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

Immunohistochemistry (IHC)
- Detection of PTEN expression

Next generation sequencing (NGS)
- Extensive coverage of mutations in 48 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
- Detects mutations in BRAF, KRAS, NRAS, and PIK3CA by MassARRAY
- More cost effective than ordering multiple single gene assays

Related tests
Solid Tumor Mutation Panel by Next Generation Sequencing 2007991
- Aids in therapeutic decisions for solid tumor cancers
- Simultaneously evaluates mutations in 48 genes, including BRAF, KRAS, NRAS, PIK3CA
- 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 colorectal cancer

KRAS Mutation Detection 0040248
- Predicts response to anti-EGFR and MAPK pathway therapies in a variety of malignancies (eg, colorectal 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 colorectal cancer
  - Also used within the Lynch syndrome reflex testing pathway (for colorectal cancer 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

PIK3CA Mutation Detection 2004510
- Predicts response to anti-EGFR and AKT/mTOR pathway therapies in a variety of malignancies (eg, colorectal, ovarian, and breast cancer)
- Detects activating PIK3CA mutations in exons 9 and 20

PTEN by Immunohistochemistry 2004115
- Detects loss of PTEN expression in tumor tissue
  - Possibly associated with relative resistance to anti-EGFR therapy

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

- CRC is one of the most commonly diagnosed malignancies worldwide
- EGFR represents an important therapeutic target in advanced CRC
- Two 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>&lt;br&gt;GTPase-encoding gene in the RAS/RAF/MAPK pathway</td>
<td>Majority of oncogenic mutations – codons 12 and 13 (&gt;90%)&lt;br&gt;Most of the remaining activating mutations – codons 61 and 146</td>
<td>Clinical sensitivity – activating KRAS mutations found in ~40% of CRCs&lt;br&gt;Analytic sensitivity/ specificity – 100%</td>
<td>Positive&lt;br&gt;• Oncogenic KRAS mutation detected&lt;br&gt;• Lack of response to therapy with antibodies targeted to EGFR is predicted</td>
<td>• Limit of detection&lt;br&gt;○ MassARRAY and pyrosequencing – 10% mutant alleles&lt;br&gt;○ NGS – 5% mutant alleles&lt;br&gt;• MassARRAY- oncogenic mutations outside of codons 12, 13, 61, 146 will not be detected&lt;br&gt;• Pyrosequencing- oncogenic mutations outside of codons 12, 13, 61 will not be detected&lt;br&gt;• 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>&lt;br&gt;Kinase-encoding gene in the RAS/RAF/MAPK pathway</td>
<td>Majority of activating mutations – codon 600&lt;br&gt;Mutually exclusive with KRAS mutations in individuals with CRC</td>
<td>Clinical sensitivity – activating BRAF mutation found in ~10% of CRCs&lt;br&gt;Analytic sensitivity/ specificity – 100%</td>
<td>Positive&lt;br&gt;• Oncogenic BRAF mutation detected&lt;br&gt;• Available data suggests resistance to anti-EGFR therapy&lt;br&gt;• Individuals appear to have a worse prognosis</td>
<td>• Limit of detection&lt;br&gt;○ MassARRAY and pyrosequencing – 10% mutant alleles&lt;br&gt;○ NGS – 5% mutant alleles&lt;br&gt;• MassARRAY and pyrosequencing – oncogenic mutations outside of codon 600 will not be detected</td>
</tr>
<tr>
<td><strong>PIK3CA</strong>&lt;br&gt;Encodes a subunit of the PI3K protein</td>
<td>Exon 9 gain-of-function mutation (codons 542 or 545) requires interaction with RAS&lt;br&gt;• May have no effect on cetuximab therapy&lt;br&gt;Exon 20 (codon 1047) mutation is independent of RAS binding&lt;br&gt;• Appears to have a negative effect on response to cetuximab</td>
<td>Clinical sensitivity – oncogenic PIK3CA mutation found in 10-20% of CRC, mostly exons 9 (60-65%) or 20 (20-25%)&lt;br&gt;Analytic sensitivity/ specificity – 100%</td>
<td>Positive&lt;br&gt;• Oncogenic PIK3CA mutation detected&lt;br&gt;○ Tumor may respond to therapies targeted at genes downstream of PI3K in the AKT/mTOR signaling cascade&lt;br&gt;○ Exon 20 (kinase domain) mutations may indicate resistance to anti-EGFR therapy in wild type KRAS tumors&lt;br&gt;○ Negative impact on the prognosis of advanced CRC</td>
<td>• Limit of detection&lt;br&gt;○ MassARRAY and pyrosequencing – 10% mutant alleles&lt;br&gt;○ NGS – 5% mutant alleles&lt;br&gt;• MassARRAY and pyrosequencing – oncogenic mutations outside of codons 542, 545, 1047 will not be detected</td>
</tr>
<tr>
<td><strong>PTEN</strong>&lt;br&gt;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&lt;br&gt;• Possible resistance to anti-EGFR therapy&lt;br&gt;Normal&lt;br&gt;• PTEN expression is intact</td>
<td></td>
</tr>
<tr>
<td>Gene</td>
<td>Mutations</td>
<td>Sensitivity/Specificity</td>
<td>Results</td>
<td>Limitations</td>
</tr>
<tr>
<td>----------------------</td>
<td>---------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>NRAS</td>
<td>GTPase-encoding gene in the RAS/RAF/MAPK pathway</td>
<td>Clinical sensitivity – oncogenic NRAS mutation found in ~3% of CRCs</td>
<td>Positive</td>
<td>Limit of detection</td>
</tr>
<tr>
<td></td>
<td>Majority of activating mutations – codon 61</td>
<td>Analytic sensitivity/ specificity – 100%</td>
<td>• Oncogenic NRAS mutation detected</td>
<td>o MassARRAY and pyrosequencing – 10% mutant alleles</td>
</tr>
<tr>
<td></td>
<td>Mutually exclusive with KRAS mutations in individuals with CRC</td>
<td></td>
<td>• Predictive of relative resistance to anti-EGFR therapy</td>
<td>o NGS – 5% mutant alleles</td>
</tr>
<tr>
<td></td>
<td>Associated with relative resistance to anti-EGFR therapy</td>
<td></td>
<td>Negative</td>
<td>MassARRAY and pyrosequencing – oncogenic mutations outside of codons 12, 13, 61 will not be detected</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• No oncogenic NRAS mutation detected</td>
<td>Presence or absence of mutations does not guarantee a response or lack of response to anti-EGFR therapy</td>
</tr>
</tbody>
</table>
