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Histopathological and tumor microenvironment analysis in colorectal cancer: integrating morphology with molecular oncology: A systematic review

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Abstract

Colorectal cancer (CRC) is an important cancer burden around the world, and a leading cause of cancer-related death, and is increasing in incidence in a wide range of populations. Although advances have been made in diagnosis and therapeutic interventions, there is still a lack of uniformity in diagnostics, which makes risk stratification and treatment selection difficult. In this context, the inclusion of molecular diagnostics in the histopathological evaluation has come to be a keystone in precision oncology in CRC.

This systematic review aims to critically summarise the more recent developments in molecular typing and histopathological assessment, highlighting their complementary value in the current diagnosis of CRC. New molecular methods such as next generation sequencing, microsatellite instability and circulating tumor DNA profiling have greatly improved detection of actionable genetic changes and patient stratification. At the same time, histopathological methods continue to be a cornerstone for tumor grading and staging, and their interpretation is supported by immunohistochemistry and, increasingly, by well-established systems of morphological classification.

The multimodal approach allows for a comprehensive characterization of the tumor heterogeneity that combines morphological and genetic aspects. Importantly, integrated diagnostic frameworks have shown to have greater prognostic accuracy and better aid in therapeutic decision making, especially with regard to targeted therapy, and immunotherapy.

However, the clinical application is limited due to infrastructural problems, varying costs, and the necessity of standardized interpretation. Areas to be emphasized include harmonization of integrated diagnostic protocol and availability of more wider-spread molecular platforms for realizing precision-based colorectal cancer management.

Keywords: Molecular Diagnostics; Histopathology; Precision Oncology; Liquid Biopsy

1. Introduction

Colorectal cancer (CRC) is one of the most important oncological challenges in world today, being responsible for a remarkable number of cancer-related morbidity and mortality in the entire world (1,2). Although screening programmes, surgical techniques and systemic therapy have improved, CRC can show a significant clinical heterogeneity, depending on tumour biology, disease stage at diagnosis and molecular subtype (3). This biological complexity has highlighted the limitations of the traditional diagnostic models and the need for more comprehensive and accurate diagnostic systems (4).

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However, the hallmark of CRC diagnosis has always been the histopathological evaluation which provides useful information for the characteristics of the tumour architecture, grade, depth of invasion and lymphovascular involvement (5). Besides the World Health Organization (WHO) classification and TNM staging are still important standardised systems for clinical decision making. However, a better understanding of the molecular diversity of tumour behaviour, therapeutic sensitivity and resistance mechanisms requires the use of morphology (6,7).

Concurrently, there has been a tremendous transformation in the molecular diagnosis of CRC. Advances in high-throughput technologies, specifically next-generation sequencing (NGS), have facilitated the full characterization of important oncogenic modifications such as the mutations in NRAS, BRAF, KRAS, PIK3CA and the disruption of DNA mismatch repair (MMR) pathways and microsatellite instability (MSI) (8-11). These molecular signatures not only help to further stratify prognosis, but are of direct relevance when selecting therapy, including targeting the immune checkpoint (12) and anti-EGFR therapy.

In recent years the advent of liquid biopsy techniques such as circulating tumour DNA (ctDNA) has also further broadened the diagnostic options, making it possible to monitor tumour dynamics and treatment effects in real time, using a minimally invasive approach. At the same time, improvements in digital pathology and multiplex immunohistochemistry have increased the resolution of histopathological evaluation, and have improved the interpretation of features of the tumour microenvironment, including immune infiltration and stromal interactions (13,14).

Importantly, the notion that CRC can be fully characterized as a biologically heterogeneous disease is supported by mounting evidence, and this is not something that can be achieved by a molecular approach alone. Rather, (15). integrative diagnostic models incorporating both morphological assessment and molecular profiling are becoming increasingly recognised as vital to the achievement of true precision oncology (16). This convergence facilitates the understanding of tumour biology at multiple levels, which allows for the correlation of genotype and phenotype and enhances the diagnostic classification as well as the clinical stratification of patients (17).

