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Relationship between microsatellite status and immune microenvironment of colorectal cancer and its application to diagnosis and treatment

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Abstract

Due to advances in understanding the immune microenvironment of colorectal cancer (CRC), microsatellite classification (dMMR/MSI-H and pMMR/MSS) has become a key biomarker for the diagnosis and treatment of CRC patients and therefore has important clinical value. Microsatellite status is associated with a variety of clinico-pathological features and affects drug resistance and the prognosis of patients. CRC patients with different microsatellite statuses have different compositions and distributions of immune cells and cytokines within their tumor microenvironments (TMEs). Therefore, there is great interest in reversing or reshaping CRC TMEs to transform immune tolerant "cold" tumors into immune sensitive "hot" tumors. This requires a thorough understanding of differences in the immune microenvironments of MSI-H and MSS type tumors. This review focuses on the relationship between CRC microsatellite status and the immune microenvironment. It focuses on how this relationship has value for clinical application in diagnosis and treatment, as well as exploring the limitations of its current application.

KEYWORDS
colorectal cancer, diagnosis and treatment, immune microenvironment, microsatellite status

1 | INTRODUCTION

The incidence of colorectal cancer (CRC) ranks third in the world among malignant tumors, and CRC is also the second leading cause of cancer-related deaths. There are about 1.8 million new cases of CRC and 881,000 deaths annually.[1] It is estimated that by 2030, the global burden of CRC will increase by 60%.[2] At present, early detection technology has greatly assisted early diagnosis and intervention of CRC, but about 25% of patients are nonetheless diagnosed as stage IV.[3] In the past 15 years, treatment strategies for metastatic colorectal cancer (mCRC) have improved, but the 5-year overall survival rate (OS) is still only 14%,[4] posing a serious threat to

public health. Immunotherapy is a new emerging tumor treatment method following surgical resection, chemoradiotherapy, and biological targeted therapy. Immunotherapy can eliminate tumor cells and inhibit tumor growth and metastasis by activating the immune system and exerting the immune capacity of the tumor microenvironment (TME). Immunotherapy is highly specific, which can not only damage normal cells but can also stimulate immune memory. Immunotherapy has become the focus of CRC treatment research in recent years. Immune checkpoint inhibitors (ICIs) are the most widely used form of immunotherapy.

At present, the FDA has approved ICI treatment for patients with mismatch repair-deficient (dMMR) / microsatellite instability-high

(MSI-H) mCRC[5] ; however, this represents only 15% of all CRC patients and only 2%–4% of stage IV mCRC patients.[6] Most patients with proficient MMR (pMMR) / microsatellite-stable (MSS) CRC cannot benefit from ICI treatment alone.[7,8] In recent years, the TME has emerged as an important source of potential therapeutic targets. The TME has an extraordinarily complex regulatory network, which plays a key role in the occurrence, progression, and treatment of tumors.[9] Reversing the inhibitory immune microenvironment of pMMR/MSS CRC and improving patient responses to ICIs have become urgent tasks.[5,10] Recent research has focused on predicting the behavior of cancer as well as studying its response to treatment by MSI detection and immune markers; however, many genetic and epigenetic factors as well as environmental and lifestyle factors can also affect immune cells, microbiota, tumor development and behavior, and response to treatment. Therefore, there are still limitations in the current application of this prediction and evaluation method, and thus, a need for further studies to be conducted in this area.

2 | MICROSATELLITE STATUS AND CRC IMMUNE MICROENVIRONMENT

dMMR occurs due to changes in MMR genes, which result in the loss of the repair function of one or several MMR proteins (MLH1, MSH2, MSH6, and PMS2), which leads to pairing errors during DNA replication.[11] These changes may be sporadic or hereditary.[12] Microsatellite instability (MSI) is the result of the accumulation of nucleotide insertions or deletions in the genome.[13] MSI can be divided into microsatellite instability-high (MSI-H), microsatellite instability-low (MSI-L),

and microsatellite-stable (MSS). At present, CRC patients are generally divided into two groups, dMMR / MSI-H type, and pMMR / MSS or MSI-L type (hereinafter referred to as pMMR/MSS type).[6]

It is well established that immune cells and cytokines in the TME can play dual roles in antagonizing or promoting tumors.[14] The body mainly achieves immune surveillance through three stages of immune elimination, immune balance, and immune escape.[15] Studies have shown that immune dysfunction caused by immunosuppression or autoimmune disease is associated with the high incidence of various cancers.[16] In addition, the infiltration of immune cells in the TME is an important factor affecting tumor heterogeneity and prognosis.[17-21] The same types of tumors have different biological characteristics and different immune microenvironments, as is the case for colorectal and rectal cancers.[22] These differences directly affect responses to ICI treatment.[23-26] The main differences in the tumor microenvironments (TMEs) of dMMR/MSI-H, and pMMR/MSS CRC patients are described below (Figure 1).

