Molecular Biomarkers for the Evaluation of Colorectal Cancer: Guideline From the American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology, and the American Society of Clinical Oncology
Introduction

• Molecular testing to select targeted and conventional therapies for patients with colorectal cancer (CRC) has been the focus of a number of recent studies and is becoming standard practice for management of patients with CRC.

• The postgenome era and the emphasis on precision genomic-based medicine are providing enormous amounts of new data and many promising new molecular cancer biomarkers that may emerge as molecular diagnostic tools that can be used to enhance successful treatment of patients with CRC and other cancers.

• There is a need for current evidence-based recommendations for the molecular testing of CRC tissues to guide EGFR-targeted therapies and conventional chemotherapy regimens.

• Therefore, the current recommendations were developed through collaboration of four societies: American Society for Clinical Pathology (ASCP), College of American Pathologists (CAP), Association for Molecular Pathology (AMP), and American Society of Clinical Oncology (ASCO).
CAP/ASCP/AMP/ASCO Guideline
Development Methodology

• This evidence-based guideline was developed following standards as endorsed by the Institute of Medicine.

• A detailed description of the methods and systematic review (including the quality assessment and complete analysis of the evidence) can be found in the Methodology Supplement, online at www.asco.org/CRC-markers-guideline
Clinical Questions

The scope of the project was to develop an evidence-based guideline to help establish standard molecular biomarker testing, guide targeted therapies, and advance personalized care for patients with CRC. The panel addressed the following key questions:

1. What biomarkers are useful to select patients with CRC for targeted and conventional therapies?
2. How should tissue specimens be processed for biomarker testing for CRC management?
3. How should biomarker testing for CRC management be performed?
4. How should molecular testing of CRC be implemented and operationalized?
5. Are there emerging genes/biomarkers that should be routinely tested in CRC?

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Target Population
Patients with CRC being considered for treatment with anti-EGFR inhibitors or conventional chemotherapy

Target Audience
Pathologists, laboratorians, oncologists and other clinicians, molecular diagnostics professionals, scientists, government agencies, non-profit organizations, patients and patient advocates, members of the public, and additional stakeholders as appropriate.
Summary of Recommendations

1. Colorectal carcinoma patients being considered for anti-EGFR therapy must receive RAS mutational testing. Mutational analysis should include KRAS and NRAS codons 12, 13 of exon 2; 59, 61 of exon 3; and 117 and 146 of exon 4 ("expanded" or "extended" RAS) (Type: recommendation; Strength of Evidence: convincing/adequate, benefits outweigh harms; Quality of Evidence: high/intermediate).

2a. BRAF p.V600 (BRAF c. 1799 (p.V600) mutational analysis should be performed in colorectal cancer tissue in patients with colorectal carcinoma for prognostic stratification (Type: recommendation, Strength of Evidence: adequate/inadequate, balance of benefits and harms; Quality of Evidence: intermediate/low).

2b. BRAF p.V600 mutational analysis should be performed in deficient MMR tumors with loss of MLH1 to evaluate for Lynch Syndrome risk. Presence of a BRAF mutation strongly favors a sporadic pathogenesis. The absence of BRAF mutation does not exclude risk of Lynch syndrome (Type: recommendation, Strength of Evidence: adequate/inadequate, balance of benefits and harms; Quality of Evidence: intermediate/low).
Summary of Recommendations

3. Clinicians should order mismatch repair status testing in patients with colorectal cancers for the identification of patients at high risk for Lynch syndrome and/or prognostic stratification (Type: recommendation; Strength of Evidence: adequate/inadequate, balance of benefits and harms; Quality of Evidence: intermediate/low).

4. There is insufficient evidence to recommend BRAF c.1799 p.V600 mutational status as a predictive molecular biomarker for response to anti-EGFR inhibitors (Type: no recommendation; Strength of Evidence: insufficient, benefits/harms balance unknown; Quality of Evidence: insufficient).

5. There is insufficient evidence to recommend PIK3CA mutational analysis of colorectal carcinoma tissue for therapy selection outside of a clinical trial (Type: no recommendation; Strength of Evidence: insufficient, benefits/harms balance unknown; Quality of Evidence: insufficient).

Note: Retrospective studies have suggested improved survival with post-operative aspirin use in patients whose colorectal carcinoma harbors a PIK3CA mutation.

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6. There is insufficient evidence to recommend PTEN analysis [expression by immunohistochemistry (IHC) or deletion by fluorescence in situ hybridization (FISH)] in colorectal carcinoma tissue for patients who are being considered for therapy selection outside of a clinical trial (Type: no recommendation; Strength of Evidence: insufficient, benefits/harms balance unknown; Quality of Evidence: insufficient).

7. Metastatic or recurrent colorectal carcinoma tissues are the preferred specimens for treatment predictive biomarker testing and should be used if such specimens are available and adequate. In their absence, primary tumor tissue is an acceptable alternative, and should be used (Type: expert consensus opinion; Strength of Evidence: inadequate/Insufficient, benefits and harms in balance; Quality of Evidence: low).