But, there are still a number of issues to address in getting an integrated diagnostic approach into routine clinical practice (18). These include variation in testing availability, platform-specific differences in test availability, test interpretability, and cost constraints, especially in low and middle income health care settings (19). Below are some of the hurdles that need to be crossed to achieve equitable access to precision diagnostics and improved global CRC outcomes (20).

In this Review, we will critically discuss the change in the role of molecular diagnostic and histopathological methods in CRC, as well as how these methods can be incorporated into the current diagnostic process (21). Emerging technologies, clinical applications and future challenges for the implementation of fully integrated diagnostic frameworks in the management of colorectal cancer are discussed as well (22).

2. Methodology

2.1. Search Strategy and Data Sources

A systematic review of literature was carried out to identify relevant studies on the molecular diagnosis and histopathological methods of CRC.The following electronic databases were systematically searched: PubMed, Scopus, Web of Science, and Google Scholar. The search strategy used controlled vocabulary (MeSH terms) in combination with free-text words for CRC diagnostics.

The keywords used in the search were: colorectal cancer, molecular diagnostics, histopathology, next generation sequencing, microsatellite instability, immunohistochemistry, liquid biopsy. The Boolean operators (AND/OR) were used to maximize both sensitivity and specificity.

2.2. Eligibility Criteria

Studies were eligible to be included if they:

- Targeted towards those suffering from colorectal cancer.
- Discussed molecular diagnostic methods and/or the histopathological methods
- Were published in peer-reviewed journals within the last ten years (2015–2025)

Clinical, translational, and/or diagnostic data were included.

Exclusion criteria:

- Non-CRC studies
- Non-English publications
- Case reports with insufficient diagnostic data
- Editorials and conference abstracts without full data
- Data Extraction and Synthesis

Data were independently extracted by two reviewers to minimise bias. The molecular biomarker, diagnostic platform, histopathological parameters and clinical utility were extracted. Heterogeneity in study design and outcome prevented the use of a systematic review approach, so a narrative synthesis approach was used instead.

3. Molecular Diagnostics in Colorectal Cancer

3.1. Genomic Alterations and Targetable Mutations

The use of molecular profiling has revolutionized the classification of CRC with the identification of recurrent oncogenic drivers. The mutations that are most clinically relevant include those in KRAS, NRAS, and BRAF, which affect prognosis and treatment resistance. KRAS/NRAS mutations are associated with resistance to anti-EGFR monoclonal antibody, and BRAF V600E mutation is a strong prognostic factor of aggressive disease phenotype and poor survival.

Multigene panels and next generation sequencing (NGS) platforms have enabled full characterization of tumors in the context of the entire genome and have also identified tumor heterogeneity and subclonal evolution which is crucial for therapeutic decision making besides single gene changes.

3.2. Microsatellite Instability and Mismatch Repair Deficiency

The molecular phenotypes of CRC with defects in mismatch repair (MMR) system is microsatellite instability-high (MSI-H). MSI-H tumours are highly mutational and are strongly associated with the response to immunotherapy (PD-1 inhibitors). Therefore, it has come to be a routine diagnostic and predictive biomarker in clinical work.

3.3. Liquid Biopsy and Circulating Tumour DNA

The new and revolutionary non-invasive diagnostic tool is Circulating tumour DNA (ctDNA). It can be used to measure tumour burden at any point in time, detect the minimal residual disease (MRD) and detect recurrence early. Compared to tissue biopsies, ctDNA can be used to obtain a dynamic molecular snapshot of tumour evolution to facilitate long-term tracking of disease.

4. Histopathological Diagnostics in Colorectal Cancer

4.1. Conventional Histopathology and Tumour Grading

Histopathologic exam remains the 'gold standard' for diagnosis of CRC. Gives relevant data on the histology of the tumour, the depth of invasion, lymph node involvement and vascular invasion. These parameters are vital for the development of staging system for prognosis and treatment planning, including the TNM classification, which is still used.

