Colorectal Cancer – Predictive Testing for Anti-EGFR Therapy

Indications for Ordering
Indicated for individuals with metastatic colorectal cancer (CRC) to guide treatment with anti-EGFR monoclonal antibodies (cetuximab and panitumumab)

Test Description
MassARRAY – matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) mass spectrometry
- Simultaneous detection of mutations in BRAF, KRAS, NRAS, PIK3CA

Pyrosequencing
- Single gene assays for detection of mutations in BRAF, KRAS, NRAS, PIK3CA

MassARRAY and pyrosequencing mutation detection
- **BRAF** – codon 600
- **KRAS** – codons 12, 13, 61 (MassARRAY also detects codon 146)
- **NRAS** – codons 12, 13, 61
- **PIK3CA** – codons 542, 545, 1047

Next generation sequencing (NGS)
- Extensive coverage of mutations in 44 genes, including **BRAF, KRAS, NRAS, PIK3CA, PTEN**
- Full gene and variant list at [www.arulab.com/ngs-oncology-mutations](http://www.arulab.com/ngs-oncology-mutations)

Tests to Consider

**Primary Tests**

**Solid Tumor Mutation Panel by Next Generation Sequencing 2007991**
- Aids in therapeutic decisions for solid tumor cancers
- Simultaneously evaluates mutations in 44 genes, including **BRAF, KRAS, NRAS, PIK3CA, PTEN**
- Predicts prognosis and therapeutic response in patients with solid tumor cancers

**KRAS Mutation Detection 0040248**
- Predicts response to anti-EGFR and MAPK pathway therapies in a variety of malignancies (eg, CRC and lung cancer)
- Does not cover extended RAS; detects mutations in codons 12, 13, and 61 only

**NRAS Mutation Detection by Pyrosequencing 2003123**
- Predicts response to anti-EGFR and MAPK pathway therapies in a variety of malignancies (eg, melanoma and CRC)
- Does not cover extended RAS; detects mutations in codons 12, 13, and 61 only

**BRAF Codon 600 Mutation Detection by Pyrosequencing 2002498**
- Use to detect activating **BRAF** mutations at codon 600
  - Can indicate resistance to anti-EGFR therapy in CRC
- Also used within the Lynch syndrome reflex testing pathway (for CRC specimens only)

**Related Tests**

**KRAS Mutation Detection with Reflex to BRAF Codon 600 Mutation Detection 2001932**
- Determine eligibility for anti-EGFR (cetuximab and panitumumab) therapy in patients with metastatic CRC

**BRAF V600E Mutation Detection in Circulating Cell-Free DNA by Digital Droplet PCR 2013921**
- Determines **BRAF** V600E mutation status in patients with solid tumors to select candidates for targeted therapy with kinase inhibitors (BRAF and/or MEK)
- Monitors response to therapy and disease progression in patients carrying **BRAF** V600E mutation
### Disease Overview

- CRC is one of the most commonly diagnosed malignancies worldwide
- EGFR represents an important therapeutic target in advanced CRC

