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REVIEW

Phenotypic and genotypic heterogeneity of Lynch syndrome: a complex diagnostic challenge

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Abstract Lynch syndrome is the hereditary disorder that most frequently predisposes to colorectal cancer as well as predisposing to a number of extracolonic cancers, most prominently endometrial cancer. It is caused by germline mutations in the mismatch repair genes. Both its phenotype and genotype show marked heterogeneity. This review gives a historical overview of the syndrome, its heterogeneity, its genomic landscape, and its implications for complex diagnosis, genetic counseling and putative implications for immunotherapy.

Keywords Lynch syndrome · Hereditary nonpolyposis colorectal cancer · Hereditary cancer · Mismatch repair · Colorectal cancer · Endometrial cancer

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Introduction

Lynch syndrome (LS) is the hereditary disorder that most frequently predisposes to colorectal cancer (CRC). An estimated one of every 35 cases of CRC is attributable to LS [1] and certain extracolonic cancers are also integral to the syndrome; endometrial cancer is the most predominant of these. It is estimated that one million individuals in the United States carry LS mutations, with only 5% of these being aware of their cancer predisposition [2].

History of Lynch syndrome

The history of LS begins in 1895 with Aldred Warthin, a pathologist, who documented three families that showed a pronounced excess of cancers, particularly involving the colorectum, stomach, and endometrium, with patterns consistent with autosomal dominant inheritance [3]. In 1962, Lynch encountered a patient from Nebraska with a similar family history. Shaw, of the University of Michigan, also had a family with strikingly similar clinical and pathology characteristics. The pedigrees from both families were published together in 1966 [4].

These early reports, along with identification of other families with similar histories, provided a more comprehensive picture of the LS phenotype and led to the establishment of clinical diagnostic criteria and management guidelines for affected families. During the 1990s, links between LS and mutations in DNA mismatch repair (MMR) genes were established [5–9]. In 2009, Ligtenberg et al. [10] demonstrated that germline deletions on the *EPCAM* gene resulted in silencing of the adjacent *MSH2* gene.

In the late 1980s, criteria for Lynch syndrome began to emerge. The Amsterdam Criteria [11] were intended to be

used to assure that collaborating researchers in different parts of the world were following common criteria in classifying research subjects rather than being intended to be used to diagnose the syndrome clinically. They were found to be rather limiting, and the less stringent Amsterdam Criteria II [12] were developed. Subsequently, an even wider approach to diagnosis of the syndrome was developed, referred to as the Bethesda Guidelines [13], which were meant to identify those who should receive microsatellite instability (MSI) testing. These were subsequently expanded by including pathology features, and since then have been known as the Revised Bethesda Guidelines [14]. However, these criteria for LS diagnosis harbor major limitations. Boland and Shike [15] determined that screening of all CRC patients meeting the Amsterdam Criteria would fail to detect half of the cases of LS, while screening all patients meeting Bethesda Guidelines for MSI testing would fail to detect about one-third of LS cases.

In 1981 [16], the Muir-Torre syndrome with sebaceous and other skin tumors was identified as a variant of LS. In 1994 [17], LS was found to include significantly increased frequencies of cancers of the stomach, small bowel, hepatobiliary system, upper urologic tract, and ovary. A follow-up study in 2008 [18] added glioblastomas to the list and found that trials of diagnostic and preventive measures could be justified for urologic tract and ovarian cancers in some LS subgroups, especially *MSH2* mutation carriers and individuals over a certain age (approaching or over age 50 for urologic tract and over 40 for ovarian cancer). More recently, cancers of the pancreas [19], breast [20], prostate [21], and the rare adrenocortical [22] tumors have been considered to be overrepresented in patients with LS. See the National Comprehensive Cancer Network Guidelines for Genetic/Familial High-Risk Assessment: colorectal cancer, page LS-B [23] for a table giving a detailed risk overview of those cancers integral to LS.

