

Japanese Society of Medical Oncology Clinical Guidelines: Molecular Testing for Colorectal Cancer Treatment, 4th edition

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JSMO

Abstract

Molecular testing to select the appropriate targeted and standard of care therapies is essential for managing patients with colorectal cancer (CRC). The Japanese Society of Medical Oncology previously published clinical guidelines for molecular testing in CRC. In the third edition published in 2018, *RAS* and *BRAF* V600E mutations should be tested prior to first-line chemotherapy to assess the benefit of anti-epidermal growth factor receptor (EGFR) antibody therapy in patients with unresectable CRC. Microsatellite instability (MSI) testing was recommended in patients with curatively resected stage II CRC because deficient mismatch repair is associated with low risk of recurrence. MSI testing was also recommended in patients with CRC suspected to be Lynch syndrome. The main aim of this fourth edition is to reflect recent advances in comprehensive genomic profiling (CGP) tests and liquid biopsy. Here, CGP tests performed on tumor tissues are strongly recommended to assess the benefit of molecular targeted drugs in patients with CRC. Circulating tumor DNA (ctDNA)-based CGP tests are also proposed. ctDNA testing is recommended to determine the optimal treatment based on the risk of recurrence for curatively resected CRC and evaluate the suitability and monitor the therapeutic effects of anti-EGFR antibodies in

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patients with unresectable CRC. While both MSI testing and immunohistochemistry are strongly recommended to determine the indication of immune checkpoint inhibitors in patients with unresectable CRC, next-generation sequencing-based tests are weakly recommended because these tests have not been validated in clinical trials.

KEYWORDS

circulating tumor DNA, colorectal cancer, comprehensive genomic profiling, guideline, microsatellite instability

1 | INTRODUCTION

Colorectal cancer (CRC) is the most common cancer in Japan. Improved molecular understanding and development of molecular targeted therapies have led to significant improvements in care for CRC patients. The Japanese Society of Medical Oncology (JSMO) is dedicated to providing guidance regarding the proper use of genomic testing for the management of CRC through the publication and dissemination of clinical practice guidelines. The first JSMO guidelines, the "Japanese Guidelines for Testing of the *KRAS* Gene Mutation in Colorectal Cancer, First Edition," was published in 2008, and then updated in 2014, with the publication of the "Japanese Society of Medical Oncology Clinical Guidelines: *RAS* (*KRAS/NRAS*) Mutation Testing in Colorectal Cancer Patients, Second Edition."¹ Both contributed to the proper use of *KRAS* and *RAS* mutation testing in clinical practice. The third edition, published in 2018, recommended *BRAF* V600E mutation testing prior to the initiation of first-line therapy for patients with unresectable advanced or recurrent CRC, and MMR deficiency testing for cases suspected of having Lynch syndrome.²

Since then, not only have tests for the *BRAF* V600E mutation and MMR deficiency received insurance coverage but there has also been approval of comprehensive genomic profiling (CGP) testing based on the rapid implementation of precision medicine. Furthermore, somatic gene testing by analyzing circulating tumor DNA (ctDNA) and ctDNA-based profiling tests has also progressed rapidly. Therefore, the JSMO established a working group in November 2018 to revise the guidelines and published the revised

Japanese version of guidelines (fourth edition) in October 2019 following the peer-review process with an external review committee and public comments from JSMO members.³ The updated guidelines set 10 basic requirements for molecular testing for CRC treatment. The degree of recommendation for each requirement was determined through votes by the working group members based on the evidence for each test and the expected balance between advantages and disadvantages for patients when the testing was performed (Table 1).

2 | BASIC REQUIREMENTS OF MOLECULAR TESTING FOR COLORECTAL CANCER TREATMENT (Table 2 and Figure 1)

2.1 | *RAS* mutation testing

RAS mutation testing is recommended prior to first-line chemotherapy to assess the benefit of anti-EGFR antibody therapy in patients with unresectable CRC.

