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Recent advances in Lynch syndrome

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Abstract

Lynch syndrome is one of the most common hereditary cancer predisposition syndromes and is associated with increased risks of colorectal and endometrial cancer, as well as multiple other cancer types. While the mechanism of mismatch repair deficiency and microsatellite instability and its role in Lynch-associated carcinogenesis has been known for some time, there have been significant advances recently in diagnostic testing and the understanding of the molecular pathogenesis of Lynch tumors. There is also an increased awareness that the clinical phenotype and cancer risk varies by specific mismatch repair mutation, which in turn has implications on surveillance strategies for patients. Even the treatment of Lynch-associated cancers has changed with the addition of immunotherapy for advanced disease. This progress report aims to review some of the many advances in epidemiology, molecular pathogenesis, diagnosis, clinical phenotype, cancer surveillance, treatment, and chemo- and immune-prevention strategies in the Lynch syndrome field over the past five years.

Background

Lynch syndrome (LS) is caused by germline alterations in the DNA mismatch repair (MMR) genes and is one of the most common hereditary cancer syndromes.¹ LS confers a markedly increased lifetime risk of colorectal cancer (CRC), endometrial cancer (EC), as well as cancers of the ovary, stomach, urothelial tract, small bowel, pancreas, biliary tract, and sebaceous neoplasms of the skin.¹ The initial discovery of the molecular phenotype now known as microsatellite instability (MSI) in 1993 and subsequent linkage of this phenotype with Lynch syndrome-associated colorectal carcinogenesis^{2–4} ultimately paved the way for what remains the primary diagnostic strategy for LS: germline MMR gene analysis in individuals with tumors demonstrating high-level MSI (MSI-H) and/or deficient MMR protein expression (MMR-D). The past 15 years have demonstrated the benefits of applying this strategy in all individuals diagnosed with CRC⁵ or EC,⁶ and such universal tumor

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screening for LS is now considered standard practice for CRC^{7–12} with multiple guidelines now recommending it as well for EC.^{13,14}

For individuals diagnosed with Lynch syndrome, the benefits of cancer screening and risk reduction have been known for some time. Frequent colonoscopic screening improves overall mortality and reduces CRC incidence;¹⁵ prophylactic hysterectomy and salpingo-oophorectomy effectively prevent Lynch-associated EC and ovarian cancer;¹⁶ and, more recently, chemoprevention with high-dose aspirin reduces the incidence of Lynch-associated CRC and other LS-associated cancers.¹⁷ MSI status has also been found to be an important biomarker predicting lack of benefit to fluoropyrimidine monotherapy in the adjuvant setting for CRC.^{18,19} In spite of this critically important knowledge base, however, several questions about LS epidemiology, pathogenesis, clinical phenotype, and cancer risk reduction remain unanswered. This review will cover progress made in these realms over the past several years.

Epidemiology

While LS has been found in about 1 out of every 35 patients with CRC (3%)^{20,21} and 1 out of every 56 patients with EC (1.8%),⁶ estimates of the overall general population frequency of LS have previously been limited to analyses of datasets of patients with this cancer history. Using population-based family data from the Colon Cancer Family Registry (CCFR), Win et al. recently estimated the prevalence of LS in the general population to actually be as high as 0.35% or 1:279, (95% CI 1:192 to 1:402).²² Of the individual MMR genes, the population prevalence of pathogenic *PMS2* variants was highest at 0.140% (1:714), followed by *MSH6* with 0.132% (1:758);²² *MLH1* and *MSH2* (which are the two most frequent MMR genes linked to LS when ascertained from CRC patients) were markedly less common in the general population at 0.051% (1:1946) and 0.035% (1:2841), respectively.²² This discrepancy highlights the variable penetrance among different MMR genes and the importance of gene-specific cancer risk assessment.

