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

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- **Professor, Departments of Pathology and Cell Biology at Columbia University**
- **AMP Co-chair, ASCP-CAP-AMP-ASCO Joint Guideline on the Evaluation of Molecular Markers for Colorectal Cancer**

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Disclosures

Dr. Sepulveda has no disclosures

Objectives

- **To establish an evidence-based guideline for the 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

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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: 4,197 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.**

Guideline Statements

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

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American Journal of Clinical Pathology (2017) 147 (3): 221-260
Journal of Molecular Diagnostics (2017) 19 (2): 187–225
Archives of Pathology and Laboratory Medicine doi: 10.5858/arpa.2016-0554-CP
Journal of Clinical Oncology DOI: 10.1200/JCO.2016.71.9807

Table 4
Guideline Statements and Strength of Recommendations

Guideline Statement	Strength of Recommendation
1. Patients with colorectal carcinoma being considered for anti-EGFR therapy must receive *RAS* mutational testing. Mutational analysis should include *KRAS* and *NRAS* codons 12 and 13 of exon 2, 59 and 61 of exon 3, and 117 and 146 of exon 4 ("expanded" or "extended" *RAS*).	Recommendation
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.	Recommendation
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 a *BRAF* mutation does not exclude risk of Lynch syndrome.	
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.	Recommendation
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.	No recommendation
5. 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 postoperative aspirin use in patients whose colorectal carcinoma harbors a PIK3CA mutation.	No recommendation
6. There is insufficient evidence to recommend PTEN analysis (expression by immunohistochemistry or deletion by fluorescence in situ hybridization) in colorectal carcinoma tissue for patients who are being considered for therapy selection outside of a clinical trial.	No recommendation
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.	Expert consensus opinion

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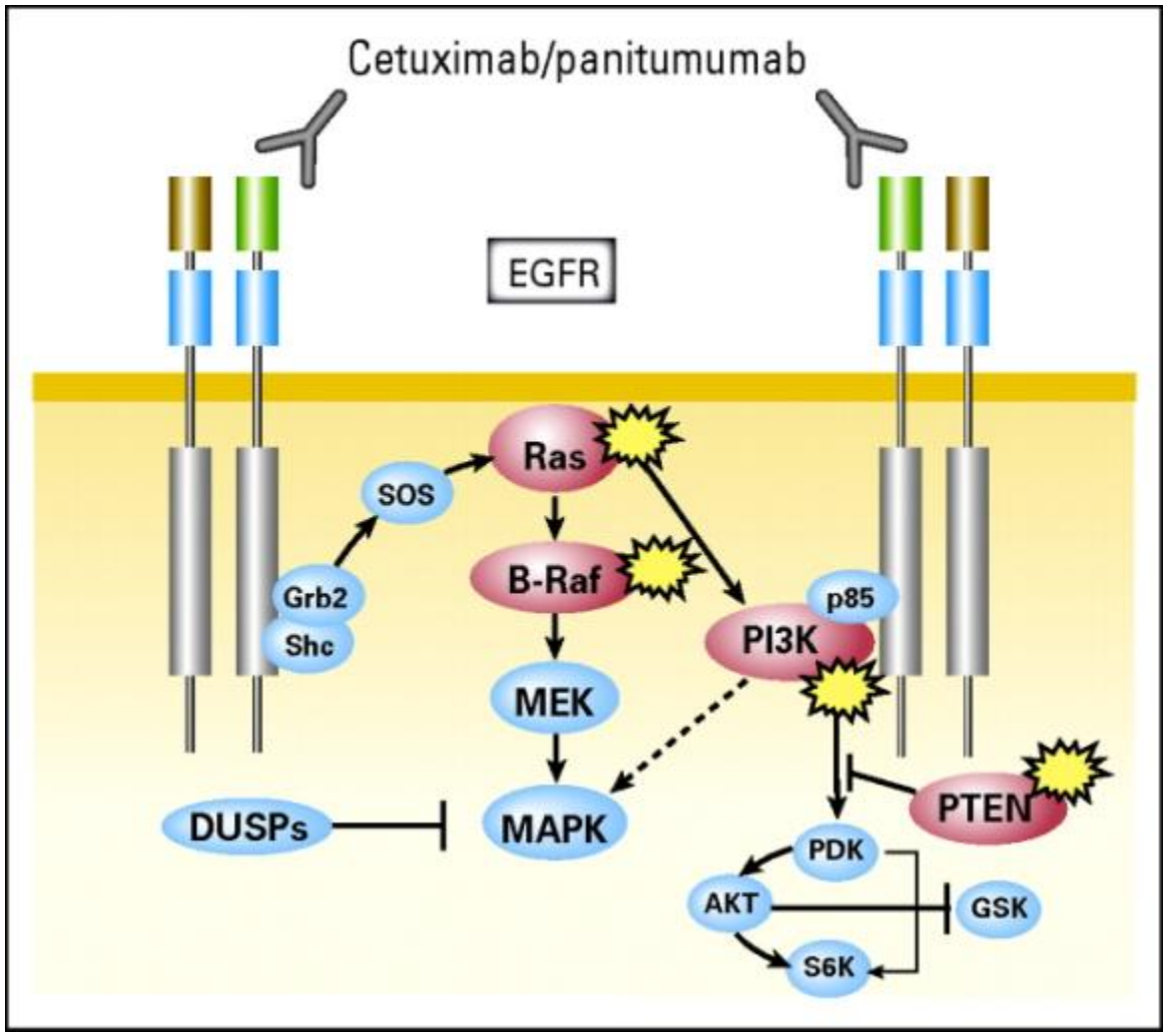
- **Key Question: What biomarkers are useful for colorectal cancer (CRC) management (selection of patients for targeted and conventional therapies)?**