### Genetics and Test Interpretation

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutations</th>
<th>Sensitivity/Specificity</th>
<th>Results</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>KRAS</strong> GTPase-encoding gene in the RAS/RAF/MAPK pathway</td>
<td>Majority of oncogenic mutations – codons 12 and 13 (&gt;90%) Most of the remaining activating mutations – codons 61 and 146</td>
<td>Clinical sensitivity – activating KRAS mutations found in ~40% of CRCs Analytic sensitivity/ specificity – 100%</td>
<td>Positive • Oncogenic KRAS mutation detected • Lack of response to therapy with antibodies targeted to EGFR is predicted</td>
<td>Limit of detection • MassARRAY and pyrosequencing – 10% mutant alleles • NGS – 5% mutant alleles • MassARRAY – oncogenic mutations outside of codons 12, 13, 61, 146 will not be detected • Pyrosequencing – oncogenic mutations outside of codons 12, 13, 61 will not be detected • A substantial portion of individuals with wild type KRAS still fail to respond to anti-EGFR agents, implicating downstream mutations</td>
</tr>
<tr>
<td><strong>BRAF</strong> Kinase-encoding gene in the RAS/RAF/MAPK pathway</td>
<td>Majority of activating mutations – codon 600 Mutually exclusive with KRAS mutations in individuals with CRC</td>
<td>Clinical sensitivity – activating BRAF mutation found in ~10% of CRCs Analytic sensitivity/ specificity – 100%</td>
<td>Positive • Oncogenic BRAF mutation detected • Available data suggest resistance to anti-EGFR therapy • Appears to be associated with a worse prognosis</td>
<td>Limit of detection • MassARRAY and pyrosequencing – 10% mutant alleles • NGS – 5% mutant alleles • MassARRAY and pyrosequencing – oncogenic mutations outside of codon 600 will not be detected</td>
</tr>
<tr>
<td><strong>PIK3CA</strong> Encodes a subunit of the PI3K protein</td>
<td>Exon 9 gain-of-function mutation (codons 542 or 545) requires interaction with RAS • May have no effect on cetuximab therapy Exon 20 (codon 1047) mutation is independent of RAS binding • Appears to have a negative effect on response to cetuximab</td>
<td>Clinical sensitivity – oncogenic PIK3CA mutation found in 10-20% of CRCs, mostly exons 9 (60-65%) or 20 (20-25%) Analytic sensitivity/ specificity – 100%</td>
<td>Positive • Oncogenic PIK3CA mutation detected • Tumor may respond to therapies targeted at genes downstream of PI3K in the AKT/mTOR signaling cascade • Exon 20 (kinase domain) mutations may indicate resistance to anti-EGFR therapy in wild type KRAS tumors • Negative impact on the prognosis of advanced CRC</td>
<td>Limit of detection • MassARRAY and pyrosequencing – 10% mutant alleles • NGS – 5% mutant alleles • MassARRAY and pyrosequencing – oncogenic mutations outside of codons 542, 545, 1047 will not be detected</td>
</tr>
<tr>
<td><strong>PTEN</strong> Tumor suppressor gene in the PI3K/AKT pathway</td>
<td>Loss has been predicted to show resistance to anti-EGFR therapy</td>
<td>Clinical sensitivity – loss of expression found in up to 50% of CRCs</td>
<td>Abnormal • Possible resistance to anti-EGFR therapy Normal • PTEN expression is intact</td>
<td>-</td>
</tr>
<tr>
<td>Gene Rearrangements by FISH</td>
<td>2011431</td>
<td>ALK (D5F3) by Immunohistochemistry with Reflex to FISH if Equivocal or Positive</td>
<td>2008414</td>
<td></td>
</tr>
<tr>
<td>-----------------------------</td>
<td>---------</td>
<td>--------------------------------------------------------------------------------</td>
<td>---------</td>
<td></td>
</tr>
<tr>
<td>EGFR Mutations Detection by Pyrosequencing</td>
<td>2002440</td>
<td>ALK, EGFR, and ROS1 fusion proteins</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Detects overexpression of c-MET protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aids in prognostic and therapeutic decisions for neoplasms where amplification has been demonstrated</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Screening test for MET gene amplification</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>RET Gene Rearrangements by FISH</td>
<td>3001312</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Detects RET gene rearrangements in solid tumors</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Does not identify the translocation partner or variant</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ROS1 by FISH</td>
<td>3001308</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Detects ROS1 gene rearrangements in solid tumors</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Does not identify the translocation partner or variant</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ROS1 with Interpretation by Immunohistochemistry with Reflex to FISH if Equivocal or Positive</td>
<td>2008414</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Detects ROS1 fusion proteins and ROS1 gene rearrangements</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Screening for immune checkpoint inhibitor therapy FDA-approved PD-L1 companion tests</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>For more information about PD-L1 testing, refer to the ARUP Consult PD-L1 Testing topic and algorithm.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PD-L1 22C3 IHC, with Tumor Proportion Score (TPS) Interpretation, pembrolizumab (KEYTRUDA)</td>
<td>2013284</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Companion diagnostic test to aid in prediction of response to pembrolizumab (KEYTRUDA) as first-line monotherapy for patients with non-small cell lung cancer (NSCLC)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Can be performed in conjunction with or instead of PD-L1 28-8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>For NSCLC specimens only</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>For gastroesophageal junction (GEJ), urothelial, cervical, or head and neck squamous cell carcinoma (HNSCC) specimens, see PD-L1 22C3 IHC with Combined Positive Score (CPS) Interpretation, pembrolizumab (KEYTRUDA)</td>
<td>3000197</td>
<td></td>
</tr>
</tbody>
</table>

