Molecular In My Pocket...™

ONCOLOGY: *Molecular Biomarkers of Colorectal Cancer*

Samples to Test: Metastatic or primary tumor can be tested*; studies have shown that mutations are similar in both primary and metastatic CRC. **Sample Types to Test:** Formalin-fixed paraffin embedded tissue (FFPE) or other type of specimens (*e.g.,* cytology, blood). ** Lynch syndrome screening is recommended for all newly diagnosed 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 (cetuximab or panitumumab) either alone or combined with other agents. Significant PFS advantage for adding anti-EGFR therapy for *KRAS* WT tumors compared to chemotherapy alone.	NGS (preferred), 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. Anti-EGFR therapy provides clinical benefit in patients with RAS WT.	NGS (preferred), pyrosequencing, Sanger sequencing, genotyping, PCR-based assays
BRAF	*BRAF* V600; V600E, V600K	Prognostic stratification	Poorer PFS and OS compared to *BRAF* WT patients.	NGS, pyrosequencing, Sanger sequencing, genotyping, PCR-based assays, IHC for V600E
		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; 1% of cancers with V600E mutations (and loss of MLH1) are Lynch Syndrome. Patients with strong family history should not be excluded from germline screening based on *BRAF* V600E mutations.	
HER2	Amplification/Overexpression	Therapy selection	Consideration of anti-HER2 therapy when *RAS* and *BRAF* are WT (1)	IHC, FISH or NGS
***NTRK* (extremely rare in CRC)**	Fusions in tumors that are pan–WT *KRAS, NRAS*, *BRAF* (1)	Therapy selection: *NTRK* inhibitors work in *NTRK* fusions, but not in point mutations.	Predicts response to NTRK targeted therapy. (1) *NTRK* fusions are more frequently found in MMRd tumors (1)	NGS, pyrosequencing, FISH, IHC, PCR-based assays
POLE/POLD1	Germline mutations predispose patients to multiple CRC and adenomas; somatic *POLE* mutations are seen in some patients with MSS/pMMR CRC	Good response to immune checkpoint inhibitor therapy	More favorable prognosis	Sanger sequencing, genotyping, PCR-based assays
RET	Fusions	Therapy selection	*RET* kinase inhibitor (1)	NGS, IHC, PCR-based assays
MSI/MMR	Loss of *MLH1, PMS2, MSH2, MSH6* expression and/or MSI-high status	Lynch syndrome screening, all newly diagnosed patients	Consideration of genetic counseling and germline testing (in the absence of *BRAF* mutation or *MLH1* promoter methylation).	IHC, NGS, 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 *MLH1* promoter	MLH1 loss by IHC	Presence of *MLH1* promoter methylation in a setting of *MLH1* loss suggests sporadic origin.	Methylation assays
TMB	Total amount of somatic coding mutations within a given coding area of the tumor genome. TMB-high is defined as ≥10 mutations/Mb	Therapy selection	Potential biomarker for response to immunotherapy (1) The NCCN Panel does not currently recommend TMB biomarker testing, unless measured as part of a clinical trial. (1)	NGS

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; TMB - Tumor Mutational Burden

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. (1)

***References*:**

1. National Comprehensive Cancer Network. Clinical practice Guidelines in Oncology. Colon Cancer. Version 4.2024 — July 3, 2024 NCCN.org. *Accessed 7/31/2024*

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