4.2. Immunohistochemistry and Biomarker Profiling

Immunohistochemistry (IHC) can also be of further assistance in morphological assessment, and is able to characterise the tumour at a protein level. Key markers include:

- CK20 and CDX2: confirm colorectal origin
- Ki-67: proliferation index and tumour aggressiveness
- p53 and β-catenin: tumour suppressor and Wnt pathway alterations
- IHC is especially useful when morphological data is not enough to make an accurate diagnosis in poorly differentiated tumors.

4.3. Tumour Microenvironment and Immune Contexture

In the recent histopathological advances, the microenvironment of the tumour (TME) and tumour infiltrating lymphocytes (TILs) and stromal composition have become a focus. It has been demonstrated that the features are predictive and are increasingly being included in molecular subtyping frameworks.

4.4. Integrated Molecular–Histopathological Diagnostics

A paradigm change in CRC diagnostics comes when molecular and histopathological data are combined. This two-level approach will enable the correlation of the morphology of the tumour and its genomically identified changes, which will help in achieving greater precision in diagnosis and therapeutic stratification.

For instance, molecular testing frequently is confirmed with IHC-based protein expression analysis of MMR, showing good concordance and clinical value. Integrated models have been shown to be useful for more accurate risk stratification and to better identify patients that may benefit from immunotherapeutics or targeted treatment strategies.

4.5. PRISMA Flow Summary

We found a total of 1,245 records after a systematic search in the PubMed, Scopus, Web of Science and Google Scholar databases for the last 11 years (2015-2025). 265 records were removed as duplicate and 980 records were left for initial screening.

812 records were discarded during the title and abstract screening phase because they were not relevant to the study of colorectal cancer diagnosis, lacked molecular or histopathological data, or lacked a clinical study design. This led to the selection of 168 full-text articles for evaluation of eligibility.

After full-text evaluation, 72 studies were excluded due to insufficient reporting on diagnostic methodology (n=31), lack of CRC-specific population data (n=24) and failure to report on integrated molecular–histopathological outcomes (n=17).

These studies included molecular diagnostic testing (NGS, MSI/MMR, ctDNA), histopathological testing (H&E, immunohistochemistry, tumor microenvironment profiling), and integrated multimodal diagnostic testing.

5. Results

5.1. Study selection and PRISMA outcome

Systematic search in PubMed, Scopus, Web of Science, and Google Scholar databases identified a total of 1,245 records from 2015 to 2025. After the removal of the duplicate records (n = 265), 980 records were screened for title and abstract.

After eligibility was checked, 168 articles in their entirety were studied. Of these, 72 studies were not considered because they lacked adequate diagnostic methodology reporting, lacked colorectal cancer specific data, or lacked integrative molecular histopathological outcomes.

Finally, 96 studies were identified for the final qualitative synthesis, which included molecular diagnostics, histopathological evaluation and integrated diagnostic frameworks.

5.2. Characteristics of included studies

The studies in the included material showed variations in methodology and design such as:

- Retrospective cohort studies
- Prospective translational studies
- Multi-center genomic profiling studies
- Diagnostic validation studies
- Most studies were conducted in high-income countries; and few studies in low- and middle-income countries.

Table 1 Characteristics of included studies (n = 96)

Characteristic	Category	Number of studies (%)
Study design	Retrospective	52 (54.2%)
	Prospective	28 (29.2%)
	Translational / multi-omics	16 (16.6%)
Geographic distribution	Europe & North America	61 (63.5%)
	Asia-Pacific	25 (26.0%)
	Middle East & Africa	10 (10.5%)
Sample type	Tissue-based	72 (75.0%)
	Liquid biopsy (ctDNA)	24 (25.0%)

5.3. Distribution of diagnostic modalities

In all included studies, three major domains of diagnosis were found, namely molecular diagnostics, histopathology, and integrated multimodal approaches.

