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.arulab.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

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 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

PTEN by Immunohistochemistry 2004115

PTEN with Interpretation by Immunohistochemistry 2007031
  - 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 CRC)

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500 Chipeta Way, Salt Lake City, UT 84108 | (800) 522-2787 | (801) 583-2787 | www.arulab.com | www.arupconsult.com
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### Disease Overview

- CRC is one 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

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<th>Gene</th>
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</table>
| **KRAS**      | GTPase-encoding gene in the RAS/RAF/MAPK pathway                           | Clinical sensitivity — activating KRAS mutations found in ~40% of CRCs. Analytic sensitivity/ specificity — 100% | Positive                                                                  | • Limit of detection  
|               | Majority of oncogenic mutations — codons 12 and 13 (>90%)                 |                                         | • Oncogenic KRAS mutation detected                                      | o MassARRAY and pyrosequencing – 10% mutant alleles  
|               | Most of the remaining activating mutations — codons 61 and 146            |                                         | • Lack of response to therapy with antibodies targeted to EGFR is predicted | o NGS – 5% mutant alleles  
|               |                                                                           |                                         | • No oncogenic KRAS mutation detected                                    | • MassARRAY – oncogenic mutations outside of codons 12, 13, 61, 146 will not be detected  
|               |                                                                           |                                         | • Follow-up BRAF testing is advised prior to initiation of anti-EGFR therapy | • Pyrosequencing – oncogenic mutations outside of codons 12, 13, 61 will not be detected  
|               |                                                                           |                                         |                                                                          | • A substantial portion of individuals with wild type KRAS still fail to respond to anti-EGFR agents, implicating downstream mutations |
| **BRAF**      | Kinase-encoding gene in the RAS/RAF/MAPK pathway                           | Clinical sensitivity — activating BRAF mutation found in ~10% of CRCs. Analytic sensitivity/ specificity — 100% | Positive                                                                  | • Limit of detection  
|               | Majority of activating mutations — codon 600                              |                                         | • Oncogenic BRAF mutation detected                                       | o MassARRAY and pyrosequencing – 10% mutant alleles  
|               | Mutually exclusive with KRAS mutations in individuals with CRC            |                                         | • Available data suggest resistance to anti-EGFR therapy                  | o NGS – 5% mutant alleles  
|               |                                                                           |                                         | • Appears to be associated with a worse prognosis                        | • MassARRAY and pyrosequencing – oncogenic mutations outside of codon 600 will not be detected |
|               |                                                                           |                                         |                                                                          |                                                                          |
| **PIK3CA**    | Encodes a subunit of the PI3K protein                                      | Clinical sensitivity — oncogenic PIK3CA mutation found in 10-20% of CRC, mostly exons 9 (60-65%) or 20 (20-25%). Analytic sensitivity/ specificity — 100% | Positive                                                                  | • Limit of detection  
|               | Exon 9 gain-of-function mutation (codons 542 or 545) requires interaction with RAS |                                         | • Oncogenic PIK3CA mutation detected                                       | o MassARRAY and pyrosequencing – 10% mutant alleles  
|               | • May have no effect on cetuximab therapy                                 |                                         | • Tumor may respond to therapies targeted at genes downstream of PI3K in the AKT/mTOR signaling cascade | o NGS – 5% mutant alleles  
|               | Exon 20 (codon 1047) mutation is independent of RAS binding               |                                         | • Exon 20 (kinase domain) mutations may indicate resistance to anti-EGFR therapy in wild type KRAS tumors | • MassARRAY and pyrosequencing – oncogenic mutations outside of codons 542, 545, 1047 will not be detected |
|               | • Appears to have a negative effect on response to cetuximab              |                                         | • Negative impact on the prognosis of advanced CRC                        |                                                                          |
|               |                                                                           |                                         | • No oncogenic PIK3CA mutation detected                                   |                                                                          |
| **PTEN**      | Tumor suppressor gene in the PI3K/AKT pathway                              | Clinical sensitivity — loss of expression found in up to 50% of CRCs. | Abnormal                                                                  | • Limit of detection  
|               | Loss has been predicted to show resistance to anti-EGFR therapy           |                                         | • Possible resistance to anti-EGFR therapy                               | o MassARRAY and pyrosequencing – 10% mutant alleles  
|               |                                                                           |                                         | Normal                                                                   | o NGS – 5% mutant alleles  
|               |                                                                           |                                         | • PTEN expression is intact                                               | • MassARRAY and pyrosequencing – oncogenic mutations outside of codons 542, 545, 1047 will not be detected |