Genomic basis of Lynch syndrome

LS is inherited in an autosomal dominant pattern, which is caused by heterozygous germline mutations in the DNA MMR genes [24]: *MLH1* [25], located on chromosome 3p21.3; *MSH2* [26] and *MSH6* [9], both located on 2p21; and *PMS2* [8], located on 7p22. Mutations in *MMR* genes decrease an individual's ability to repair base pair mismatches that occur during cell division, thereby predisposing carriers to high lifetime risks of developing MMR-deficient cancer(s). A proportion of patients without an identified genetic mutation within a MMR gene have a germline deletion spanning the 3′ end of the epithelial cell adhesion molecule (*EPCAM*) gene that is located immediately 5′ of *MSH2* [27] and which silences the *MSH2* gene in epithelial cells [10, 28]. Approximately one-third of suspected LS cases have no identifiable pathogenic mutation. An alternative mechanism in a portion of these cases is constitutional epimutation of *MLH1* or *MSH2* [29] which is characterized by methylation and transcriptional inactivation of an allele. *MSH2* and some *MLH1* epimutations have been linked to genetic alterations and show a dominant inheritance pattern; other *MLH1* epimutations are reversible between generations and show non-Mendelian inheritance patterns [29].

MSI is a consequence and a characteristic of MMR-deficient tumors. MSI involves the accumulation of length variations in repetitive DNA sequences referred to as microsatellites. MSI indicates a defect in MMR which can be characterized by a hypermutable phenotype with a high mutational load.

The identification of MSI in LS-associated tumors and the use of immunohistochemistry to detect losses of expression of the MMR proteins have revolutionized the diagnosis of LS and enabled the possibility of testing all CRC and endometrial cancers to determine the probability of LS being present in the patient [1, 30, 31]. MSI and/or MMR protein expression status are now used as the first step in so-called "universal testing" schemes to identify patients with an increased risk of having LS.

Win et al. [32] estimated population carrier frequency of pathogenic MMR mutations to be 1/714 for *PMS2*, 1/758 for *MSH6*, 1/1,946 for *MLH1*, and 1/2,841 for *MSH2*. These authors calculated that these figures would lead to a population estimate of 1/279 for any MMR gene (note: they found no *EPCAM* mutation carriers in their cohort).

It has usually been reported that an estimated 80–90% of LS involves mutations in *MLH1* or *MSH2*, while mutations in *MSH6* or *PMS2* account for 10–20% [27]. Up to 3% of LS is caused by an *EPCAM* mutation. However, these frequencies have been reported in patients ascertained via fulfillment of the Amsterdam or Bethesda clinical criteria for a presentation of CRC or endometrial cancer, which may have been biased against the identification of *MSH6* and *PMS2* mutations [33, 34].

Soares et al. [35] have found next-generation sequencing to be an efficient strategy that reduces time and expense in identifying mutations in *MMR* genes.

Differential diagnosis

Hereditary nonpolyposis colorectal cancer (HNPCC) was historically synonymous with LS, but it is now possible to differentiate among familial nonpolyposis CRC syndromes on the basis of a mutated gene [36]. Differentiation of HNPCC disorders can be aided by molecular analysis of tumors and germline mutation testing, and has implications for their diagnosis and management

Table 1 Familial nonpolyposis colorectal cancer syndromes

Term	Synonyms	Definition
Lynch syndrome		Families that have a monoallelic germline mutation in DNA MMR or *EPCAM* genes [101]
Muir-Torre syndrome		A variant of Lynch syndrome manifesting sebaceous and other skin tumors [102]
HNPCC (hereditary nonpolyposis colorectal cancer)		Families that satisfy the Amsterdam I or II criteria clinically but do not have a germline mutation in DNA mismatch repair (MMR) or *EPCAM* genes [101]
Lynch-like syndrome	Tumor Lynch	Families where testing of associated tumor(s) show MMR deficiency but a mutation in DNA MMR or *EPCAM* gene is not present upon germline testing [101]
Familial colorectal cancer type X	MSS-HNPCC	HNPCC-defined families that have microsatellite stable (MSS) tumors; heterogeneous group with dominant inheritance of cancer but intact MMR [103]
Familial colorectal cancer		Families that contain an unusual aggregation of members with cancer of the colon or rectum but that do not appear to have a known inherited colorectal cancer syndrome. Familial colorectal cancer (FCC) is essentially a descriptive term and does not point to a particular underlying cause. [104]
Constitutional mismatch repair deficiency (CMMRD)	Biallelic mismatch repair deficiency; homozygous mismatch repair mutations	Biallelic mutations in one of the MMR genes that cause constitutional mismatch repair deficiency; a rare recessively inherited syndrome mainly characterized by café-au-lait spots and a broad spectrum of childhood malignancies, primarily hematological, brain, and intestinal tract tumors [105]

