Cancer Biol Med 2025. doi: 10.20892/j.issn.2095-3941.2025.0030

PERSPECTIVE

Current status of multiple markers in precision immunotherapy for colorectal cancer

Chao Liu[1,2,3]*, Ya Lan[1,3]*, Hong Wang[1,3], Yanqiao Zhang[1,2,3]

[1]Department of Gastrointestinal Medical Oncology, Harbin Medical University Cancer Hospital, Harbin 150000, China; [2]Clinical Research Center for Colorectal Cancer in Heilongjiang, Harbin 150000, China; [3]Key Laboratory of Tumor Immunology in Heilongjiang, Harbin 150000, China

Colorectal cancer (CRC) is the third most common cancer worldwide and the second leading cause of cancer-related mortality. While early-stage CRC patients generally exhibit favorable overall survival (OS) rates, the prognosis for metastatic CRC (mCRC) remains poor, with a survival rate < 15%. Targeted combination therapy remains the main treatment strategy for mCRC, with a median OS (mOS) of only 25–30 months. With the widespread use of immune checkpoint inhibitors (ICIs), significant progress has been made in microsatellite instability-high (MSI-H) mCRC. However, patients with deficient mismatch repair (dMMR)/MSI-H only account for 5% of all CRC patients and 95% of microsatellite stable (MSS) mCRC patients are not recommended to receive direct immunotherapy. Given the heterogeneity of tumors, MSI status alone is insufficient to accurately predict the efficacy of immunotherapy in CRC. Specifically, some MSI-H CRC patients may exhibit primary resistance to treatment, while some MSS CRC patients could potentially benefit from immunotherapy. Therefore, there is an urgent need to identify new immunotherapy biomarkers to enhance the precision of immunotherapy for CRC.

*Chao Liu and Ya Lan contributed equally to this work.
Correspondence to: Yanqiao Zhang
E-mail: yanqiaozhang@ems.hrbmu.edu.cn
ORCID ID: https://orcid.org/0000-0002-6206-8364
Received January 16, 2025; accepted February 20, 2025;
published online March 26, 2025.
Available at www.cancerbiomed.org
©2025 The Authors. Creative Commons Attribution-NonCommercial 4.0 International License

Immune microenvironment characteristics across different pathologic CRC subtypes

CRC predominantly consists of adenocarcinomas, although other more rare histologic subtypes, such as mucinous adenocarcinoma (MC), signet-ring cell carcinoma (SRCC), adenosquamous carcinoma (ASC), and medullary carcinoma (MeC), also exist. These subtypes are distinguished by unique histopathologic features and distinct immune microenvironment profiles. These differences will likely affect the efficacy of immunotherapy but this issue has received little attention in previous studies.

MC

MC accounts for approximately 10% of CRC cases. MC is characterized by abundant mucin production and the presence of mucin pools, which are believed to have a critical role in tumor progression, invasion, survival, and evasion of host immune responses. Notably, some studies have reported that MC exhibits increased lymphocyte infiltration, enhanced programmed death-ligand-1 (*PD-L1*) expression, and a higher frequency of MSI compared to non-MC. These features may be linked to Lynch syndrome and the CpG island methylator phenotype (CIMP), suggesting a more immunogenic tumor microenvironment (TME) in a subset of these cases[1].

SRCC

SRCC is a highly aggressive CRC subtype with a poor prognosis. Studies have shown that hypermethylated SRCC is characterized by a high tumor mutation burden (TMB), MSI-H,

elevated *PD-L1* expression, and increased immune cell infiltration, such as $CD8^+$ T cells, which suggests that this subtype may be sensitive to immunotherapy. However, current clinical observations have shown that SRCC patients exhibit dismal clinical outcomes and derive limited benefit from immunotherapy with the underlying mechanisms requiring further investigation[2].

ASC

ASC is an uncommon CRC subtype that combines glandular and squamous *PD-L1* histologic features. The ASC immune microenvironment exhibits a dual nature with a pronounced immunogenicity counterbalanced by substantial immunosuppressive elements. High expression of immune checkpoint molecules, such as is frequently observed in ASC, contributes to establishing an immunosuppressive microenvironment that limits immune response efficacy. Moreover, most reported cases of ASCs are MSS mCRCs, which may partly explain the poor response to immunotherapy[3].