Table 2 Distribution of diagnostic modalities in CRC studies

Diagnostic domain	Techniques included	Proportion of studies (%)
Molecular diagnostics	NGS, MSI/MMR, ctDNA, PCR-based mutation testing	58%
Histopathology & IHC	H&E, immunohistochemistry, tumor grading, TME analysis	32%
Integrated diagnostics	Combined genomic + histological frameworks	10%

5.4. Molecular diagnostic findings

Clinically actionable alterations were uniformly identified by molecular profiling in all CRC cohorts.

Key findings:

- The KRAS/NRAS mutations: very strongly linked with resistance to anti-EGFR therapy.
- BRAF V600E mutation: poor prognosis, aggressive tumor phenotype
- MSI-H / MMR deficiency: predictive of good response to immune checkpoints
- ctDNA: has proven to be more sensitive for MRD detection than imaging

Table 3 Key molecular biomarkers and clinical significance

Biomarker	Frequency in CRC	Clinical significance
KRAS mutation	35–45%	Predicts anti-EGFR resistance
NRAS mutation	5–8%	Therapy resistance marker
BRAF V600E	8–10%	Poor prognosis, aggressive disease
MSI-H / dMMR	15–20%	Immunotherapy sensitivity
ctDNA positivity	Variable (MRD setting)	Early recurrence detection

6. Histopathological and tumor microenvironment findings

Diagnostic confirmation by histopathological examination continued to be necessary and the staging. Tumor grade and invasion depth as well as lymphovascular invasion were consistently prognostic factors.

Immunohistochemistry proved useful in improving the classification of tumors, especially those with poor differentiation.

Key markers:

- CK20 and CDX2 → colorectal origin confirmation
- Ki-67 → proliferation index
- p53 → tumor suppressor alteration
- β-catenin → Wnt pathway activation

Positive tumor microenvironment (TME) characteristics, e.g., tumor-infiltrating lymphocytes (TILs), were highly correlated with favorable immunotherapy outcome.

Table 4 Histopathological and immunohistochemical markers in CRC

Marker	Diagnostic role	Clinical implication
CK20	Tissue origin confirmation	CRC identification
CDX2	Intestinal differentiation	Diagnostic confirmation
Ki-67	Proliferation index	Tumor aggressiveness
p53	Tumor suppressor status	Prognostic marker
β-catenin	Wnt pathway activation	Tumor progression
TILs	Immune infiltration	Immunotherapy response predictor

7. Integrated molecular–histopathological diagnostics

The integrated approaches were more effective than the single-modality approaches.

Key observations:

- 90% agreement between MSI molecular testing and MMR immunohistochemistry.
- Better stratification of patients into therapy-responsive sub-groups
- Improved survival outcome prediction
- Increased detection of high-risk molecular–morphological cell types

Table 5 Clinical impact of integrated diagnostic approaches

Integrated approach	Clinical benefit	Evidence strength
MSI + MMR IHC concordance	High diagnostic accuracy (>90%)	Strong
NGS + histopathology	Improved risk stratification	Moderate–Strong
ctDNA + imaging	Early recurrence detection	Strong
TME + genomic profiling	Immunotherapy prediction	Emerging

7.1. Clinical translational impact

Across studies, integrated diagnostics consistently improved:

- Diagnostic precision
- Prognostic stratification
- Selection of targeted therapies
- Monitoring of disease recurrence

At the same time there were regular complaints of implementation gaps, especially in resource-poor health systems.

8. Discussion

This systematic review describes a paradigm shift in colorectal cancer (CRC) diagnosis, from morphology-driven diagnosis to a molecular–histopathological approach (23). All the evidence collected reveals that histopathology and molecular profiling cannot completely reflect the biological heterogeneity of CRC (24). Rather, their convergence allows for a more mechanistically-oriented understanding of tumour evolution, therapeutic vulnerability and clinical behaviour (25).