[36] (see Table 1). Conditions associated with defective DNA MMR include LS (germline MMR mutation), constitutional MMR deficiency syndrome (biallelic germline MMR mutations) [37], Lynch-like syndrome (some cases caused by biallelic somatic MMR mutations, some due to unknown causes) [38], and sporadic CRC with MSI (somatic biallelic methylation of *MLH1*) [39]. An HNPCC condition with proficient DNA MMR is known as familial CRC type X [40], which is familial nonpolyposis CRC that meets the Amsterdam criteria for HNPCC but is microsatellite stable (MSS) as well as MMR proficient.

Garre et al. [41] compared familial CRC type X (which they refer to as MSS-HNPCC), LS, and sporadic CRC. Similar to previous studies, they found MSS-HNPCC to have earlier age at CRC diagnosis and increased CRC incidence in relatives when compared with sporadic CRC, as well as distal tumor preference and more frequent presence of tumors when compared with LS. New findings were increased extracolonic cancers and improved overall survival in MSS-HNPCC when compared with sporadic CRC. In MSS-HNPCC families, incidences of CRC and endometrial cancer were higher than in sporadic CRC relatives but lower than in LS families; kidney and stomach cancers were higher than in sporadic CRC and similar to LS.

The majority of MSI is found in a subset of sporadic tumors, where it is caused by methylation of *MLH1*. Testing for *BRAF* mutation is often used to rule out LS in these cases, since *BRAF* V600E is closely associated with somatic *MLH1* methylation. However, Adar et al. [42] have proposed a hybrid approach utilizing both *BRAF* genotyping and testing for *MLH1* methylation, which could significantly reduce the number of methylation assays performed and reduce the referral rate for genetic testing.

Boulagnon et al. [43] have discussed the application of immunohistochemistry and molecular morphology relevant to their importance in the detection of *BRAF* mutation in CRC to enable the discrimination between sporadic and LS-related CRC. Therein, BRAF-specific antibody can be used effectively on tissue microarray in order to screen *BRAF*-mutated CRCs [42].

Heterogeneity

According to Vogelstein et al. [44], four types of genetic heterogeneity can be involved in tumorigenesis: (1) intratumoral: heterogeneity among the cells of one tumor; (2) intermetastatic: heterogeneity among different metastasized lesions in the same individual; (3) intrametastatic: heterogeneity among the cells of an individual metastasis; and (4) interpatient: heterogeneity among the tumors of different individuals. Interpatient heterogeneity accounts for no two cancer patients having identical clinical courses. Some of the difference could be attributable to host factors such as germline mutations, some to nongenetic factors, and some to variation of somatic mutations occurring within tumors. This interpatient heterogeneity makes development of uniform cancer treatments difficult and drives research into individualized treatments through genome-based medicine. Because of intrametastatic heterogeneity, most patients will need to be treated with at least two drugs which target different pathways.

