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

Tests to Consider

Primary test
Colon Cancer Gene Panel, Somatic 2011616
- Use for individuals with metastatic CRC to guide treatment with anti-EGFR monoclonal antibodies (cetuximab and panitumumab)
- Detects mutations in BRAF, KRAS, NRAS, extended KRAS, and PIK3CA

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

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

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)

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

NRAS Mutation Detection by Pyrosequencing 2003123
- Predicts response to anti-EGFR and MAPK pathway therapies in a variety of malignancies (eg, melanoma and CRC)
**Disease Overview**

- CRC is 1 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></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&lt;br&gt; 0 MassARRAY and pyrosequencing – 10% mutant alleles&lt;br&gt; 0 NGS – 5% mutant alleles&lt;br&gt; 0 MassARRAY ovonogenic mutations outside of codons 12, 13, 61, 146 will not be detected&lt;br&gt; 0 Pyrosequencing – oncogenic mutations outside of codons 12, 13, 61 will not be detected&lt;br&gt; 0 A substantial portion of individuals with wild type KRAS still fail to respond to anti-EGFR agents, implicating downstream mutations</td>
</tr>
<tr>
<td>GTPase-encoding gene in the RAS/RAF/MAPK pathway</td>
<td>Most of the remaining activating mutations – codons 61 and 146</td>
<td>Analytic sensitivity/ specificity – 100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BRAF</strong></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&lt;br&gt; 0 MassARRAY and pyrosequencing – 10% mutant alleles&lt;br&gt; 0 NGS – 5% mutant alleles&lt;br&gt; 0 MassARRAY and pyrosequencing – oncogenic mutations outside of codon 600 will not be detected</td>
</tr>
<tr>
<td>Kinase-encoding gene in the RAS/RAF/MAPK pathway</td>
<td>Mutually exclusive with KRAS mutations in individuals with CRC</td>
<td>Analytic sensitivity/ specificity – 100%</td>
<td></td>
<td></td>
</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&lt;br&gt; 0 MassARRAY and pyrosequencing – 10% mutant alleles&lt;br&gt; 0 NGS – 5% mutant alleles&lt;br&gt; 0 MassARRAY and pyrosequencing – oncogenic mutations outside of codons 542, 545, 1047 will not be detected</td>
</tr>
<tr>
<td>Encodes a subunit of the PI3K protein</td>
<td>Most of the remaining activating mutations – codons 61 and 146</td>
<td>Analytic sensitivity/ specificity – 100%</td>
<td></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&lt;br&gt; 0 MassARRAY and pyrosequencing – 10% mutant alleles&lt;br&gt; 0 NGS – 5% mutant alleles&lt;br&gt; 0 MassARRAY and pyrosequencing – oncogenic mutations outside of codons 542, 545, 1047 will not be detected</td>
</tr>
<tr>
<td>Tumor suppressor gene in the PI3K/AKT pathway</td>
<td></td>
<td></td>
<td>Abnormal</td>
<td></td>
</tr>
</tbody>
</table>

© 2013 ARUP LABORATORIES | Content Review April 2018 | Last Update April 2018
### 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

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutations</th>
<th>Sensitivity/Specificity</th>
<th>Results</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRAS GTPase-encoding gene in the RAS/RAF/MAPK pathway</td>
<td>Majority of activating mutations – codon 61 Mutually exclusive with KRAS mutations in individuals with CRC Associated with relative resistance to anti-EGFR therapy</td>
<td>Clinical sensitivity – oncogenic NRAS mutation found in ~3% of CRCs Analytic sensitivity/specificity – 100%</td>
<td>Positive • Oncogenic NRAS mutation detected • Predictive of relative resistance to anti-EGFR therapy Negative • No oncogenic NRAS mutation detected</td>
<td>• 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</td>
</tr>
</tbody>
</table>

<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 GTPase-encoding gene in the RAS/RAF/MAPK pathway</td>
<td>Majority of activating mutations – codon 61 Mutually exclusive with KRAS mutations in individuals with CRC Associated with relative resistance to anti-EGFR therapy</td>
<td>Clinical sensitivity – oncogenic KRAS mutation found in ~15% of CRCs Analytic sensitivity/specificity – 100%</td>
<td>Positive • Oncogenic KRAS mutation detected • Predictive of relative resistance to anti-EGFR therapy Negative • No oncogenic KRAS mutation detected</td>
<td>• 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</td>
</tr>
</tbody>
</table>

#### 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 20113082

- Aids in prognostication and therapeutic decisions for neoplasms where amplification has been demonstrated
- Screening test for MET gene amplification

#### RET Gene Rearrangements by FISH 2012654

- 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

#### 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

#### PD-L1 22C3 IHC for NSCLC with Interpretation, pembrolizumab (KEYTRUDA) 2013284

- Aid in prediction of response to pembrolizumab (KEYTRUDA) as first- or second-line therapy for patients with nonsmall cell lung cancer (NSCLC)
- Use for NSCLC specimens only
- Can be performed in conjunction with or instead of PD-L1 28-8
- For gastroesophageal junction (GEJ) specimens, see PD-L1 22C3 IHC with Combined Positive Score (CPS) Interpretation, pembrolizumab (KEYTRUDA) 3000197

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutations</th>
<th>Sensitivity/Specificity</th>
<th>Results</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR GTPase-encoding gene in the RAS/RAF/MAPK pathway</td>
<td>Majority of activating mutations – codon 61 Mutually exclusive with KRAS mutations in individuals with CRC Associated with relative resistance to anti-EGFR therapy</td>
<td>Clinical sensitivity – oncogenic EGFR mutation found in ~15% of CRCs Analytic sensitivity/specificity – 100%</td>
<td>Positive • Oncogenic EGFR mutation detected • Predictive of relative resistance to anti-EGFR therapy Negative • No oncogenic EGFR mutation detected</td>
<td>• 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</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutations</th>
<th>Sensitivity/Specificity</th>
<th>Results</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALK (D5F3) with Interpretation by Immunohistochemistry 2007324</td>
<td>Detects ALK fusion proteins</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutations</th>
<th>Sensitivity/Specificity</th>
<th>Results</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALK (D5F3) by Immunohistochemistry with Reflex to ALK Gene Rearrangements by FISH 2011431</td>
<td>Detects ALK fusion proteins and ALK gene rearrangements in solid tumors</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutations</th>
<th>Sensitivity/Specificity</th>
<th>Results</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALK Gene Rearrangements by FISH, Lung 2006102</td>
<td>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</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutations</th>
<th>Sensitivity/Specificity</th>
<th>Results</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR Mutation Detection by Pyrosequencing 2002440</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
PD-L1 28-8 pharmDx by Immunohistochemistry with Interpretation, nivolumab (OPDIVO) 2013684
- Aid in prediction of response to nivolumab for patients with nonsquamous NSCLC or melanoma
- Can be performed in conjunction with or instead of PD-L1 22C3

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>ALK</td>
<td>IHC</td>
<td>Positive – uniform membranous staining in tumor cells</td>
<td></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></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Does not identify translocation partner</td>
<td></td>
</tr>
</tbody>
</table>
## Single Gene Testing

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