2.1 | Tumor mutation burden

According to the latest classification of CRC consensus molecular subtypes (CMSs), MSI-H belongs to the CMS1 type and accounts for 14% of CRC cases.[27,28] Several studies have shown high tumor mutation burden (TMB) in the TME of dMMR / MSI-H CRC.[6] Common mutations include widespread hypermethylation, BRAF mutations, and mutations in genes encoding DNA mismatch repair proteins. These unique highly mutated genomic structures can be regarded as new antigens; this makes them more sensitive to ICI therapy regardless of
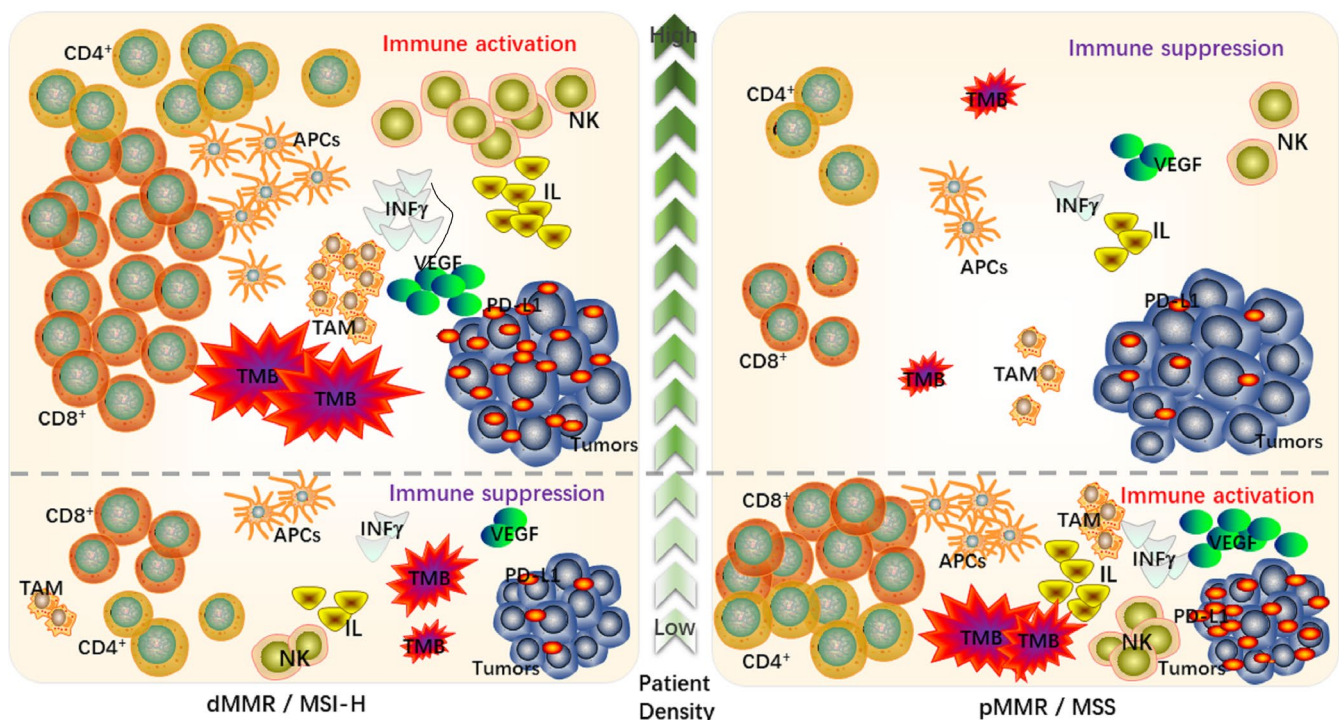

FIGURE 1 The main differences of TME among different MSI types

the cancer tissue type.[29-31] In addition, high TMB can stimulate the presentation efficiency of antigen-presenting cells (APCs), increase the diversity of MHC phenotypes, and affect the prognoses of patients.[32,33] Among these, increased expression of MHC-I molecules can promote the differentiation of CD8+ T cells into CD8+ cytotoxic T lymphocytes (CTLs) and directly produce tumor cell killing effects.[34] Furthermore, upregulation of MHC-II can induce CD4+ T helper cells to indirectly activate other immune cells.[35,36] Therefore, a high TMB dMMR / MSI-H phenotype has become an important biomarker to suggest that ICI treatment will be effective.[12,37]

pMMR/MSS type CRC accounts for about 95% of all CRC cases. The level of TMB in the TME of MSS patients is significantly lower than that in MSI-H patients; this greatly limits MHC expression on the surface of APCs, preventing an effective anti-tumor immune response and limiting the effectiveness of ICIs.[38,39] However, the response of pMMR / MSS patients to ICI treatment is very heterogeneous. There are some MSS patients with TMB levels close to MSI-H CRC patients, and these patients have more abundant T cell antigen receptors (TCRs), which may activate anti-tumor immune responses by regulating the TCR-MHC signaling pathway. These patients have better prognoses than other MSS patients.[40]

