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 American Society of Clinical Oncology

Objectives

- **To establish an evidence-based guideline for molecular biomarker testing for the evaluation of colorectal cancer.**

Background

The CAP, ASCP, AMP, and ASCO convened an expert panel to systematically review published documents and develop an evidence-based guideline to:

- **Establish evidence-based recommendations for the molecular testing of CRC tissues to guide targeted therapies and conventional chemotherapy regimens**

- **Summarize emerging molecular testing approaches for CRC and provide insight on needed studies**

Guideline Expert Panel

Co-chairs

Wayne Grody, MD – ASCP

Stanley Hamilton, MD – CAP

Antonia Sepulveda, MD, PhD – AMP*

Carmen J. Allegra, MD – ASCO

Expert Panel Members
Allison Marie Cushman-Vokoun, MD,PhD
Veena M Singh, MD, ACMG
Joseph Willis, MD
Bruce Minsky, MD
Jan Anthony Nowak, MD, PhD
Daniel J. Sargent, PhD
Noralane M Lindor, MD
William K. Funkhouser, MD, PhD
Federico Alberto Monzon, MD
Christopher Lieu, MD
Scott Kopetz, MD

Advisory Panel Members
Charles David Blanke, MD

Jean-Francois Flejou, MD, PhD

Heather Lynn Hampel, MS

Joel Randolph Hecht, MD

Kazunori Kanehira, MD

Fay Kastrinos, MD, MPH

Carla Beltran MacLeod, MD

Pamela McAllister, PhD (Patient Advocate)

Peter J O'Dwyer, MD

Shuji Ogino, MD, PhD

Kim Ryan (Patient Advocate)

Weijing Sun, MD

Josep Tabernero, MD, PhD

Laura H Tang, MD, PhD

Mary Kay Washington, MD, PhD

Staff
Christina Ventura, MLS(ASCP) – CAP
Lisa Fatheree, SCT(ASCP) – CAP
Carol Colasacco, MLIS, SCT(ASCP) – CAP
Jennifer Clark, SCT(ASCP)^{cm} MB^{cm} – ASCP
Shiwen Song, MD, PhD – ASCP
Robyn Temple-Smolkin, PhD – AMP
R. Bryan Rumble – ASCO Methodologist

*Lead Co-chair

4

ASCP STRONGERTOGETHER   COLLEGE of AMERICAN PATHOLOGISTS   AMP ASSOCIATION FOR MOLECULAR PATHOLOGY   ASCO American Society of Clinical Oncology

Institute of Medicine Standards

- **Establishing transparency**
- **Management of conflict of interest (COI)**
- **Guideline development group composition**
- **Clinical practice guideline–systematic review intersection**
- **Establishing evidence foundations for and rating strength of recommendations**

- **Articulation of recommendations**
- **External Review**
- **Updating**

[Clinical Practice Guidelines We Can Trust: IOM Report](#)

Grades for Strength of Recommendation

Designation	Recommendation	Rationale
Strong Recommendation	**Recommend for or against a particular molecular testing practice for colorectal cancer (Can include must or should)**	**Supported by convincing (high) or adequate (intermediate) quality of evidence and clear benefit that outweighs any harms**
Recommendation	**Recommend for or against a particular molecular testing practice for colorectal cancer (Can include should or may)**	**Some limitations in quality of evidence (adequate [intermediate] or inadequate [low]), balance of benefits and harms, values, or costs but panel concludes that there is sufficient evidence and/or benefit to inform a recommendation**
Expert Consensus Opinion	**Recommend for or against a particular molecular testing practice for colorectal cancer (Can include should or may)**	**Serious limitations in quality of evidence (inadequate [low] or insufficient), balance of benefits and harms, values or costs, but panel consensus is that a statement is necessary**
No Recommendation	**No recommendation for or against a particular molecular testing practice for colorectal cancer**	**Insufficient evidence or agreement of the balance of benefits and harms, values, or costs to provide a recommendation**

