who demonstrated primary resistance.[135] Thus, mutations which lead to a downregulation in antigen presentation appear to be the major pathway of resistance.

Previously, outside the confines of immunotherapeutic treatment, *B2M* mutations were reported to be present in 15.8% of MSI adenomas and 27.9% of MSI CRCs, but were not detected in patients with metastases.[141] Of note, there are multiple routes to loss of HLA Class I expression and while deleterious *B2M* mutations predominate in Lynch syndrome, the inactivation of various antigen-presenting machinery components, namely *TAP1* or *TAP2*, represents the predominant mechanism in sporadic MSI cancers.[142] Disparate mechanisms for HLA Class I loss or downregulation in Lynch versus sporadic MSI CRCs may have consequences on the expression levels of additional HLA classes, as well as on the ability of natural killer (NK) T-cells to engage tumor cells. Thus, means to overcome acquired resistance may differ in Lynch-associated versus sporadic MSI cancers.

While the mechanisms of primary and acquired resistance are still incompletely understood, there would appear to be multiple opportunities to overcome primary, and perhaps acquired, resistance to PD-1 blockade in MSI tumors. Multiple immune inhibitory checkpoints and enzymes exist which act in the dynamic balance of immune regulation. Many of these are simultaneously upregulated in MSI CRCs, including CTLA-4, LAG-3, and IDO-1.[143] Logically, one strategy to counter resistance to immunotherapy might include the simultaneous blockade of multiple immune checkpoints. PD-1 inhibition has largely gained

traction, not due to being the first exploitable target of this type, but rather, due to its greater efficacy and tolerability in comparison to monotherapy CTLA-4 blockade.[144] As multiple drugs targeting these processes come into clinical use, such combinatorial approaches are well within reach. For instance, dual CTLA-4 and PD-1 inhibition is approved in melanoma due to the demonstration of improved responses and survival compared to PD-1 monotherapy.[145] In this vein, preliminary data in MSI CRC utilizing the CTLA-4 targeting monoclonal antibody ipilimumab in combination with nivolumab appears promising. Responses have been seen in 55% of patients at latest report with stable disease or response in 79% of the patients.[146] These findings merit further investigation.

Additional novel strategies are available to augment immunologic targeting of Lynch-associated cancers. As mentioned earlier, cancers with MSI develop multiple frameshift-induced neoantigens. Consequently, personalized neoantigen-based therapies hold promise, both in the treatment of early stage tumors, as well as for advanced disease. Multiple groups have demonstrated that neoantigen-targeted vaccine-based approaches are feasible and induce measurable immune responses.[130] Preliminarily provocative data suggests clinical benefit when administered independently or prior to immune checkpoint targeting therapies.[147, 148] Additional opportunities might involve the use of adoptive T-cell receptor transfer which utilizes T-cells engineered to target specific mutated cancer proteins. *Transforming growth factor receptor-β receptor II* (*TGFBR2*) frameshift mutations are present in 90% of MSI CRCs, including most Lynch syndrome CRCs,[149] as well as a sizable portion of other MSI cancers. Frameshift mutations are also present in *BAX*, *MSH3* and *MSH6* in most gastrointestinal cancers with MSI.[150] At this point, a patient vaccinated with a 23-mer TGFBR2 peptide has demonstrated highly durable benefit; in vivo studies have also demonstrated the benefit of an engineered TGFBR2-mutant specific T-cell receptor in murine models.[151] Thus, truly personalized immune treatments appear to be on the horizon, but will require rigorous evaluation. However, given that in tumors with dMMR activity there is potential for the development of multiple, independent malignant clones with heterogeneous drivers, as well as the evolution of diverse treatment-driven, pro-tumorigenic mutations, neoantigen specific approaches may have their limitations, at least initially.[152]

Additional aberrations have been noted preferentially in MSI CRC which may represent therapeutic candidates. Somatic *BRCA2* mutations were recently reported in a high proportion (50%) of MSI CRCs compared with MSS CRCs (14%).[153] *BRCA1* and *BRCA2* mutations are crucial for the repair of double-stranded DNA breaks (DSBs). Poly(ADP-ribose) polymerase 1 and 2 (PARP1 and PARP2) are instrumental components of the DNA damage response, binding damaged DNA at single-strand DNA breaks (SSBs).[154] PARP inhibition (PARPi) in *BRCA*-mutant cancer represents a synthetic lethal interaction. In addition, PARPi upregulates PD-L1 expression, and the addition of PD-L1 blockade to PARPi confers added benefit pre-clinically.[155] While the PARP inhibitor olaparib failed in a small trial of MSS and MSI CRCs previously, the selection of *BRCA1/2* mutant CRCs with or without PD-1 inhibition may represent a promising means to revisit this target in MSI cancers.[156]