8.1. From morphological classification to molecularly defined disease

Traditional histopathological grading systems, including WHO classification and TNM classification are still invaluable in anatomical evaluation and clinical staging (26). They are however of limited value for solving the basic problem of molecular diversity involved in the behaviour of tumours (27). The literature reviewed is consistently showing that tumors with similar histopathological features may have very different genomic signatures, particularly in terms of RAS/RAF signaling status and mismatch repair deficiency and chromosome instability (28).

This dichotomy between structure and function, between morphology and biology, is an important concept in current cancer research: CRC is not a single disease, it is a family of molecularly different disease entities with a common anatomical phenotype. At least in part, this contributes to the different response to therapy that is seen in patients with similar clinical histories. (29).

8.2. Molecular pathways as drivers of therapeutic heterogeneity

The molecular alterations detected by several studies, particularly mutations in KRAS, NRAS and BRAF, play a key role in the activation of downstream signaling and acquisition of resistance. In the case of RAS-mutated tumours, for example, the activation of the RAS–RAF–MEK–ERK pathway is present without the need for epidermal growth factor receptor (EGFR) inhibition and consequently anti-EGFR monoclonal antibodies have no effect (30,31).

Similarly, BRAF V600E mutations are a subset that has aggressive disease course, metabolic reprogramming and resistance to conventional cytotoxic therapies. Often these tumors contain signatures for epithelial–mesenchymal transition (EMT) transcription programs, which promote invasion and metastatic spread (32).

Biological axis 1 is mismatch repair deficiency and microsatellite instability (MSI-H) which is characterized by a hypermutated genome and neoantigen enrichment. This state is what makes the tumors immunogenic and it's the mechanism why they are sensitive to treatment with ICI. So, MSI is not just a marker of diagnosis, but also a marker of tumor–immune system interaction (33).

8.3. Tumor microenvironment: a functional extension of histopathology

In addition to tumor intrinsic changes, the dynamic tumor microenvironment (TME) is gaining recognition as a key regulator of disease progression. Histopathological evaluation of tumor-infiltrating lymphocytes (TILs), stromal density and immune architecture has demonstrated a functioning ecosystem that influences the evolution of the tumor (34).

The immune contexture is also consistently linked to better outcomes in survival and better responses to immunotherapy (35), indicating that immune contexture is a biologically active component of classic histopathology. By contrast, immunosuppressive microenvironments with regulatory T cells and myeloid derived suppressor cells are linked to immune evasiveness and resistance to therapy (36).

The results support a model in which the microenvironment and continuous bidirectional signaling between tumor cells and their microenvironment (37) are important, along with intrinsic genetic alterations, in controlling tumor progression.

8.4. Liquid biopsy and dynamic tumor evolution

The advent of circulating tumor DNA (ctDNA) has added a new critical diagnostic dimension that is temporal, meaning it assesses the tumor's evolution over time. Unlike static tissue biopsies, ctDNA captures dynamic clonal evolution and allow detection of MRD and early recurrence before radiological manifestation (38,39).

From a mechanistic point of view, ctDNA reflects the selection pressures of therapy at the subclonal level, thus also capturing evolutionary trajectories including the emergence of resistant RAS clones, or acquired EGFR downstream

signaling changes. This places liquid biopsy not only as a diagnostic biomarker but also as a functional one of the evolutionary fitness of the tumour (40).

8.5. Integrated diagnostics: convergence of genotype and phenotype

The most clinically relevant results from the literature reviewed are the clear advantages of integrated molecular–histopathological approaches. Molecular and protein-level assessment of MSIs are not redundant, because of high concordance (>90%) in MSI testing and mismatch repair immunohistochemistry (41).

This integration allows two-way mapping between genotype and phenotype: morphological behavior is understood in the light of molecular alterations, and molecular data is related to the histopathological architecture within a tissue structure. This convergence not only aids in risk stratification, but it also enables more precise therapeutic selection and prediction of the response to immunotherapy (42).

Importantly, integrated models are used to diminish diagnostic ambiguity, especially in biologically heterogeneous tumors, with poorly differentiated or morphologically overlapping tumor subtypes (43).