[Strong recommendation]

Anti-EGFR antibody therapy is not effective in patients with *KRAS* exons 2, 3 and 4 and *NRAS* exons 2, 3 and 4 mutations, regardless of the type of anti-EGFR antibody (cetuximab or panitumumab), treatment lines, use of combined chemotherapy or type

TABLE 1 Degrees of recommendation and decision criteria

Degree of recommendation	Decision criteria
Strong recommendation	Sufficient evidence and the benefits of testing outweigh the losses
Recommendation	Evidence considering the balance between benefits and losses
Expert consensus opinion	Consensus obtained although not enough evidence and information
No recommendation	Not recommended owing to the lack of evidence

Note: Sufficient evidence, consistent evidence from randomized control trials (RCT) without important limitations or exceptionally strong evidence from observational studies; evidence, evidence from RCT with important limitations or strong evidence from observational studies; consensus, evidence for at least 1 critical outcome from observational studies, case series, or RCT with serious flaws or indirect evidence.

TABLE 2 Summary of basic requirements

	Recommendation
RAS mutation testing	
RAS mutation testing is recommended prior to first-line chemotherapy to assess the benefit of anti-EGFR antibody therapy in patients with unresectable CRC.	Strong recommendation
RAS mutation testing can determine the optimal perioperative chemotherapy based on the presumed recurrence risk in patients with resectable CRC.	Expert Consensus Opinion
BRAF mutation testing	
BRAF V600E mutation testing is recommended prior to first-line chemotherapy to determine the optimal treatment based on the prognosis of patients with unresectable CRC.	Strong recommendation
BRAF V600E mutation testing is recommended to determine the optimal perioperative chemotherapy based on the presumed recurrence risk in patients with resectable CRC.	Recommendation
BRAF V600E mutation testing is recommended to help diagnose Lynch syndrome.	Recommendation
Testing for mismatch repair deficiency	
MMR deficiency testing is recommended to evaluate the benefit of immune checkpoint inhibitors in patients with unresectable CRC.	Strong recommendation
MMR deficiency testing is recommended to assess the risk of recurrence and to stratify optimal perioperative chemotherapy in patients with resectable CRC.	Recommendation
MMR deficiency testing is recommended to screen for Lynch syndrome.	Strong recommendation
The following methods are recommended when assessing for MMR deficiency:	
MSI testing	Strong recommendation
IHC testing	Strong recommendation
NGS-based testing	Recommendation
Next-generation sequencing-based comprehensive genomic profiling tests	
Comprehensive genomic profiling tests are recommended to assess the benefits of molecular targeted drugs in patients with unresectable CRC.	Strong recommendation
Liquid biopsy	
ctDNA testing is recommended to determine the optimal perioperative chemotherapy based on the presumed recurrence risk of patients with resectable CRC.	Recommendation
ctDNA testing is recommended to evaluate the suitability of and to monitor the therapeutic effects of anti-EGFR antibody therapy in patients with unresectable CRC.	Recommendation
ctDNA-based comprehensive genomic profiling tests are recommended to assess the benefits of molecular targeted drugs for patients with unresectable CRC.	Recommendation
Angiogenic factors	
Measurement of VEGF-D level is performed to identify the appropriate angiogenesis inhibitors for patients with unresectable CRC.	Expert Consensus Opinion
Samples for molecular testing	
FFPE tissue is suitable for genomic testing of somatic mutations. It is recommended to confirm that the samples have an adequate amount of tumor cells and expect sufficient quality of nucleic acids by assessing the matched reference hematoxylin and eosin stained slides. Selection of FFPE samples, decision on the need for macrodissection, and assessment of tumor cell content should be performed by a pathologist.	Strong recommendation
In ctDNA testing, use of collection tubes and the preservation and adjustment of plasma after blood collection should be performed in accordance with the manufacturer's instructions.	Strong recommendation
Quality assurance requirements for testing	
Genomic testing for CRC treatment should be carried out under a quality assurance system.	Strong recommendation

Abbreviations: CRC, colorectal cancer; ctDNA, circulating tumor DNA; EGFR, epidermal growth factor receptor; FFPE, formalin-fixed paraffin-embedded; IHC, immunohistochemistry; MMR, mismatch repair; NGS, next-generation sequencing; VEGF, vascular endothelial growth factor.