Targeting the EGFR Pathway



Bardelli, A. et al. *J Clin Oncol*; 28:1254-1261 2010

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*).**

Guideline Statements 4, 5, 6

No Recommendation: There is insufficient evidence to recommend *BRAF* V600, *PIK3CA*, mutational status and PTEN IHC as predictive molecular biomarkers for response to anti-EGFR inhibitors

Guideline Statement 1, continued

Rationale: **311 primary studies with 74,546 patients that reported treatment outcomes in metastatic CRC**

comparing *RAS* mutation vs. *RAS* nonmutated(nm)/wild type(wt) in earlier studies of mostly KRAS exon 2 mutations

- **Survival advantage for patients treated with anti-EGFR MoAb with *KRAS* nm/wt vs. *KRAS* mutation tumors**
- **Studies reported an overall response rate (ORR) & progression free survival (PFS) advantage for adding anti-EGFR MoAb to chemotherapy for patients with *KRAS* nm/wt**

Guideline Statement 1, continued
Rationale:

- **There is also conclusive evidence that other *RAS* mutations in *KRAS* and *NRAS* are associated to nonresponse of metastatic CRC to anti-EGFR monoclonal antibody therapy (Sorich MJ et al. 2015)**
- **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 (Sorich MJ et al. 2015)**

Table 6 Outcomes of *RAS* Mutations and Anti-EGFR Therapy[12]

	Overall Survival		Progression-Free Survival	
Characteristic	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
RAS nm vs *RAS* mutation, *RAS* nm superior	0.72 (0.56-0.92)	<.01	0.60 (0.48-0.76)	<.001
KRAS exon 2 mutant vs new *RAS* mutant		ns		ns
KRAS nm exon 2, anti-EGFR vs no anti-EGFR	0.90 (0.83-0.98)	ns	0.68 (0.58-0.80)	<.001
KRAS exon 2 mutant, anti-EGFR vs no anti-EGFR	1.05 (0.95-1.17)	ns	1.14 (0.95-1.36)	ns
RAS nm, anti-EGFR vs no anti-EGFR	0.87 (0.77-0.99)	<.04	0.62 (0.50-0.76)	<.001
Any *RAS* mutant, anti-EGFR vs no anti-EGFR	1.08 (0.97-1.21)	ns	1.12 (0.94-1.34)	ns

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Prevalence of new *RAS* mutations across studies