Grades for Strength of Evidence

Designation	Description	Quality of Evidence
Convincing	High confidence that available evidence reflects true effect. Further research is very unlikely to change the confidence in the estimate of effect.	High/Intermediate quality evidence
Adequate	Moderate confidence that available evidence reflects true effect. Further research is likely to have an important impact on the confidence in estimate of effect and may change the estimate.	Intermediate/Low quality of evidence
Inadequate	Little confidence that available evidence reflects true effect. Further research is very likely to have an important impact on the confidence in the estimate of effect and is likely to change the estimate.	Low/Insufficient evidence and expert panel uses formal consensus process to reach recommendation
Insufficient	Evidence is insufficient to discern net effect. Any estimate of effect is very uncertain.	Insufficient evidence and expert panel uses formal consensus process to reach recommendation

Adapted by permission from BMJ Publishing Group Limited. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. Guyatt GH, et al; GRADE Working Group. 2008;336(7650):924-926.[9]

Systematic Evidence Review

- **Identify Key Questions**

- **Literature search**

- **Data extraction**

- **Develop proposed recommendations**

- **Open comment period**

- **Considered judgment process**

Molecular Testing Guidelines for Colorectal Cancer: Overarching Key Questions

- **What biomarkers are useful for colorectal cancer (CRC) management (selection of patients for targeted and conventional therapies)?**

- **How should tissue specimens be processed for biomarker testing for CRC management?**

- **How should biomarker testing for CRC management be performed?**

- **How should molecular testing of CRC be implemented and operationalized?**

- **Should other genes/biomarkers be routinely tested in CRC?**

Systematic Review

- **Systematic literature search: Initial dates from Jan 1, 2008 through Aug 1, 2013 with a literature refresh with dates covering through February 12, 2015**

- **Title-Abstract Screen: 4,497 abstracts**

- **Full-text Article Review: 866 articles**

- **Data Extraction: 123 articles for data extraction and qualitative analysis; Over 70+ systematic reviews and meta-analyses analyzed**

Systematic Review, continued

- **All expert panel members participated in the systematic review of the literature.**

- **The expert panel convened to review the extracted data and drafted recommendations.**

- **The draft recommendations were available for public commentary in April 2015.**

- **Draft recommendations were updated based on public commentary in July 2015.**

STRONGER TOGETHER

COLLEGE of AMERICAN PATHOLOGISTS

AMP Association for Molecular Pathology

ASCO American Society of Clinical Oncology

Guideline Statements

- **Key Question:** **What biomarkers are useful for colorectal cancer (CRC) management (selection of patients for targeted and conventional therapies)?**

Guideline Statement 1

Recommendation: Patients with CRC 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*).

Rationale: 311 primary studies with 74,546 patients that reported treatment outcomes between *RAS* mutation vs. *RAS* nonmutated(nm)/wild type(wt)

- Studies reported a pooled survival advantage for patients treated with anti-EGFR monoclonal antibody (MoAb) with *KRAS* nm/wt vs *KRAS* mutation tumors.

- Significant progression free survival (PFS) advantage for adding anti-EGFR MoAb therapy for *KRAS* nm/wt tumors compared to chemotherapy alone.

Guideline Statement 1, continued

Recommendation: **Patients with CRC 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*).**

Rationale:

- **Studies reported an overall response rate (ORR) advantage for adding EGFR monoclonal antibody to chemotherapy for patients with *KRAS* non-mutated/wild type.**

- **There is also conclusive evidence regarding other *RAS* mutations in *KRAS* exons 2, 3, and 4 and *NRAS* exons 2, 3, and 4 associated to nonresponse of metastatic CRC to anti-EGFR monoclonal antibody therapy.**

Guideline Statement 1, continued

Recommendation: **Patients with CRC 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*).**

Rationale:

- **Patients with colorectal cancers that are *KRAS* exon 2 nm/wt but harbor *RAS* mutations in *KRAS* exons 3 and 4 or *NRAS* exons 2, 3, and 4 also have significantly inferior anti-EGFR treatment outcomes benefit compared with those without any *RAS* mutations.**

- **No progression free survival (PFS) or overall survival (OS) benefit with use of anti-EGFR mAbs for tumors harboring any *RAS* mutation (Sorich MJ et al. 2014).**

Guideline Statement 2A

Recommendation: *BRAF* V600 (*BRAF* c.1799 [p.V600]) position mutational analysis should be performed in CRC tissue in selected patients with colorectal carcinoma for prognostic stratification.

