

See below for Additional Technical Information topics

Colorectal Cancer – Predictive Testing for Anti-EGFR Therapy Lung Cancer Molecular Markers

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
<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 CRC, 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"> • PTEN expression is intact 	

Gene	Mutations	Sensitivity/Specificity	Results	Limitations
<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

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Lung Cancer Molecular Markers

Indications for Ordering

Mutation testing to aid in selection of tyrosine kinase inhibitor (TKI) and/or immune checkpoint inhibitor therapy

Tests to Consider

Typical testing strategy

Minimum initial recommendation – lung cancer panel that includes simultaneous ordering for mutations in *ALK*, *EGFR*, and *ROS1* genes

Primary tests

Determine eligibility for TKI therapy (panel tests)

[Lung Cancer Panel 2008894](#)

- Screening panel detects
 - EGFR* mutations
 - ALK and ROS1 fusion proteins

[Lung Cancer Panel with *KRAS* 2008895](#)

- Screening panel detects
 - EGFR* and *KRAS* mutations
 - ALK and ROS1 fusion proteins

Determine eligibility for TKI therapy (single tests)

[ALK \(D5F3\) with Interpretation by Immunohistochemistry 2007324](#)

- Detects ALK fusion proteins

[ALK \(D5F3\) by Immunohistochemistry with Reflex to ALK Gene Rearrangements by FISH 2011431](#)

- Detects ALK fusion proteins and *ALK* gene rearrangements in solid tumors

[ALK Gene Rearrangements by FISH, Lung 2006102](#)

- Screening test for all ALK fusions
- Use this test if the companion diagnostic test for crizotinib is required
- Does not identify the translocation partner or variant

[EGFR Mutation Detection by Pyrosequencing 2002440](#)

[KRAS Mutation Detection 0040248](#)

- Predicts response to anti-EGFR and MAPK pathway therapies in a variety of malignancies (eg, colorectal and lung cancer)

[c-MET by Immunohistochemistry 2008652](#)

- Detects overexpression of c-MET protein

[MET Gene Amplification by FISH 2013082](#)

- Aids in prognostication and therapeutic decisions for neoplasms where amplification has been demonstrated
- Screening test for *MET* gene amplification

[RET Gene Rearrangements by FISH 2012654](#)

- Detects RET gene rearrangements in solid tumors
- Does not identify the translocation partner or variant

[ROS1 by FISH 2008418](#)

- Detects ROS1 gene rearrangements in solid tumors
- Does not identify the translocation partner or variant

[ROS1 with Interpretation by Immunohistochemistry with Reflex to FISH if Equivocal or Positive 2008414](#)

- Detects ROS1 fusion proteins and ROS1 gene rearrangements

Screening for immune checkpoint inhibitor therapy FDA-approved PD-L1 companion tests

[PD-L1 22C3 pharmDx by Immunohistochemistry with Interpretation, pembrolizumab \(KEYTRUDA\) 2013284](#)

- Aid in prediction of response to pembrolizumab (KEYTRUDA), as first- or second-line therapy, for patients with non-small cell lung cancer (NSCLC)
- Can be performed in conjunction with or instead of PD-L1 28-8

[PD-L1 28-8 pharmDx by Immunohistochemistry with Interpretation, nivolumab \(OPDIVO\) 2013684](#)

- Aids in prediction of response to nivolumab for patients with non-squamous NSCLC or melanoma
- Can be performed in conjunction with or instead of PD-L1 22C3

Monitor for EGFR T790M resistance

[EGFR T790M Mutation Detection in Circulating Tumor DNA by Digital Droplet PCR 2012868](#)

- Monitor blood plasma or cerebrospinal fluid (CSF) for
 - Development of *EGFR* T790M drug-resistant mutation in patients administered TKI therapy for *EGFR*-mutant NSCLC
 - Response to therapy and disease progression in patients receiving *EGFR* T790M-specific TKIs