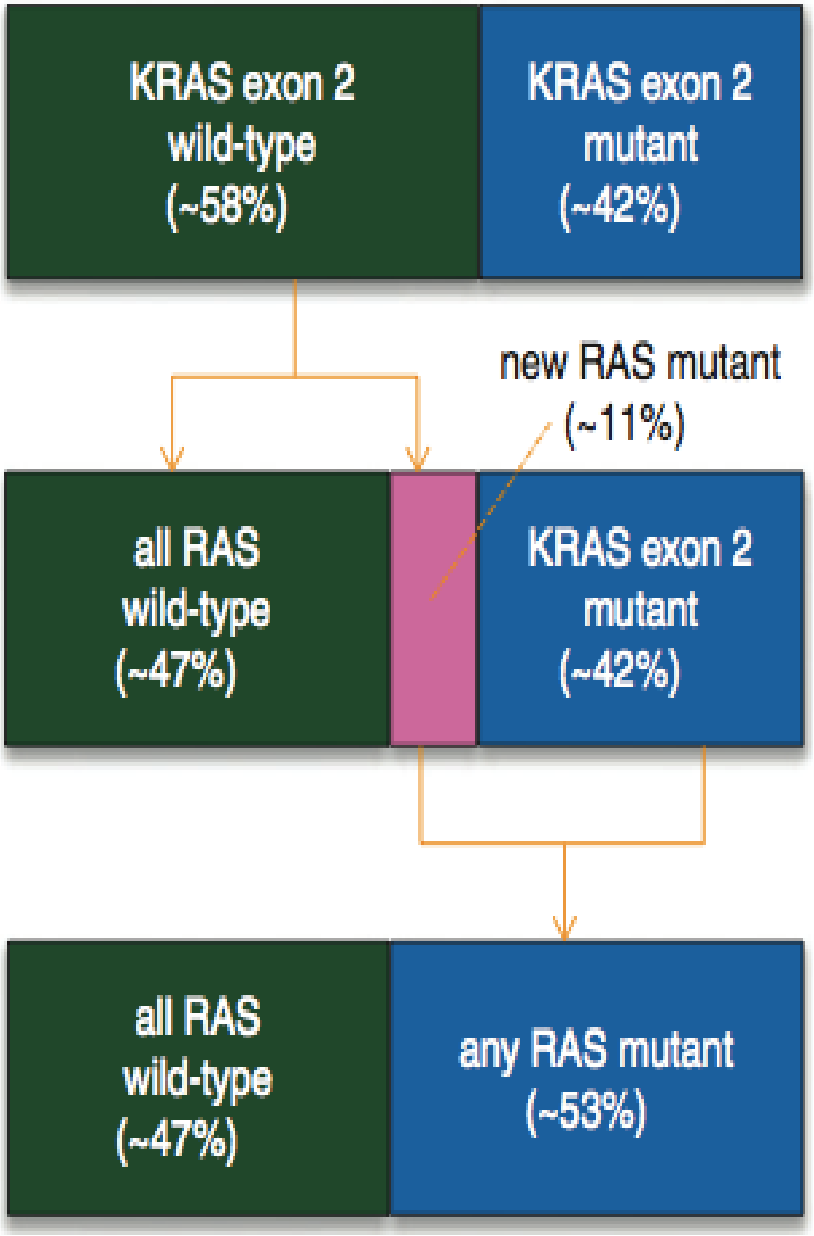
	New RAS Total[a]	KRAS Exon 3[a] (59, 61)	KRAS Exon 4[a] (117, 146)	NRAS Exon 2[a] (12, 13)	NRAS Exon 3[a] (59, 61)	NRAS Exon 4[a] (117, 146)
OPUS	26.3%	5.9%	9.3%	6.8%	5.1%	0.8%
PICCOLO	9.8%	NR[b]	3.7%[c]	6.3%[d]	NR[b]	NE
20020408	17.6%	4.8%[b]	5.0%	4.2%	3.0%[b]	1.1%
20050181	20.5%	4.6%	7.9%	2.3%	5.8%	0.0%
PRIME	17.4%	3.7%[b]	5.6%	3.4%	4.1%[b]	0.0%
FIRE-3	16.0%	4.3%[b]	4.9%[c]	3.8%	2.0%[b]	0.0%
PEAK	20.1%	4.1%	7.7%	5.4%	5.9%	0.0%
COIN	8.4%	2.1%[b]	NE	0.9%[e]	3.0%[b]	NE
CRYSTAL	14.7%	3.3%	5.6%	3.5%	2.8%	0.9%
SUMMARY[f]	19.9% (16.7%, 23.4%)	4.3% (3.3%, 5.5%)	6.7% (5.7%, 7.9%)	3.8% (3.0%, 4.8%)	4.8% (3.4%, 6.8%)	0.5% (0.2%, 1.2%)

a: proportion of the *KRAS* exon 2 wild-type group

Sorich MJ, et al. *Ann Oncol.* 2015;26:13–21.

All *RAS* Mutant CRC:

KRAS exon 2 c12 & c13 mutations and extended *RAS* mutations



Sorich MJ, et al. *Ann Oncol.* 2015;26:13–21.

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 of 463 patients with *KRAS* 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 it showed 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.**

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:

- **CRC patients with BRAF mutation have worse outcome relative to nm patients**
- **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**

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 epigenetic silencing of** ***MLH1***
- **Testing may help to further refine the risk of Lynch syndrome for patients with germline-based dMMR.**

