

# 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](http://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](#)

#### [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 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

Gene	Mutations	Sensitivity/Specificity	Results	Limitations
<i>KRAS</i> GTPase-encoding gene in the RAS/RAF/MAPK pathway	Majority of oncogenic mutations – codons 12 and 13 (>90%) Most of the remaining activating mutations – codons 61 and 146	Clinical sensitivity – activating <i>KRAS</i> mutations found in ~40% of CRCs Analytic sensitivity/specificity – 100%	Positive <ul style="list-style-type: none"> <li>• Oncogenic <i>KRAS</i> mutation detected</li> <li>• Lack of response to therapy with antibodies targeted to EGFR is predicted</li> </ul> Negative <ul style="list-style-type: none"> <li>• No oncogenic <i>KRAS</i> mutation detected</li> <li>• Follow-up <i>BRAF</i> testing is advised prior to initiation of anti-EGFR therapy</li> </ul>	<ul style="list-style-type: none"> <li>• Limit of detection <ul style="list-style-type: none"> <li>○ MassARRAY and pyrosequencing – 10% mutant alleles</li> <li>○ NGS – 5% mutant alleles</li> </ul> </li> <li>• MassARRAY- oncogenic mutations outside of codons 12, 13, 61, 146 will not be detected</li> <li>• Pyrosequencing- oncogenic mutations outside of codons 12, 13, 61 will not be detected</li> <li>• A substantial portion of individuals with wild type <i>KRAS</i> still fail to respond to anti-EGFR agents, implicating downstream mutations</li> </ul>
<i>BRAF</i> Kinase-encoding gene in the RAS/RAF/MAPK pathway	Majority of activating mutations – codon 600 Mutually exclusive with <i>KRAS</i> mutations in individuals with CRC	Clinical sensitivity – activating <i>BRAF</i> mutation found in ~10% of CRCs Analytic sensitivity/specificity – 100%	Positive <ul style="list-style-type: none"> <li>• Oncogenic <i>BRAF</i> mutation detected</li> <li>• Available data suggests resistance to anti-EGFR therapy</li> <li>• Individuals appear to have a worse prognosis</li> </ul> Negative <ul style="list-style-type: none"> <li>• No oncogenic <i>BRAF</i> mutation detected</li> </ul>	<ul style="list-style-type: none"> <li>• Limit of detection <ul style="list-style-type: none"> <li>○ MassARRAY and pyrosequencing – 10% mutant alleles</li> <li>○ NGS – 5% mutant alleles</li> </ul> </li> <li>• MassARRAY and pyrosequencing – oncogenic mutations outside of codon 600 will not be detected</li> </ul>
<i>PIK3CA</i> Encodes a subunit of the PI3K protein	Exon 9 gain-of-function mutation (codons 542 or 545) requires interaction with RAS <ul style="list-style-type: none"> <li>• May have no effect on cetuximab therapy</li> </ul> Exon 20 (codon 1047) mutation is independent of RAS binding <ul style="list-style-type: none"> <li>• Appears to have a negative effect on response to cetuximab</li> </ul>	Clinical sensitivity – oncogenic <i>PIK3CA</i> mutation found in 10-20% of CRC, mostly exons 9 (60-65%) or 20 (20-25%) Analytic sensitivity/specificity – 100%	Positive <ul style="list-style-type: none"> <li>• Oncogenic <i>PIK3CA</i> mutation detected <ul style="list-style-type: none"> <li>○ Tumor may respond to therapies targeted at genes downstream of PI3K in the AKT/mTOR signaling cascade</li> <li>○ Exon 20 (kinase domain) mutations may indicate resistance to anti-EGFR therapy in wild type <i>KRAS</i> tumors</li> <li>○ Negative impact on the prognosis of advanced CRC</li> </ul> </li> </ul> Negative <ul style="list-style-type: none"> <li>• No oncogenic <i>PIK3CA</i> mutation detected</li> </ul>	<ul style="list-style-type: none"> <li>• Limit of detection <ul style="list-style-type: none"> <li>○ MassARRAY and pyrosequencing – 10% mutant alleles</li> <li>○ NGS – 5% mutant alleles</li> </ul> </li> <li>• MassARRAY and pyrosequencing – oncogenic mutations outside of codons 542, 545, 1047 will not be detected</li> </ul>
<i>PTEN</i> Tumor suppressor gene in the PI3K/AKT pathway	Loss has been predicted to show resistance to anti-EGFR therapy	Clinical sensitivity – loss of expression found in up to 50% of CRCs	Abnormal <ul style="list-style-type: none"> <li>• Possible resistance to anti-EGFR therapy</li> </ul> Normal <ul style="list-style-type: none"> <li>• <i>PTEN</i> expression is intact</li> </ul>	

Gene	Mutations	Sensitivity/Specificity	Results	Limitations
<p><i>NRAS</i> GTPase-encoding gene in the RAS/RAF/MAPK pathway</p>	<p>Majority of activating mutations – codon 61 Mutually exclusive with <i>KRAS</i> mutations in individuals with CRC Associated with relative resistance to anti-EGFR therapy</p>	<p>Clinical sensitivity – oncogenic <i>NRAS</i> mutation found in ~3% of CRCs Analytic sensitivity/specificity – 100%</p>	<p>Positive</p> <ul style="list-style-type: none"> <li>• Oncogenic <i>NRAS</i> mutation detected</li> <li>• Predictive of relative resistance to anti-EGFR therapy</li> </ul> <p>Negative</p> <ul style="list-style-type: none"> <li>• No oncogenic <i>NRAS</i> mutation detected</li> </ul>	<ul style="list-style-type: none"> <li>• Limit of detection <ul style="list-style-type: none"> <li>○ MassARRAY and pyrosequencing – 10% mutant alleles</li> <li>○ NGS – 5% mutant alleles</li> </ul> </li> <li>• MassARRAY and pyrosequencing – oncogenic mutations outside of codons 12, 13, 61 will not be detected</li> <li>• Presence or absence of mutations does not guarantee a response or lack of response to anti-EGFR therapy</li> </ul>