of chemotherapy. While the addition of anti-EGFR antibody significantly improved the overall survival (OS) and progression-free survival (PFS) in patients with RAS wild-type left-sided colon cancer, anti-EGFR antibody therapy was not beneficial in those with right-sided colon cancer. The “Japanese Society for Cancer of the

Colon and Rectum Guidelines 2019 for the Treatment of Colorectal Cancer” and the “Pan-Asian Adapted ESMO Consensus Guidelines for the Management of Patients with Metastatic Colorectal Cancer” stratified the treatment strategies based on primary tumor location and RAS mutation status.⁴

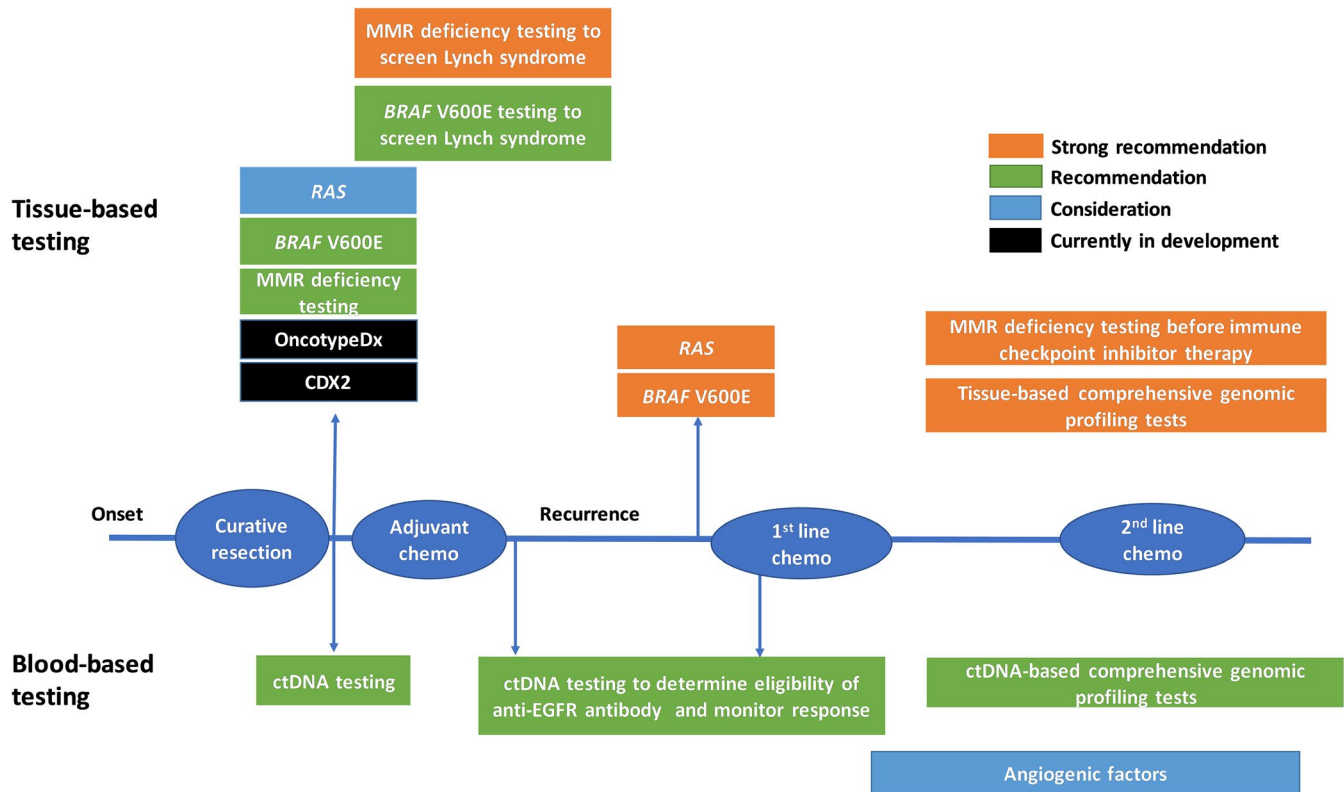


FIGURE 1 Timing for each genomic test

RAS mutation testing can determine the optimal perioperative chemotherapy based on the presumed recurrence risk in patients with resectable CRC.