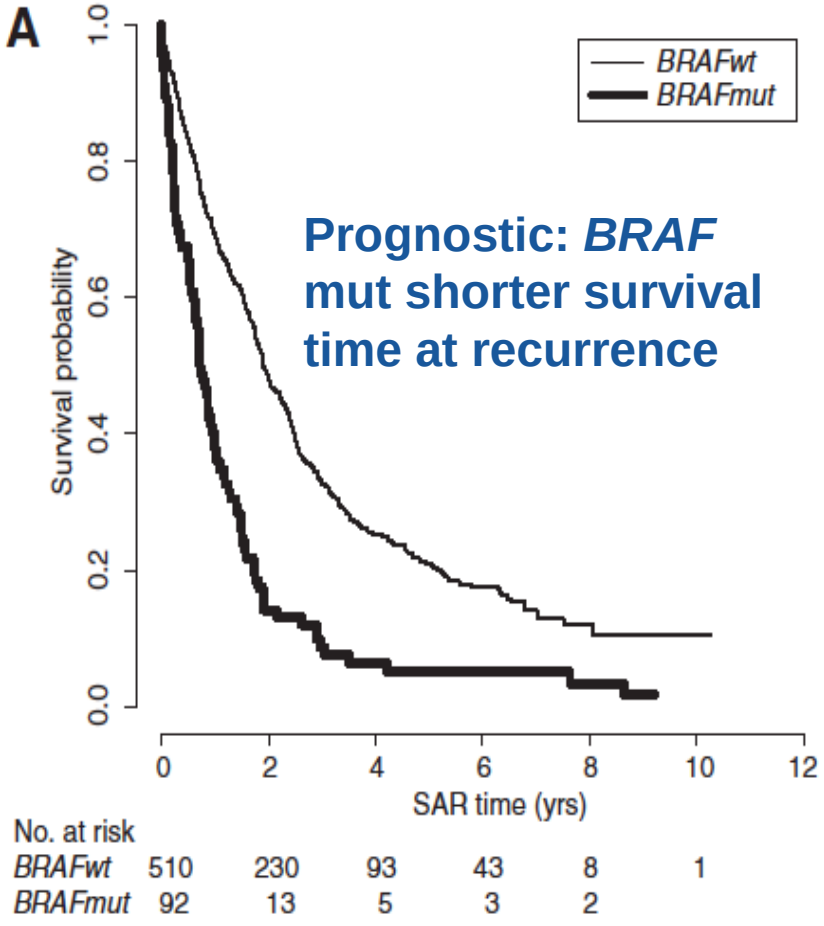
Guideline Statement 3

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.

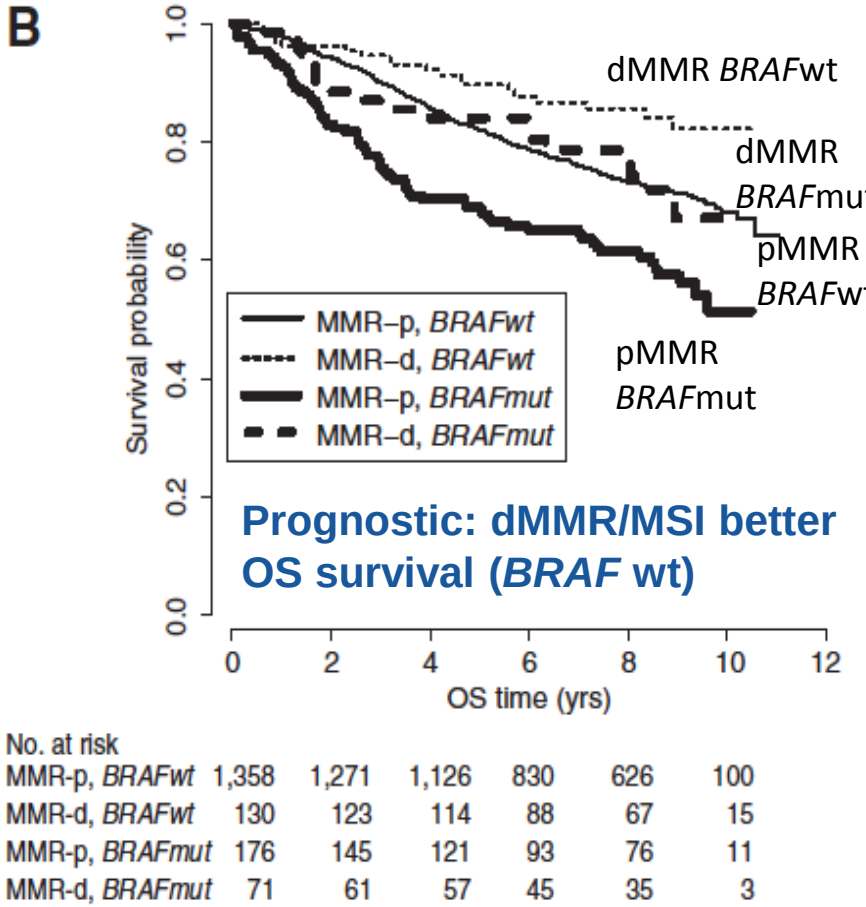
Rationale:

