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

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)

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

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 ○ 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 *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 suggest resistance to anti-EGFR therapy • Appears to be associated with a worse prognosis Negative • No oncogenic *BRAF* mutation detected	• Limit of detection ○ MassARRAY and pyrosequencing – 10% mutant alleles ○ 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 CRCs, mostly exons 9 (60-65%) or 20 (20-25%) Analytic sensitivity/ specificity – 100%	Positive • Oncogenic *PIK3CA* mutation detected ○ 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 *KRAS* tumors ○ Negative impact on the prognosis of advanced CRC Negative • No oncogenic *PIK3CA* mutation detected	• Limit of detection ○ 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
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	

Gene	Mutations	Sensitivity/Specificity	Results	Limitations
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 ○ 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

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

ROS 1 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 IHC for NSCLC with Interpretation, pembrolizumab (KEYTRUDA) 2013284
- Companion diagnostic test to aid in prediction of response to pembrolizumab (KEYTRUDA) as first- or second-line monotherapy for patients with non-small cell lung cancer (NSCLC)
- Can be performed in conjunction with or instead of PD-L1 28-8
- For NSCLC specimens only
  - For gastroesophageal junction (GEJ), urothelial, and cervical specimens, see PD-L1 22C3 IHC with Combined Positive Score (CPS) Interpretation, pembrolizumab (KEYTRUDA) 3000197

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

- FDA-approved complementary codiagnostic test to aid in prediction of response to nivolumab (OPDIVO) for patients with nonsquamous NSCLC, melanoma, urothelial carcinoma, and head and neck squamous cell carcinoma (HNSCC)

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: February 2018

Single Gene			
Gene	**Testing method**	**Test result**	
ALK	IHC	Positive – uniform membranous 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

Single Gene			
Gene	**Testing method**	**Test result**	
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