### Lung Cancer Molecular Markers

#### Indications for Ordering

Mutation testing to aid in selection of tyrosine kinase inhibitor (TKI) and/or immune checkpoint inhibitor therapy

#### Tests to Consider

**Typical Testing Strategy**
Minimum initial recommendation – lung cancer panel that includes simultaneous ordering for mutations in ALK, EGFR, and ROS1 genes

**Primary Tests**
Determine eligibility for TKI therapy (panel tests)

**Lung Cancer Panel 2008894**
- Screening panel detects
  - EGFR mutations
  - ALK and ROS1 fusion proteins

**Lung Cancer Panel with KRAS 2008895**
- Screening panel detects
  - EGFR and KRAS mutations
  - ALK and ROS1 fusion proteins

Determine eligibility for TKI therapy (single tests)

**ALK (DSF3) with Interpretation by Immunohistochemistry 2007324**
- Detects ALK fusion proteins

**ALK (DSF3) by Immunohistochemistry with Reflex to ALK Gene Rearrangements by FISH 2011431**
- Detects ALK fusion proteins and ALK gene rearrangements in solid tumors

**ALK Gene Rearrangements by FISH, Lung 3001302**
- Screening test for all ALK fusions
  - Use this test if the companion diagnostic test for crizotinib is required
  - Does not identify the translocation partner or variant

**KRAS Mutation Detection 0040248**
- Predicts response to anti-EGFR and MAPK pathway therapies in a variety of malignancies (eg, colorectal and lung cancer)

**c-MET by Immunohistochemistry 2008652**
- Detects overexpression of c-MET protein

**MET Gene Amplification by FISH 3001313**
- Aids in prognostic and therapeutic decisions for neoplasms where amplification has been demonstrated
- Screening test for MET gene amplification

**RET Gene Rearrangements by FISH 3001312**
- Detects RET gene rearrangements in solid tumors
- Does not identify the translocation partner or variant

**ROS1 by FISH 3001308**
- Detects ROS1 gene rearrangements in solid tumors
- Does not identify the translocation partner or variant

**ROS1 with Interpretation by Immunohistochemistry with Reflex to FISH if Equivocal or Positive 2008414**
- Detects ROS1 fusion proteins and ROS1 gene rearrangements

Screening for immune checkpoint inhibitor therapy FDA-approved PD-L1 companion tests

For more information about PD-L1 testing, refer to the ARUP Consult PD-L1 Testing topic and algorithm.

**PD-L1 22C3 IHC, with Tumor Proportion Score (TPS) Interpretation, pembrolizumab (KEYTRUDA) 2013284**
- Companion diagnostic test to aid in prediction of response to pembrolizumab (KEYTRUDA) as first-line monotherapy for patients with non-small cell lung cancer (NSCLC)
- Can be performed in conjunction with or instead of PD-L1 28-8
- For NSCLC specimens only
  - For gastroesophageal junction (GEJ), urothelial, cervical, or head and neck squamous cell carcinoma (HNSCC) specimens, see PD-L1 22C3 IHC with Combined Positive Score (CPS) Interpretation, pembrolizumab (KEYTRUDA) 3000197
PD-L1 28-8 pharmDx by Immunohistochemistry with Interpretation, nivolumab (OPDIVO) 2013684

- FDA-approved complementary codiagnostic test to aid in prediction of response to nivolumab (OPDIVO) for patients with non-squamous NSCLC, melanoma, urothelial carcinoma, and HNSCC

Monitor for EGFR T790M resistance

**EGFR T790M Mutation Detection in Circulating Tumor DNA by Digital Droplet PCR**

- Monitor blood plasma or cerebrospinal fluid (CSF) for
  - Development of EGFR T790M drug-resistant mutation in patients administered TKI therapy for EGFR-mutant NSCLC
  - Response to therapy and disease progression in patients receiving EGFR T790M-specific TKIs

