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REPORT

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Japanese Society of Medical Oncology Clinical Guidelines: Molecular Testing for Colorectal Cancer Treatment, Third Edition

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The Japanese Society of Medical Oncology (JSMO) previously published 2 editions of the clinical guidelines: "Japanese guidelines for testing of *KRAS* gene mutation in colorectal cancer" in 2008 and "Japanese Society of Medical Oncology Clinical Guidelines: *RAS* (*KRAS*/*NRAS*) mutation testing in colorectal cancer patients" in 2014. These guidelines have contributed to the proper use of *KRAS* and *RAS* mutation testing, respectively. Recently, clinical utility, particularly for colorectal cancer (CRC) patients with *BRAF* V600E mutation or DNA mismatch-repair (MMR) deficiency, has been established. Therefore, the guideline members decided these genetic alterations should also be involved. The aim of this revision is to properly carry out testing for *BRAF* V600E mutation and MMR deficiency in addition to *RAS* mutation. The revised guidelines include the basic requirements for testing for these genetic alterations based on recent scientific evidence. Furthermore, because clinical utility of comprehensive genetic testing using next-generation sequencing and somatic gene testing of analyzing circulating tumor DNA has increasingly evolved with recent advancements in testing technology, we noted the current situation and

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prospects for these testing technologies and their clinical implementation in the revised guidelines.

KEYWORDS
BRAF, colorectal cancer, DNA mismatch repair, guideline, *RAS*

1 | INTRODUCTION

With the recent advances in molecular biological research, genetic abnormalities that affect the clinical outcomes of colorectal cancer (CRC) are being understood. Western studies showed that *KRAS* exon 2 mutation and, subsequently, *KRAS/NRAS* mutation (in exons 2, 3 and 4) were established as negative predictive biomarkers for efficacy of anti-epidermal growth factor receptor (EGFR) antibody therapy. The Japanese Society of Medical Oncology (JSMO) published "Japanese guidelines for testing of *KRAS* gene mutation in colorectal cancer, first edition" in November 2008 and "JSMO Clinical Guidelines: *RAS* (*KRAS*/*NRAS*) mutation testing in colorectal cancer patients, second edition" in April 2014 in order to determine basic requirements for the proper use of *KRAS* and *RAS* testing in Japan.[1] Since the release of the latest edition, *BRAF* V600E mutation and DNA mismatch-repair (MMR) deficiency, in addition to *RAS* mutation, became established as important genetic alterations that affect prediction of a prognosis and selection of the optimal treatment. Recent advances in testing technology have also led to rapid developments of comprehensive gene testing using next-generation sequencing (NGS) and somatic gene testing of analyzing circulating tumor DNA (ctDNA), so-called liquid biopsy.

JSMO established a working group to revise the guidelines in February 2016, and published the revised Japanese version of guidelines (third edition) in November 2016 through the peer-review process by an external review committee and by public comments from JSMO members. Objectives of the revised guidelines are to provide basic requirements to physicians and laboratory professionals for the proper use of testing for *BRAF* V600E mutation and MMR deficiency in addition to *RAS* mutation (Tables 1-3). We also provided information regarding the current status and future prospects for the emerging new testing technologies. Degrees of recommendation for each requirement were determined through votes by the working group members based on the evidence for each test and the expected balance between benefits and disadvantages for patients when the testing is carried out (Table 1). At almost the same timing as the publication of these revised guidelines, MMR deficiency was recognized as a biomarker for anti-programmed death 1 (PD-1) antibody therapy. Here, we summarize the new clinical guidelines. The English-translated full text is available in supplementary materials (Data S1).