Related test

[Solid Tumor Mutation Panel by Next Generation Sequencing 2007991](#)

- Aids in therapeutic decisions for solid tumor cancers
- Simultaneously evaluates mutations in multiple genes, including AKT1, ALK, BRAF, EGFR, ERBB2, ERBB4, KRAS, NRAS, and PIK3CA
- Does not detect translocations

Test Methodology

ALK

- Immunohistochemistry (IHC) using ALK clone D5F3
- Fluorescence in situ hybridization (FISH)

EGFR – polymerase chain reaction (PCR) and pyrosequencing

- Detects mutations at codons 719 (exon 18), 768 and 790 (exon 20), 858 and 861 (exon 21)
- Detects deletions in exon 19

EGFR T790M (serum) – digital droplet PCR

KRAS – PCR and pyrosequencing

- Detects mutations at codons 12, 13 (exon 2), and 61 (exon 3)

c-MET – IHC

MET – FISH

PD-L1 – IHC

RET – FISH

- Detects all *RET* gene fusions

ROS1 – IHC (using *ROS1* clone D4D6) with FISH reflex if equivocal

Test Interpretation

Results

Single gene testing (includes genes in panels) – see table

Limitations

- Results must be interpreted in the context of clinical findings and morphological and other relevant data
- Results may be compromised if the recommended tissue-fixation procedures have not been followed

Disease Overview

Incidence

Lung cancer is the second most common cancer in U.S.

Treatment issues

- Lung cancer has poor response to traditional chemotherapy agents
 - ◦ Dismal 5-year outcome when using these agents
 - ◦ Newer targeted agents have better response rates in specific individuals and tumor types
- Tumor response rates to targeted therapy are closely associated with mutation status of the tumor, especially for adenocarcinoma or mixed adenocarcinoma subtypes
- Mutation status
 - Determines TKI therapy eligibility
 - Confers resistance to TKIs (eg, *EGFR* T790M mutation)
 - Monitoring in serum for *EGFR* T790M mutation may detect TKI resistance sooner and alter treatment plans
- PD-L1 expression
 - May predict response to immune checkpoint inhibitor therapy

References

- Francis G, Stein S. Circulating cell-free tumour DNA in the management of cancer. *Int J Mol Sci.* 2015;16;14122-14142
- NCCN Clinical Practice Guidelines in Oncology, Lung Cancer Screening. National Comprehensive Cancer Network. Fort Washington: Pennsylvania [Accessed: January 25, 2016]

Single Gene Testing			
Gene	Testing method	Test result	
ALK	IHC	Positive – cytoplasmic staining in tumor cells Equivocal – very weak cytoplasmic staining visible only on higher power by microscopy Negative – no cytoplasmic staining in tumor cells	May predict response to TKI therapy
	FISH	Positive – ALK gene rearrangements detected in ≥15% of nuclei • Does not identify translocation partner	May predict response to TKI therapy
EGFR	PCR/pyrosequencing	Positive – mutation detected	May predict response to TKI therapy
	EGFR T790M (serum) Digital droplet PCR	Positive – mutation detected • Expressed as percentage	Predicts resistance to TKI therapy
KRAS	PCR/pyrosequencing	Positive – mutation detected	May predict response to TKI therapy
MET	FISH	Positive – detects gene amplification	<ul style="list-style-type: none"> • May predict response to crizotinib TKI therapy • Associated with acquired resistance to EGFR inhibitors in 5-20% of patients with EGFR-mutated tumors
RET	FISH	Positive – gene rearrangements detected • Does not identify translocation partner	May predict response to TKI therapy
ROS1	IHC FISH reflex	Positive – uniform membranous staining in tumor cells Equivocal – any degree of cytoplasmic staining or focal/weak membranous staining in tumor cells • Reflexes to FISH for confirmation ○ Does not identify translocation partner	May predict response to TKI therapy