2.2 | Tumor infiltrating lymphocytes

CRC patients with different MSI types have different compositions and distributions of immune cells and cytokines within their TMEs.[6] MSI-H type tumors have significantly increased recruitment of tumor infiltrating lymphocytes, including activated cytotoxic T lymphocytes (CTLs), Th1 cells, and CD4+ T cells, as well as NK cells and macrophages.[41] Local tumor infiltration of CTLs is a prerequisite for response to ICIs.[42] In addition, MSI-H type tumors also have increased secretion of tumor necrosis factor, perforin, granzyme, IL-1, IL-6, IFN-γ, and other related cytokines in the TME,[6,43] and these cytokines regulate the TME immune "activation" or "inhibition" state.[44,45] At the same time, a variety of inflammatory mediators infiltrate to form an inflammatory TME, and continuous inflammatory stimulation leads to exhaustion of T lymphocytes, which upregulate inhibitory receptors such as PD-1, CTLA-4, TIM3, and LAG-3.[46,47] These immunosuppressive receptors bind to the corresponding ligands in the TME and regulate the anti-tumor immune response.[33] It has been shown that increased interferon expression is associated with better prognosis and can induce the secretion of chemokines and induce adaptive immune responses.[6,33,48] Thus, high TIL concentrations in MSI-H CRC patients indicate a better survival outcome.

Endogenous anti-tumor T-cell immunity is mainly achieved by CTLs with high PD-1 expression. Compared with most MSI-H CRCs, the TME of MSS type patients usually conveys an immune rejection or immune desert phenotype. It is manifested by low TIL infiltration and lack of CTLs or insufficient CTL activity[38,39]; this is also an important potential mechanism of resistance to PD-1/PD-L1 inhibitors. Some studies have found that the level of TIL infiltration in the TME directly affects the recruitment of CTLs and the ability to recognize

malignant cells, and high levels of TILs are usually conducive to ICI treatment.[49,50] Pamplona et al. also showed that CD8A expression (an indicator of TIL infiltration) can be used as a biomarker to evaluate the prognosis of patients with MSS tumors.[51] Recently, Dahna et al. found that pembrolizumab can not only restore the cytotoxic function of T lymphocytes but can also promote the recruitment of other immune cells to tumor sites by blocking the interaction between PD-1 and PD-L1.[52] Therefore, reshaping the TME and increasing the degree of TIL infiltration may be important new directions for the treatment of MSS CRC patients.

2.3 | PD-L1 expression

Korehisa et al[53] found that 5.4% of CRC tumor cells in MSS patients and 36.1% of CRC tumor cells in MSI-H patients were PD-L1 positive, and 27% of stromal cells in MSS patients and 72.2% of stromal cells in MSI-H patients were PD-L1. Expression of PD-L1 in MSI-H type patients in both tumor cells and stromal cells is much higher than in MSS type patients, which indicates that PD-1 / PD-L1 blockers have more targets and higher sensitivity in the TME of MSI-H CRC patients. In addition, when MSI-H CRC is about to invade and metastasize, the expression of PD-L1 on tumor cells in the TME and CD68 / CD163+(M2) macrophages in the stroma is upregulated, which induces immune escape.[54,55] This suggests that PD-1 / PD-L1 inhibitors have great potential in the treatment of MSI-H CRC patients with high PD-L1 expression and can effectively inhibit tumor progression in these patients.

PD-L1 expression in most MSS CRC patients is significantly lower than in MSI-H CRC patients, but this is not absolute. Llosa et al. showed that some patients with pMMR / MSS tumors have TMEs similar to dMMR/MSI-H patients. For example, the TMEs of some patients have high PD-L1 expression and high infiltration of PD-1+ CD8 cytotoxic lymphocytes without inhibitory Th17 cells; these factors relate to the benefit of patients receiving pembrolizumab.[56] Recently, Nicolas et al. also showed that dMMR/MSI-H CRC is not the only subgroup that benefits from ICI treatment. Anti PD-1 combined with anti CTLA-4 therapy can enhance the immunogenicity of some pMMR / MSS CRC tumors, thus activating the anti-tumor immune response and improving the patient's prognosis and survival.[7] Therefore, the use of checkpoint blockade therapy—to save effector T cells from exhaustion or induce Treg depletion—can help prevent PD-1/PD-L1 binding, reverse the TME in MSS patients, and inhibit immune escape.[5,13,57]

2.4 | VEGF expression

Vascular endothelial growth factor (VEGF) is the strongest and most specific pro-angiogenic growth factor. It can stimulate tumor growth and metastasis by stimulating the growth of tumor microvessels.[58] Miyamoto et al[59] reported that MSI-H, and MSS tumors utilize different carcinogenic pathways, including the

abnormal expression of angiogenesis-related genes. Sun et al[60] also found that MSI-H CRC, and MSS CRC may use two different angiogenesis pathways. High VEGF expression in CRC patients is associated with blood metastasis, lymph node metastasis, advanced TNM stage and depth of invasion. Lower VEGF expression in the TME of MSI-H tumors is associated with lower invasion and better prognosis.

VEGF expression in the TME of most MSS type CRC patients is upregulated, which leads to increased recruitment of myeloid-derived suppressor cells (MDSC), downregulation of IL-12 and upregulation of IL-10 in macrophages, and M1 macrophage polarization into M2 macrophages[61]; these events induce the formation of the inhibitory TME, which is conducive to the growth, invasion, and metastasis of tumors.[62] Therefore, VEGF inhibitors combined with PD-1 / PD-L1 inhibitors can exert a synergistic effect, simultaneously blocking the activation of VEGF-related pathways and PD-1 / PD-L1-related pathways in the TME and reducing tumor neovascular density. This combination can reduce the occurrence of immune escape, improving the prognosis of patients with MSS CRC.