[Expert Consensus Opinion]

The benefit of cetuximab has not been shown in resectable CRC. In two phase III trials comparing FOLFOX with or without cetuximab as adjuvant therapy for curatively resected stage III colon cancer, the addition of cetuximab did not improve the recurrence-free survival (RFS) or OS, even in patients with wild-type *KRAS* exon 2.^{5,6} In contrast, patients with *KRAS* mutations have significantly worse outcomes. Among patients with resected metastatic lesions such as liver metastases, patients with *RAS* mutations had shorter RFS and OS than those with *RAS* wild-type.⁷ Association was also reported between *KRAS* mutation and lung metastasis after curative resection in patients with stage II/III colon cancer.⁸

2.2 | BRAF mutation testing

BRAF V600E mutation testing is recommended prior to first-line chemotherapy to determine the optimal treatment based on the prognosis of patients with unresectable CRC.

[Strong recommendation]

The *BRAF* V600E mutation is a poor prognostic factor of advanced CRC. Recent studies demonstrated favorable therapeutic effects of FOLFOXIRI with bevacizumab as the first-line treatment for patients with advanced CRC harboring the *BRAF* V600E mutation.⁹ In addition, the BEACON CRC phase III trial showed that the combination of BRAF inhibitor with MEK inhibitor and anti-EGFR antibody significantly improved OS and objective response compared with standard care in patients with *BRAF* V600E mutant advanced CRC.¹⁰ Therefore, the *BRAF* V600E mutation status is important in determining the optimal first-line regimen in patients with metastatic CRC (mCRC).

BRAF V600E mutation testing is recommended to determine the optimal perioperative chemotherapy based on the presumed recurrence risk in patients with resectable CRC.

[Recommendation]

The presence of the *BRAF* V600E mutation is associated with poor prognosis, especially in patients with microsatellite stable (MSS) resectable CRC. In a meta-analysis of phase III trials of adjuvant chemotherapy in patients with stage II/III CRC, the presence of the *BRAF* V600E mutation was a risk factor for recurrence.¹¹ Moreover, the influence of the *BRAF* V600E mutation was examined between patients with microsatellite instability-high (MSI-H) and those with MSS tumors. MSS/*BRAF* mutant was a poor prognostic factor, while MSS/*BRAF* wild-type and MSI-H/*BRAF* wild-type were

comparable and favorable, respectively, and MSI-H/*BRAF* mutant was moderate.¹²

BRAF V600E mutation testing is recommended to help diagnose Lynch syndrome.

[Recommendation]

BRAF V600E mutations are dominantly observed in patients with sporadic deficient mismatch repair (dMMR) CRC. Lynch syndrome harbors germline mutations in MMR genes, while most of the sporadic dMMR CRC are caused by promoter methylation, such as of the *MLH1* gene. Among dMMR tumors, Lynch syndrome can be excluded with high probability if the *BRAF* V600E mutation is present, especially concomitant loss of *MLH1* expression.

2.3 | Testing for mismatch repair deficiency

Mismatch repair deficiency testing is recommended to evaluate the benefit of immune checkpoint inhibitors in patients with unresectable CRC.

[Strong recommendation]

Pembrolizumab was approved in Japan in December 2018 for the treatment of MSI-H advanced solid tumors, including CRC, with an MSI testing kit (FALCO) as its companion diagnostic tool. For CRC, pembrolizumab is approved as a second-line or subsequent treatment. Nivolumab also demonstrated favorable therapeutic effects in pre-treated patients with MSI-H recurrent or metastatic CRC.¹³ However, patients with MSI-H CRC have poor prognoses, regardless of the presence of the *BRAF* V600E mutation. To avoid missing opportunities to use immune checkpoint inhibitors, it is recommended that MMR deficiency testing be assessed at an early stage of mCRC treatment. Notably, MMR deficiency and *RAS/BRAF* mutations are not mutually exclusive.

Mismatch repair deficiency testing is recommended to assess the risk of recurrence and to stratify optimal perioperative chemotherapy in patients with resectable CRC.