Of note, LS prevalence likely varies by population, and over 50 founder mutations in MMR genes have been recently identified in Icelandic, French Canadian, African American, Polish and Latin American groups, among others.^{23–28} In Iceland, for example, due to three founder mutations (two in *MSH6*, one in *PMS2*), LS is more common than what was calculated from the CCFR data, with an estimated general population prevalence of 0.442% (1:226).^{24,28} Despite this higher prevalence, however, the frequency of CRC related to LS in this population is just 2.3%,^{24,28} again likely reflecting the lower penetrance of *MSH6* and *PMS2* in CRC.

Molecular pathogenesis:

MMR-D has previously been theorized to be a late event in the development of CRC, whereby polyps in LS first develop similar to sporadic polyps (such as via an *APC*-mediated mechanism),²⁹ with biallelic MMR loss then occurring afterwards;³⁰ the resultant MMR dysfunction leads to an accumulation of somatic mutations (i.e. MSI) which, in turn, accelerates progression to invasive cancer. This model is supported by the finding that in

pre-malignant polyps in LS patients, the likelihood of MMR-D was associated with increased polyp size (suggesting that the smaller – and presumably earlier – polyps have not yet had the necessary ‘second hit’ to actually become MMR-D).³⁰

Advances in histopathology and sequencing, however, have led to other potential models of LS-associated colorectal carcinogenesis. For example, Ahadova et al. found MMR-D crypt foci (histologically normal and apparently non-neoplastic intestinal crypts with absent MMR protein expression) adjacent to MMR-D adenomas, suggesting a role for MMR-D in adenoma initiation.³¹ These investigators have proposed a novel and provocative pathway for LS-associated colorectal neoplasia that completely bypasses adenomatous precursors altogether.³¹ Their data suggest that the MMR-D crypt foci, known to be reasonably common in the intestinal epithelium of healthy cancer-free LS carriers,³² may actually acquire somatic mutations in *TP53* or *CTNNB1* that could lead to immediate invasive cancer growth, and that this direct process may explain interval colorectal cancers that develop between short-interval screening colonoscopies.^{31,33,34}

Diagnosis of Lynch syndrome: universal tumor screening, clinical history and germline testing

As outlined above, current standard of care is to test all CRC specimens for MSI-H/MMR-D as a screen for LS,^{7–12} with universal testing of EC also now recommended by many professional guidelines.^{13,14} The traditional thinking has been that essentially all MSI-H/MMR-D CRC and ECs (aside from those arising from somatic hypermethylation of the *MLH1* promoter) were presumed to be due to LS, even if a germline MMR gene variant could not be found on clinical testing. Some studies have referred to this phenomenon of tumors with unexplained MMR-D by the term “Lynch-like syndrome.”³⁵ Recent data, however, have demonstrated that roughly 70% of these “Lynch-like” MMR-D tumors arise from biallelic somatic MMR gene inactivation, including sequence variants and epigenetic silencing, and are thus truly sporadic malignancies for which LS-related screening recommendations are not indicated.^{36–38} The use of upfront next-generation tumor sequencing (as an alternative to PCR-based MSI testing or MMR protein IHC) to screen for LS can effectively distinguish between LS-associated MSI-H/MMR-D cancers and those with sporadic MSI-H/MMR-D cancers and, in one recent study, was demonstrated to have improved sensitivity over MSI and MMR IHC for identifying LS probands.³⁹

While universal tumor screening is an important tool for identifying LS probands, its efficacy is inherently limited only to individuals who have actually been affected with cancer and a tumor sample is available. For families where a clinical suspicion exists, but no tumor sample is available for analysis, clinical prediction models, such as PREMM (<http://premm.dfci.harvard.edu/>), can be efficiently incorporated into routine clinical workflows as a way to screen for LS among cancer-free individuals with worrisome family histories and/or when tumor assessment of microsatellite instability is not possible.⁴⁰ The newly developed PREMM₅ is the first such model to effectively predict the likelihood of carrying a germline variant in any of the 5 genes linked to LS (AUC 0.81; 95% CI 0.75–0.82), although predicting *PMS2* variants remains a particular challenge due to the attenuated phenotype.⁴¹

Guidelines recommend formal genetic evaluation for any individual predicted to have a 5% likelihood of LS with prior versions of PREMM or other models such as MMRPro, and 2.5% threshold for PREMM₅.^{7–9,41}