TABLE 1 Degrees of recommendation and decision criteria

Degree of recommendation	Decision criteria
Strong recommendation	There is sufficient evidence and the benefits of testing outweigh the losses for patients
Recommendation	There is certain evidence, considering the balance between benefits and losses for patients
Expert consensus opinion	A certain consensus has been obtained although evidence and information that shows patient benefits cannot be said to be sufficient
No recommendation	There is no evidence

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

2 | BASIC REQUIREMENTS OF MOLECULAR TESTING FOR CRC TREATMENT

2.1 | *RAS (KRAS/NRAS)* mutation testing is recommended prior to the initiation of anti-EGFR antibody therapy for patients with unresectable advanced or recurrent CRC. [Strong recommendation]

Additional analyses in several randomized clinical trials showed that anti-EGFR antibody therapy is unlikely to benefit patients with *KRAS* exons 3 and 4, and *NRAS* exons 2, 3, and 4 mutations, in addition to those with *KRAS* exon 2 mutation. Reproducibility of this tendency was observed regardless of the type of anti-EGFR antibody (cetuximab or panitumumab), treatment lines, use of combined chemotherapy, or types of chemotherapy, which was confirmed in a large-scale meta-analysis. Therefore, testing for *RAS* mutation is strongly recommended prior to anti-EGFR antibody therapy for patients with unresectable advanced or recurrent CRC. In addition, in "Japanese Society for Cancer of the Colon and Rectum (JSCCR) Guidelines 2016 for the Treatment of Colorectal Cancer", cetuximab and panitumumab are indicated for *RAS* wild-type, unresectable advanced or recurrent CRC.[2]

TABLE 2 Basic requirements

Basic requirements	Recommendation
(1) *RAS (KRAS/NRAS)* mutation testing is recommended prior to the initiation of anti-EGFR antibody therapy for patients with unresectable advanced or recurrent CRC	Strong recommendation
(2) Methods with confirmed analytical validity such as in vitro diagnostics are recommended as *RAS* mutation testing using tumor tissue samples	Strong recommendation
(3) *BRAF* V600E mutation testing is recommended prior to the initiation of first-line therapy for patients with unresectable advanced or recurrent CRC	Recommendation
(4) Direct sequencing method (combined with manual microdissection) or a PCR-based method is recommended as *BRAF V600E* mutation testing using tumor tissue samples	Recommendation
(5) MMR-deficiency testing using tumor tissue samples is recommended for patients with CRC suspected to have Lynch syndrome	Strong recommendation
(6) Microsatellite instability testing and immunohistochemistry for MMR proteins are recommended for tumor MMR-deficiency testing	Strong recommendation
(7) MMR-deficiency testing using tumor tissue samples is recommended for patients with Stage II colon cancer who underwent curative resection	Recommendation
(8) MMR-deficiency testing using tumor tissue samples is considered prior to the initiation of first-line therapy for patients with unresectable advanced or recurrent CRC	Expert consensus opinion
(9) FFPE tissue blocks are recommended for use in somatic gene testing. It is recommended to confirm that the unstained, thin-sliced section contains sufficient tumor cells in which the quality of nucleic acids is expected to be maintained histologically by a pathologist, using paired H&E staining	Strong recommendation
(10) Genetic testing for CRC treatment should be carried out under a quality-assured system	Strong recommendation

anti-EGFR, anti-epidermal growth factor receptor; CRC, colorectal cancer; FFPE, formalin-fixed, paraffin-embedded; MMR, mismatch repair.

TABLE 3 Subject and timing for each genetic test

	RAS mutation testing	*BRAF* V600E mutation testing	MSI testing, immunohistochemistry for MMR proteins	
Stage 0	—	—	—	If clinicopathological information indicates Lynch syndrome [SR]
Stage I	—	—	—	
Stage II	—	—	After curative resection [R]	
Stage III	—	—	—	
Stage IV	—	—	—	
Unresectable	Before anti-EGFR antibody therapy [SR]	Before starting first-line therapy [R]	Before starting first-line therapy [ECO]	

anti-EGFR, anti-epidermal growth factor receptor; ECO, expert consensus opinion; MMR, mismatch repair; MSI, microsatellite instability; R, recommendation; SR, strong recommendation; –, no recommended timing.

