See below for Additional Technical Information topics

Colorectal Cancer – Predictive Testing for Anti-EGFR Therapy

Lung Cancer Molecular Markers

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.aruplab.com/ngs-oncology-mutations](http://www.aruplab.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 (e.g., 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 (e.g., 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>KRAS</td>
<td>Majority of oncogenic mutations – codons 12 and 13 (&gt;90%)</td>
<td>Clinical sensitivity – activating KRAS mutations found in ~40% of CRCs</td>
<td>Positive</td>
<td>Limit of detection</td>
</tr>
<tr>
<td></td>
<td>Most of the remaining activating mutations – codons 61 and 146</td>
<td>Analytic sensitivity/specificity – 100%</td>
<td>Oncogenic KRAS mutation detected</td>
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<td></td>
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<td></td>
<td>Lack of response to therapy with antibodies targeted to EGFR is predicted</td>
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<td></td>
<td>No oncogenic KRAS mutation detected</td>
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<td></td>
<td></td>
<td></td>
<td>Follow-up BRAF testing is advised prior to initiation of anti-EGFR therapy</td>
<td></td>
</tr>
<tr>
<td>BRAF</td>
<td>Majority of activating mutations – codon 600</td>
<td>Clinical sensitivity – activating BRAF mutation found in ~10% of CRCs</td>
<td>Positive</td>
<td>Limit of detection</td>
</tr>
<tr>
<td></td>
<td>Mutually exclusive with KRAS mutations in individuals with CRC</td>
<td>Analytic sensitivity/specificity – 100%</td>
<td>Oncogenic BRAF mutation detected</td>
<td></td>
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<td></td>
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<td></td>
<td>Available data suggest resistance to anti-EGFR therapy</td>
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<td></td>
<td>Appears to be associated with a worse prognosis</td>
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<td></td>
<td></td>
<td></td>
<td>No oncogenic BRAF mutation detected</td>
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<tr>
<td>PIK3CA</td>
<td>Exon 9 gain-of-function mutation (codons 542 or 545) requires interaction</td>
<td>Clinical sensitivity – oncogenic PIK3CA mutation found in 10-20% of CRCs, mostly</td>
<td>Positive</td>
<td>Limit of detection</td>
</tr>
<tr>
<td></td>
<td>with KRAS</td>
<td>exons 9 (60-65%) or 20 (20-25%)</td>
<td>Oncogenic PIK3CA mutation detected</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• May have no effect on cetuximab therapy</td>
<td></td>
<td>o Tumor may respond to therapies targeted at genes downstream of PIK3 in</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Exon 20 (codon 1047) mutation is independent of RAS binding</td>
<td></td>
<td>the AKT/mTOR signaling cascade</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Appears to have a negative effect on response to cetuximab</td>
<td></td>
<td>o Exon 20 (kinase domain) mutations may indicate resistance to anti-EGFR</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>therapy in wild type KRAS tumors</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>o Negative impact on the prognosis of advanced CRC</td>
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<td></td>
<td></td>
<td></td>
<td>No oncogenic PIK3CA mutation detected</td>
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<tr>
<td>PTEN</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</td>
<td>Limit of detection</td>
</tr>
<tr>
<td>Tumor suppressor gene in the PI3K/AKT pathway</td>
<td></td>
<td></td>
<td>Possible resistance to anti-EGFR therapy</td>
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<td></td>
<td></td>
<td></td>
<td>Normal</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>PTEN expression is intact</td>
<td>MassARRAY and pyrosequencing – oncogenic mutations outside of codons 542, 545, 1047 will not be detected</td>
</tr>
<tr>
<td>Gene Rearrangements by FISH 2011431</td>
<td>ALK (D5F3) by Immunohistochemistry with Reflex to FISH if Equivocal or Positive 2008414</td>
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<td>------------------------------------------------------------------------------------------------</td>
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<tr>
<td>Majority of activating mutations – codon 61</td>
<td>Clinical sensitivity – oncogenic NRAS mutation found in ~3% of CRCs</td>
<td></td>
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<tr>
<td>Mutually exclusive with KRAS mutations in individuals with CRC</td>
<td>Analytic sensitivity/specificity – 100%</td>
<td></td>
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<tr>
<td>Associated with relative resistance to anti-EGFR therapy</td>
<td>Positive</td>
<td></td>
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<tr>
<td></td>
<td>Oncogenic NRAS mutation detected</td>
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<tr>
<td></td>
<td>Predictive of relative resistance to anti-EGFR therapy</td>
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<tr>
<td></td>
<td>No oncogenic NRAS mutation detected</td>
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<tr>
<td></td>
<td>Limit of detection</td>
<td></td>
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<tr>
<td></td>
<td>MassARRAY and pyrosequencing – 10% mutant alleles</td>
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<td>NGS – 5% mutant alleles</td>
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<tr>
<td></td>
<td>MassARRAY and pyrosequencing – oncogenic mutations outside of codons 12, 13, 61 will not be detected</td>
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<tr>
<td></td>
<td>Presence or absence of mutations does not guarantee a response or lack of response to anti-EGFR therapy</td>
<td></td>
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</tr>
</tbody>
</table>

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**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

**EGFR Mutation Detection by Pyrosequencing 2002440**

**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 prognostication 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- or second-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, and cervical specimens, see PD-L1 22C3 IHC with Combined Positive Score (CPS) Interpretation, pembrolizumab (KEYTRUDA) 3000197

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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 nonsquamous NSCLC, melanoma, urothelial carcinoma, and head and neck squamous cell carcinoma (HNSCC)

**Monitor for EGFR T790M resistance**
EGFR T790M Mutation Detection in Circulating Tumor DNA by Digital Droplet PCR 2012868
- 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 (eg, 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>May predict response to TKI therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ALK</strong></td>
<td>IHC</td>
<td>Positive – uniform membranous staining in tumor cells</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Equivocal – very weak cytoplasmic staining visible only on higher power by microscopy</td>
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<td></td>
<td></td>
<td>Negative – no cytoplasmic staining in tumor cells</td>
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<td></td>
<td></td>
<td>Detects all ALK gene rearrangements detected in ≥15% of nuclei</td>
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<td></td>
<td></td>
<td>Does not identify translocation partner</td>
<td></td>
</tr>
</tbody>
</table>

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