[Recommendation]

Survival of dMMR was better than that of MSS among patients with curatively resected stage II and III CRC. However, fluoropyrimidine-based adjuvant chemotherapy could increase the risk of recurrence for dMMR stage II colon cancer.¹⁴ *BRAF* V600E mutations are more frequently observed in patients with dMMR than in those with mismatch repair-proficient (pMMR) cancer. When *BRAF* V600E or *KRAS* exon 2 mutations are present with pMMR, the recurrence risk is significantly higher with poor prognosis in patients with stage III colon cancer.¹⁵ Therefore, MMR deficiency testing is recommended to determine the regimen and duration of adjuvant chemotherapy

stratified by the risk of recurrence in patients with resected colon cancer.

Mismatch repair deficiency testing is recommended to screen for Lynch syndrome.

[Strong recommendation]

Lynch syndrome is an autosomal dominant inherited disorder caused by germline mutations in one of the MMR genes: *MLH1*, *MSH2*, *MSH6* and *PMS2*. Lynch syndrome is a rare disease occurring in 2%–4% of Caucasian and 0.7% of Japanese patients with CRC. However, patients and their families have an increased risk of many types of malignancies. Therefore, MMR deficiency testing is strongly recommended in patients with CRC with suspected Lynch syndrome. Notably, MMR deficiency is also observed in a subset of sporadic CRC, such as tumors with hypermethylation of the *MLH1* promoter.

The following methods are recommended when assessing for MMR deficiency:

MSI testing	(Strong recommendation)
Immunohistochemistry (IHC) testing	(Strong recommendation)
Next-generation sequencing (NGS)-based testing	(Recommendation)

The typical methods for evaluating MMR deficiency include MSI testing, assessing the MSI by the shift of microsatellite markers, IHC testing, assessing the expression of MMR proteins (*MLH1*, *MSH2*, *MSH6* and *PMS2*) in cancer tissues, and NGS-based evaluation of mismatch repair function.

Although dinucleotide markers, including the Bethesda panel, are valid for determining MSI-low, it sometimes fails to detect MSI-H in Lynch syndrome, caused by germline mutations in *MSH6* or *PMS2*. In contrast, mononucleotide markers are more sensitive and specific than dinucleotide markers for the detection of MSI and are also less influenced by polymorphisms. Moreover, a mononucleotide marker panel identified patients with *MSH6* deficiency at a relatively high rate (62.5%).¹⁶ The MSI test kit (FALCO) determines the MSI status based on five mononucleotide markers.

In IHC testing, tumors without MMR deficiency express all four proteins, while proteins that correspond to the inactivated MMR genes are not expressed in patients with dMMR tumors. The results of testing showed high concordance between IHC testing and MSI testing. Currently, IHC is used in screening Lynch syndrome; however, it is expected to become a companion diagnostic assay for immune checkpoint inhibitors (ICI). In a pooled analysis of the five KEYNOTE studies and the CheckMate 142 trial, the efficacy of anti-PD-1 antibody was demonstrated in patients with dMMR determined by IHC.¹⁷

FoundationOne CDx detects MSI status by evaluating 95 in-tronic microsatellite markers, showing a more than 95% concordance rate with MSI testing and IHC. There are other algorithms to analyze MSI status, such as the MSIsensor algorithm in MSK-IMPACT and the MOSAIC and MANTIS algorithms with whole exome sequencing. Each method uses different microsatellite

markers and algorithms. Although these NGS-based tests are found to be useful, the tests were not validated in clinical trials of ICIs.

2.4 | Next-generation sequencing-based comprehensive genomic profiling tests

Comprehensive genomic profiling (CGP) tests are recommended to assess the benefits of molecular targeted drugs in patients with unresectable CRC.

[Strong recommendation]

Patients with driver mutations receiving a matched targeted agent showed better PFS and OS than those whose tumors did not harbor druggable driver mutations.¹⁸ The CGP test can identify rare driver abnormalities in CRC, such as *NTRK* fusion, *HER2* amplification, *BRAF* non-V600E mutations and *ALK* translocations. Currently, CGP tests are approved for use in CRC patients with disease progression after receiving standard chemotherapy. Although off-label drug use based on CGP results is not permitted in the Japanese healthcare system, patients harboring these mutations may be eligible for clinical trials.