While both tumor screening and clinical history are useful to identify patients with possible LS, the diagnosis is ultimately confirmed by the presence of a pathogenic germline MMR gene variant on genetic testing. A number of recent studies have demonstrated the utility of using multi-gene panel testing, rather than syndrome-specific germline testing, in the identification of patients with LS in both unselected and high risk population cohorts.^{21,42–45} These studies also identified patients that actually had pathologic variants in non-LS genes, including both other CRC predisposition syndromes (such as mutations in *APC* or biallelic *MUTYH*) as well as in genes not classically associated with CRC risk (such as *BRCA1/2*) (see Table 1).^{21,42–45} While there are certainly potential risks to the routine use of larger panels for routine clinical genetic testing (e.g. identification of variants in low-/moderate-penetrance genes which often lack clear management guidelines, germline variants of uncertain significance; risk of mismanagement/misinterpretations), their ability to identify a wide diversity of inherited cancer risk has made them a highly appealing alternative to syndrome-specific gene testing.^{46–48}

Importantly, the spectrum of cancer associated with LS also continues to expand as the use of next-generation sequencing technology grows. Schwark et al. analyzed germline and somatic data from >15,000 tumors (encompassing >50 cancer types) and found that half of all MSI-H/MMR-D cancers in patients with germline MMR gene variants were non-CRC and non-EC primary cancers and included various malignancies not classically linked to LS such as soft tissue sarcoma, germ cell tumors, mesothelioma, melanoma, and CNS tumors, among others.⁴⁹ Of these non-CRC/non-EC LS-associated MSI-H/MMR-D cases, 54.5% (18/33) would not have met testing clinical criteria for genetic testing.⁴⁹ These important results emphasize the need for germline evaluation of all MSI-H/MMR-D tumors, even in cancer types not typically associated with LS, unless biallelic somatic MMR gene inactivation cancer has been identified.⁴⁹

Clinical phenotype:

The cumulative cancer risk in LS has been challenging to truly quantify given study limitations and challenges from ascertainment bias.⁵⁰ As described previously, however, given variable penetrance of the MMR genes, it is perhaps more important to consider gene-specific risk (see Table 2) in order to better tailor clinical recommendations, though gene-specific approaches to LS cancer screening and prevention remain unstudied.

The cancer risk for carriers of pathogenic *PMS2* variants, in particular, has historically been difficult to estimate given limited numbers of carriers included in most studies (in part because of its low penetrance as well as due to sequencing challenges caused by the presence of multiple pseudogenes), although collaborative efforts from various registries are beginning to tackle this challenge. A recent multinational study analyzing data from 284 families with pathogenic germline *PMS2* variants confirmed the attenuated phenotype demonstrated by this subset of LS families, finding a cumulative risk of CRC to age 80 years

of 13% (95% CI, 7.9-22%) and 12% (95% CI 6.7-21%) for males and females, respectively, and a similar cumulative risk of endometrial cancer to 80 years of 13% (95% CI 7.0-24%).⁵¹ This study observed no significant increased risk of other LS-associated cancers beyond CRC and EC, including ovarian, gastric, hepatobiliary, bladder, renal, brain, breast, prostate, or small bowel cancer cancers.⁵¹ Furthermore, among the *PMS2* carriers who underwent routine screening colonoscopies, no colorectal cancers were identified.⁵¹ These data led the authors to postulate that *PMS2* mutation carriers might safely be managed with later-onset and less frequent colonoscopic screening (beginning at age 35-40, rather than age 20-25, and continuing every 2-3 years instead of every 1-2 years) than what is typically recommended in LS, though the safety and efficacy of such a reduced intensity screening approach for this specific LS subgroup has not been studied.⁵¹

Cancer surveillance

Screening guideline recommendations for LS-associated tumors (and especially for non-CRC cancers) continue to be limited. In the United States, current guidelines recommend colonoscopy for CRC surveillance every 1-2 years starting at age 20-25.⁷⁻⁹ There is less clear evidence for other cancer screenings. Many guidelines include recommendations for annual endometrial biopsy and transvaginal ultrasound, and for at least a baseline EGD with gastric biopsy and *H. pylori* testing, with the caveat that these screenings have no proven mortality benefit and must be balanced with the undesirable effects and risk of the procedures.⁷⁻¹² Gene-specific approaches to screening and risk-reduction remain unproven.

