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
- Detects mutations in *BRAF, KRAS*, *NRAS*, and *PIK3CA* by MassARRAY
- More cost effective than ordering multiple single gene assays

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 colorectal cancer

KRAS Mutation Detection 0040248
- Predicts response to anti-EGFR and MAPK pathway therapies in a variety of malignancies (eg, colorectal 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 colorectal cancer
- Also used within the Lynch syndrome reflex testing pathway (for colorectal cancer 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

PIK3CA Mutation Detection 2004510
- Predicts response to anti-EGFR and AKT/mTOR pathway therapies in a variety of malignancies (eg, colorectal, ovarian, and breast cancer)
- Detects activating *PIK3CA* mutations in exons 9 and 20

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 colorectal cancer)

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

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 suggests resistance to anti-EGFR therapy • Individuals appear to have 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 CRC, 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

Gene	Mutations	Sensitivity/Specificity	Results	Limitations
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	
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

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
  - o *EGFR* mutations
  - o ALK and ROS1 fusion proteins

Lung Cancer Panel with *KRAS* 2008895
- Screening panel detects
  - o *EGFR* and *KRAS* mutations
  - o 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 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 *PIKC3CA*
- 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	• 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 – any degree of membranous staining in tumor cells Equivocal – any degree of cytoplasmic staining in tumor cells • Reflexes to FISH for confirmation o Does not identify translocation partner	May predict response to TKI therapy