Rationale:

- Studies show that patients with advanced CRC with a *BRAF* mutation show poorer progression free survival (PFS), overall survival (OS), and a decreased response rate to anti-EGFR therapy.

- Patients with *BRAF* mutation showed modest beneficial impact from the use of anti-EGFR agents relative to those patients with *RAS* mutation.

- CRC patients with *BRAF* mutation have worse outcome relative to non-mutated patients.

Guideline Statement 2B

Recommendation: *BRAF* p.V600 mutational analysis should be performed in dMMR 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.

Rationale:

- Testing for *BRAF* mutations may help distinguish between germline from epigenetic dMMR, especially in the cases where the dMMR is the result of the epigenetic silencing of *MLH1*.
- Testing may help to further refine the risk of Lynch syndrome for patients with germline-based dMMR.

Guideline Statement 3

Recommendation: 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.

Rationale:

- Diagnosis of Lynch Syndrome is important to allow patients to actively manage cancer risks to benefit gene mutation carriers.

- Emerging data indicate that MMR status may have predictive value in some settings, specifically in patients with advanced disease being considered for anti-programmed cell death protein-1 (PD-1)/ programmed cell death ligand protein-1 (PD-L1) immune checkpoint inhibitor therapy.

Guideline Statement 4

No Recommendation: **There is insufficient evidence to recommend *BRAF* c.1799 p.V600 mutational status as a predictive molecular marker for response to anti-EGFR inhibitors.**

Rationale:

- Studies used nonrandomized cohorts which makes the evaluation of the potential predictive value of the *BRAF* p.V600 mutation difficult to determine.

- With the low mutation prevalence , the evaluation of the relative benefit of anti-EGFR inhibitors is also difficult to determine.

- A meta-analysis (M-A) of 463 patients with *KRAS* wild-type (wt) and *BRAF* p.600 mutation did not provide sufficient evidence to determine the magnitude of benefits seen in *KRAS/BRAF* wt tumors.

- Another M-A showed that EGFR monoclonal antibody therapy in *BRAF* p.600 mutation patients was not associated with significant OS (p=.43), although shows a better PFS (p=.07).

Guideline Statement 5

No Recommendation: **There is insufficient evidence to recommend** *PIK3CA* **mutational analysis of colorectal carcinoma tissue for therapy selection outside of a clinical trial.**

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

Rationale:

- **Comprehensive** *PIK3CA* **testing would increase response rate in the first-line setting by only 1%.**

- **The prognostic impact of** *PIK3CA* **in stage I to III disease has been inconsistent.**

- **Multiple prospective observational studies have demonstrated an association between aspirin use and decreased CRC mortality.**

Guideline Statement 6

No Recommendation: 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.

Rationale:

- **There is evidence suggesting that PTEN is a critical factor in cancer development, but the association between PTEN expression and predictive/prognostic value remains controversial.**

- **Several studies suggesting an association with poorer prognosis and others finding no association at all.**

- **Due to the discordant studies, the prognostic or predictive role of PTEN in CRC is still unknown.**

- **Key Question:** How should tissue specimens be processed for biomarker testing for CRC management?

Guideline Statement 7

Expert Consensus Opinion: 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.

Rationale:

- Despite high concordance between the primary and metastatic or recurrent, discordant mutational status *may still* happen in some cases, therefore metastatic or recurrent tissue is preferred.

- If the metastatic or recurrent tissue is unavailable, the primary tissue may be used for testing.