Serrated adenocarcinoma (SAC)

SAC, a variant of colorectal adenocarcinoma by the World Health Organization (WHO), is considered the ultimate progression of serrated lesions. The SAC immune microenvironment exhibits distinct characteristics. Current research suggests that the SAC TME may impair recruitment or survival of cytotoxic T lymphocytes, potentially leading to a relatively weakened antitumor immune response. Reduced expression of β2-microglobulin (*B2M*) in SAC tumor cells, which results in diminished tumor neoantigen presentation, has been identified as a key mechanism contributing to the formation of an immunosuppressive microenvironment in SACs[4].

MeC

MeC is a rare, poorly differentiated CRC subtype that is often misdiagnosed but with a relatively good prognosis. MeCs have a unique immune microenvironment with extensive lymphocyte infiltration and high interferon-gamma (*IFN-γ*) pathway activation. Analysis of 47 MeC patients showed a high prevalence of dMMR/MSI-H, *ARID1A* mutations and *ASCL2* gene amplification. Genes related to T-cell activation were upregulated, while oncogenic pathways were downregulated. There was abundant immune cell infiltration, especially clonally expanded $CD8^+$ T cells. Both MSI-H and MSS MeC had more immune cell infiltration than MSI-H non-MeC. In a retrospective study involving 47 MeC patients treated with ICIs, 30 had a partial or complete response and 10 had stable disease. The median progression-free survival was 14.33 months and the mOS was 38.33 months, which was far better than non-MeC[5]. There was no significant response difference between MSI-H and MSS MeCs, suggesting that even MSS MeCs (traditionally "cold") benefit from immunotherapy due to the active immune microenvironment. MeC is a "hot" CRC subtype that is highly responsive to immunotherapy independent of MSI status. This feature shows the potential for CRC immunotherapy and the need to determine its immunogenicity and improve the diagnosis and applications.

Immunotherapy biomarkers in CRC

Recent research on biomarkers that predict immunotherapy efficacy in CRC is summarized in **Table 1**. These biomarkers mainly focus on the mutation status of specific genes or the composition and characterization of tumor immune cells [e.g., tumor infiltrating lymphocytes (TILs)[6]] The application of these biomarkers is expected to improve the precision of immunotherapy for CRC.

TMB

The TMB measures the total mutations per megabase in tumor cells with a high TMB linked to increased neoantigen production and enhancing immune recognition. The TMB is a potential biomarker for predicting immunotherapy response across cancers, including mCRC. Recent studies highlight the TMB predictive value. Tremelimumab combined with durvalumab was used as a last-line treatment for patients with refractory CRC in the CTGCO.26 trial. The TMB status was assessed through plasma testing. Patients with TMB > 28 mutations/Mb could benefit from immunotherapy[7]. Further analysis from the KEYNOTE 177 clinical trial revealed that, even for MSI-H CRC, higher TMB levels often indicate higher responsiveness to immune checkpoint blockade (ICB) therapy[8]. Therefore, the TMB is a marker of great clinical value for CRC. The only remaining challenge is to determine a more accurate cut-off value.

Table 1 Current research on biomarkers for precision immunotherapy in CRC

Biomarkers	Microsatellite status	Efficacy indicator	Detection method	Significance
TMB	MSS/MSI-H	OS/PFS	WES	High TMB is associated with better immunotherapy efficacy but is not sufficient for independent prediction[7,8].
POLE/POLD1 mutation	MSS	OS	WES	MSS CRC with *POLE* mutations show elevated TILs, upregulated *PD-L1*, and durable clinical benefits from ICB therapy[9].
Epigenetic gene mutation	MSS	OS/PFS/ ORR	WES	Studies showed that epigenetic mutations (e.g., *ARID1A* and *KMT2C*) had higher ORRs and improved PFS and OS with ICI treatment[10,11].
DDR gene mutation	MSS	NA	WES	DDR mutations in MSS-CRC are associated with enhanced immune activity, including more cytotoxic cell infiltration, alleviative $CD8^+$ T-cell exhaustion, and elevated *IFN-γ* scores[11].
Immunoscore	MSS	OS/PFS	IHC	Higher immunoscores are associated with better prognosis, more active anti-tumor immune microenvironment, and potentially greater sensitivity to immunotherapy[12].
PD-L1	MSI-H	ORR	IHC	The KEYNOTE-016 and Checkmate 142 trials showed no link between tumor cell *PD-L1* expression and immunotherapy response, but high *PD-L1* expression on immune cells improved ORR[9].
B2M; JAK1/2	MSI-H	NA	NGS	*B2M* and *JAK1/2* mutations are more common in MSI-H CRC patients, and CRC patients carrying these mutations can still benefit from anti-*PD-1* therapy[13].
TILs	MSI-H	OS/PFS	IHC	Treatment response and survival benefits of dMMR/MSI CRC patients are significantly associated with high TILs infiltration[6].
$CD8^+$ MeTIL score	MSI-H	NA	MSP	Patients with MSI-H and abundant $CD8^+$ TILs had the best overall survival, despite MSI-H having lower $CD8^+$ MeTIL scores than the MSI-L/MSS groups[15].
GEP	MSS/MSI-H	NA	NanoString	High T cell–inflamed GEP indicates enhanced T cell activity and potentially better immunotherapy efficacy[16,17].