To further investigate the optimal interval between colonoscopies in LS, the German HNPCC Consortium, the Dutch Lynch Syndrome Collaborative Group, and the Finnish Lynch Syndrome Registry recently reported prospective data from 2,747 LS patients (*MLH1*, *MSH2*, and *MSH6* carriers only), representing 23,309 person-years of cumulative observation time, since these three countries follow different national guidelines regarding the recommended frequency of colonoscopic surveillance for LS carriers (annually in Germany; every 1-2 years in the Netherlands; every 2-3 years in Finland).⁵² In this analysis, there was no significant difference in cumulative CRC incidence between countries, even when controlling for age, sex, MMR gene, or adenoma on index colonoscopy, nor was there any significant association between development of stage III/IV CRC with colonoscopy interval.⁵² However, prospective data from another large multinational registry of 1,942 LS patients (including the three countries from the aforementioned study) found that 25% of all incident CRCs developed in individuals who were 12-23 months out from their last colonoscopy, which may argue in favor of annual screenings.⁵³

Therapy of LS-associated malignancies

Given that LS-associated cancers almost invariably harbor MSI-H/MMR-D, recent advances in the use of immune checkpoint inhibitors have had profound implications for the medical management of LS-associated cancers (as well as sporadic MSI-H/MMR-D malignancies). MSI is characterized by the accumulation of frameshift mutations at hotspot repeat sequences, and in turn lead to the development of immunogenic neopeptides/neoantigens, which are recognized by CD8+ tumor infiltrating lymphocytes.⁵⁴ These features led

naturally to the hypothesis that MSI-H/MMR-D cancers may be particularly susceptible to immune-based therapies, and such treatments have indeed now proven to be exquisitely effective in treatment-refractory advanced/metastatic MSI-H/MMR-D malignancies with both encouraging and durable responses (see Table 3).^{55–57} To date, such studies suggest that the efficacy of immune checkpoint inhibitor therapy in MSI-H/MMR-D cancers is independent of whether or not the affected individual has LS. Randomized clinical trials using immune checkpoint inhibitors in the first-line treatment of metastatic MSI-H/MMR-D CRC and in the adjuvant treatment of resected stage III MSI-H/MMR-D colon cancer are ongoing (NCT02563002 and NCT02912559, respectively).

Chemoprevention

A secondary analysis of LS patients enrolled in the landmark CAPP2 (Colorectal Adenoma/ carcinoma Prevention Programme) chemoprevention trial (which demonstrated a markedly reduced incidence of CRC in LS carriers taking aspirin 600 mg/day for 2 years)¹⁷ found obesity (BMI ≥ 30 kg/m²) to be a significant risk factor for CRC development in LS carriers with a continuous hazard ratio of 1.07 per kg/m² (95% CI 1.02–1.13).⁵⁸ Interestingly, the obesity-related risk of CRC in this population was limited to participants who had been randomized to placebo rather than aspirin in CAPP2, thereby suggesting that aspirin chemoprevention and BMI reduction might each be independent interventions for CRC risk reduction in LS patients.⁵⁸

Immunoprevention

Another intriguing but as yet theoretical prevention strategy exploits the phenomenon of innate immunosurveillance known to occur in LS patients, where even cancer-free LS patients have been found to harbor circulating cytotoxic T-cells targeted against MSI-induced frameshift neoantigens.^{59,60} Such biology has led to abundant speculation that immune-based therapies (e.g. immune checkpoint inhibitors; vaccination) could be used as a means of primary cancer prevention in LS individuals, possibly providing protection across a wide spectrum of cancer types. Clinical trials (e.g. NCT03631641) are beginning to investigate the efficacy and safety of such approaches.^{61,62}

Conclusions

The last five years have brought about tremendous advances in our understanding of LS, particularly with regards to its genetic epidemiology, variable clinical phenotype, diagnosis, immunotherapy, and medical prevention. The next line of critical unanswered questions in LS involve uncertainties about gene-specific (and perhaps even variant- or family-specific) risk assessment and management, challenges in identifying LS among cancer-free individuals, and furthering knowledge about how to leverage the syndrome's unique immunobiology to further the treatment – and hopefully the primary prevention – of LS-associated cancers.