3 | APPLICATION OF MICROSATELLITE STATUS IN THE DIAGNOSIS AND TREATMENT OF CRC

CRC is a molecularly heterogeneous disease characterized by three carcinogenic pathways, including chromosome instability (CIN), microsatellite instability (MSI), and CpG island methylation phenotype (CIMP).[63] Studies have shown that about 85%–90% of hereditary non-polyposis CRC and about 10%–15% of sporadic CRC patients have high expression of MSI-H.[64,65] Moreover, it has been shown that MMR gene deletions in CRC patients are mainly caused by gene mutation or promoter methylation; of these, MSH2 and MLH1 gene mutations account for more than 90% of all gene mutations.[66] In 2018, the National Comprehensive Cancer Network (NCCN) guidelines recommended that MSI status should be considered in CRC patients regardless of tumor type, especially in stage II patients. Thus, the classification of the CRC microsatellite status is significant for the clinical diagnosis and treatment of patients.

3.1 | Guiding Lynch syndrome screening

Lynch syndrome (LS) is the most common hereditary CRC syndrome.[67] It is a familial disease of autosomal dominant inheritance, which is clinically similar to sporadic MSI-H CRC. In contrast to sporadic CRC patients, LS carriers or family members usually develop CRC or other Lynch-related tumors when they are young.[68] CRC and endometrial cancers are the most common cancer affecting LS patients. When the patient is diagnosed, LS increases the lifetime risk of CRC to about 80%.[69] Parag et al[70] performed

a retrospective meta-analysis studying the significance of MSI detection of colorectal adenomas for LS screening and found that 69.5% of patients in the LS cohort could be diagnosed through detection of MSI status of their routine adenomas. Thus, the dMMR/MSI-H adenoma phenotype is a risk factor for CRC among LS patients, and MSI detection has especially important application value for early LS screening.

3.2 | Guiding the evaluation of prognosis

The prognosis of CRC patients is closely related to the age of diagnosis, gender, disease stage, tumor location, degree of differentiation, pathological type and other characteristics, while the invasion, metastasis, and prognosis of CRC are significantly related to the classification of the status of the microsatellite.[5,71] Many studies have shown that sporadic CRC with the MSI-H phenotype is more common in women and may be related to estrogen secretion.[72,73] Hormone replacement therapy can reduce the risk of MSI-H CRC.[74,75] The increased DNA methylation caused by MSI-H is also related to the age of onset, and menopausal women have a higher risk of developing sporadic MSI-H CRC. In addition, most patients with MSI-H CRC have primary tumors located in the proximal colon[76]; which accounts for 15% of stage II-III tumors and 4%–5% of stage IV tumors.[77] Moreover, MSI-H tumors are usually poorly differentiated or mucinous adenocarcinoma with characteristic lymphocytic infiltration.[78] However, Watanabe et.al have confirmed that CRC-specific survival was significantly better in patients with MSI cancer than in those with MSS (p = .02). They also found that MSI was strongly associated with a decreased likelihood of lymph node and distant organ metastases at diagnosis (all p < .001).[79] Therefore, dMMR / MSI-H CRC may indicate a better prognosis, which may be related to high infiltration of lymphocytes and high sensitivity to immunotherapy.

3.3 | Guiding diagnosis of post-colonoscopy CRC

Colorectal endoscopy is considered to be the gold standard for the diagnosis of CRC, but it is not infallible. Post-colonoscopy CRC (PCCRC) is defined as a CRC diagnosed 6–36 months after a negative result from a colonoscopy. Although the number of these patients is very small, it is very important for clinics.[80] Arain et al[81] reported that, after adjusting for tumor location, MSI-H was independently associated with PCCRC (odds ratio: 2.7; 95% CI: 1.1–6.8). Sawhney et al[82] also showed that the probability of MSI in PCCRC was 3.7 times higher than that in noninterval / detected cancers. In 2019, Samadder et al[83] found that MSI was observed in 32% of PCCRC and only 13% of detected CRC (p = .005) in a cross-sectional study based on CRC cases in Utah, and they concluded that PCCRC was associated with MSI (odds ratio was 4.20; 95% CI was 1.58–11.14). Therefore, MSI detection also has important application value in the field of CRC diagnosis.

3.4 | Guiding adjuvant chemotherapy

Many studies have found that when 5-fluorouracil (5-FU) is used in patients with MSI-H and MSS type CRCs, 5-FU adjuvant chemotherapy for MSI-H is unfavorable to the survival of patients.[84] However, patients with MSI-H CRC seem to respond well to irinotecan treatment.[85,86] It has been suggested that the difference in patient response to 5-FU and irinotecan treatment may be due to the fact that in MSI-H patients, cell death induced by 5-FU treatment requires the MMR system to function, whereas irinotecan induced DNA damage can be lethal directly.[87] In addition, some scholars have found that MSI-H tumors highly express thymidylate synthase, which may also lead to resistance to 5-FU.[88] However, in a multicenter international trial (MOSAIC) study on the efficacy of oxaliplatin/fluorouracil/calcium leucovorin in adjuvant treatment of colon cancer, researchers analyzed the MSI status of CRC patients and followed up for 10 years; they found FOLFOX4 adjuvant chemotherapy can improve OS in patients with dMMR / MSI-H type III CRC.[89] The MSI status may affect the effectiveness of adjuvant chemotherapy in patients with CRC, but additional clinical trials are needed to determine the role of MSI classification in the selection of an adjuvant chemotherapy regimen.