8. Formalin fixed paraffin embedded tissue is an acceptable specimen for molecular biomarker mutational testing in colorectal carcinoma. Use of other specimens (e.g. cytology specimens) will require additional adequate validation, as would any changes in tissue processing protocols (Type: expert consensus opinion; Strength of Evidence: inadequate/Insufficient, benefits and harms in balance; Quality of Evidence: low).
Summary of Recommendations

9. Laboratories must use validated colorectal carcinoma molecular biomarker testing methods with sufficient performance characteristics for the intended clinical use. Colorectal carcinoma molecular biomarker testing validation should follow accepted standards for clinical molecular diagnostics tests (Type: strong recommendation; Strength of Evidence: Convincing/adequate, benefits outweigh harms; Quality of Evidence: high/intermediate).

10. Performance of molecular biomarker testing for colorectal carcinoma must be validated in accordance with best laboratory practices (Type: strong recommendation; Strength of Evidence: Convincing/adequate, benefits outweigh harms; Quality of Evidence: high/intermediate).

11. Laboratories must validate the performance of IHC testing for colorectal carcinoma molecular biomarkers (currently IHC testing for MLH1, MSH2, MSH6, and PMS2) in accordance with best laboratory practices (Type: strong recommendation; Strength of Evidence: Convincing/adequate, benefits outweigh harms; Quality of Evidence: high/intermediate).

12. Laboratories must provide clinically appropriate turnaround times and optimal utilization of tissue specimens by using appropriate techniques (e.g. multiplexed assays) for clinically relevant molecular and immunohistochemical biomarkers of colorectal cancer (Type: expert consensus opinion; Strength of Evidence: inadequate/Insufficient, benefits and harms in balance; Quality of Evidence: low).
Summary of Recommendations

13. Molecular and IHC biomarker testing in colorectal carcinoma should be initiated in a timely fashion based upon the clinical scenario and in accordance with institutionally accepted practices (Type: expert consensus opinion; Strength of Evidence: inadequate/Insufficient, benefits and harms in balance; Quality of Evidence: low).
Note: Test ordering can occur on a case-by-case basis or by policies established by the medical staff.

14. Laboratories should establish policies to ensure efficient allocation and utilization of tissue for molecular testing, particularly in small specimens (Type: expert consensus opinion; Strength of Evidence: inadequate/Insufficient, benefits and harms in balance; Quality of Evidence: low).

15. Members of the patient’s medical team, including pathologists, may initiate colorectal carcinoma molecular biomarker test orders in accordance with institutionally accepted practices (Type: expert consensus opinion; Strength of Evidence: inadequate/Insufficient, benefits and harms in balance; Quality of Evidence: low).

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Summary of Recommendations

16. Laboratories that require send out of tests for treatment predictive biomarkers should process and send colorectal carcinoma specimens to reference molecular laboratories in a timely manner (Type: expert consensus opinion; Strength of Evidence: inadequate/Insufficient, benefits and harms in balance; Quality of Evidence: low). Note: It is suggested that a benchmark of 90% of specimens should be sent out within 3 working days.

17. Pathologists must evaluate candidate specimens for biomarker testing to ensure specimen adequacy taking into account tissue quality, quantity, and malignant tumor cell fraction. Specimen adequacy findings should be documented in the patient report (Type: expert consensus opinion; Strength of Evidence: inadequate/Insufficient, benefits and harms in balance; Quality of Evidence: low).

18. Laboratories should use colorectal carcinoma molecular biomarker testing methods that are able to detect mutations in specimens with at least 5% mutant allele frequency, taking into account the analytical sensitivity of the assay (limit of detection or LOD) and tumor enrichment (e.g. microdissection) (Type: expert consensus opinion; Strength of Evidence: inadequate/Insufficient, benefits and harms in balance; Quality of Evidence: low). Note: It is recommended that the operational minimal neoplastic carcinoma cell content tested should be set at least 2 times the assay’s LOD.
Summary of Recommendations

19. Colorectal carcinoma molecular biomarker results should be made available as promptly as feasible in order to inform therapeutic decision-making, both prognostic and predictive (Type: expert consensus opinion; Strength of Evidence: inadequate/Insufficient, benefits and harms in balance; Quality of Evidence: low).

Note: It is suggested that a benchmark of 90% of reports available within 10 working days from date of receipt in the molecular diagnostics laboratory.

20. Colorectal carcinoma molecular biomarker testing reports should include a results and interpretation section readily understandable by oncologists and pathologists. Appropriate Human Genome Variation Society (HGVS) and Human Genome Organisation (HUGO) nomenclature must be used in conjunction with any historical genetic designations (Type: expert consensus opinion; Strength of Evidence: inadequate/Insufficient, benefits and harms in balance; Quality of Evidence: low).

21. Laboratories must incorporate colorectal carcinoma molecular biomarker testing methods into their overall laboratory quality improvement program, establishing appropriate quality improvement monitors as needed to assure consistent performance in all steps of the testing and reporting process. In particular, laboratories performing colorectal carcinoma molecular biomarker testing must participate in formal proficiency testing programs, if available, or an alternative proficiency assurance activity (Type: strong recommendation; Strength of Evidence: Convincing/adequate, benefits outweigh harms; Quality of Evidence: high/intermediate).

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Additional Resources

More information, including a Data Supplement, a Methodology Supplement, slide sets, and clinical tools and resources, is available at

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Patient information is available at www.cancer.net
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