Significant inter-patient heterogeneity exists among patients with LS and this poses challenges for diagnosis and clinical management. Some of this heterogeneity may be attributed to which of the *MMR* genes is mutated. Lifetime risk of CRC for *MLH1* or *MSH2* mutation carriers is estimated to be 52–85%, while that for *MSH6* mutation carriers is 10–22% and that for *PMS2* mutation carriers is 15–20%. Lifetime risk of endometrial cancer for *MLH1* or *MSH2* mutation carriers is 25–60%, while this risk is 16–26% for *MSH6* and 15% for *PMS2*. (It is essential to observe that even these lower risks are substantially higher than those for the general population, which are 4.5% for CRC and 2.7% for endometrial cancer.) [23]. For patients with an *EPCAM* deletion, the lifetime risk for CRC is equivalent to patients with an *MSH2* mutation, but the lifetime risks for extracolonic cancers is lower (~12% for endometrial cancer), unless the deletion extends into *MSH2* [45, 46]. This difference has been attributed to the tissue-specific effects of the *EPCAM* deletion on downstream inactivation of *MSH2*, whereby *MSH2* inactivation occurs predominantly in epithelial cells including the colonic mucosa [10]. The mean age of onset for the major LS cancers also differs by gene mutated, with cancer diagnoses occurring at a younger age in carriers of *MLH1* and *MSH2* mutations. The phenotypic heterogeneity by gene mutated has led to recommendations for a new classification for LS, whereby the gene mutated is now designated, as well as tailored clinical management of the varied cancer risks. Patients with *MLH1*- or *MSH2*-LS are advised to undergo colonoscopy every 1–2 years from age 20–25 and have additional surveillance of endometrium, ovaries and urinary tract, whereas carriers of mutations in *MSH6* or *PMS2* are advised to have a colonoscopy every 1–2 years from age 25–30 and additional surveillance of the endometrium and ovaries [47].

Møller et al. [48], used an observational international multi-center study with the objective of determining prospectively observed incidences of cancers and survival in pathogenic MMR mutation carriers up to 75 years of age. The conclusion was that as carriers of different pathologic MMR variants aged, they showed distinct patterns of cancer risk and survival. Therein, estimates for counseling and planning of surveillance strategies should be tailored to each patient from the perspectives of age, gender, and the pathologic MMR variant.

There is heterogeneity even among family members sharing the same mutation. Clearly the MMR mutation is not the whole story and other factors, such as environmental or polygenic factors, may influence phenotypic expression.

Watson et al. [49] found an association between tobacco use and CRC in LS, but did not find a phenotypic association involving alcohol use. Burn et al. [50] identified a long-term reduction of cancer associated with aspirin use. Donald et al. [51] noted that LS has a variable phenotypic expression that remains largely unexplained and suggest that more investigation is warranted. They concluded that currently there is no consistent evidence of low penetrance genetic modifiers that affect the LS phenotype.

In terms of diagnosis, Yamano et al. [34] note that patients with LS may be overlooked as a result of the syndrome's heterogeneity. Historically, *MSH6* and *PMS2* cases may have been underdiagnosed because they did not fulfill the clinical criteria of the Amsterdam or Bethesda guidelines due to their lower penetrance and later age of cancer onset. It is also pointed out by ten Broeke et al. [52] that technical difficulties have possibly led to underreporting of *PMS2* mutations, although new strategies have reduced this difficulty; these authors found significantly raised standardized incidence ratios for cancers of the small bowel, ovaries, breast, and renal pelvis in carriers of *PMS2* mutations, with lower rates of CRC and endometrial cancer when compared with carriers of other MMR mutations. In an earlier study, Senter et al. [53] found *PMS2* mutations to have lower penetrance than other MMR genes, although they determined that the incidence of CRC among *PMS2* mutation carriers was 5.2-fold higher than the general population and the incidence of endometrial cancer was 7.5-fold higher.