2.2 | Methods with confirmed analytical validity such as in vitro diagnostics are recommended as *RAS* mutation testing using tumor tissue samples. [Strong recommendation]

Methods for detecting *RAS* mutations include: (i) PCR-based techniques for specific detection, such as allele-specific PCR that specifically amplify mutant alleles and reverse sequence-specific oligonucleotide with polymerase chain reaction (PCR-rSSO) that amplifies multiple regions using PCR and exhaustively detects gene alterations by fluorotyping; and (ii) a technique that amplifies the target gene region in the tumor DNA and sequences directly. Because the existence of *RAS* mutation is important to determine the indication for anti-EGFR antibody therapy, testing is strongly recommended using methods, such as approved in vitro diagnostics, in which sufficient analytical validity was confirmed.

2.3 | *BRAF* V600E mutation testing is recommended prior to the initiation of first-line therapy for patients with unresectable advanced or recurrent CRC. [Recommendation]

BRAF V600E mutation was shown to be an independent strong predictor for poor prognosis in patients with CRC. Recently, the possibility was reported that triplet chemotherapy combining fluorouracil (5-FU), oxaliplatin and irinotecan (FOLFOXIRI) with bevacizumab is more effective than other chemotherapies for unresectable advanced or recurrent CRC patients with *BRAF* V600E mutation, and both

European Society For Medical Oncology (ESMO) consensus guidelines and pan-Asian adapted ESMO consensus guidelines recommend FOLFOXIRI plus bevacizumab as the preferred choice for these patients.[3,4] Therefore, *BRAF* V600E mutation testing is recommended prior to starting first-line therapy for patients with unresectable advanced or recurrent CRC. In addition, therapies with *BRAF* inhibitors in combination with anti-EGFR antibody showed promise in early clinical trials.[5,6]

2.4 | Direct sequencing method (combined with manual microdissection) or a PCR-based method is recommended as *BRAF* V600E mutation testing using tumor tissue samples. [Recommendation]

Polymerase chain reaction using sequence-specific probes or direct sequencing method is recommended for *BRAF* V600E mutation testing. Mutation detection limits for the testing methods used in previous studies are 10%-25% mutant alleles for direct sequencing and approximately 1%-10% mutant alleles for the other methods. Considering that these various testing methods showed consistent results for an indicator of poor prognosis, a detection limit ranging from 1% to 10% is recommended for detecting *BRAF* V600E mutation.

2.5 | MMR-deficiency testing using tumor tissue samples is recommended for patients with CRC suspected to have Lynch syndrome. [Strong recommendation]

Lynch syndrome is an autosomal dominant inherited disorder caused by germline mutation in 1 of the MMR genes: *MLH1*, *MSH2*, *MSH6*, and *PMS2*. Although it is a rare disease accounting for 2%-4% in the West and 0.7% in Japan of overall patients with CRC, the diagnosis of Lynch syndrome is clinically significant because patients and their families have an increased risk of many types of malignant tumors.[7] Therefore, MMR-deficiency testing is strongly recommended for patients with CRC suspected of having Lynch syndrome. However, it should be noted that MMR deficiency is not specific to Lynch syndrome, and is also observed in a subset of sporadic CRC such as tumor with hypermethylation of the *MLH1* promoter.

2.6 | Microsatellite instability testing and immunohistochemistry for MMR proteins are recommended for tumor MMR-deficiency testing. [Strong Recommendation]

Tests for tumor MMR deficiency include either PCR-based microsatellite instability (MSI) testing or immunohistochemistry (IHC) for MMR proteins. The function of MMR proteins in the tumor is determined through MSI testing by assessing the presence or absence of microsatellites of different lengths resulting from abnormal repeats in the microsatellite regions, or through IHC for MMR proteins by determining whether MMR proteins are missing. High-level or high-frequency MSI (MSI-H) or absent MMR protein expressions are described as MMR deficient (dMMR), whereas low-frequency MSI (MSI-L), microsatellite stable (MSS), or positive MMR protein expressions are described as MMR proficient (pMMR).