- **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**

BRAF and dMMR/MSI: Prognostic and Predictive Markers for Stage II/III CRC

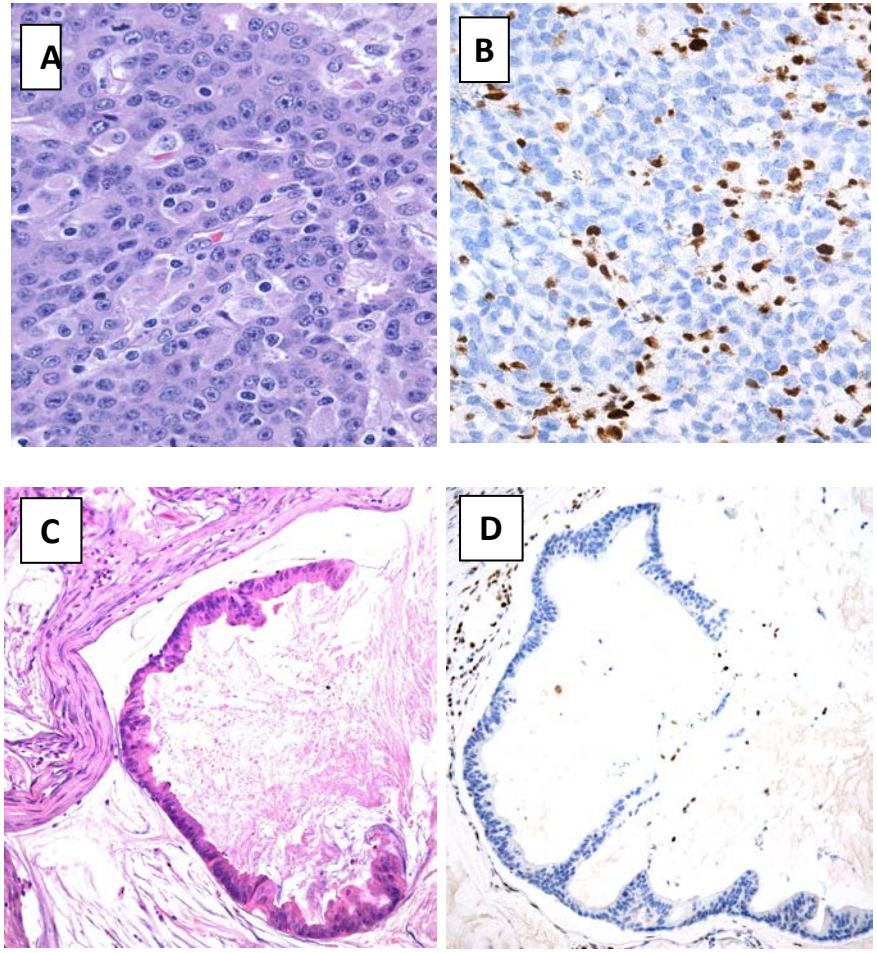


Prognostic: *BRAF* mut shorter survival time at recurrence



Prognostic: dMMR/MSI better OS survival (*BRAF* wt)

Gavin PG, et al. *Clin Cancer Res*. 2012;18:6531– 6541.



- Poorly differentiated adenocarcinoma with medullary features & prominent TILs
- Immunohistochemistry for MLH1: Loss of expression in tumor cell nuclei

- Mucinous adenocarcinoma
- Immunohistochemistry for MLH1: loss of expression in tumor

Molecular Pathology of Gastrointestinal Neoplasia Springer, LLC, New York, NY. AR Sepulveda and JP Lynch (eds.). 2013.

CRC emerging molecular biomarkers

- **MSI/MMR status may have predictive value in patients with advanced CRC being considered for anti-PD-1/PD-L1 immune checkpoint inhibitor therapy**

- DNA MMR status tested by MSI DNA test
- Pembrolizumab IV
- 82% had HNPCC germline detected

Le DT et al. *N Eng J Med* 2015; 372: 2509-20

Table 2. Objective Responses According to RECIST Criteria.

Type of Response	Mismatch Repair–Deficient Colorectal Cancer (N=10)	Mismatch Repair–Proficient Colorectal Cancer (N=18)	Mismatch Repair–Deficient Noncolorectal Cancer (N=7)
Complete response — no. (%)	0	0	1 (14)*
Partial response — no. (%)	4 (40)	0	4 (57)†
Stable disease at week 12 — no. (%)	5 (50)	2 (11)	0
Progressive disease — no. (%)	1 (10)	11 (61)	2 (29)
Could not be evaluated — no. (%)‡	0	5 (28)	0
Objective response rate (95% CI) — %	40 (12–74)	0 (0–19)	71 (29–96)
Disease control rate (95% CI) — %§	90 (55–100)	11 (1–35)	71 (29–96)
Median duration of response — wk	Not reached	NA¶	Not reached
Median time to response (range) — wk	28 (13–35)	NA¶	12 (10–13)

- **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

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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 (e.g. cytology specimens) will require additional adequate validation, as would any changes in tissue processing protocols.