TMB, tumor mutational burden; DDR, DNA damage response; TILs, tumor-Infiltrating lymphocytes; $CD8^+$ MeTIL, DNA methylation-based signature of $CD8^+$ tumor-infiltrating lymphocytes; GEP, gene expression profiles; OS, overall survival; PFS, progression-free survival; ORR, objective response rate; WES, whole-exome sequencing; IHC, immunohistochemistry; NGS, next-generation sequencing; MSP, methylation-specific PCR.

Polymerase epsilon (*POLE*)/polymerase delta 1 (*POLD1*) mutations

DNA *POLE* subunit 1 and *POLD1* mutations are considered significant predictive biomarkers for immunotherapy efficacy in MSS CRCs. DNA *POLE* subunit 1 and *POLD1* mutations are frequently associated with a high TMB. *POLE*-mutated CRCs (approximately 1% of CRCs) exhibit enhanced tumor immunogenicity that is characterized by increased TIL levels, upregulated *PD-L1* expression, and elevated cytotoxic T-cell markers and effector cytokines, which results in durable clinical benefits from ICB therapy[9]. *POLD1* mutations, which have a critical role in cell cycle regulation and DNA damage repair (DDR), are often associated with microsatellite instability, suggesting that patients with *POLD1*-mutated CRCs may also benefit from immunotherapy. Currently, *POLE* and *POLD1* mutations are recommended as markers for predicting benefit from immunotherapy in patients with MSS CRCs[6].

Epigenetic gene mutations

Mutations in epigenetic regulation genes have been increasingly shown to be closely associated with immunotherapy. For example, *ARID1A* mutations define an immunologically active subgroup in MSS CRCs that is characterized by a higher TMB, increased frameshift mutations, and enhanced immune responses, such as T-cell infiltration and *IFN-γ* pathway activation. These traits suggest that MSS CRC patients with *ARID1A* mutations may respond better to immunotherapy[10]. Another study showed that *KMT2C/D* loss-of-function mutations are associated with a higher TMB, enhanced immune responses (e.g., increased *PD-L1* expression and $CD8^+$ T cell

infiltration), and improved efficacy of programmed death-1 (*PD-1*)/*PD-L1* therapy in CRC, suggesting the potential as predictive biomarkers for immunotherapy[11].

DDR mutations

Genes involved in DDR, including *ATM, BRCA2, ERCC2/4, FANCA*, and *CHEK1/2*, and MMR-related genes, such as *MLH1*, *MSH2*, and *MSH6*, are integral to maintaining genomic integrity. Mutations in these genes can promote the generation of neoantigens, thereby increasing tumor immunogenicity and enhancing the TMB. For instance, DDR mutations have been linked to the development of hypermutator phenotypes. Studies suggest that MSS-CRC patients harboring DDR mutations exhibit stronger immune responses and greater sensitivity to ICIs compared to MSS-CRC patients without such mutations. Nevertheless, the pathogenicity and prevalence of specific DDR mutations in MSS-CRCs remain poorly defined and are significantly lower than cancers, such as endometrial carcinoma[11].

