

ONCOLOGY: Molecular Biomarkers of Colorectal

Samples to Test: Metastatic or recurrent tumor is preferable if available and adequate*; primary tumor is an acceptable alternative. **Sample Types to Test:** Formalin fixed paraffin embedded tissue (FFPE) or other type of specimens (e.g., cytology). * *Lynch syndrome screening is recommended for all primary colorectal cancers.*

Biomarker	Specific Alterations/ Alternative Names	Indications	Result Interpretation/ Significance	Assay Techniques
KRAS	Mutations in codons 12, 13 of exon 2; codons 59, 61 of exon 3; codons 117, 146 of exon 4	Consideration of anti-EGFR therapy Should be performed in all patients with metastatic CRC	Patients with these mutations should not be treated with anti-EGFR therapy. Significant PFS advantage for adding anti-EGFR therapy for <i>KRAS</i> WT tumors compared to chemotherapy alone	NGS, pyrosequencing, Sanger sequencing, genotyping, PCR-based assays
NRAS	Mutations in codons 12, 13 of exon 2; codons 59, 61 of exon 3; codons 117, 146 of exon 4	Consideration of anti-EGFR therapy Should be performed in all patients with metastatic CRC	Patients with these mutations should not be treated with anti-EGFR therapy	NGS, pyrosequencing, Sanger sequencing, genotyping, PCR-based assays
BRAF	<i>BRAF</i> V600; V600E, V600K	Prognostic stratification	Poorer PFS and OS compared to <i>BRAF</i> WT patients	NGS, pyrosequencing, Sanger sequencing, genotyping, PCR-based assays, IHC
		Consideration of anti-EGFR therapy	Unlikely response to anti-EGFR therapy unless given with a BRAF inhibitor (1)	
		In MMRd tumors with MLH1 loss	Presence of mutation strongly favors sporadic tumor; the presence of BRAF mutations does not exclude the risk of Lynch Syndrome	
HER2	Amplification	Therapy selection	Consideration of anti-HER2 therapy when <i>RAS</i> and <i>BRAF</i> are wildtype (1)	IHC, FISH or NGS
NTRK	Fusions	Therapy selection	Predicts response to NTRK targeted therapy (1)	NGS, pyrosequencing, FISH, IHC, PCR-based assays
MSI/ MMR	Loss of <i>MLH1</i> , <i>PMS2</i> , <i>MSH2</i> , <i>MSH6</i> expression and/or MSI-high status	Lynch syndrome screening, all newly diagnosed patients	Consideration of genetic counseling and germline testing (in the absence of <i>BRAF</i> mutation or <i>MLH1</i> promoter methylation)	IHC, PCR-based assays
	MSI-high	Therapy selection (stage II patients)	Improved prognosis and no benefit from 5-FU adjuvant therapy Consideration of immune checkpoint inhibitor therapy	
MLH1 promoter methylation	Methylation of <i>MLH1</i> promoter	MLH1 loss by IHC	Presence of <i>MLH1</i> promoter methylation in a setting of <i>MLH1</i> loss suggests sporadic origin	Methylation assays

Abbreviations: CRC - colorectal cancer; NGS - next generation sequencing; PFS - progression free survival; OS - overall survival; WT - wild type (non-mutant); MMRd - mismatch repair deficient; MSI - microsatellite instability; IHC - immunohistochemistry

Note: Insufficient evidence to recommend *PIK3CA* mutational analysis for therapy selection outside of clinical trial. Insufficient evidence to recommend *PTEN* testing (IHC or FISH) for therapy selection outside of clinical trial.

Where to Test: Testing should be performed in the laboratories that are certified under Clinical Laboratory Improvement Amendments of 1988 (CLIA-88) as qualified to perform high complexity (molecular pathology) testing.

References:

1. National Comprehensive Cancer Network. Clinical practice Guidelines in Oncology. Colon Cancer. Version 1.2022 – February 25, 2022NCCN.org. accessed 6/27/2022



Prepared by the Association for Molecular Pathology Training and Education Committee
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