Rationale:

- **The use of FFPE tissue or 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**

Table 13 Comparison of Test Performing Characteristic of Assays for *KRAS* Mutation Detection

Author, Year	No.	Comparison	Testing Method	Codons	Tissue Site	Procedure	Sample Type
Ma et al, 2009[130]	100	Sequencing	HRM	12, 13	Primary	NR	FFPE
Pinto et al, 2011[131]	372	Consensus‡	Sequencing	12, 13	NR	NR	FFPE
	184		DxS				
	182		HRM				
	372		Snapshot				
Tol et al, 2010[132]	511	Sequencing	DxS	12, 13	Primary	Resection	Frozen
Buxhofer-Ausch et al, 2013[133]	60	Sequencing	SA	12, 13	Primary	NR	Biopsy
Chang et al, 2010[136]	60	Sequencing	MPCR PE	12, 13, 61	Primary	NR	Frozen
Chen et al, 2009[137]	90	Sequencing	SSCP	12, 13	Primary	NR	Fresh
Chow et al, 2012[138]	204	Sequencing	ASP	12, 13	NR	NR	FFPE
Sundstrom et al, 2010[142]	100	DxS	Pyro	12, 13, 61	Primary or met	Biopsy	
Franklin et al, 2010[128]	59	Sequencing	HRM	12, 13	Primary	Resection	FFPE
	59	Sequencing	ARMS	12, 13		NR	
Laosinchai-Wolf et al, 2011[129]	86	Sequencing	BMA	12, 13	Primary	NR	FFPE
Carotenuto et al, 2010[134]	540	Sequencing	DxS	12, 13	Primary	NR	FFPE
	540	Sequencing	Sanger				
Cavallini et al, 2010[135]	112	DxS	SA	12, 13	NR	NR	FFPE
	112	DxS	PCR-RFLP				
Kristensen et al, 2010[139]	61	COLD-PCR	DxS	12, 13	Primary	Resection	FFPE
	61	PCR	MCA				
Kristensen et al, 2012[140]	100	CADMA	DxS	12, 13	Primary	Resection	FFPE
	100	DxS	CADMA				
Lang et al, 2011[141]	125	Sequencing	ASP	12, 13	Primary	Resection	FFPE

(table continues)

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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**

Table 13 (continuation)