The targeting of cell cycle checkpoints may also represent an opportunity for the exploitation of a vulnerability in MSI CRCs. Cyclin-dependent kinases 4 and 6 (*CDK4*/6)

are key drivers of the cell cycle and crucial in numerous malignancies. *CDK4/6* inhibitors upregulate B2M and MHC class I proteins and increase the expression of antigen. In preclinical models, this inhibition is associated with increased tumor infiltrating CD3+ T-cells and decreased regulatory T-cells (T-regs). Of relevance, CD8 neutralization abrogates the in vivo anti-tumor activity of *CDK4/6* inhibition; PD-L1 inhibition augments the effects of CDK4/6 inhibition in breast and CRC models.[157] As such, a combinatorial strategy of *CDK4/6* and PD-1/L1 targeting holds promise, potentially with attention to tumors with decreased expression of B2M or MHC Class I proteins.

Summary

The management of familial CRC has advanced from the description a group of poorly-understood polyposis syndromes and suspected non-polyposis forms of familial CRC to our current appreciation of dominant and recessively-inherited polyposis syndromes and Lynch Syndrome. The former conditions are relatively rare, whereas Lynch Syndrome—as defined by the carriage of a germline mutation in a DNA MMR gene—appears to be present in 1/279 to 1/226 in some populations. One key clinical implication is that the polyposis syndromes are best managed endoscopically or surgically, and when cancers occur, they are clinically and biologically similar to common, sporadic CRCs. On the other hand, Lynch syndrome represents a disease that develops through the MSI pathway, cancers evolve relatively quickly from adenomas or possibly even normal-appearing tissue, frequently elicit strong immunological responses, and may be highly responsive to immune checkpoint therapies—occasionally with dramatic responses. The discovery of a multitude of genes responsible for familial CRC has been countered with the development of multi-gene diagnostic panels. This has greatly reduced the problem of guessing which gene might be responsible for diseases, which often display a high degree of clinical heterogeneity.

Our expectations for the future include an expansion of our understanding of how to deal with the ability of hypermutated tumors to escape immune detection. A second expectation is that we should develop non-invasive approaches to surveillance in Lynch syndrome, which has an accelerated appearance of CRCs in colons that appeared to be free of neoplasia within the prior year. Finally, it is imperative to gain a better understanding of what modulates the age of onset and tumor spectrum in the lower penetrance familial CRC genes. One might say we have a robust future ahead.

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Abbreviations

B2M β_2-microglobulin

CCFR	colon cancer familial registry
CRC	colorectal cancer
EC	endometrial cancer
IHC	immunohistochemistry
MMR	DNA mismatch repair
dMMR	defective MMR activity
pMMR	proficient MMR activity
MSI	microsatellite instability (always assumes high level MSI, MSI-H)
MSS	microsatellite stable
NCCN	National Colorectal Cancer Network
NGS	next-generation sequencing (of DNA)

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Table 1

Summary of Current Understanding of Lynch Syndrome and Key Recent Advances

Epidemiology	• 3% of colorectal cancers and 2% of endometrial cancers • Estimated 1:279 general population prevalence; *PMS2* mutations most common in general population, but confer lower cancer risk • Increased prevalence in certain populations (Icelanders, French Canadians, Ashkenazi Jews, others) due to founder mutations • BMMRD is a rare pediatric cancer susceptibility syndrome caused by biallelic inheritance of germline mutations in the same MMR gene (most commonly *PMS2*, likely due to high background population prevalence)
Germline and Tumor/Somatic Testing	• Increasing availability of NGS multigene panels for hereditary CRC risk assessment; some individuals with clinical histories suggestive of Lynch syndrome will have germline mutations in other cancer susceptibility genes (see Table 2) • Virtually all Lynch-associated cancers demonstrate dMMR and MSI (see Table 3 regarding universal tumor testing and diagnostic strategies) • Somatic NGS panels can detect dMMR/MSI by assessing overall mutational burden of a cancer • Conflicting results about whether loss of MMR activity is an early or late event in Lynch syndrome adenomas and carcinogenesis; dMMR/MSI detectable in non-neoplastic crypts in the small and large intestines of Lynch syndrome patients, and some Lynch-associated CRCs appear to arise directly from colonic epithelium without forming a polyp/adenoma
Surveillance and Prevention	• Colonoscopic screening every 1-2 years shown to reduce CRC incidence and associated mortality in Lynch carriers • Hysterectomy with salpingo-oophrectomy have been shown to reduce risk of Lynch-associated endometrial and ovarian cancers in *MLH1*, *MSH2*, and *MSH6* carriers, though survival benefits are uncertain; no proven efficacy to screening with ultrasound, endometrial biopsy, or CA-125 surveillance • Minimal data on efficacy of screening for other Lynch-associated cancers • Aspirin 600 mg/day for at least 2 years reduces risk of CRC (HR 0.41) and all Lynch-associated cancers (HR 0.45) in Lynch syndrome patients; ongoing clinical trial investigating different doses • Limited data suggest possible chemopreventive benefit to ibuprofen, multivitamins, supplemental calcium, and progestins in Lynch syndrome patients
Treatment and Precision Medicine for Lynch-Associated CRC	• Lack of benefit from adjuvant 5-fluorouracil monotherapy for stage II/III colon cancer; FOLFOX/CAPOX the accepted standard for high risk stage II and stage III colon cancer, though oxaliplatin benefit not fully established • Total colectomy preferred for resecting Lynch-associated colon cancer to reduce risk of metachronous colon cancer • ~30-40% objective response rate and ~70% disease control rate with PD-1 inhibitors (pembrolizumab or nivolumab) in refractory metastatic dMMR/MSI CRC due to high burden of somatic frameshift mutations leading to production of immunogenic neoantigens • Ongoing clinical trials investigating PD-1 inhibitors with other immune checkpoint inhibitors (e.g., CTLA-4 inhibitor ipilimumab) in dMMR/MSI CRC • Somatic inactivation in immune response and antigen presenting genes (e.g., *JAK1, JAK2*, *B2M*) appears to confer resistance to immune checkpoint inhibitors