A recent study by Espenschied et al. [33] presented data that led them to suggest that some *MSH6* and *PMS2* mutation carriers may present with a hereditary breast-ovarian cancer (HBOC)-like phenotype and are more likely to be missed by current LS screening and testing, which tends to concentrate on occurrences of CRC and endometrial cancer. Espenschied et al. [33] reviewed the clinical histories of patients who had undergone multigene panel testing for a diagnosis of CRC and/or endometrial

cancer, and/or breast cancer, and/or ovarian cancer. Of their 528 MMR mutation carriers identified, 22.2% met *BRCA1* and *BRCA2* (*BRCA1/2*) testing criteria but not LS criteria while 5.1% met neither *BRCA1/2* nor LS testing criteria. *MSH6* and *PMS2* mutations were more frequent than *MLH1* and *MSH2* mutations among patients who met *BRCA1/2* testing criteria but did not meet LS testing criteria ($P = 4.3 \times 10^{-7}$). It was noted that 11.9% (63) of the 528 MMR mutation carriers had breast cancer only, while 27.3% (144) had CRC only, and 27.5% presented with breast or ovarian cancer as their first primary cancer. Of further interest, mutations of *MSH6* or *PMS2* were significantly more frequent than mutations of *MLH1* or *MSH2* in patients with breast cancer only compared with patients with CRC only ($P = 2.3 \times 10^{-5}$) [33]. A similar trend was found for ovarian cancer only, but this did not reach statistical significance. The authors noted that patients with HBOC were over-represented in this high-risk cohort, which may have incurred some bias in the mutation frequencies detected by multigene panel testing. Furthermore, the rates of *MSH6* and *PMS2* mutations detected in their cancer cohort were only marginally higher than the population-based rates estimated by Win et al. [32]. Nevertheless, Espenschied et al. note that their data may give insight into why *MSH6* and *PMS2* mutations have been under-represented in previous reports and suggest they may be under-identified in general [33].

Kloor et al. [54] gave emphasis to CRC being a heterogeneous tumor type with regard to molecular pathogenesis and genetic instability. Therein, the majority of CRCs display chromosomal instability which follows the classical adenoma-carcinoma sequence of tumor progression. The authors note that a subset of approximately 15% of CRCs display DNA MMR deficiency and a high level of MSI (MSI-H). Therein, MSI-H CRCs can be either sporadic tumors or LS-related CRCs. These observations harbor important clinical relevance. Specifically, the MSI-H phenotype poses a hallmark of LS-associated cancers which is of diagnostic relevance with respect to the identification of LS mutation carriers. MSI-H CRCs are characterized by distinct clinical behavior which may result from their particular molecular pathogenesis and therefore gives rise to MSI testing for its clinical significance wherein the MSI-H phenotype shows association with "…proximal tumor localization, a dense local lymphocyte infiltration, and a low frequency of distant organ metastasis. Moreover, MSI-H colorectal cancers have a better prognosis than their microsatellite-stable counterparts." Furthermore, these authors concluded that "…the clinical characteristics of MSI-H cancers are closely linked to their molecular pathogenesis and research on the molecular alteration characteristic of MSI-H cancers may provide a basis for novel diagnostic or therapeutic approaches." [54].

Management

Surveillance for CRC in MMR mutation carriers has the potential to be effective and considerably more cost effective than foregoing surveillance [55–57]. Because of the early age of CRC onset in LS and the predominance of proximal colon involvement, full colonoscopy should be initiated by age 20–25. Because of accelerated carcinogenesis compared to non-MMR CRCs (CRC in 2–3 years compared with 8–10 years for non-MMR CRCs) [58], colonoscopy should be performed at least every 1–2 years. The early age of onset, right-sided predominance, and accelerated carcinogenesis lead to the conclusion stated by Kravochuck and Church [59] that, with respect to colonoscopy in LS patients, there is "no room for error."

Haanstra et al. [60] have reviewed studies of new techniques for identifying carcinoma and precancer in the colorectum in LS patients, inclusive of narrow-band imaging [61], chromoendoscopy (topical application of stains during endoscopy to improve polyp detection) [62–65], autofluorescence endoscopy [66], and I-SCAN (digital enhancement of surfaces and contrast) and endomicroscopy. At the time of their review, none of these new techniques had been found to have clear and convincing superiority over conventional colonoscopy, although chromoendoscopy has advantages as the equipment is inexpensive and the procedure is easily performed.