Immunoscore

Studies have shown that the immunoscore, by quantifying the density of $CD3^+$ and $CD8^+$ T-cells in tumors and invasive margins, effectively predicts the risk of recurrence and survival rates in CRC patients. A high immunoscore is significantly associated with a lower recurrence risk, higher disease-free survival, and overall survival. The predictive ability of a high immunoscore is independent of TNM staging and MSI status. As a reflection of the antitumor immune microenvironment, the immunoscore can assist in identifying patients suitable for immunotherapy[12]. A study from the 2024 European Society for Medical Oncology (ESMO) conference reported that chemotherapy combined with targeted and immune treatment for patients with proficient mismatch repair (pMMR)/MSS CRC and high immune scores achieved an objective response rate (ORR) of 74% and a disease control rate (DCR) of 100%.

PD-L1 expression

While high *PD-L1* expression is often linked to better responses to ICB therapy in some tumor types, such as non-small cell lung cancer (NSCLC) and gastric cancer, the predictive value in CRC, especially in MSS patients, is not satisfactory. *PD-L1* expression in CRC was shown not to be associated with response or survival in the registration studies[9]. The challenges in using *PD-L1* as a CRC biomarker include sample heterogeneity, risk of false negatives, and the dynamic nature of *PD-L1* expression influenced by the TME. Further research is needed to determine the predictive value of *PD-L1* expression in immunotherapy for CRC, as well as to investigate the potential of combining *PD-L1* with other biomarkers, such as immune scores, TMB, $CD4^+$ T cell frequency, and the $CD4^+/CD8^+$ ratio.

B2M and Janus kinases 1 and 2 (*JAK1/2*) mutations

B2M has a crucial role in tumor antigen presentation and mutations in *JAK1/2* can impair the transmission of signals through the interferon pathway. Typically, mutations in *B2M* and *JAK1/2* are associated with resistance to ICB therapy, as occurs in malignant melanoma. However, an apparently paradoxical finding has emerged in patients with MSI-H CRC. A post-hoc analysis of the KEYNOTE-177 study cohort revealed that patients with *B2M* mutations or loss of expression could achieve sustained therapeutic responses to ICB therapy[8]. Similarly, a study conducted in China reported comparable results. Notably, MSI-H CRC patients with *JAK1/2* mutations exhibit superior responses to anti-*PD-1* therapy compared to MSI-H CRC patients without such mutations[13]. Researchers believe that these discrepant findings of CRC with *B2M* or *JAK1/2* mutations are mainly due to changes in the immune microenvironment and the TMB. In MC38 *B2M*-knockout tumors, the number of type 1 conventional DCs (cDCs1) decreases while type 2 conventional DCs (cDCs2) increases. cDCs2 partially compensate for the loss of cDC1 function by activating $CD4^+$ T cells[14]. Additionally, $CD4^+$ T cells limit the differentiation and proliferation of macrophages into an immunosuppressive M2 state, thereby promoting the anti-tumor immune response. Moreover, MSI-H CRC patients carrying *B2M* or *JAK1/2* mutations have a higher TMB[13], which implies that tumor cells may generate more tumor neoantigens, making the tumor cells more easily recognized and attacked by the immune system. Deeper mechanisms deserve further exploration.

$CD8^+$ MeTIL score

DNA methylation detection is an important method for the molecular diagnosis of tumors, especially in the early diagnosis

of cancer, but it also has great potential in predicting the effect of antitumor treatment. A study analyzed the genome-wide DNA methylation profiles of immune cells and colonic epithelial cells to identify $CD8^+$ T cell-specific differentially methylated positions (DMPs) and developed the $CD8^+$ MeTIL score to assess $CD8^+$ TILs and survival outcomes in CRC. The results showed that the DNA methylation signature for the $CD8^+$ TIL ($CD8^+$ MeTIL) score was significantly lower in the MSI-H group than the microsatellite instability-low (MSI-L)/MSS group and patients with abundant $CD8^+$ TILs (low $CD8^+$ MeTIL score) had the best OS in both MSI-H and MSS cohorts. The $CD8^+$ MeTIL signature has the potential to be a useful biomarker, including immunotherapy[15].