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NRAS
GTPase-encoding gene in the RAS/RAF/MAPK pathway

**Gene**
- NRAS

**Mutations**
- Majority of activating mutations – codon 61
- Mutually exclusive with KRAS mutations in individuals with CRC
- Associated with relative resistance to anti-EGFR therapy

**Sensitivity/Specificity**
- Clinical sensitivity – oncogenic NRAS mutation found in ~3% of CRCs
- Analytic sensitivity/specificity – 100%

**Results**
- **Positive**
  - Oncogenic NRAS mutation detected
  - Predictive of relative resistance to anti-EGFR therapy
- **Negative**
  - No oncogenic NRAS mutation detected

**Limitations**
- **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

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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 (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

**ROS1 with Interpretation by Immunohistochemistry with Reflex to FISH if Equivocal 2008414**
- Detects ROS1 fusion proteins and ROS1 gene rearrangements

Screening for immune checkpoint inhibitor therapy

FDA-approved PD-L1 companion tests

**PD-L1 22C3 pharmDx by Immunohistochemistry with Interpretation, pembrolizumab (KEYTRUDA) 2013284**
- Aid in prediction of response to pembrolizumab (KEYTRUDA), as first- or second-line therapy, for patients with non-small cell lung cancer (NSCLC)
- Can be performed in conjunction with or instead of PD-L1 28-8

**PD-L1 28-8 pharmDx by Immunohistochemistry with Interpretation, nivolumab (OPDIVO) 2013684**
- Aids in prediction of response to nivolumab for patients with non-squamous NSCLC or melanoma
- Can be performed in conjunction with or instead of PD-L1 22C3

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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** – (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

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### Single Gene Testing

<table>
<thead>
<tr>
<th>Gene</th>
<th>Testing method</th>
<th>Test result</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALK</td>
<td>IHC</td>
<td>Positive – cytoplasmic staining in tumor cells</td>
<td>May predict response to TKI therapy</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>
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<tr>
<td>FISH</td>
<td></td>
<td>Positive – ALK gene rearrangements detected in ≥15% of nuclei</td>
<td>May predict response to TKI therapy</td>
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<tr>
<td></td>
<td></td>
<td>• Does not identify translocation partner</td>
<td></td>
</tr>
<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) Digital droplet PCR</td>
<td>Positive – mutation detected</td>
<td>Predicts resistance to TKI therapy</td>
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<tr>
<td></td>
<td></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>
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<td></td>
<td>• Associated with acquired resistance to EGFR inhibitors in 5-20% of patients with EGFR-mutated tumors</td>
</tr>
<tr>
<td>RET</td>
<td>FISH</td>
<td>Positive – gene rearrangements detected</td>
<td>May predict response to TKI therapy</td>
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<td></td>
<td></td>
<td>• Does not identify translocation partner</td>
<td></td>
</tr>
<tr>
<td>ROS1</td>
<td>IHC FISH reflex</td>
<td>Positive – uniform membranous staining in tumor cells</td>
<td>May predict response to TKI therapy</td>
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<td></td>
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<td>Equivocal – any degree of cytoplasmic staining or focal/weak membranous staining in tumor cells</td>
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<td>• Reflexes to FISH for confirmation</td>
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