3.5 | Guiding targeted therapy

Some studies have shown that promoter methylation and genome amplification or mutation of HER2, MET, PTEN, or PIK3CA are common in MSI-H tumors. These lead to decreased expression of EGFR ligands, decreased efficacy of EGFR inhibitors, and resistance to EGFR therapy.[90,91] For example, cetuximab treatment of MSI-H CRC usually has adverse reactions, while bevacizumab can reduce immunosuppressive cells and enhance anti-tumor immune responses by inhibiting angiogenesis and promoting vascular normalization. The CALGB/SWOG 80405 study compared the efficacy of first-line (FOLFOX or FOLFIRI) combined with bevacizumab or cetuximab in the treatment of mCRC. The results showed that the median OS of the MSI-H group and the MSS group was 30 months versus 11.9 months, and the median OS of MSS mCRC patients treated with cetuximab and bevacizumab was similar (*n* = 586; median OS: 30.7 months vs. 30.3 months).[92] In addition, a subgroup analysis of the NASBP C-08 study found that MSI-H patients in stage II-III CRC who received FOLFOX+bevacizumab had better outcomes than those treated with chemotherapy alone.[93] Recently, Zaanan et al. retrospectively analyzed data of 128 patients with MSI-H / dMMR mCRC who received first-line chemotherapy alone or combined with anti-EGFR treatment from 2007 to 2017; they found that the addition of anti-EGFR to chemotherapy significantly improved progression-free survival (PFS) in patients with familial mCRC.[94] Therefore, in the era of precision treatment, MSI testing for patients with sporadic or hereditary CRC can help guide patients in choosing a suitable targeted therapy plan and predicting the benefits of targeted therapy.

3.6 | Guiding immunotherapy

The long-term clinical efficacy of ICIs in treating refractory malignant solid tumors has revolutionized cancer treatment.[95,96] In five clinical trials of prembrolizumab for CRC, KEYNOTE-016[97]、KEYNOTE-164[98]、KEYNOTE-012[99]、KEYNOTE-028,[100] and KEYNOTE-158,[101] a higher overall remission rate was observed, which further indicates that pembrolizumab is effective in treating MSI-H tumors.[102] Although anti-PD-1/PDL-1 immunotherapy is generally ineffective for CRC,[37,103,104] definite clinical responses have been observed in patients with dMMR / MSI-H CRC.[105,106] Although not all MSI-H CRC patients can respond to immunotherapy,[37,97] PD-1 / PD-L1 blockade therapy reactivates effector T cells, inhibits immune escape, and shapes the activated immune TME.[107] These factors make PD-1/PD-L1 blockade therapy likely to become an important CRC treatment modality in the future. Therefore, MSI detection is not only a directional "landmark" for immunotherapy in CRC patients, but it is also a predictive marker for the efficacy of ICI treatment.[108]

4 | MICROSATELLITE STATUS AND APPLICATION PROGRESS OF ICIS

Samstein et al. showed that patients with CRC in the MSS/MSI-L group are not sensitive to ICI treatment[95]; however, due to the complexity of anti-tumor immune responses and the heterogeneity between tumor and metastasis, dMMR / MSI-H status alone may not be enough to accurately identify those responsive to ICI treatment.[109] In recent years, to further clarify the effectiveness immunotherapy for CRC, many studies on ICI treatment have been carried out worldwide. Recent preclinical and clinical studies have shown that ICIs combined with chemotherapy, molecular targeted therapy, radiotherapy, or new immunomodulators can act synergistically and extend the application of ICIs to MSS type CRC.[7,110,111]

4.1 | dMMR/MSI-H type CRC and ICIs

4.1.1 | Single drug research

The most recent KEYNOTE 177 study[112] is an international, randomized, open phase III clinical trial of MSI-H / dMMR mCRC, comparing the role of pembrolizumab and chemotherapy in the first-line treatment of MSI-H / dMMR stage IV CRC. This clinical trial is estimated to be completed in December 2021. The latest follow-up results show that compared to those treated with chemotherapy, patients receiving pembrolizumab as a first-line treatment had significant improvements in PFS. This may change the first-line treatment for patients with dMMR mCRC.[113,114]

4.1.2 | Combined targeting

A phase II clinical trial, CheckMate-142 (NCT02060188),[115] is studying the efficacy and safety of nivolumab (3 mg/kg every 2 weeks) or nivolumab combined with ipilimumab (1 mg/kg every 3 or 6 weeks) in the first-line treatment of dMMR / MSI-H CRC. This study is expected to be completed in July 2022. Follow-up results thus far have been encouraging.[29,106,116,117]

4.1.3 | Combined chemotherapy ± targeting treatment

A phase III randomized COMMIT study (NCT02997228)[118] is also in progress. At present, 347 patients with MSI-H / dMMR MCRC have been randomly assigned to mFOLFOX6 / bevacizumab combined with or without atezolizumab or atezolizumab combined with chemotherapy as the first-line treatment.[78] This clinical trial is still in progress and is expected to be completed in April 2022. The results are highly anticipated.