Concordance between primary and metastases

Genes Tested	Concordance Rate (%)
KRAS (n=117)	91.0
KRAS, NRAS, BRAF (n=84)	98.8
PIK3CA (n=117)	94.0
PIK3CA (n=84)	92.8
PTEN IHC (n=117)	66.0

Guideline Statement 8

Expert Consensus Opinion: **Formalin fixed paraffin embedded (FFPE) tissue is an acceptable specimen for molecular marker mutational testing in colorectal carcinoma. Use of other specimens (eg, cytology specimens) will require additional adequate validation, as would any changes in tissue processing protocols.**

Rationale:

- The use of FFPE cell blocks allows for the evaluation of tumor cell content and viability.
- Cytology specimens may be adequate for testing but will require proper validation.
- Note: Laboratories need to establish the minimum tumor cell content for specimens based on the performance characteristics of their validated assay.

Key Questions

- **How should biomarker testing for CRC management be performed?**

- **How should molecular testing of CRC be implemented and operationalized?**

Guideline Statement 9

Strong Recommendation: **Laboratories must use validated colorectal carcinoma molecular marker 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.**
Rationale:

- **Validation should be performed to ensure all molecular marker testing methods, such as those used for colorectal carcinoma, are ready for implementation in the clinical laboratory.**

Guideline Statement 10

Strong Recommendation: Performance of molecular biomarker testing for colorectal carcinoma must be validated in accordance with best laboratory practices.

Rationale:

- Validation of CRC biomarker testing is important to ensure appropriate patient care. If validation is inadequate, this can lead to erroneous results and improper diagnosis, prognosis, and/or therapeutic intervention.

- Thorough validation of preanalytical (specimen type and processing), analytical (assay performance), and postanalytical (bioinformatics, annotation, and reporting) steps is important.

- Assay validation should be done in accordance with CLIA (42 CFR 493.1253(b)(2), also known as Title 42 Chapter IV Subchapter G Part 493 Subpart K§493.1253)111 as applicable to the assay type.

Guideline Statement 10, continued

Strong Recommendation: **Performance of molecular biomarker testing for colorectal carcinoma must be validated in accordance with best laboratory practices.**

Rationale:

- **Validation of assays used in CRC molecular testing is important for accuracy of reporting and proper patient care.**

Guideline Statement 11

Strong Recommendation: 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).

Rationale:

- Development of anti-MMR protein antibody staining protocols follows a standard:
  - Demonstration of absent background noise with secondary antibody alone;
  - Optimization of the signal-to-noise ratio by testing different antibody concentrations, antigen retrieval buffers, and reaction conditions, taking advantage of internal control cells, including lymphocytes, stromal cells, and other nonneoplastic nuclei.

Guideline Statement 11, continued

Strong Recommendation: 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).

Rationale:

- Validation of the final staining protocol is required prior to implementation for clinical use.

- Concordance with internal or external known comparator tests is required.

- Once the protocol is defined and validated for a given primary antibody clone and antigen retrieval conditions, a known positive external control is routinely run in parallel with each unknown.

Guideline Statement 12

Expert Consensus Opinion: **Laboratories must provide clinically appropriate turnaround times and optimal utilization of tissue specimens by using appropriate techniques (eg, multiplexed assays) for clinically relevant molecular and immunohistochemical biomarkers of CRC.**

Rationale:

- **Laboratories should have in place a process on how to optimally utilize tissue specimens for testing.**
  - **In cases where there is a small amount of tumor tissue, the laboratories should section tissue appropriately, with sufficient sections reserved for molecular and immunohistochemical methods.**
- **Results should be available to the clinician within 10 working days of receipt in the molecular diagnostics laboratory in order to initiate appropriate therapy.**

Guideline Statement 13

- **Recommendation:** **Molecular and IHC marker testing in colorectal carcinoma should be initiated in a timely fashion based upon the clinical scenario and in accordance with institutionally accepted practices.**

  Note: **Test ordering can occur on a case-by-case basis or by policies established by the medical staff.**

  Rationale:

  - **Predictive markers should be initiated in a timely manner to help guide therapy options.**

  - **Institutional policies and practices that recommend the rapid initiation of appropriate molecular biomarker testing should be put in place.**

Guideline Statement 14

Expert Consensus Opinion: Laboratories should establish policies to ensure efficient allocation and utilization of tissue for molecular testing, particularly in small specimens.

Rationale:

- It is important to have laboratory protocols in place for handling small specimens to ensure efficient allocation and utilization of tissue for molecular testing.