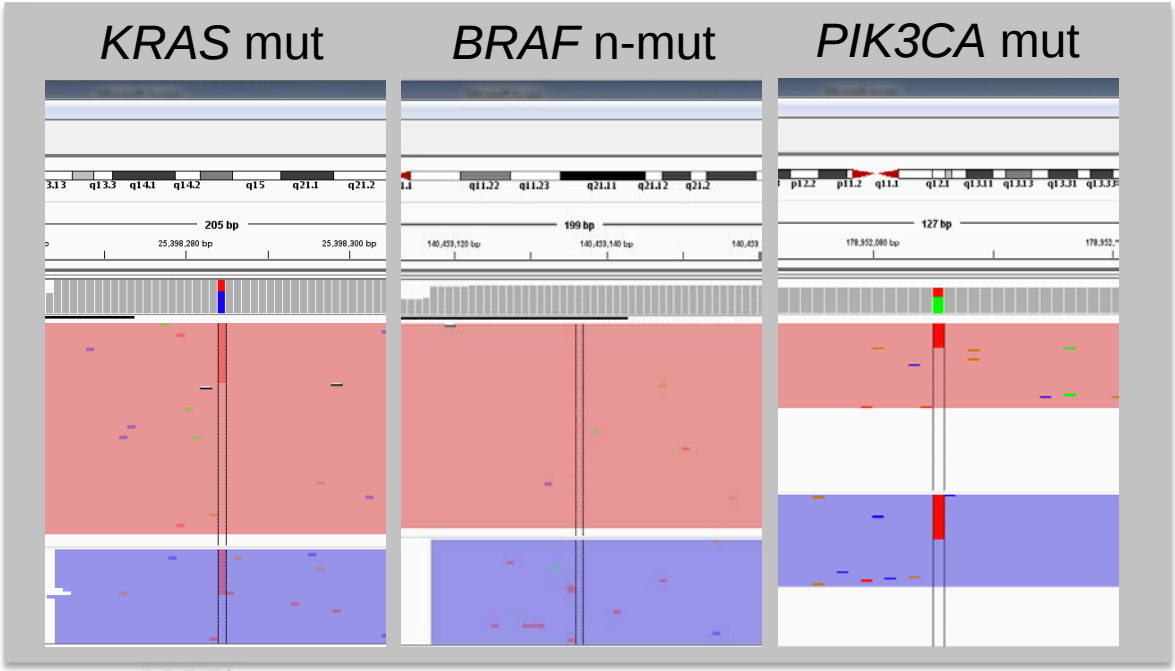
Testing Method	Population Sensitivity of Testing Method, %*	Sensitivity of Assay	Analytical Sensitivity, % (Mutant Allele Fraction)	Specificity, %	PPV, %	NPV, %	Minimal Tumor, %	Concordance Between Assays, %
HRM	59	Increased† (>100)	5-10	98	NR	NR	30	95
Sequencing	36.4	84.4‡	15-20	NR	NR	NR	>50	NR
DxS	43.1	96	1	NR	NR	NR		NR
HRM	42.7	98	3-10	NR	NR	NR		NR
Snapshot	43.3	99	5	NR	NR	NR		NR
DxS	39.4	96.5	1	99.7	99.5	97.2	3-90	95.30
SA	47.0	100	1	100	NR	NR	At least 50	100
MPCR PE	34.0	100	NR§	100	100	100	NR	100
SSCP	36.0	100	NR	100	100	100	NR	100
ASP	40.7	100	1.25-2.5	100	100	100	NR	NR
Pyro	39.0	91	1.25-2.5; 1.25	NR	NR	NR	NR	NR
HRM	54.0	100	1	87	81	100	1-90	NR
ARMS	43	100	5	71	66	100	1-90	93
BMA	45.0	100	1	100	100	100	NR	NR or M
DxS	38.6	95.8	1	100	100	97.3	<30 vs >70	Variable¶
Sanger		98.6	NR	100	100	99.1	NR	NR
SA		92.5-100	NR	100	NR	NR	70	NR
PCR-RFLP		92.5-100	NR	100	NR	NR	NR	NR
DxS	NR	93	0.1-5	100	NR	NR	NR	‖
MCA		97	5-10	100	NR	NR	NR	
DxS	44.4	98	0.50	98	NR	NR	NR	95.9
CADMA		99	NR	100	NR	NR	NR	NR
ASP	36.8	95.7§	1	NR	NR	NR	>50	NR

Sepulveda AR et al.

The Journal of Molecular Diagnostics, Vol. 19, No. 2, March 2017

Next Generation Sequencing (NGS) Targeted Gene Panels

- **There was strong evidence showing that NGS targeted gene panels are able to meet the sensitivity of detection used in CRC clinical trials (detecting at least 5% mutant alleles), with otherwise adequate performing characteristics, and permitting simultaneous testing of hundreds of mutations, including those in extended *RAS*, *BRAF* and *PIK3CA* mutation testing.**



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 (e.g. 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.**

  - **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 in place laboratory protocols 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 (e.g. 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 strong evidence of negative predictors of benefit (mutated *KRAS, NRAS*) to anti-EGFR therapies**
- **There is prognostic value in testing for MMR and *BRAF***
- ***BRAF* is associated with poor outcomes for patients with advanced CRC**

Conclusions, continued

- **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

http://www.amp.org/committees/clinical_practice/AMPclinicalpracticeguidelines/CRCMMGuideline.cfm

http://jmd.amjpathol.org/article/S1525-1578(16)30224-0/fulltext

Save the Date for Upcoming Complimentary CAP PHC Webinars

DATE	TOPIC	SPEAKERS
April 20 11 AM CT	The Cancer Protocols and Changes in Tumor Staging	Thomas P. Baker, MD, FCAP
June 14 11 AM CT	Emerging Concepts on Liquid Biopsy Testing	Abhijit A. Patel, MD, PhD Pranil Chandra, DO, FCAP