2.7 | MMR-deficiency testing using tumor tissue samples is recommended for patients with Stage II colon cancer who underwent curative resection. [Recommendation]

Based on the meta-analysis of phase III studies comparing surgery alone with postoperative 5-FU therapy, dMMR is currently recognized as a predictive factor for the low risk of recurrence in patients with Stage II/III colon cancer, particularly in Stage II patients. Many studies indicated that postoperative 5-FU monotherapy is ineffective or worsens the recurrence risk or survival, thus dMMR is recognized to be a predictive biomarker for the lack of efficacy from postoperative 5-FU monotherapy. Therefore, MMR-deficiency testing is recommended to predict the risk of recurrence and prognosis of patients with Stage II colon cancer treated with curative resection and to predict the lack of efficacy of postoperative 5-FU monotherapy for patients for whom postoperative adjuvant therapy is being considered.

2.8 | MMR-deficiency testing using tumor tissue samples is considered prior to the initiation of first-line therapy for patients with unresectable advanced or recurrent CRC. [Expert consensus opinion]

Some reports demonstrated that Stage IV or unresectable advanced or recurrent CRC patients with dMMR tended to have poorer survival compared with pMMR among patients with wild-type *BRAF* V600E. Therefore, when starting first-line therapy in patients with unresectable advanced or recurrent CRC, MMR-deficiency testing in combination with *BRAF* V600E testing can be considered for predicting the prognosis. More recently, anti-PD-1 antibodies showed promising efficacy results for patients with unresectable advanced or recurrent MMR-deficiency CRC, and US Food and Drug Administration (FDA) approved pembrolizumab and nivolumab for these patients in 2017.

2.9 | Formalin-fixed, paraffin-embedded (FFPE) tissue blocks are recommended for use in somatic gene testing. It is recommended to confirm that the unstained, thin-sliced section contains sufficient tumor cells in which the quality of nucleic acids is expected to be maintained histologically by a pathologist, using paired H&E staining. [Strong recommendation]

Formalin-fixed, paraffin-embedded tissue is suitable for somatic genetic testing because it can be easily obtained and enables morphological observation of tumor tissue with H&E staining.

Proportion of tumor cells vs normal cells should be assessed using a section stained with H&E, and specimens should be used for genetic testing when the contained tumor cells are sufficient. Along with the amount of tumor cells, specimens should be used when the quality of nucleic acids is expected to be maintained histologically.

2.10 | Genetic testing for CRC treatment should be carried out under a quality assured system. [Strong recommendation]

Requirements for quality assurance testing should be discussed from the aspect of facility certifications, testing itself, levels and testing qualifications of laboratory workers, education of personnel and risk management. Clinical laboratories should secure the accuracy and reliability of testing by obtaining and maintaining an external certificate such as International Organization for Standardization (ISO) 15189, specified requirements for quality and competence in medical laboratories, or one by College of American Pathologists (CAP). Quality assurance testing itself and laboratory workers should adhere to both "OECD Guidelines for Quality Assurance in Molecular Genetic Testing (http://www.oecd.org/sti/biotech/38839788.pdf)" and "Japanese Best Practice Guidelines for Genetic Testing; Commentary Edition (http://www.jccls.org/active/MM6-A1.pdf)".

3 | TESTING TECHNOLOGY CURRENTLY UNDER DEVELOPMENT AND FUTURE PROSPECTS

3.1 | Comprehensive genetic testing using NGS

Comprehensive genetic testing using NGS dramatically improved sequencing capability and enabled a massive amount of genomic sequencing data to be generated at a super-high speed compared with the conventional technique. Because comprehensive genetic testing for Cancer Panel using NGS has the possibility of detecting any patterns of mutation within the amplified region, it is being introduced to clinical practice as a diagnostic and screening method of genetic alterations targeted for molecular target therapies. Recently, Clinical Practice Guidance for Next Generation Sequencing in Cancer Diagnosis and Treatment First Edition (Japanese only) has been published on October 11, 2017 as the joint consensus of (JSMO; https://www.jsmo.or.jp/about/doc/20171011_01.pdf), Japan Society of Clinical Oncology (JSCO), and Japanese Cancer Association (JCA). For recommendations for comprehensive genetic testing for Cancer Panel using NGS, refer to requirements in the above guideline.