Rahmi et al. [67] in a multicenter trial compared standard colonoscopy with standard colonoscopy followed by chromoscopy while screening 78 LS patients. Significantly more patients with at least one adenoma were identified by chromocolonoscopy (32/78 [41%]) than by standard colonoscopy (18/78 [23%]; $P < 0.001$). However, it is difficult to ascertain if improved adenoma detection was due to chromoendoscopy, or was merely the result of a "second pass" of the colonoscope.

Bisschops et al. [68] accounted for the second pass effect while comparing I-SCAN with standard colonoscopy. LS patients were randomized to either standard colonoscopy followed by I-SCAN (n = 31) or I-SCAN first followed by standard colonoscopy (n = 30). When standard colonoscopy was performed first, five adenomas were detected and removed and a second pass with I-SCAN detected a further eight adenomas. When I-SCAN was used first, 15 adenomas were removed and subsequent standard colonoscopy detected two additional adenomas. The adenoma miss rate was significantly higher for standard colonoscopy (62%) compared with I-SCAN (12%; RR 0.44, 95% confidence interval 0.21–0.87; P = 0.007).

Subtotal colectomy in LS patients at the time of first CRC has been shown to decrease incidence of metachronous CRCs [69] and the need for subsequent abdominal surgery when compared with more limited surgery [70].

Lynch et al.[71] have strongly advocated offering prophylactic subtotal colectomy to patients who have tested positive for a germline mutation for LS, or who are obligate mutation carriers. They cite the limitations of colonoscopy, the potential for the rapid rate of cancer progression, and the high penetrance of the germline mutation. Subtotal colectomy as a prophylactic measure among LS patients remains controversial because the risk of metachronous CRCs must be considered against the possible negative consequences of the more extensive surgery [72]. It must involve genetic counseling, as well as input from the surgeon, to aid patients in assessing various management strategies. Using a decision analysis model for Lynch patients at age 25 years, Syngal et al. [73] calculated life expectancy improvements of 13.5 years for colonoscopy, and 15.6 years for prophylactic proctocolectomy (compared with no intervention). Prophylactic colectomy has been suggested [74, 75] as an option for patients likely to show poor compliance with colonoscopy.

Liska and Kalady [76] point out that there are no prospective clinical studies evaluating the potential survival benefit of prophylactic colectomy in LS and should only be offered in special circumstances: patients who have a colon that is technically difficult to examine endoscopically, those with poor compliance with screening recommendations, and those who have severe psychological fear of developing colorectal cancer, and it is also to be considered for patients in families with severe penetrance of disease or early-age onset of CRC.

Clinical practice guidelines for LS from the US Multi-Society Task Force on Colorectal Cancer guidelines [77] include colectomy with ileorectal anastomosis as the primary treatment for LS patients with CRC or colon neoplasia not removable by endoscopy. They note that less-extensive surgery could be considered in patients older than 60–65 years of age and those with underlying sphincter dysfunction. Church [78] notes that in cases where genotype, phenotype, and family history increase CRC risk, earlier and more aggressive surgery is appropriate; a balance of cancer prevention with lifestyle considerations should be maintained.

Endometrial cancer is the most frequently occurring extracolonic cancer in LS and women with a LS germline mutation have an increased incidence rate for ovarian cancer. Because of limited effectiveness of surveillance for endometrial [79] and, especially, ovarian cancer, prophylactic hysterectomy and bilateral salpingo-oophorectomy can be considered after childbearing is completed in germline MMR mutation carriers [80]. Such surgery has been shown to effect a significant reduction in endometrial and ovarian cancer among LS patients [81]. Clinical practice guidelines from the US Multi-Society Task Force on Colorectal Cancer guidelines [77] recommend prophylactic hysterectomy and bilateral salpingo-oophorectomy for women with LS who have finished childbearing, or at 40 years of age. In a recent study involving 1942 LS mutation carriers, the Mallorca Group found good survival of endometrial and ovarian cancer, but it is not clear whether this was due to surveillance or to more favorable tumor characteristics in LS-associated cancers when compared with sporadic disease [82]. Each of LS's integral cancers may harbor significant attributes for early detection and prevention which will affect genetic counseling, surveillance and management [23]. Møller et al. [83] studied subsequent cancers in patients with LS and found that favorable survival validated the importance of continuous follow-up after subsequent cancers, with the primary mission of cancer prevention and decrease of death from cancer.