Gene expression profiles (GEPs)

GEP is often used in tumor immunology research to describe the expression characteristics of gene sets associated with T-cell inflammation in the TME, particularly genes related to tumor antigen presentation, cytokine signaling, and adaptive immune responses. A study analyzing the immune GEP of patients with NSCLC, head and neck squamous cell carcinoma (HNSCC), and melanoma (SKCM) following *PD-1* blockade therapy reported that immune GEP was associated with treatment efficacy[16]. An evaluation of patient samples from 22 tumor types across 4 KEYNOTE clinical trials revealed that the T-cell-inflamed GEP can independently predict responses to pembrolizumab and holds promise for guiding precision immunotherapy. For example, it was noted that some MSI-H CRC patients with low GEPs may be associated with ineffective immunotherapy and while most MSS CRCs have a low GEP, a subset of high-GEP patients may still benefit from immunotherapy[17].

Precision immunotherapy in CRC

Precision immunotherapy of CRC hinges on in-depth research and application of immunotherapy biomarkers. ICIs are highly effective in MSI-H CRC patients, yet this subgroup accounts for only approximately 5% of CRC cases, making it crucial to expand immunotherapy applicability. Recent studies have uncovered emerging biomarkers, such as TMB, *POLE/POLD1* mutations, epigenetic changes (e.g., *ARID1A* and *KMT2D*), and DDR gene mutations, therefore presenting new treatment chances for MSS patients. Notably, MSS patients with a high TMB or *POLE/POLD1* mutations respond well to immunotherapy. Moreover, the CRC pathologic heterogeneity and complex immune microenvironment pose challenges. MeC, for example, has unique immune activation features, such as lymphocytic infiltration, elevated *IFN-γ* pathway activity, increased MMR protein deficiency, and a 10% *POLE* mutation rate, making MeC highly responsive to immunotherapy. This immune-active phenotype exhibits strong antitumor immunity and immunotherapeutic efficacy independent of MSI status (**Figure 1**). Beyond focusing on the biomarkers' guiding implications, the correlations with other elements in the TME should be studied. Whether biomarkers cooperate with cytokines, such as tumor necrosis factor-α, during the immune response[18] and interfere with the epithelial-mesenchymal transition[19] to affect tumor recurrence and metastasis should be determined. In addition, whether biomarkers can influence efficacy through associations with the gut microbiota and microbial metabolic profile should also be determined[20].

In addition, advanced detection technologies, including single-cell sequencing, spatial transcriptomics, dynamic liquid biopsy, immune repertoire sequencing, and multi-omics integrated analysis, offer significant advantages. These technologies can effectively address issues related to sample heterogeneity, which enables precise identification of the functional states of key cell subsets. Moreover, the technologies allow for real-time monitoring of marker dynamic changes, which is crucial for early prediction of immunotherapy responses and drug resistance. Ultimately, these capabilities provide a robust scientific basis for personalized treatment and precision immunotherapy. When leveraging these markers to direct precision immunotherapy, it is of utmost importance to comprehensively acknowledge that the therapeutic implications of the same biomarker can exhibit substantial disparities among diverse tumor types. For example, high *PD-L1* expression generally indicates a favorable response to immunotherapy in NSCLC and gastric cancer, while in CRC high *PD-L1* expression needs to be comprehensively interpreted in combination with the MSI status. *B2M* and *JAK1/2* mutations are associated with a better prognosis in CRC and are closely linked to immunotherapy resistance in melanoma. Similarly, colorectal MeC has a relatively good prognosis, but in thyroid cancer MeC represents the subtype with the highest degree of malignancy. Therefore, in clinical research these markers should be gradually incorporated into the process of protocol design and implementation. Through combined and stratified analysis, the populations

