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 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 44 genes, including BRAF, KRAS, NRAS, PIK3CA, PTEN
- 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

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

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| **KRAS**      | GTPase-encoding gene in the RAS/RAF/MAPK pathway                            |                                             | Has been predicted to show resistance to anti-EGFR therapy              | Limit of detection                           o MassARRAY and pyrosequencing – 10% mutant alleles
 o NGS – 5% mutant alleles
 o MassARRAY – oncogenic mutations outside of codons 12, 13, 61, 146 will not be detected
 o Pyrosequencing – oncogenic mutations outside of codons 12, 13, 61 will not be detected
 o 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                            |                                             | Has been predicted to show resistance to anti-EGFR therapy              | Limit of detection                           o MassARRAY and pyrosequencing – 10% mutant alleles
 o NGS – 5% mutant alleles
 o MassARRAY and pyrosequencing – oncogenic mutations outside of codon 600 will not be detected |
| **PIK3CA**    | Encodes a subunit of the PI3K protein                                       |                                             | Has been predicted to show resistance to anti-EGFR therapy              | Limit of detection                           o MassARRAY and pyrosequencing – 10% mutant alleles
 o NGS – 5% mutant alleles
 o MassARRAY and pyrosequencing – oncogenic mutations outside of codons 542, 545, 1047 will not be detected |
| **PTEN**      | Tumor suppressor gene in the PI3K/AKT pathway                               |                                             | Has been predicted to show resistance to anti-EGFR therapy              | Limit of detection                           o MassARRAY and pyrosequencing – 10% mutant alleles
 o NGS – 5% mutant alleles
 o MassARRAY and pyrosequencing – oncogenic mutations outside of codons 542, 545, 1047 will not be detected |
| **NRAS**      | GTPase-encoding gene in the RAS/RAF/MAPK pathway                            |                                             | Has been predicted to show resistance to anti-EGFR therapy              | Limit of detection                           o MassARRAY and pyrosequencing – 10% mutant alleles
 o NGS – 5% mutant alleles
 o MassARRAY and pyrosequencing – oncogenic mutations outside of codons 12, 13, 61 will not be detected
 o Presence or absence of mutations does not guarantee a response or lack of response to anti-EGFR therapy |

**Notes:**
- Percentages may vary from lab to lab, and some mutations may fall outside of the listed percentages.
- KRAS mutations not captured by pyrosequencing include codons 51, 53, 62, 104, and 106.
- **Clinical sensitivity/resistance:**
  - Normal: KRAS mutation not detected
  - Positive: Oncogenic KRAS mutation detected
  - Negative: No oncogenic KRAS mutation detected
- **Analytic sensitivity:**
  - MassARRAY: >90% mutant alleles
  - Pyrosequencing: 10% mutant alleles
  - NGS: 5% mutant alleles
- **Limit of detection:**
  - MassARRAY and pyrosequencing: 10-100% mutant alleles
  - NGS: <5% mutant alleles
- **Limit of sensitivities:**
  - MassARRAY and pyrosequencing: >10% mutant alleles
  - Pyrosequencing: >5% mutant alleles
  - NGS: >1% mutant alleles
- **PIK3CA mutations not captured by pyrosequencing include:**
  - Codons 542, 545, 1047
  - MassARRAY: 10% mutant alleles
  - Pyrosequencing: 5% mutant alleles
  - NGS: <1% mutant alleles
- **PTEN mutations not captured by MassARRAY and pyrosequencing include:**
  - Exons 9
  - MassARRAY: 10% mutant alleles
  - Pyrosequencing: 5% mutant alleles
  - NGS: <1% mutant alleles
- **NRAS mutations not captured by MassARRAY and pyrosequencing include:**
  - Codons 51, 53, 62, 104, 106
  - MassARRAY: 10-100% mutant alleles
  - Pyrosequencing: 5-100% mutant alleles
  - NGS: <5-50% mutant alleles