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)
  • For use in non-small cell lung cancer, see the Non-Small Cell Lung Cancer Molecular Markers Test Fact Sheet

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

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| **KRAS**        | Majority of oncogenic mutations – codons 12 and 13 (>90%)                  | Clinical sensitivity – activating KRAS mutations found in ~40% of CRCs                  | Positive                                                                                | * Limit of detection*  
|                 | Most of the remaining activating mutations – codons 61 and 146            | Analytic sensitivity/ specificity – 100%                                                | • Oncogenic KRAS mutation detected                                                             | o MassARRAY and pyrosequencing – 10% mutant alleles  
|                 |                                                                          |                                                                                        | • 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**        | Majority of activating mutations – codon 600                               | Clinical sensitivity – activating **BRAF** mutation found in ~10% of CRCs               | Positive                                                                                | * Limit of detection*  
|                 | Mutually exclusive with **KRAS** mutations in individuals with CRC         | Analytic sensitivity/ specificity – 100%                                                | • Oncogenic **BRAF** mutation detected                                                        | o MassARRAY and pyrosequencing – 10% mutant alleles  
|                 |                                                                          |                                                                                        | • 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**      | Exon 9 gain-of-function mutation (codons 542 or 545) requires interaction  | Clinical sensitivity – oncogenic **PIK3CA** mutation found in 10-20% of CRCs, mostly  | Positive                                                                                | * Limit of detection*  
|                 | with **RAS**                                                              | exons 9 (60-65%) or 20 (20-25%)                                                       | • Oncogenic **PIK3CA** mutation detected                                                        | o MassARRAY and pyrosequencing – 10% mutant alleles  
|                 | • May have no effect on cetuximab therapy                                  | Analytic sensitivity/ specificity – 100%                                                | • 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           |                                                                                        | o 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                                           |                                                                                                |
| **PTEN**        | Loss has been predicted to show resistance to anti-EGFR therapy           | Clinical sensitivity – loss of expression found in up to 50% of CRCs                    | Abnormal                                                                                | * Limit of detection*  
|                 |                                                                          |                                                                                        | • Possible resistance to anti-EGFR therapy                                                    | o MassARRAY and pyrosequencing – 10% mutant alleles  
|                 |                                                                          |                                                                                        | Normal                                                                                       | o NGS – 5% mutant alleles  
<p>|                 |                                                                          |                                                                                        | • PTEN expression is intact                                                                  | MassARRAY and pyrosequencing – oncogenic mutations outside of codons 542, 545, 1047 will not be detected |</p>
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| NRAS | Majority of activating mutations – codon 61 | Clinical sensitivity – oncogenic NRAS mutation found in ~3% of CRCs | Positive  
  • Oncogenic NRAS mutation detected  
  • Predictive of relative resistance to anti-EGFR therapy | • 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 |
|       | Mutually exclusive with KRAS mutations in individuals with CRC | Analytic sensitivity/ specificity – 100% | Negative  
  • No oncogenic NRAS mutation detected | |
|       | Associated with relative resistance to anti-EGFR therapy | | | |

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

CRC: Colorectal cancer.

EGFR: Epidermal growth factor receptor.