Related Test

**Solid Tumor Mutation Panel by Next Generation Sequencing 2007991**

- Aids in therapeutic decisions for solid tumor cancers
- Simultaneously evaluates mutations in multiple genes, including AKT1, ALK, BRAF, EGFR, ERBB2, ERBB4, KRAS, NRAS, and PIKC3CA
- Does not detect translocations

Test Methodology

**ALK**
- Immunohistochemistry (IHC) using ALK clone D5F3
- Fluorescence in situ hybridization (FISH)

**EGFR** – polymerase chain reaction (PCR) and pyrosequencing
- Detects mutations at codons 719 (exon 18), 768 and 790 (exon 20), 858 and 861 (exon 21)
- Detects deletions in exon 19

**EGFR T790M (serum)** – digital droplet PCR

**KRAS** – PCR and pyrosequencing
- Detects mutations at codons 12, 13 (exon 2), and 61 (exon 3)

**c-MET** – IHC

**MET** – FISH

**PD-L1** – IHC

**RET** – FISH

- Detects all RET gene fusions

ROS1 – IHC (using ROS1 clone D4D6) with FISH reflex if equivocal

**Test Interpretation**

**Results**

Single gene testing (includes genes in panels) – see table

**Limitations**

- Results must be interpreted in the context of clinical findings and morphological and other relevant data
- Results may be compromised if the recommended tissue-fixation procedures have not been followed

**Disease Overview**

**Incidence**

Lung cancer is the second most common cancer in U.S.

**Treatment Issues**

- Lung cancer has poor response to traditional chemotherapy agents
  - Dismal 5-year outcome when using these agents
  - Newer targeted agents have better response rates in specific individuals and tumor types
- Tumor response rates to targeted therapy are closely associated with mutation status of the tumor, especially for adenocarcinoma or mixed adenocarcinoma subtypes
- Mutation status
  - Determines TKI therapy eligibility
  - Confers resistance to TKIs (e.g., EGFR T790M mutation)
  - Monitoring in serum for EGFR T790M mutation may detect TKI resistance sooner and alter treatment plans
- PD-L1 expression
  - May predict response to immune checkpoint inhibitor therapy

**References**

- Francis G, Stein S. Circulating cell-free tumour DNA in the management of cancer. Int J Mol Sci. 2015:16;14122-14142

<table>
<thead>
<tr>
<th>Gene</th>
<th>Testing method</th>
<th>Test result</th>
<th>Test result</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALK</td>
<td>IHC</td>
<td>Positive – uniform membranous staining in tumor cells</td>
<td>May predict response to TKI therapy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Equivocal – very weak cytoplasmic staining visible only on higher power by microscopy</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative – no cytoplasmic staining in tumor cells</td>
<td></td>
</tr>
<tr>
<td>FISH</td>
<td></td>
<td>Positive – ALK gene rearrangements detected in ≥15% of nuclei</td>
<td>May predict response to TKI therapy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Does not identify translocation partner</td>
<td></td>
</tr>
</tbody>
</table>
## Single Gene

<table>
<thead>
<tr>
<th>Gene</th>
<th>Testing method</th>
<th>Test result</th>
<th>Additional Information</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EGFR</strong></td>
<td>PCR/pyrosequencing</td>
<td>Positive – mutation detected</td>
<td>May predict response to TKI therapy</td>
</tr>
<tr>
<td></td>
<td><strong>EGFR T790M (serum)</strong> Digital droplet PCR</td>
<td>Positive – mutation detected</td>
<td>Predicts resistance to TKI therapy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Expressed as percentage</td>
<td></td>
</tr>
<tr>
<td><strong>KRAS</strong></td>
<td>PCR/pyrosequencing</td>
<td>Positive – mutation detected</td>
<td>May predict response to TKI therapy</td>
</tr>
</tbody>
</table>
| **MET**   | FISH            | Positive – detects gene amplification | • May predict response to crizotinib TKI therapy  
|           |                 |                                  | • Associated with acquired resistance to EGFR inhibitors in 5-20% of patients with EGFR-mutated tumors |