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
- 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)

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

Gene	Mutations	Sensitivity/Specificity	Results	Limitations
KRAS 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 *KRAS* mutations found in ~40% of CRCs Analytic sensitivity/ specificity – 100%	Positive • Oncogenic *KRAS* mutation detected • Lack of response to therapy with antibodies targeted to EGFR is predicted Negative • No oncogenic *KRAS* mutation detected • Follow-up *BRAF* testing is advised prior to initiation of anti-EGFR therapy	• Limit of detection o MassARRAY and pyrosequencing – 10% mutant alleles o NGS – 5% mutant alleles • MassARRAY – oncogenic mutations outside of codons 12, 13, 61, 146 will not be detected • 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	Majority of activating mutations – codon 600 Mutually exclusive with *KRAS* mutations in individuals with CRC	Clinical sensitivity – activating *BRAF* mutation found in ~10% of CRCs Analytic sensitivity/ specificity – 100%	Positive • Oncogenic *BRAF* mutation detected • Available data suggest resistance to anti-EGFR therapy • Appears to be associated with a worse prognosis Negative • No oncogenic *BRAF* mutation detected	• Limit of detection o MassARRAY and pyrosequencing – 10% mutant alleles o NGS – 5% mutant alleles • MassARRAY and pyrosequencing – oncogenic mutations outside of codon 600 will not be detected
PIK3CA Encodes a subunit of the PI3K protein	Exon 9 gain-of-function mutation (codons 542 or 545) requires interaction with RAS • May have no effect on cetuximab therapy Exon 20 (codon 1047) mutation is independent of RAS binding • 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%) Analytic sensitivity/ specificity – 100%	Positive • Oncogenic *PIK3CA* mutation detected o Tumor may respond to therapies targeted at genes downstream of PI3K in the AKT/mTOR signaling cascade o Exon 20 (kinase domain) mutations may indicate resistance to anti-EGFR therapy in wild type *KRAS* tumors o Negative impact on the prognosis of advanced CRC Negative • No oncogenic *PIK3CA* mutation detected	• Limit of detection o MassARRAY and pyrosequencing – 10% mutant alleles o NGS – 5% mutant alleles • MassARRAY and pyrosequencing – oncogenic mutations outside of codons 542, 545, 1047 will not be detected
PTEN 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 • Possible resistance to anti-EGFR therapy Normal • PTEN expression is intact	

Gene	Mutations	Sensitivity/Specificity	Results	Limitations
NRAS GTPase-encoding gene in the RAS/RAF/MAPK pathway	Majority of activating mutations – codon 61 Mutually exclusive with *KRAS* mutations in individuals with CRC Associated with relative resistance to anti-EGFR therapy	Clinical sensitivity – oncogenic *NRAS* mutation found in ~3% of CRCs Analytic sensitivity/ specificity – 100%	Positive • Oncogenic *NRAS* mutation detected • Predictive of relative resistance to anti-EGFR therapy Negative • No oncogenic *NRAS* mutation detected	• Limit of detection o MassARRAY and pyrosequencing – 10% mutant alleles o 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