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Table 1:

Prevalence of LS and other cancer pre-disposition genes by multi-gene panel testing

Reference	Study population	Germline pathogenic mutation carriers n (% of total cohort)	Prevalence of LS n (% of total cohort)	Prevalence of non-LS mutations n (% of total cohort)	VUS rate n (%)
Colon cancer patients (unselected by age, personal/family history, MSI/MMR-D status)					
Yurgelun (2017) ²¹	1058 patients with CRC, clinic-based cohort	105 (9.9%)	33 (3.1%) <i>MLH1</i> : 13 (1.2%) <i>MSH2</i> : 7 (0.7%) <i>MSH6</i> : 6 (0.6%) <i>PMS2</i> : 7 (0.7%)	Overall: 74 (7%)* <i>APC</i> : 5 (0.5%); biallelic <i>MUTYH</i> : 3 (0.3%) <i>BRCA1</i> : 3 (0.3%); <i>BRCA2</i> : 8 (0.8%); <i>PALB2</i> : 2 (0.2%); <i>CKDN2A</i> : 1 (0.1%); <i>TP53</i> : 1 (0.1%)	330 (31.2%)
Early-onset colon cancer patients (age < 50 years)					
Pearlman (2017) ⁴⁴	450 early-onset CRC patients, population-based cohort	72 (16%)	Overall: 37 (8.2%) <i>MLH1</i> : 13 (2.9%) <i>MSH2</i> : 17 (3.8%) <i>MSH6</i> : 2 (0.4%) <i>PMS2</i> : 5 (1.1%)	Overall: 34 (7.6%)* <i>APC</i> : 6 (1.3%); biallelic <i>MUTYH</i> : 4 (0.9%); <i>SMAD4</i> : 1 (0.7%) <i>ATM</i> : 4 (0.9%); <i>BRCA1</i> : 2 (0.4%); <i>BRCA2</i> : 4 (0.9%); <i>CDKN2A</i> : 1 (2%); <i>CHEK2</i> : 1 (0.7%); <i>PALB2</i> : 2 (0.4%)	145 (32.2%)
Stoffel (2018) ⁴⁵	430 early-onset CRC patients, clinic-based cohort	79 (18.4%)	56 (13%) <i>MLH1</i> : 24 (5.6%) <i>MSH2</i> : 25 (5.8%) <i>MSH6</i> : 5 (1.2%) <i>PMS2</i> : 2 (0.5%)	Overall: 23 (5.3%) <i>APC</i> : 10 (2.3%); <i>MUTYH</i> : 8 (1.9%) <i>BRCA1</i> : 1 (0.2%); <i>CHEK2</i> : 1 (0.2%); <i>SMAD4</i> : 2 (0.5%); <i>TP53</i> : 1 (0.2%),	21 (4.9%)
High risk patients (personal history of LS-associated cancer and/or colorectal polyps)					
Yurgelun (2015) ⁴²	1260 patients referred for LS testing, commercial testing laboratory cohort	182 (14.4%)	114(9.0%) <i>MLH1</i> : 31 (27%) <i>MSH2</i> : 40 (35%) <i>MSH6</i> : 26 (23%) <i>PMS2</i> : 14 (12%) <i>EPCAM</i> : 3 (3%)	Overall: 71 (5.6%)* <i>APC</i> : 5 (0.4%); biallelic <i>MUTYH</i> : 3 (0.2%); <i>STK11</i> : 1 (0.1%) <i>BRCA1</i> : 6 (0.5%), <i>BRCA2</i> : 9 (0.7%)	479 (38%)
Endometrial cancer patients (unselected by age, personal/family history, MSI/MMR status)					
Ring (2016) ⁴³	381 EC patients, clinic-based cohort	35 (9%)	22 (6%) <i>MLH1</i> : 3 (0.8%) <i>MSH2</i> : 5 (1.3%) <i>MSH6</i> : 6 (1.6%) <i>PMS2</i> : 6 (1.6%) <i>EPCAM-MSH2</i> : 2 (0.5%)	Overall: 13 (3%) <i>APC</i> : 1 (0.3%); <i>ATM</i> : 1 (0.3%); <i>BARD1</i> : 1 (0.3%); <i>BRCA1</i> : 1 (0.3%); <i>BRCA2</i> : 1 (0.3%); <i>BRIP1</i> : 1 (0.3%); <i>CHEK2</i> : 4 (1%); <i>NBN</i> : 1 (0.3%); <i>PTEN</i> : 1 (0.3%); <i>RAD51C</i> : 1 (0.3%)	Not reported