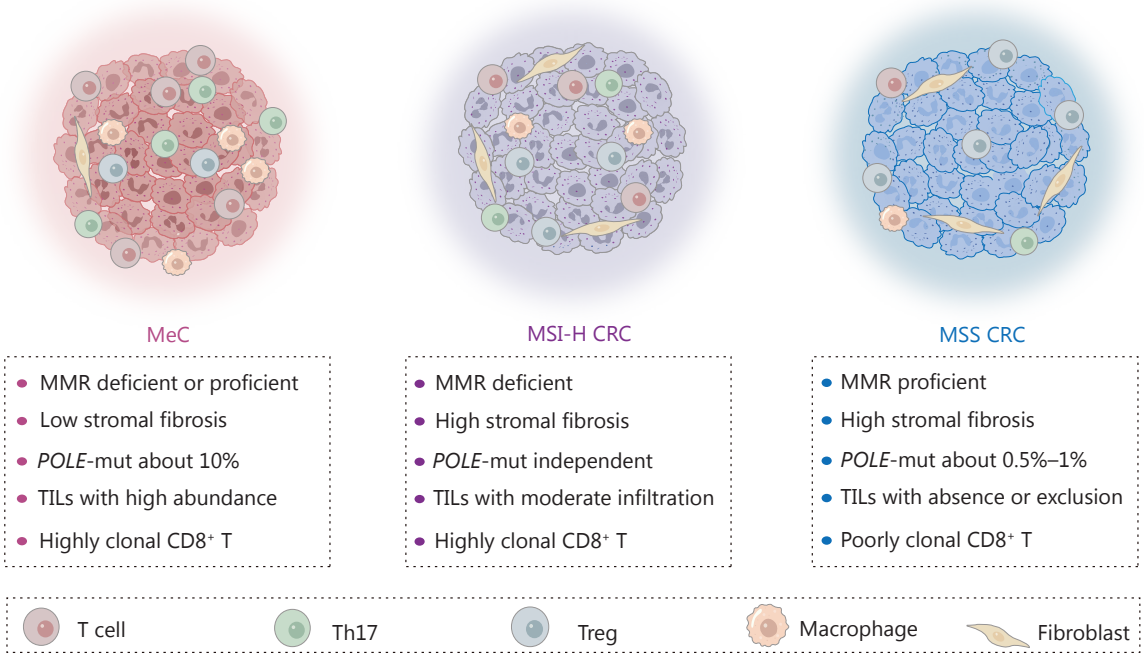


Figure 1 MeC exhibits unique immune activation characteristics independent of MSI status. MeC, medullary carcinoma; MMR, mismatch repair; TILs, tumor-infiltrating lymphocytes; MSI-H, microsatellite instability-high; MSS, microsatellite stable.

with advantages for immunotherapy can be screened out to pursue a higher treatment efficiency. This approach not only contributes to enhancing the clinical accessibility of biomarkers but also further promotes the application and development of precision immunotherapy in the clinical practice of CRC.

Grant support

This study was supported by grants from the National Natural Science Foundation of China (Nos. U22A20330 and 82373372), the Key Project of Research and Development Plan in Heilongjiang Province (Nos. 2022ZX06C01 and JD2023SJ40), the Natural Science Funding of Heilongjiang (No. YQ2022H017), and the Haiyan Foundation of Harbin Medical University Cancer Hospital (No. JJJQ 2024-02).

Conflicts of interest statement

No potential conflicts of interest are disclosed.

Author contributions

Conceived and designed the analysis: Yanqiao Zhang, Chao Liu, Ya Lan, Hong Wang.
Wrote the paper: Chao Liu and Ya Lan.

References

1. Duggan WP, Kisakol B, O'Connell E, Matveeva A, O'Grady T, McDonough E, et al. Multiplexed immunofluorescence imaging reveals an immune-rich tumor microenvironment in mucinous rectal cancer characterized by increased lymphocyte infiltration and enhanced programmed cell death protein 1 expression. Dis Colon Rectum. 2022; 66: 914-22.
2. Alvi MA, Loughrey MB, Dunne P, McQuaid S, Turkington R, Fuchs M-A, et al. Molecular profiling of signet ring cell colorectal cancer provides a strong rationale for genomic targeted and immune checkpoint inhibitor therapies. Br J Cancer. 2017; 117: 203-9.
3. Parente P, Mastracci L, Vanoli A, Businello G, Paudice M, Angerilli V, et al. Colorectal adenosquamous carcinoma: peculiar morphologic features and distinct immunoprofiles in squamous and glandular components. Pathol Res Pract. 2022; 236: 153967.