4.2 | pMMR/MSS type CRC and ICIs

Because pMMR/MSS CRC patients respond poorly to single-agent ICIs, current research mainly focuses on combination therapy.

4.2.1 | Combined radiotherapy

The ongoing PEMREC trial (NCT04109755)[119] evaluates the feasibility of a neoadjuvant regimen without chemotherapy for patients with locally advanced pMMR CRC. The enrolled patients use radiotherapy combined with pembrolizumab as a neoadjuvant treatment regimen.

Other studies on the safety and efficacy of radiotherapy combined with pembrolizumab in MSS type mCRC, especially patients with liver metastases, such as NCT02837263[120] and NCT02437071[121] are under active development.

4.2.2 | Combined radiotherapy ± targeting

A phase II clinical trial of neoadjuvant radiotherapy for rectal cancer combined with atezolizumab and bevacizumab, the Tarzan trial (NCT04017455),[122] is currently recruiting patients and is expected to be completed in August 2024.

4.2.3 | Combined targeting

At present, NCT03442569 trial[123] is exploring the potential synergistic effect of anti-EGFR and ICIs, as well as the safety and efficacy of the combined application of panitumumab, nivolumab, and ipilimumab in KRAS / NRAS / BRAF wild-type MSS MCRC.

REGONIVO(EPOC1603) study is an open-label, dose-escalation, dose-expansion, and phase Ib study for patients with advanced gastric cancer or CRC. A total of 25 CRC patients (24 MSS and 1 MSI-H) were enrolled in the CRC cohort to receive regorafenib combined with nivolumab. The results of this study showed that eight (33%) of the 24 patients with MSS mCRC achieved objective remission, which indicated that regorafenib 80 mg combined with nivolumab is controllable and has encouraging anti-tumor activity in patients with MSS.[124]

In addition, a phase I / II clinical trial (NCT03657641[125] and NCT03797326[126]) on regorafenib or lenvatinib in combination with anti-PD-1/PD-L1 in mCRC patients will study whether regorafenib or levatinib interacts synergistically with anti-PD-1/PD-L1 treatment.[127]

4.2.4 | Combined immunotherapy

In a phase II trial to explore the combination of a cancer vaccine (GVAX colon vaccine) with pembrolizumab and cyclophosphamide in the treatment of pMMR / MSS type CRC, biochemical reactions were observed in 41% of patients. This indicates that GVAX can regulate the anti-tumor immune response.[128]

Cibisatamab (CEA-CD3-TCB; RG7802, RO6958688) is a T cell bispecific antibody (TCB). In an ongoing study, the activity of CEA-TCB combined with atezolizumab was enhanced and the toxicity was controllable. In the clinical treatment of pMMR / MSS mCRC patients, ORR was 18% and DCR was 82%, and the results were encouraging.[129]

The niche study (NCT03026140[130]) carried out by Chalabi et al. identified dMMR and pMMR in patients with early-stage colon cancer and divided them into two groups. The results showed that 4 / 15 (27%; 95% CI: 8%–55%) of pMMR tumors showed pathological responses, with three MPRs and one partial response. After treatment, the infiltration of CD8+ T cells significantly increased, indicating that the anti-tumor immune function was partially activated.

5 | LIMITATIONS OF MSI STATUS AND IMMUNE MARKERS IN PREDICTING CANCER BEHAVIOR AND TREATMENT RESPONSE

At present, MSI status and immune markers are widely used as the foundation of immunotherapy, especially for CRC due to its molecular characteristics. Not only can this predict the prognosis of patients as well as their response to certain intervention measures, but it can also help identify causal relationships and optimize prevention strategies by examining the relationship between a certain etiology and different molecular subtypes.[131] However, in the process of clinical application we often find that the predictive power

of MSI detection and immune marker detection (such as PD-L1) varies between patients.[132] Therefore, scholars have increasingly suggested that, in addition to the molecular characteristics and immune markers of a tumor, various other factors might also affect tumor evolution and response to treatment. The development, behavior, and response of tumor cells to TME treatment might be affected by the following factors: genetic and epigenetic factors, environmental factors and lifestyle, dietary habits, microbial factors, and the application of some anti-inflammatory drugs.[133] This also explains why some PD-L1 + tumor patients do not respond to ICIs while some PD-L1 – tumor patients respond strongly to ICIs.[134]

5.1 | Genetic and epigenetic factors

Colorectal cancer is a heterogeneous disease with different genetic and epigenetic variations in which genetic factors (such as SNP or family history) have a significant impact on the tumor antigen landscape.[135] Nonsynonymous mutations and insertions as well as deletions in protein coding genes are both the main sources of tumor new antigens (TNA) and important targets of tumor specific CD8 + cytotoxic T lymphocytes (CTLs).[136] Therefore, gene sequence changes caused by genetic factors have been shown to affect the clinical outcomes in CRC patients.