- Protocols that allow upfront ordering of required tissue testing may help limit tissue wasting and improve the turnaround time of final results.

Guideline Statement 15

Expert Consensus Opinion: **Members of the patient's medical team, including pathologists, may initiate colorectal carcinoma molecular marker test orders in accordance with institutionally accepted practices.**

Rationale:

- Following institutionally accepted protocols, test ordering should be ordered as efficiently as possible.
- Algorithms and reflex testing may help with the efficient test ordering of appropriate molecular biomarker testing for CRC.

Guideline Statement 16

Expert Consensus Opinion: **Laboratories that require send out of tests for treatment predictive markers should process and send colorectal carcinoma specimens to reference molecular laboratories in a timely manner.**

Note: It is suggested that a benchmark of 90% of specimens should be sent out within 3 working days.

Rationale:

- It is important to provide results of molecular biomarker tests in a timely fashion to initiate needed therapy.
- Result delays are associated with worse outcomes.
- Laboratories that send out molecular testing should have in place a process to ensure that tissues are sent out within 3 days from the test order.

Guideline Statement 17

Expert Consensus Opinion: **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.**

Rationale:

- **The total amount of tissue and the fraction of malignant tumor cells – it is critical that the pathologist selects the appropriate sections for testing.**

- **Tumor genetic heterogeneity may be present in samples.**

- **Tumor necrosis and degeneration can lead to errors.**

Guideline Statement 18

Expert Consensus Opinion: **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 (eg, microdissection).**

Note: **It is recommended that the operational minimal neoplastic carcinoma cell content tested should be set at least 2 times the assay's LOD.**

Rationale:

- **Laboratories should establish minimum acceptable tumor cell content.**

- **Minimum tumor cell content should be at least 2x the lower limit of detection of the assay being utilized.**

Guideline Statement 19

Expert Consensus Opinion: Colorectal carcinoma molecular biomarker results should be made available as promptly as feasible in order to inform therapeutic decision-making, both prognostic and predictive.

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.

Rationale:

- Molecular biomarker results inform therapeutic decision-making, and delays in resulting cause delays in therapy.

Guideline Statement 20

Expert Consensus Opinion: **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.**

Rationale:

- A report that is easily readable and understandable is beneficial to clinicians and patients.

- Molecular biomarker reports can be complex; these reports need to use standard nomenclature (HGVS/HUGO), and also include elements of result interpretation, variant classification, assay limit of detection, and other limitations that may help the clinicians.

Guideline Statement 21

Strong Recommendation: 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 marker testing must participate in formal proficiency testing programs, if available, or an alternative proficiency assurance activity.

Rationale:

- Participation in proficiency testing allows assessment and comparison of test performance among different laboratories.

Guideline Statement 21, continued

Rationale:

- **Proficiency testing (PT) allows for verification of accuracy and reliability of test results.**

- **PT is a requirement in the United States and similar requirements of external quality assurance are in place in other countries.**

- **In the absence of formal PT, laboratories may exchange specimens with other laboratories.**

Conclusions

- **Evidence supports mutational testing of specific genes in the EGFR signaling pathway, since they provide clinically actionable information for targeted therapy of CRC with anti-EGFR monoclonal antibodies.**

- **There is prognostic value in testing for MMR and *BRAF*.**

- **There is strong evidence of negative predictors of benefit (*KRAS, NRAS*) to anti-EGFR therapies.**

STRONGERTOGETHER · COLLEGE of AMERICAN PATHOLOGISTS · AMP Association for Molecular Pathology · ASCO American Society of Clinical Oncology

Conclusions, continued

- *BRAF* is associated with poor outcomes for patients with advanced CRC.

- Laboratories must operationalize testing for molecular biomarkers (eg, assay selection, specimen selection, test ordering, turnaround times, external quality assurance) to ensure accuracy and timeliness of the diagnosis and therapy selection.

Link to Guideline

Molecular Markers for the Evaluation of Colorectal Cancer

- **http://dx.doi.org/10.1016/j.jmoldx.2016.11.001**