Immunotherapy

A promising avenue of research is linking cancer genetics to immunotherapy. Approximately 15% of sporadic CRCs and most LS-associated CRCs show MSI. Llosa et al. [84] examined the immune microenvironment of CRCs. They found that a subset, virtually all of which had MSI, showed high infiltration with activated $CD8^+$ cytotoxic T lymphocyte (CTL) and activated Th1 cells. This is the first link described between a genetically defined subset of cancer and the corresponding expression of immune checkpoints; MSI-H tumors showed expression of at least five checkpoint molecules (PD-1, PD-L1, CTLA-4, LAG-3, and IDO) that are targeted by inhibitors that are currently being clinically tested.

Since almost all tumors in patients with LS are deficient in MMR, those with metastatic disease can benefit from this therapy along with patients who have sporadic MMR deficiency. This therapy does not attempt to kill cancer cells directly but instead blocks a pathway that protects cancer cells from the body's immune system which is able to fight cancer. One pathway includes the programmed death-1 (PD-1) protein which is expressed on the surface of immune cells and the programmed death ligand-1 (PD-L1) which is expressed on cancer cells. When PD-1 and PD-L1 join together, they protect tumor cells from being destroyed by the immune system by forming a shield that does not allow the immune system to recognize and attack the tumor cell. Therefore, blocking PD-1 or PD-L1 can interrupt that shield and allow the immune system to destroy the tumor [85]. See Fig. 1.

"Both ligands are expressed in response to γ-chain cytokines, Type I interferons, and interferon-γ. GM-CSF and IL-4 can also induce expression of PD-L2. Expression of PD-L1 occurs not only on immune cells, but also epithelial cells including cancer cells. Binding of ligands

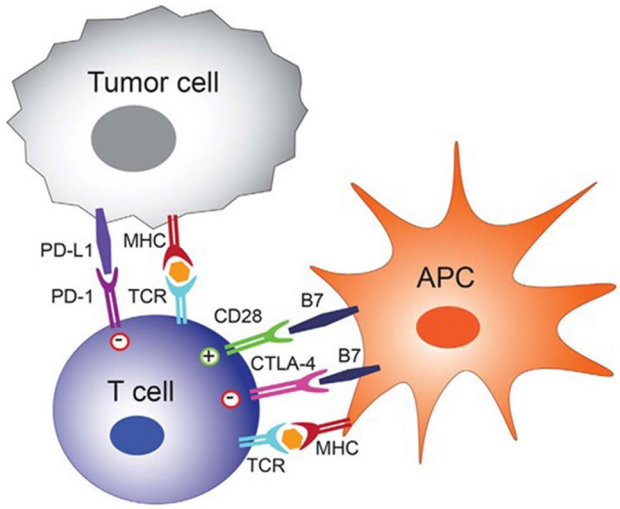

Fig. 1 Binding of the TCR to the peptide:MHC complex alone is not sufficient to activate T cells. Costimulation is necessary from the binding of B7-1/B7-2 to CD28. Inhibitory receptors such as PD-1 and CTLA-4 have been discovered, which blunt costimulation, prevent T cell activation, and result in T cell anergy and/or apoptosis. *TCR* T cell receptor, *MHC* major histocompatibility, *APC* antigen presenting cell. Reprinted from Pharmacological Research. Vol. 120, pp. 1–9. Sweis and Luke [85], Mechanistic and pharmacologic insights on immune checkpoint inhibitors. Copyright 2017, with permission from Elsevier

to PD-1 inhibits T cell activation, and thus dampens the immune response." [85].

The first clinical study of ipilimumab was published in 2003 and included 14 patients with metastatic melanoma. In that study, ipilimumab was administered by IV and was effective [86].

