# 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 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 therapeutic response in patients with solid tumor cancers

*KRAS* Mutation Detection 0040248
- Predicts response to anti-EGFR and MAPK pathway therapies in a variety of malignancies (eg, CRC and lung cancer)
- Does not cover extended *RAS*; detects mutations in codons 12, 13, and 61 only

*NRAS* Mutation Detection by Pyrosequencing 2003123
- Predicts response to anti-EGFR and MAPK pathway therapies in a variety of malignancies (eg, melanoma and CRC)
- Does not cover extended *RAS*; detects mutations in codons 12, 13, and 61 only

*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)

### Related Tests

*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

*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

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

| Gene | Mutations | Sensitivity/Specificity | Results | Limitations |
|---|---|---|---|---|
| *NRAS*<br>GTPase-encoding gene in the RAS/RAF/MAPK pathway | Majority of activating mutations – codon 61<br>Mutually exclusive with *KRAS* mutations in individuals with CRC<br>Associated with relative resistance to anti-EGFR therapy | Clinical sensitivity – oncogenic *NRAS* mutation found in ~3% of CRCs<br>Analytic sensitivity/ specificity – 100% | Positive<br>• Oncogenic *NRAS* mutation detected<br>• Predictive of relative resistance to anti-EGFR therapy<br>Negative<br>• No oncogenic *NRAS* mutation detected | • Limit of detection<br>○ MassARRAY and pyrosequencing – 10% mutant alleles<br>○ NGS – 5% mutant alleles<br>• MassARRAY and pyrosequencing – oncogenic mutations outside of codons 12, 13, 61 will not be detected<br>• Presence or absence of mutations does not guarantee a response or lack of response to anti-EGFR therapy |