* Includes some moderate and lower penetrant genes not listed in this table

APC, adenomatous polyposis coli; *ATM*, ataxia-telangiectasia mutated; *BARD1*, BRCA1 associated RING domain 1; *BRCA1*, breast cancer DNA repair-associated gene 1; *BRCA2*, breast cancer DNA repair-associated gene 2; *BRIP1*, BRCA1 interacting protein C-terminal helicase 1; *CDKN2A*, cyclin-dependent kinase Inhibitor 2A; *CHEK2*, checkpoint kinase 2; *CRC*, colorectal cancer; *EC*, endometrial cancer; *EPCAM*, epithelial cellular adhesion molecule; *LS*, Lynch syndrome; *MLH1*, MutL homolog 1; *MSH2*, MutS homolog 2; *MSH6*, MutS Homolog 6; *PALB2*, partner and localizer of BRCA2; *PMS2*, PMS1 homolog 2; *PTEN*, phosphatase and tensin homolog; *MUTYH*, mutY DNA glycosylase; *NBN*, nibrin; *PTEN*, phosphatase and tensin homolog; *RAD51C*, RAD51 paralog C; *SMAD4*, SMAD family member 4; *STK11*, serine/threonine kinase 11; *VUS*, variant of undetermined significance

Table 2:

Cumulative risk (CR) of CRC and/or EC by MMR gene (studies from 2013-2018)

Reference	Number of families or patients	Genes	70-year CR of CRC % (95% CI) *	70-year CR of EC % (95% CI) *
Dowty (2013) ⁶³	166 <i>MLH1</i> and 224 <i>MSH2</i> families (CCFR)	<i>MLH1</i> <i>MSH2</i>	<i>MLH1</i> - (M): 34% (25-50) - (F): 36% (25-51) <i>MSH2</i> - (M): 47% (36-60) - (F): 37% (27-50)	<i>MLH1</i> : 18% (9.1-34) <i>MSH2</i> : 30% (18-45)
Møller 2018) ⁶⁴	3119 LS patients (PLSD, European)	<i>MLH1</i> <i>MSH2</i> <i>MSH6</i> <i>PMS2</i>	<i>MLH1</i> : 40.1% (33.5-46.7) <i>MSH2</i> : 40.8% (31.6-50.1) <i>MSH6</i> : 15.0% (3.3 to 26.6) <i>PMS2</i> : 0	<i>MLH1</i> : 40.3% (31.5-49.1) <i>MSH2</i> : 52.7% (38.7-66.8) <i>MSH6</i> : 46.3% (27.3-65.0) <i>PMS2</i> : 26.4% (0.8-51.9)
Sanchez (2018) ⁶⁵	1,108 LS patients (Spain)	<i>MLH1</i> <i>MSH2</i> <i>MSH6</i> <i>PMS2</i>	<i>MLH1</i> : 25.6% (13.2-38.2) <i>MSH2</i> : 22.1% (11.3 – 35.1) <i>MSH6</i> : 6.3% (0-12.8) <i>PMS2</i> : 25.9% (7-71)	Not studied
ten Broeke (2018) ⁵¹	284 families with <i>PMS2</i> variants (European, Ohio State, CCFR)	<i>PMS2</i>	<i>PMS2</i> : * - (M): 13% (7.9-22) - (F): 13% (7.0-24)	<i>PMS2</i> : * 13% (7.0-24)