Le et al. [87] showed that MMR status in gastrointestinal cancers predicted whether immune checkpoint blockade with pembrolizumab would provide clinical benefit.

The majority of MMR-deficient endometrial tumors have been found to be PD-L1 positive in at least a subset of tumor cells [88]. Furthermore, it was found that tumoral PD-L1 expression is more common in LS-associated endometrial cancers relative to *MLH1* hypermethylated and MMR-intact tumors, although sporadic cancers often show PD-L1 positive immune staining. These data suggest that MMR deficiency may be a better predictor of response to PD-1/PD-L1 inhibitor therapy than tumor grade in endometrial cancer and that the potential benefit may be based on the molecular mechanism of MMR defects [88].

Once CTLA-4 and PD-1/PD-L1 were determined to be negative regulators of anti-tumor immunity, clinical exploration of antibodies targeting these pathways ensued between 2011 and 2017 for immune checkpoint inhibitors were approved for six different diseases [85].

Within a tumor microenvironment, this requires uptake of peptide fragments by specialized antigen presenting cells (APCs) driven by Type I interferons which cross present them to T cells in the tumor draining lymph nodes [85].

Boland noted that previously it had been shown that MSI CRCs do not respond favorably to conventional cytotoxic adjuvant chemotherapy with 5-fluorouricil (5-FU) and that this treatment may even worsen outcome [89]. However, in a study by Bertagnolli et al. [90], MSI-H CRCs had improved outcomes when compared with MS-stable tumors when irinotecan was added to 5-fluorouracil/leucovorin in the adjuvant setting, suggesting that MSI-H/LS-associated CRCs may have improved response at least to irinotecan as a cytotoxic agent.

Boland [91] has developed a discussion of what he refers to as immunotherapy for LS dealing with a matter of speculation as to whether MMR-deficient crypts may be precursors of genuinely neoplastic tissues. However, investigators have demonstrated that the causal frameshift mutations give rise to immunogenic neoantigens [92] with neoantigenic peptides occurring in most MMR-defective neoplasms, and appearing repeatedly in an individual with LS. These neoantigens induce an antibody response [93] and tumor infiltrating lymphocyte (TIL) response typical of MMR-defective tumors [94]. Therein, these TILs are activated $CD8^+$ T cells associated with early-stage tumors and the absence of lymph node metastases [95]. It has been proposed that immunizing LS patients with frameshift peptides may prevent cancer in such individuals [96]. Westdorp et al. [97] noted that neoantigen-based vaccination currently is being studied both in LS and in advanced-stage sporadic MSI CRC.

Predictive biomarkers are central to the concept of precision cancer medicine. Such validated predictive biomarkers such as *BRCA1, BRCA2*, and the MMR germline mutations in LS, among others, can often be effectively employed in the selection of individual patients for targeted treatment. For example, patients with *BRCA* mutations respond better to PARP inhibitors [98] and LS metastatic patients respond better to anti PD-1 [99]. On the other hand, a prognostic biomarker such as cancer stage or grade will be limited to providing statistical probability inclusive of survival estimates.

Conclusion

Precision medicine, which will include personalized medicine and genomic medicine as well as individualized medicine, is becoming a triumph for certain hereditary disorders inclusive of LS. With the continued advancements of immunotherapy and genomics, we should explore ways to combine various therapies to cure cancer. Significant challenges include research on predictive biomarkers as well as insight into the management of immune related toxicities

and, finally, reversing mechanisms of primary and secondary resistance [85]. Full application of genomic and personalized medicine in health care will require dramatic changes in regulatory and reimbursement policies, as well as legislative protections for privacy for its system-wide adoption. Thus, there are challenges from both a scientific and a policy perspective, but they will be met with the certainty that the science behind genomic medicine is sound and the practice of medicine that it informs is evidence based [100]. Recognition of LS patients who can fully benefit from the personalized management and treatment options for these patients will have the potential of being lifesaving.

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