

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

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"> Oncogenic <i>KRAS</i> mutation detected Lack of response to therapy with antibodies targeted to EGFR is predicted Negative <ul style="list-style-type: none"> No oncogenic <i>KRAS</i> mutation detected Follow-up <i>BRAF</i> testing is advised prior to initiation of anti-EGFR therapy 	<ul style="list-style-type: none"> Limit of detection <ul style="list-style-type: none"> MassARRAY and pyrosequencing – 10% mutant alleles 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 <i>KRAS</i> still fail to respond to anti-EGFR agents, implicating downstream mutations
<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"> Oncogenic <i>BRAF</i> mutation detected Available data suggest resistance to anti-EGFR therapy Appears to be associated with a worse prognosis Negative <ul style="list-style-type: none"> No oncogenic <i>BRAF</i> mutation detected 	<ul style="list-style-type: none"> Limit of detection <ul style="list-style-type: none"> MassARRAY and pyrosequencing – 10% mutant alleles NGS – 5% mutant alleles MassARRAY and pyrosequencing – oncogenic mutations outside of codon 600 will not be detected
<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"> May have no effect on cetuximab therapy Exon 20 (codon 1047) mutation is independent of RAS binding <ul style="list-style-type: none"> Appears to have a negative effect on response to cetuximab 	Clinical sensitivity – oncogenic <i>PIK3CA</i> mutation found in 10-20% of CRCs, mostly exons 9 (60-65%) or 20 (20-25%) Analytic sensitivity/specificity – 100%	Positive <ul style="list-style-type: none"> Oncogenic <i>PIK3CA</i> mutation detected <ul style="list-style-type: none"> Tumor may respond to therapies targeted at genes downstream of PI3K in the AKT/mTOR signaling cascade Exon 20 (kinase domain) mutations may indicate resistance to anti-EGFR therapy in wild type <i>KRAS</i> tumors Negative impact on the prognosis of advanced CRC Negative <ul style="list-style-type: none"> No oncogenic <i>PIK3CA</i> mutation detected 	<ul style="list-style-type: none"> Limit of detection <ul style="list-style-type: none"> MassARRAY and pyrosequencing – 10% mutant alleles NGS – 5% mutant alleles MassARRAY and pyrosequencing – oncogenic mutations outside of codons 542, 545, 1047 will not be detected
<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"> Possible resistance to anti-EGFR therapy Normal <ul style="list-style-type: none"> <i>PTEN</i> expression is intact 	
<i>NRAS</i> GTPase-encoding gene in the RAS/RAF/MAPK pathway	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	Clinical sensitivity – oncogenic <i>NRAS</i> mutation found in ~3% of CRCs Analytic sensitivity/specificity – 100%	Positive <ul style="list-style-type: none"> Oncogenic <i>NRAS</i> mutation detected Predictive of relative resistance to anti-EGFR therapy Negative <ul style="list-style-type: none"> No oncogenic <i>NRAS</i> mutation detected 	<ul style="list-style-type: none"> Limit of detection <ul style="list-style-type: none"> MassARRAY and pyrosequencing – 10% mutant alleles 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