Epigenetic changes are also closely related to the prognosis of patients.[131,137] Previous studies have found that there is a specific tumor phenotype, CpG island methylator phenotype (CIMP), which is characterized by widespread CpG island hypermethylation, and is affected by a variety of factors that lead to the poor prognosis of a tumor.[138] Other studies have found that DNA hypomethylation at the LINE-1 repeat element can also lead to poor prognosis of colon cancer. LINE-1 hypomethylation may provide alternative promoter activation [215] and help regulate the expression of non-coding RNA in many genes.[139] Additionally, one carbon metabolism plays a major role in DNA synthesis and methylation.[140] DNA demethylation activated retrotransposons may transpose throughout the genome, leading to gene destruction and chromosomal instability (CIN). Although these epigenetic changes are usually reversible, they can be passed on to cell progeny and affect the proliferation, invasion, and metastasis of tumor cells as well as their resistance to treatment.[141]

5.2 | Environmental factors and lifestyle

Traditional epidemiological studies have confirmed that the formation of a tumor is a complex and multifactorial process in which environmental factors are also involved. People will form different lifestyle habits due to their local environmental factors (such as weather, terrain, etc.).[131] There are currently some studies that analyze how lifestyle factors (such as physical exercise, smoking, and obesity) are related to the occurrence and prognosis of CRC.[142] In 2008, Ogino s et al. studied the interaction between obesity and

fatty acid synthetase (FASN) and its impact on the prognosis of colon cancer. It was found that obesity had an adverse effect on the prognosis of patients with FASN positive colon cancer, but obesity did not affect the prognosis of patients with FASN negative colon cancer.[143] These data suggest that excessive energy in obese patients may promote the growth and proliferation of tumor cells through FASN activation. It was also found that energy balance has a relationship with many signal transduction pathways impacting tumor invasion such as activation of STMN1, PI3 K, and Wnt.[144,145]

5.3 | Dietary factors

It is known that regular consumption of red processed meat, low dietary fiber intake, excessive alcohol consumption, and vitamin B and D deficiency increase the risk of CRC.[146,147] However, the exact mechanisms by which these items increase this risk remains to be clarified.[148] As for preventive dietary items, the potential for fish oil or omega-3 polyunsaturated fatty acids (PUFA) to prevent cancer has been debated.[149] The results of Song, et al[150] showed that high omega-3 PUFA intake can reduce the risk of CRC in patients with a high Foxp3 + regulatory T cell (Treg) count, but does not reduce the risk of cancer in patients with a low Foxp3 + Treg count. Some studies have also found that certain diets can reduce the risk of CRC by inhibiting *Fusobacterium nucleatum*. The so-called "cautious diet" which is rich in whole grains and fiber is associated with a low risk of CRC. The level of *Clostridium nucleatum* can be detected, but it is not associated with low cancer risk.[151] Trans fatty acids and salt in one's diet can also affect inflammation. Researchers have shown that high levels of salt can make macrophages display a pro-inflammatory phenotype and promote the differentiation of CD4 + T cells into Th-17 cells.[152] Ultimately, drastic dietary changes can lead to detectable changes in the structure of the intestinal microbial community over a relatively short period of time. These changes can serve to regulate and affect metabolism and the immune system response and thus affect the prognosis of the tumor and the efficacy of different anti-tumor treatments.[153]

5.4 | Microbiological factors

Evidence from many preclinical models and population cohorts shows that the diversity and composition of intestinal flora plays an important role in both the pathogenesis of tumors in the gastrointestinal tract as well as in other parts of the body.[154,155] Host microbiota factors may affect patients' responses to different forms of cancer treatment and treatment-related toxicity.[156,157] This points to the potential use of intestinal flora as a biomarker of cancer treatment response.[158]

At present, *Salmonella typhi* and *Helicobacter pylori* in cholangiocarcinoma as well as *H. pylori* in gastric cancer have been identified as carcinogenic enterobacteria.[159,160] *F. nucleatum* has been proven to play a role in the formation and progression of colon serrated

adenoma and colon cancer.[161] It can also be detected in lymph nodes and distant metastasis in patient samples,[162] which is related to many clinicopathological and molecular characteristics. The metabolic components of *F. nucleoatum*, including Fad adhesion complex (FadAc), can activate the Wnt /β-Catenin signaling pathway in human colon cancer cell lines and induce changes in carcinogenic transcription.[163]

Some microorganisms produce metabolites that can promote the growth of tumors, while some compounds or metabolites derived from microorganisms can play the role of tumor inhibitors and immune regulators and thus can be used in the treatment of tumors. A number of studies have reported that there is a strong relationship between the gut microbiota and the response to immune checkpoint blocking therapy.[164-166] Regulating the gut microbiota can enhance the therapeutic response, and it can also regulate the drug toxicity in anti-tumor therapy. For example, the common diarrhea response to Irinotecan (topoisomerase I inhibitor) is mediated by the symbiotic bacterial β – glucuronidase. Selective enzyme inhibition may protect the microbiota from the toxicity induced by Irinotecan.[167] Ultimately, changing the microbial community may be a new direction for targeted therapy to explore in the future.

