### 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
- **KRAS**
  - Mutations in codons 12, 13 of exon 2; codons 59, 61 of exon 3; codons 117, 146 of exon 4
- **NRAS**
  - Mutations in codons 12, 13 of exon 2; codons 59, 61 of exon 3; codons 117, 146 of exon 4
- **BRAF**
  - BRAF V600; V600E, V600K
- **MSI/MMR**
  - Loss of MLH1, PMS2, MSH2, MSH6 expression and/or MSI-high status
  - MSI-high
- **MLH1 promoter methylation**
  - Methylation of MLH1 promoter

### Indications
- **KRAS**
  - Consideration of anti-EGFR therapy
  - Should be performed in all patients with metastatic CRC
- **NRAS**
  - Consideration of anti-EGFR therapy
  - Should be performed in all patients with metastatic CRC
- **BRAF**
  - Prognostic stratification
  - Consideration of anti-EGFR therapy
  - In MMRd tumors with MLH1 loss
- **MSI/MMR**
  - Lynch syndrome screening
  - Therapy selection (stage II patients)
- **MLH1 promoter methylation**
  - Methylation of MLH1 promoter

### Result Interpretation/Significance
- **KRAS**
  - Patients with these mutations should not be treated with panitumumab and cetuximab
  - Significant PFS advantage for adding anti-EGFR therapy for KRAS WT tumors compared to chemotherapy alone
- **NRAS**
  - Patients with these mutations should not be treated with panitumumab and cetuximab
- **BRAF**
  - Poorer PFS and OS compared to BRAF WT patients
  - Unlikely response to panitumumab and cetuximab (2)
  - Insufficient evidence (1)
- **MSI/MMR**
  - Presence of mutation strongly favors sporadic tumor; absence of BRAF mutation does not exclude risk of Lynch syndrome
  - Improved prognosis and no benefit from 5-FU adjuvant therapy
- **MLH1 promoter methylation**
  - Presence of MLH1 promoter methylation in a setting of MLH1 loss suggests sporadic origin

### Assay Techniques
- **KRAS**
  - NGS, pyrosequencing, Sanger sequencing, genotyping, PCR-based assays
- **NRAS**
  - NGS, pyrosequencing, Sanger sequencing, genotyping, PCR-based assays
- **BRAF**
  - NGS, pyrosequencing, Sanger sequencing, genotyping, PCR-based assays
- **MSI/MMR**
  - IHC, PCR-based assays
- **MLH1 promoter methylation**
  - 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