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
### 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></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 – MassARRAY and pyrosequencing – 10% mutant alleles</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>
<td>o NGS – 5% mutant alleles</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>• Lack of response to therapy with antibodies targeted to EGFR is predicted</td>
<td>MassARRAY – oncogenic mutations outside of codons 12, 13, 61, 146 will not be detected</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• No oncogenic KRAS mutation detected</td>
<td>Pyrosequencing – oncogenic mutations outside of codons 12, 13, 61 will not be detected</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Follow-up BRAF testing is advised prior to initiation of anti-EGFR therapy</td>
<td>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></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</td>
<td>Positive</td>
<td>Limit of detection – MassARRAY and pyrosequencing – 10% mutant alleles</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Analytic sensitivity/ specificity – 100%</td>
<td>• Oncogenic BRAF mutation detected</td>
<td>o NGS – 5% mutant alleles</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>• Available data suggest resistance to anti-EGFR therapy</td>
<td>MassARRAY and pyrosequencing – oncogenic mutations outside of codon 600 will not be detected</td>
</tr>
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<td></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>Negative</td>
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<td></td>
<td></td>
<td></td>
<td>• No oncogenic BRAF mutation detected</td>
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</tr>
<tr>
<td><strong>PIK3CA</strong></td>
<td>Exon 9 gain-of-function mutation (codons 542 or 545) requires interaction with RAS</td>
<td>Clinical sensitivity – oncogenic PIK3CA mutation found in 10-20% of CRCs, mostly exons 9 (60-65%) or 20 (20-25%)</td>
<td>Positive</td>
<td>Limit of detection – MassARRAY and pyrosequencing – 10% mutant alleles</td>
</tr>
<tr>
<td></td>
<td>• May have no effect on cetuximab therapy</td>
<td>Analytic sensitivity/ specificity – 100%</td>
<td>• Oncogenic PIK3CA mutation detected</td>
<td>o NGS – 5% mutant alleles</td>
</tr>
<tr>
<td></td>
<td>• Exon 20 (codon 1047) mutation is independent of RAS binding</td>
<td></td>
<td>o Tumor may respond to therapies targeted at genes downstream of PI3K in the AKT/mTOR signaling cascade</td>
<td>MassARRAY and pyrosequencing – oncogenic mutations outside of codons 542, 545, 1047 will not be detected</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 therapy in wild type KRAS tumors</td>
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</tr>
<tr>
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<td>o Negative impact on the prognosis of advanced CRC</td>
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<td></td>
<td>Negative</td>
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<td></td>
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<td></td>
<td>• No oncogenic PIK3CA mutation detected</td>
<td></td>
</tr>
<tr>
<td><strong>PTEN</strong></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 – MassARRAY and pyrosequencing – 10% mutant alleles</td>
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<tr>
<td></td>
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<td></td>
<td>• Possible resistance to anti-EGFR therapy</td>
<td>o NGS – 5% mutant alleles</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>Normal</td>
<td>MassARRAY and pyrosequencing – oncogenic mutations outside of codons 542, 545, 1047 will not be detected</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• PTEN expression is intact</td>
<td></td>
</tr>
<tr>
<td>Gene</td>
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<td>-----------------------------------------------------------------------------</td>
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</tbody>
</table>
| NRAS                 | Majority of activating mutations – codon 61 | Clinical sensitivity – oncogenic NRAS mutation found in ~3% of CRCs | Positive                        | Limit of detection  
  - MassARRAY and pyrosequencing – 10% mutant alleles  
  - NGS – 5% mutant alleles  
  - MassARRAY and pyrosequencing – oncogenic mutations outside of codons 12, 13, 61 will not be detected  
  - Presence or absence of mutations does not guarantee a response or lack of response to anti-EGFR therapy |
| GTPase-encoding gene in the RAS/RAF/MAPK pathway | Mutually exclusive with KRAS mutations in individuals with CRC  
  - Associated with relative resistance to anti-EGFR therapy | Analytic sensitivity/ specificity – 100% |                      | |

### 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 2006102**
  - 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 2012654**
    - Aids in prognostication and therapeutic decisions for neoplasms where amplification has been demonstrated  
    - Screening test for MET gene amplification
  - **RET Gene Rearrangements by FISH 2013082**
    - Detects RET gene rearrangements in solid tumors  
    - Does not identify the translocation partner or variant

- **ROS1 by FISH 2008418**
  - Detects ROS1 gene rearrangements in solid tumors  
  - Does not identify the translocation partner or variant

- **ROS 1 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

- **PD-L1 22C3 IHC for NSCLC with 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  
    - Use this test if the companion diagnostic test for crizotinib is required  
    - Does not identify the translocation partner or variant

  - **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 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

| Gene | Testing method | Test result | Gene
| --- | --- | --- | --- |
| ALK | IHC | Positive – uniform membranous staining in tumor cells | RET – FISH
| | | Equivocal – very weak cytoplasmic staining visible only on higher power by microscopy | Detects all RET gene fusions
| | | Negative – no cytoplasmic staining in tumor cells | ROS1 – IHC (using ROS1 clone D4D6) with FISH reflex if equivocal
| | | May predict response to TKI therapy | **May predict response to TKI therapy**
| | | | **May predict response to TKI therapy**
| | | | **May predict response to TKI therapy**
| FISH | Positive – ALK gene rearrangements detected in ≥15% of nuclei | C-MET – IHC
| | Does not identify translocation partner | | **May predict response to TKI therapy**

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<table>
<thead>
<tr>
<th>Gene</th>
<th>Testing method</th>
<th>Test result</th>
<th>Additional information</th>
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<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><em>EGFR T790M (serum)</em> Digital droplet PCR</td>
<td>Positive – mutation detected • Expressed as percentage</td>
<td>Predicts resistance to TKI therapy</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>
<tr>
<td><strong>MET</strong></td>
<td>FISH</td>
<td>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</td>
<td></td>
</tr>
</tbody>
</table>