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
Colorectal 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

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 therapeutically in patients with solid tumor cancers

KRAS Mutation Detection with Reflex to BRAF Codon 600 Mutation Detection 2001392
- 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
- 2 anti-EGFR monoclonal antibodies (cetuximab and panitumumab) are available for treatment of advanced CRC
- KRAS, BRAF, and possibly PIK3CA, PTEN, and NRAS mutations are associated with resistance to anti-EGFR therapy

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</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 MassARRAY and pyrosequencing – 10% mutant alleles</td>
</tr>
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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>
<td>o NGS – 5% mutant alleles</td>
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<td></td>
<td>Follow-up BRAF testing is advised prior to initiation of anti-EGFR therapy</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></td>
<td>Pyrosequencing – oncogenic mutations outside of codons 12, 13, 61 will not be detected</td>
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<td></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</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>o MassARRAY and pyrosequencing – 10% mutant alleles</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Available data suggest resistance to anti-EGFR therapy</td>
<td>o NGS – 5% mutant alleles</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Appears to be associated with a worse prognosis</td>
<td>MassARRAY and pyrosequencing – oncogenic mutations outside of codon 600 will not be detected</td>
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<tr>
<td><strong>PIK3CA</strong></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></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>Analytic sensitivity/specificity = 100%</td>
<td>o Tumor may respond to therapies targeted at genes downstream of PIK3 in the AKT/mTOR signaling</td>
<td></td>
</tr>
<tr>
<td></td>
<td>* Exon 20 (codon 1047) mutation is independent of RAS binding</td>
<td></td>
<td>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 therapy in wild type KRAS</td>
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</tr>
<tr>
<td></td>
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<td>o Negative impact on the prognosis of advanced CRC</td>
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<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</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Possible resistance to anti-EGFR therapy</td>
<td>o MassARRAY and pyrosequencing – 10% mutant alleles</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Normal</td>
<td>o NGS – 5% mutant alleles</td>
</tr>
<tr>
<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>
</tbody>
</table>

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<tr>
<td>NRAS</td>
<td>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>
</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 (D5F3) with Interpretation by Immunohistochemistry 2007324**
- Detects ALK fusion proteins

**ALK (D5F3) 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 2013082**
- 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

**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
  - For gastroesophageal junction (GEJ), urothelial, and cervical 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 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**


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