5.5 | Use of anti-inflammatory drugs

The inflammatory microenvironment of tumor cells can change the tissue homeostasis to build an internal environment suitable for tumor growth and metastasis.[168] It is known that frequent use of aspirin or non-steroidal anti-inflammatory drugs can affect the development of malignant tumors. The effect of these drugs on the anti-tumor immune process of the immune system has shown to be especially important in the prevention of CRC.[169,170] Randomized trials have confirmed that frequent use of aspirin or other PTGS2 inhibitors reduces the risk of colorectal adenoma as well as increases levels of hpgd mRNA expression in adjacent normal colon tissues.[171-173] There is also experimental evidence that the overexpression of PTGS2 (COX-2) is related to the aggressive behavior of tumor. PTGS2 plays an important role in the development of CRC. Regular aspirin use in patients with confirmed CRC can significantly reduce the mortality rate of PTGS2 positive cancer patients.[174] Other research found that regular aspirin use was associated with longer survival in patients with the PIK3CA mutation but not in patients with wild-type PIK3CA. This suggests that the PIK3CA mutation may be a predictive biomarker of aspirin response, which may be related to the interaction between phosphatidylinositol-4,5-diphosphate 3-kinase (PI3 K) and the PTGS2 pathway.[175] Additionally, many experimental results have shown that the incidence rate and survival rate of patients with CRC diagnosed with low expression of TIL and CD274 (PD-L1) were strongly correlated with aspirin use. This correlation may be due to the fact that anti-inflammatory drugs are another means of changing microbial composition and reducing microbial diversity.[176,177] Some studies have found that the use of antibiotics may reduce the anti-tumor effect of immune checkpoint inhibitors.[178,179]

6 | APPLICATION STATUS AND PROSPECT OF MOLECULAR PATHOEPIDEMIOLOGY

Molecular pathological epidemiology (MPE), first proposed by Ogino, is a relatively new research field based on the molecular typing of cancer.[180] It integrates molecular pathology, immune response, and clinical results of cancer, using epidemiological research design methods to analyze the impact of lifestyle habits and changes in an individual's molecular environment have on disease development, prognosis, and outcome. MPE is widely used as a combination of pathology and epidemiology.[181] Not only can it be used to enhance the etiology and heterogeneity of almost all human diseases, but it can also be combined with other multidisciplinary fields.[182] For example, MPE can be integrated into immunology, life cycle epidemiology, microbiology, pharmacology, and social sciences to assess intermediate biomarkers that can predict future disease outbreaks.[183-186]

Currently, genome-wide association studies (GWAS) and immunology are combined with MEP studies to form GWAS-MPE or Immune-MPE, respectively. These multidisciplinary studies evaluate the effects of exogenous and endogenous factors on carcinogenesis.[137,187,188] The development of these comprehensive fields can fill the research gaps between tumor genetics, immunology, and epidemiology, and can also help us clarify the carcinogenic mechanisms of some exposure factors.[186,189] The study of MPE can provide a reasonable explanation for the differences in prognosis and treatment responses among patients. Further research in this field can also further improve the accuracy of evaluating the prognosis and the prediction to the response to treatment, which are the future directions of tumor research with far-reaching significance.

7 | CONCLUSION

The development and gradual maturation of immunotherapy has revolutionized CRC treatment. Microsatellite status has also played an indispensable role in the clinical diagnosis and treatment of patients and is especially useful as a predictor immunotherapy effectiveness. Previous studies have found that ICIs can bring long-term survival benefits to patients with MSI-H tumors, but they are not the only group to benefit. In this paper, the differences between MSI-H and MSS were discussed, including expression of TMB, TILs, PD-L1, and VEGF in the TME of CRC patients; these are important factors affecting patient response to ICI treatment. Although MSI status along with some immune markers detection results are used as the premise of immunotherapy as well as other anti-tumor treatments globally, this method of prediction has its limitations and can be affected by genetic and epigenetic factors, environmental and lifestyle factors, dietary habits, microbiological factors, and drug factors. But despite these limitations, MPE offers a new horizon

of possibilities for further improving the accuracy of prognosis. It is thus necessary to use GWAS-MPE or Immuno-MPE combination methods to develop an in-depth understanding of molecular mechanisms of CRC immunoreactivity and how they relate to cancer treatment. Furthermore, it is imperative to develop effective TME regulatory drugs or better combination therapies, overcome primary and secondary drug resistance in CRC treatment, and achieve truly individualized precision therapies.

CONFLICT OF INTEREST

None of the authors has any financial support or relationships that may pose a conflict of interest.

AUTHOR CONTRIBUTIONS

Chen Hongsheng made substantial contributions to the conception and design of this study. Bai Junge wrote the first draft of the manuscript. All authors made substantial contributions to the acquisition or analysis of data used in this article. Bai Xuefeng revised the manuscript for the purpose of important intellectual content.

INFORMED CONSENT

Informed consent is not required for this study.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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