2.5 | Liquid biopsy

Circulating tumor DNA (ctDNA) testing is recommended to determine the optimal perioperative chemotherapy based on the presumed recurrence risk of patients with resectable CRC.

[Recommendation]

Circulating tumor DNA (genetically mutated allele) has an extremely short half-life in plasma (ie, within 2 hours). NGS-based ctDNA analysis is being developed to assess minimal residual disease (MRD) and to monitor recurrence in cancer. Postoperative ctDNA analysis was useful for the stratification of recurrence risk independent of conventional clinicopathological risk factors.¹⁹ Moreover, longitudinal ctDNA analysis after surgery could identify CRC recurrence earlier than radiologic imaging. Therefore, ctDNA testing for the detection of MRD is recommended to identify patients at high risk of recurrence and to optimize the treatment strategies for patients with resectable CRC.

Circulating tumor DNA testing is recommended to evaluate the suitability of and to monitor the therapeutic effects of anti-EGFR antibody therapy in patients with unresectable CRC.

[Recommendation]

The fraction of *KRAS*-mutated alleles quantified by digital PCR was inversely correlated with response to anti-EGFR therapy. Furthermore, the rechallenge treatment of cetuximab and

irinotecan after resistance to first-line irinotecan and cetuximab-based therapy was effective only in patients with *RAS* and *BRAF* wild-type mCRC confirmed by ctDNA analysis.²⁰ The OncoBEAM *RAS* CRC kit, which uses BEAMing to detect *RAS* mutations in ctDNA, showed an 86% concordance rate with *RAS* testing by tissue analysis, and the kit was approved for use in July 2019.

Circulating tumor DNA-based comprehensive genomic profiling tests are recommended to assess the benefits of molecular targeted drugs for patients with unresectable CRC.

[Recommendation]

Circulating tumor DNA-based CGP analysis detects multiple genomic abnormalities that are associated with resistance to anti-EGFR therapy, including *EGFR*, *KRAS*, *NRAS* and *BRAF* mutations, and *HER2* and *MET* amplifications. In addition, ctDNA-negative patients showed a better response to rechallenge treatment with anti-EGFR antibody therapy.²¹ ctDNA-based CGP analyses were used to screen eligible patients and/or assess the efficacy of molecular targeted agents in clinical trials. For example, the reduction of allele frequency after treatment was correlated with the degree of response in a phase 1b trial of vemurafenib with irinotecan and cetuximab in patients with *BRAF* V600E mutant mCRC.²²

2.6 | Angiogenic factors

Measurement of vascular endothelial growth factor (VEGF)-D levels is performed to identify the appropriate angiogenesis inhibitors for patients with unresectable CRC.

[Expert Consensus Opinion]

The phase III RAISE trial demonstrated that ramucirumab, a VEGF receptor 2 binding monoclonal antibody, plus 5-fluorouracil, leucovorin and irinotecan (FOLFIRI) significantly improved OS and PFS compared with placebo plus FOLFIRI as second-line treatment.²³ Biomarker analysis showed that VEGF-D is a potential predictive biomarker for ramucirumab efficacy.

2.7 | Samples for molecular testing

Formalin-fixed paraffin-embedded (FFPE) tissue is suitable for genomic testing of somatic mutations. It is recommended to confirm that the samples have an adequate amount of tumor cells and expect sufficient quality of nucleic acids by assessing the matched reference hematoxylin and eosin stained slides. Selection of FFPE samples, decision on the need for

macrodissection, and assessment of tumor cell content should be performed by a pathologist.

[Strong recommendation]

In ctDNA testing, use of collection tubes and the preservation and adjustment of plasma after blood collection should be performed in accordance with the manufacturer's instructions.

[Strong recommendation]

2.8 | Quality assurance requirements for testing

Genomic testing for CRC treatment should be carried out under a quality assurance system.

[Strong recommendation]

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CONFLICT OF INTEREST

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randomised, double-blind, multicentre, phase 3 study. *Lancet Oncol.* 2015;16:499-508.

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