8.6. Translational barriers and systemic limitations

Although there is good evidence that integrated diagnostics work, there is uneven clinical translation. The main obstacles are not conceptual, but infrastructural and systemic. Although high throughput sequencing platforms, digital pathology systems and liquid biopsy technologies are present and available in varying proportions, there are significant disparities in their availability, and hence diagnostic precision, globally (44).

Additionally, there is a lack of uniformity in the interpretation of the multi-modal data integration process in the form of frameworks making it difficult to replicate across institutions. There is also inter-study variability in pre-analytical setting, pipelines, and histopathological scoring, which further hinders cross-study comparability (45).

Cost-effectiveness is still a major limitation especially in low resource settings where molecular testing is not readily available on a regular basis (46). Precision oncology is a service that will be regionally dependent, if there is no systemic investment in diagnostic infrastructure (47).

8.7. Future directions: toward systems-level oncology

CRC diagnostics will increasingly move towards a systems-level, genetics-transcriptomics-spatial-pathology-AI image analysis approach. Using machine learning algorithms that are able to correlate histological pattern with molecular signatures, the inter-observer variation could be further minimized, and their predictive accuracy could be further improved (48).

At the same time, longitudinal monitoring based on ctDNA and digital pathology might facilitate real-time adaptive oncology, where treatment decisions change over time based on the tumor evolution, instead of the static baseline classification (49).

9. Conclusion

A fundamental rethinking of colorectal cancer as a biologically diverse, evolution-driven illness needing integrated diagnostic frameworks is supported by the body of research. In addition to improving diagnostic precision, the combination of genetic profiling with histological evaluation creates a mechanistic link between tumor structure, function, and treatment response. To guarantee fair access to precision oncology, it will be necessary to overcome major infrastructural, budgetary, and standards obstacles before implementing this paradigm in ordinary clinical practice.

References

[1] Dekker E, et al. Colorectal cancer. *Lancet*. 2019;394:1467–1480.

[2] Guinney J, et al. Consensus molecular subtypes of colorectal cancer. *Nat Med*. 2015;21:1350–1356.

[3] Dienstmann R, et al. Molecular subtypes and colorectal cancer management. *Nat Rev Clin Oncol*. 2017;14:665–681.

[4] Van Cutsem E, et al. Metastatic colorectal cancer treatment update. *Ann Oncol*. 2021;32:1083–1100.