Abbreviations: BMMRD, biallelic mismatch repair deficiency; CRC, colorectal cancer; dMMR, mismatch repair deficiency; MSI, high-level microsatellite instability; NGS, next-generation sequencing

Table 2

Key studies on multigene panel testing in Lynch syndrome/hereditary colorectal cancer

Reference	Germline panel type	Study population	Key germline findings
High-risk patient cohorts ascertained from commercial testing labs			
Yurgelun MB, et al. *Gastroenterology* 2015[56]	Commercial 25-gene panel	Laboratory-based cohort of 1260 patients referred for Lynch syndrome germline testing	14.4% prevalence of any mutation; 9.0% Lynch syndrome prevalence; 5.6% prevalence of other mutations (most commonly *APC*, mono- and biallelic *MutYH*, and *BRCA1/2*)
Espenchied CR, et al. *J Clin Oncol* 2017[58]	Various commercial panels (9-49 genes)	Laboratory-based cohort of 34,981 patients referred for multigene panel germline testing for a variety of clinical indications	Overall 1.5% Lynch syndrome prevalence; *MSH6* mutations most common among carriers with ovarian or endometrial cancer; *PMS2* mutations most common among carriers with breast cancer only
Cohorts of colorectal cancer patients not pre-selected by personal or family history of cancer			
Yurgelun MB, et al. *J Clin Oncol* 2017[25]	Commercial 25-gene panel	Single-site clinic-based cohort of 1058 colorectal cancer patients	9.9% prevalence of any mutation; 3.1% Lynch syndrome prevalence; 7.0% prevalence of other mutations (most commonly *APC*, mono- and biallelic *MutYH*, and *BRCA1/2*)
Pearlman R, et al. *JAMA Oncol* 2017[55]	Commercial 25-gene panel	Multi-site population-based cohort of 450 colorectal cancer patients age <50 years	16.0% prevalence of any mutation; 8.4% Lynch syndrome prevalence; 8.0% other mutations (most commonly *APC*, mono- and biallelic *MutYH*, and *BRCA1/2*)

Table 3

Strategies for clinically identifying individuals for germline Lynch syndrome testing

Diagnostic strategy	Advantages	Disadvantages
Clinical history (e.g., Amsterdam criteria; Bethesda guidelines; etc.)	Inexpensive; can be used for individuals with or without personal histories of cancer	Poor sensitivity and specificity; inconsistent usage; difficult to remember complex criteria
Universal tumor testing with MSI and/or MMR IHC	Cost-effective; highly sensitive for Lynch syndrome screening in colorectal and endometrial cancer patients	Cannot be used in individuals without a personal history of cancer; sensitivity/specificity for cancer/tumors other than colorectal and endometrial cancer poorly defined; poor specificity, since testing will detect a number of individuals who have non-Lynch forms of MSI-H/MMR-D; patient and provider follow through on abnormal tumor test results may be variable
Clinical prediction models (e.g., $PREMM_5$)	Cost-effective; can be used for individuals with or without personal histories of cancer	Models do not account for MSI/MMR IHC results; model performance in endometrial cancer patients is likely suboptimal; model performance in populations of patients unaffected by cancer is not known

Abbreviations: MMR IHC, mismatch repair protein immunohistochemistry; MMR-D, mismatch repair deficient; MSI, microsatellite instability; MSI-H, high-level microsatellite instability