* ten Broeke series reported cumulative risk at 80 years, not 70

CCFR, Colon Cancer Family Registry; CI, confidence interval; CRC, colorectal cancer; EC, endometrial cancer; F, female; M, male; *MLH1*, MutL homolog 1; *MSH2*, MutS homolog 2; *MSH6* MutS Homolog 6; PLSD, Prospective Lynch Syndrome Database; *PMS2*, PMS1 homolog 2

Table 3:

Immunotherapy trials in MSI-H/MMR-D Cancer

Phase 2: completed studies					
Reference	Trial	Regimen	Patient population	Outcomes: % (95% CI)	FDA approval
Le et al. (2015) ⁶⁶	NCT01876511 Phase 2 (n=41)	Pembrolizumab	Treatment refractory metastatic: MMR-D CRC (n=11); MMR-P CRC (n=21); MMR-D non-CRC (n=9)	• ORR for MMR-D CRC: 40% (12-74) • mOS for MMR-D CRC: not met	5/2017 for refractory metastatic MSI-H/ MMR-D cancers, regardless of primary site
Le et al. (2017) ⁶⁷	NCT01876511 Phase 2 (n=86)	Pembrolizumab	Treatment refractory metastatic MMR-D/MSI-H tumors (12 cancer types)	• ORR: 53% (42-54) • mOS: not met • 2 year OS: 64% (53-78)	As above
Overman et al. (2017) ⁵⁷	CheckMate -142 NCT02060188 Phase 2 (n=74)	Nivolumab	1 line prior therapy recurrent/metastatic MMR-D CRC	• ORR: 69% (57-79) • mOS: not met • 1 year OS: 73% (62-82)	7/2017 for refractory MMR-D/ MSI-H mCRC
Overman et al. (2017) ⁵⁶	CheckMate -142 NCT02060188 Phase 2 (n=119)	Nivolumab + Ipilimumab	1 line prior therapy recurrent/metastatic MMR-D CRC	• ORR (55%. 45.2-63.8) • mOS: not met • 1 year OS: 85% (CI 77.0-90.2)	7/2018 for 2 nd line MMR-D/MSI-H mCRC

Phase 3: ongoing trials				
Trial	Regimen	Patient population	Outcomes (pending)	Expected completion
KEYNOTE-17 NCT02563002 Phase 3 (n=308)	Pembrolizumab vs standard of care (6 regimens)	1 st line MMR-D/MSI-H mCRC	• PFS • OS • ORR	September 19, 2019
NCT02912559 Phase 3 (n=700)	Atezolizumab + FOLFOX vs FOLFOX alone	Stage III MMR-D CRC (adjuvant)	• DFS • OS • AE	July 1, 2020

Immunotherapy: mechanism of action		
Name	Brand name (company)	Mechanism of action
Atezolizumab	Tecentriq (Genentech/Roche)	humanized, monoclonal antibody that binds to Programmed Death Ligand 1 (PD-L1)
Ipilimumab	Yervoy (Bristol-Myers Squibb)	humanized, monoclonal antibody that binds to Programmed Death Ligand 1 (PD-L1)
Nivolumab	Opdivo (Bristol-Myers Squibb)	humanized IgG4 antibody targeting the immune checkpoint programmed death receptor-1 (PD-1)
Pembolizumab	Keytruda (Merck and Co. Inc.)	humanized monoclonal antibody against PD-1 receptor.

CI, confidence interval; CRC, colorectal cancer; DFS, disease free survival; F, female; FOLFOX, combination chemotherapy: 5-fluorouracil (5-FU), leucovorin, oxaliplatin; M, male; mCRC: metastatic colorectal cancer; MMR-D, mismatch repair deficient; MMR-P, mismatch repair proficient; mOS, median overall survival; MSI-H, microsatellite instability – high; ; ORR, objective response rate; PFS, progression free survival;