3.2 | Genetic testing analyzing ctDNA for patients with CRC

Liquid biopsy has emerged as an excellent molecular diagnostic tool for assessing predominant spatial and temporal intratumoral heterogeneity with minimal invasiveness. Previous studies have indicated that genomic alterations in *RAS*, *BRAF*, *ERBB2*, and *MET*, as well as other cancer-related genes associated with resistance to anti-EGFR therapy can be analyzed by ctDNA analysis with high diagnostic accuracy. Furthermore, by longitudinally monitoring ctDNAs during anti-EGFR therapy, the emergence of genomic alterations can be detected as acquired resistance mechanisms in specific genes, mainly those associated with the MAPK signaling pathway. Some ctDNA assays, particularly for detecting *KRAS* or *RAS* mutation in unresectable advanced or recurrent CRC, have indicated clinical validity and utility; however, there is no evidence of clinical validity and utility to suggest that ctDNA assays are useful for early detection of CRC in asymptomatic individuals and populations outside of a clinical trial. It is highly likely that evidence will shortly emerge to enable better assessment of the clinical validity and utility of ctDNA assays.[8]

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CONFLICTS OF INTEREST

K.Y. received honoraria for lectures from Takeda Pharmaceutical Co., Ltd, Chugai Pharmaceutical Co., Ltd, and Taiho Pharmaceutical Co., Ltd. H.T. received honoraria for lectures from Takeda Pharmaceutical Co., Ltd, Taiho Pharmaceutical Co., Ltd, and received research funding from Takeda Pharmaceutical Co., Ltd. T.Y. received honoraria for lectures from Taiho Pharmaceutical Co., Ltd, Eli Lilly Japan K.K., and Chugai Pharmaceutical Co., Ltd, and received research funding from GlaxoSmithKline K.K., and Nippon Boehringer Ingelheim Co., Ltd. K.N. received research funding from Sysmex Co., Ltd. K.M. received honoraria for lectures from Yakult Honsha Co., Ltd, Chugai Pharmaceutical Co., Ltd, Takeda Pharmaceutical Co., Ltd, Taiho Pharmaceutical Co., Ltd, Merck Serono Co., Ltd, and Eli Lilly Japan K.K., and received research funding from MSD K.K., Ono Pharmaceutical Co., Ltd, Daiichi-Sankyo Co., Ltd, Kyowa Hakko Kirin Co., Ltd, Shionogi Co., Ltd, and Gilead Science K.K. Y.Y. received honoraria for lectures from MSD K.K., and Chugai Pharmaceutical Co., Ltd. K.Y. received honoraria for lectures from Chugai Pharmaceutical Co., Ltd, Yakult Honsha Co., Ltd, Taiho Pharmaceutical Co., Ltd, Takeda Pharmaceutical Co., Ltd, Merck Serono Co., Ltd, Eli Lilly Japan K.K., and Bristol-Myers Squibb Co., Ltd, and received research funding from AstraZeneca Co., Ltd, IQVIA Co., Ltd, Sanofi K.K., Bristol-Myers Squibb Co., Ltd, Merck Serono Co., Ltd, Ono Pharmaceutical Co., Ltd, Sumitomo Dainippon Pharma Co., Ltd, Taiho Pharmaceutical Co., Ltd, Eli Lilly Japan K.K., Daiichi-Sankyo Co., Ltd, Chugai Pharmaceutical Co., Ltd, and Boehringer Ingelheim Co., Ltd. K.T. received honoraria for lectures from Takeda Pharmaceutical Co., Ltd. All remaining authors have no conflicts of interest to declare.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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