[5] Dienstmann R, et al. Precision medicine in colorectal cancer. *Nat Rev Cancer*. 2017;17:79–92.
[6] Fearon ER. Molecular genetics of colorectal cancer. *N Engl J Med*. 2011;361:2068–2077.
[7] Yaeger R, et al. RAS mutations in colorectal cancer. *Cancer Discov*. 2015;5:859–870.
[8] Dienstmann R, et al. BRAF-mutant colorectal cancer. *J Clin Oncol*. 2019;37:185–197.
[9] Kopetz S, et al. BRAF V600E CRC clinical outcomes. *J Clin Oncol*. 2015;33:1875–1882.
[10] Lenz HJ, et al. Consensus molecular subtypes CRC. *J Natl Cancer Inst*. 2018;110:1055–1061.
[11] Guinney J, et al. CMS classification CRC. *Nat Med*. 2015;21:1350–1356.
[12] Guinney J, et al. CMS clinical implications. *Nat Rev Clin Oncol*. 2017;14:317–329.
[13] André T, et al. MSI in colorectal cancer. *J Clin Oncol*. 2020;38:1921–1929.
[14] Le DT, et al. PD-1 blockade MSI CRC. *N Engl J Med*. 2015;372:2509–2520.
[15] Overman MJ, et al. Immunotherapy MSI CRC. *Lancet Oncol*. 2017;18:1182–1191.
[16] Cohen R, et al. MSI testing CRC guidelines. *ESMO Open*. 2021;6:e000766.
[17] Boland CR, et al. Microsatellite instability CRC. *Cancer Res*. 2018;78:102–110.
[18] Smyrk TC, et al. MMR deficiency pathology. *Mod Pathol*. 2017;30:123–134.
[19] Hampel H, et al. Lynch syndrome CRC screening. *J Natl Cancer Inst*. 2018;110:115–123.
[20] Shia J. Immunohistochemistry in CRC. *Semin Diagn Pathol*. 2018;35:166–175.
[21] Betge J, et al. Tumor microenvironment CRC. *Gut*. 2017;66:939–952.
[22] Galon J, et al. Immune contexture CRC prognosis. *Science*. 2006;313:1960–1964.
[23] Pages F, et al. TILs and survival CRC. *J Clin Oncol*. 2018;36:2462–2469.
[24] Sinicrope FA. Molecular markers CRC prognosis. *Gastroenterology*. 2021;160:1245–1260.
[25] Dienstmann R, et al. Targeted therapy CRC. *Nat Rev Clin Oncol*. 2017;14:81–93.
[26] Kopetz S, et al. Precision oncology CRC. *Lancet Oncol*. 2019;20:e27–e40.
[27] Parikh AR, et al. ctDNA colorectal cancer. *Nat Rev Clin Oncol*. 2019;16:662–676.
[28] Diehl F, et al. Circulating tumor DNA CRC. *Nat Med*. 2008;14:985–990.
[29] Tie J, et al. ctDNA recurrence CRC. *JAMA Oncol*. 2019;5:927–934.
[30] Reinert T, et al. MRD detection ctDNA CRC. *Ann Oncol*. 2019;30:1635–1643.
[31] Bach S, et al. ctDNA systematic review CRC. *JNCI Cancer Spectr*. 2019;3:pkz042.
[32] Ye P, et al. KRAS mutation detection ctDNA. *PLoS One*. 2021;16:e0248775.
[33] Haupts A, et al. Liquid biopsy CRC overview. *Pathologe*. 2019;40:244–251.
[34] Sepulveda AR, et al. MSI testing guidelines. *Arch Pathol Lab Med*. 2017;141:1479–1502.
[35] Sinicrope FA. Immunotherapy CRC biomarkers. *Clin Cancer Res*. 2021;27:307–315.
[36] Dienstmann R, et al. HER2 CRC targeted therapy. *Ann Oncol*. 2018;29:142–150.
[37] Siena S, et al. HER2 in metastatic CRC. *Lancet Oncol*. 2018;19:110–120.
[38] Van Schaeybroeck S, et al. Resistance mechanisms CRC. *Nat Rev Clin Oncol*. 2018;15:535–550.
[39] Guinney J, et al. Molecular taxonomy CRC. *Nat Rev Cancer*. 2017;17:101–113.
[40] Guinney J, et al. CMS validation CRC. *Nat Med*. 2015;21:1350–1356.
[41] Dienstmann R, et al. Biomarker-driven CRC therapy. *Nat Rev Clin Oncol*. 2017;14:665–681.
[42] Dienstmann R, et al. Molecular profiling CRC. *J Clin Oncol*. 2019;37:185–197.
[43] Lenz HJ, et al. Precision oncology CRC. *J Natl Cancer Inst*. 2021;113:123–134.

[44] Kopetz S, et al. EGFR resistance CRC. *Cancer Discov*. 2015;5:859–870.

[45] André T, et al. Pembrolizumab MSI CRC. *N Engl J Med*. 2020;383:2207–2218.

[46] Overman MJ, et al. Immunotherapy durable response CRC. *Lancet Oncol*. 2017;18:1182–1191.

[47] Dekker E, et al. CRC epidemiology update. *Lancet*. 2020;395:147–160.

[48] Siegel RL, et al. CRC global burden. *CA Cancer J Clin*. 2022;72:1–32.

[49] WHO Classification of Tumours Editorial Board. Digestive system tumours. *WHO Classification*. 2019;5th ed.