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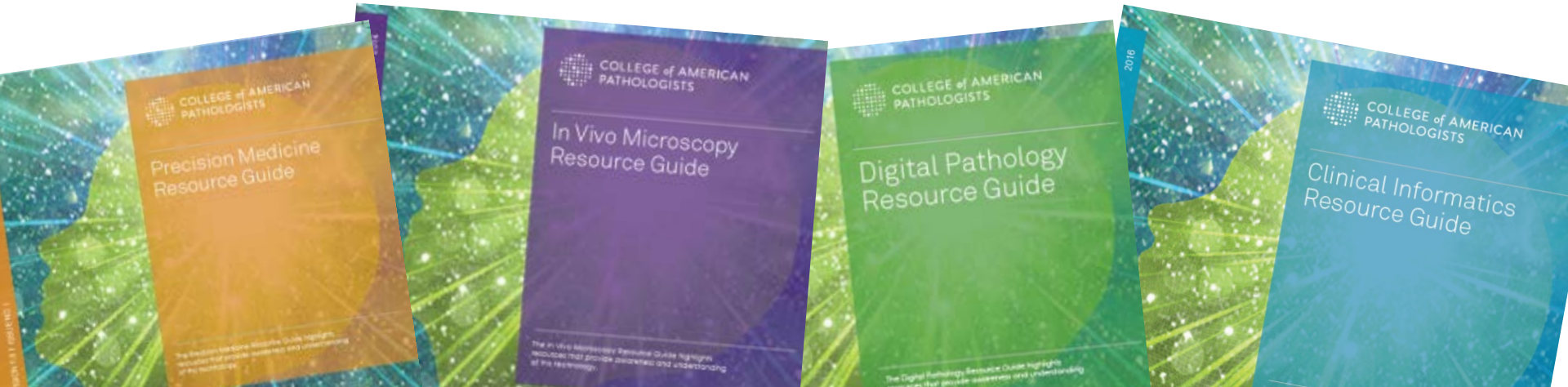
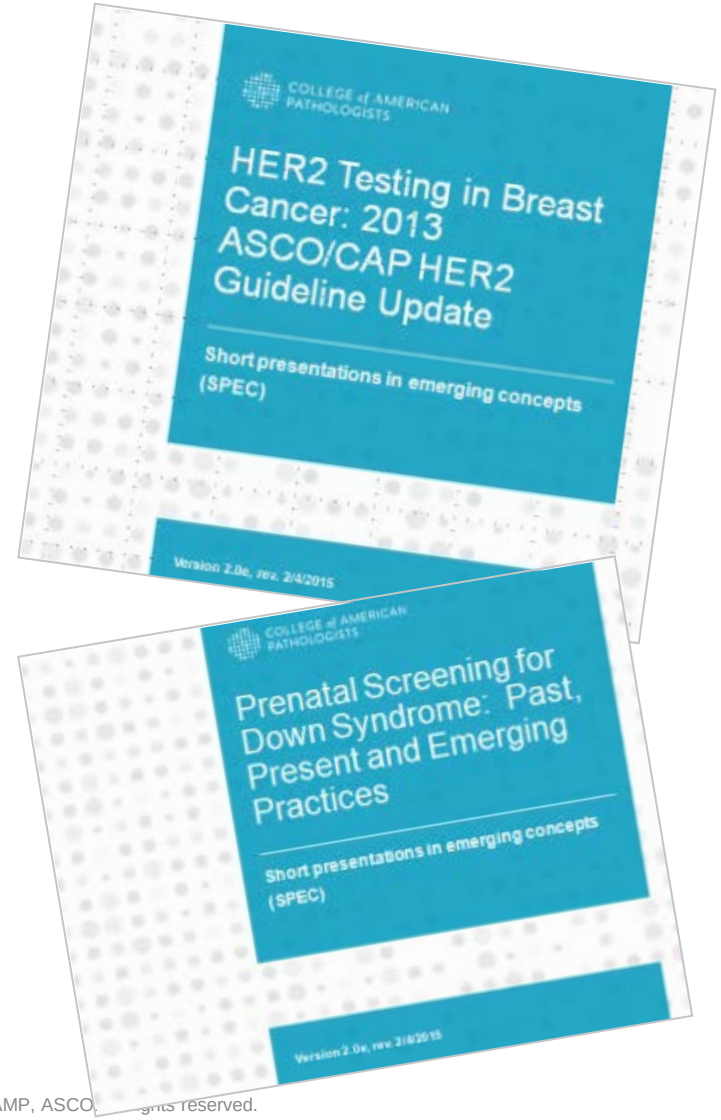
  - **Printed guides are now available for members ($39) and non-members ($69)**
  - **The digital copy of the Resource Guides are a complimentary member benefit**
  - **Access them www.cap.org > Resources and Publications**



Short Presentations on Emerging Concepts (SPECS)

- **Pathology SPECs are:**
  - **short PowerPoints, created for pathologists**
  - **Focused on diseases where molecular tests play a key role in patient management**
- **New topics are Renal Tumors, cell free DNA (cfDNA), and PD-L1 as well as other emerging topics**
- **Access them www.cap.org > Resources and Publications**



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THANK YOU!

- Thank you for attending our webinar, **“Molecular Biomarkers for the Evaluation of Colorectal Cancer: New evidence-based guideline from ASCP, CAP, AMP and ASCO” by Antonia R. Sepulveda, MD, PhD, FCAP**
- For comments about this webinar or suggestions for upcoming webinars, please contact phcwebinars@cap.org.

- **NOTE:** There is no CME/CE credit available for today’s free webinar. The PDF of the presentation will be sent out in a week.

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