4. Yılmaz O, Crabbe A, Neyaz A, Pankaj A, Lee SH, Hosseini S, et al. Clinical, pathological genetics and intratumoral immune milieu of serrated adenocarcinoma of the colon. Histopathology. 2022; 81: 380-8.
5. Liu C, Zou H, Ruan Y, Fang L, Wang B, Cui L, et al. Multiomics reveals the immunologic features and the immune checkpoint blockade potential of colorectal medullary carcinoma. Clin Cancer Res. 2025; 31: 773-86.
6. Loupakis F, Depetris I, Biason P, Intini R, Prete AA, Leone F, et al. Prediction of benefit from checkpoint inhibitors in mismatch repair deficient metastatic colorectal cancer: role of tumor infiltrating lymphocytes. Oncologist. 2020; 25: 481-7.
7. Loree JM, Titmuss E, Topham JT, Kennecke HF, Feilotter H, Virk S, et al. Plasma versus tissue tumor mutational burden as biomarkers of durvalumab plus tremelimumab response in patients with metastatic colorectal cancer in the CO.26 Trial. Clin Cancer Res. 2024; 30: 3189-99.
8. Bortolomeazzi M, Keddar MR, Montorsi L, Acha-Sagredo A, Benedetti L, Temelkovski D, et al. Immunogenomics of colorectal cancer response to checkpoint blockade: analysis of the KEYNOTE 177 trial and validation cohorts. Gastroenterology. 2021; 161: 1179-93.
9. Ganesh K, Stadler ZK, Cercek A, Mendelsohn RB, Shia J, Segal NH, et al. Immunotherapy in colorectal cancer: rationale, challenges and potential. Nat Rev Gastroenterol Hepatol. 2019; 16: 361-75.
10. Mehrvarz Sarshekeh A, Alshenaifi J, Roszik J, Manyam GC, Advani SM, Katkhuda R, et al. *ARID1A* mutation may define an immunologically active subgroup in patients with microsatellite stable colorectal cancer. Clin Cancer Res. 2021; 27: 1663-70.
11. Liu R, Niu Y, Liu C, Zhang X, Zhang J, Shi M, et al. Association of *KMT2C/D* loss-of-function variants with response to immune checkpoint blockades in colorectal cancer. Cancer Sci. 2023; 114: 1229-39.
12. Pagès F, Mlecnik B, Marliot F, Bindea G, Ou F-S, Bifulco C, et al. International validation of the consensus Immunoscore for the classification of colon cancer: a prognostic and accuracy study. Lancet. 2018; 391: 2128-39.
13. Zhang C, Li D, Xiao B, Zhou C, Jiang W, Tang J, et al. *B2M* and *JAK1/2*-mutated MSI-H colorectal carcinomas can benefit from anti-*PD-1* therapy. J Immunother. 2022; 45: 187-93.
14. Torrejon DY, Galvez M, Abril-Rodriguez G, Campbell KM, Medina E, Vega-Crespo A, et al. Antitumor immune responses in *B2M*-deficient cancers. Cancer Immunol Res. 2023; 11: 1642-55.
15. Zou Q, Wang X, Ren D, Hu B, Tang G, Zhang Y, et al. DNA methylation-based signature of $CD8^+$ tumor-infiltrating lymphocytes enables evaluation of immune response and prognosis in colorectal cancer. J Immunother Cancer. 2021; 9: e002671.
16. Prat A, Navarro A, Paré L, Reguart N, Galván P, Pascual T, et al. Immune-related gene expression profiling after *PD-1* blockade in non-small cell lung carcinoma, head and neck squamous cell carcinoma, and melanoma. Cancer Res. 2017; 77: 3540-50.
17. Cristescu R, Mogg R, Ayers M, Albright A, Murphy E, Yearley J, et al. Pan-tumor genomic biomarkers for *PD-1* checkpoint blockade-based immunotherapy. Science. 2018; 362: eaar3593.
18. Manohar SM. At the crossroads of *TNF α* signaling and cancer. Curr Mol Pharmacol. 2024; 17: e060923220758.
19. Glaviano A, Lau HS, Carter LM, Lee EHC, Lam HY, Okina E, et al. Harnessing the tumor microenvironment: targeted cancer therapies through modulation of epithelial-mesenchymal transition. J Hematol Oncol. 2025; 18: 6.
20. Lin A, Jiang A, Huang L, Li Y, Zhang C, Zhu L, et al. From chaos to order: optimizing fecal microbiota transplantation for enhanced immune checkpoint inhibitors efficacy. Gut Microbes. 2025; 17: 2452277.

Cite this article as: Liu C, Lan Y, Wang H, Zhang Y. Current status of multiple markers in precision immunotherapy for colorectal cancer. Cancer Biol Med. 2025; 22: 205-211. doi: 10.20892/j.issn.2095-3941.2025.0030