See below for Additional Technical Information topics

***MET* Gene Amplification by FISH**

Lung Cancer Molecular Markers

MET Gene Amplification by FISH

Indications for Ordering

Aids in prognostication and therapeutic decisions for neoplasms where amplification has been demonstrated

Test methodology

FISH

- Fluorescence in situ hybridization (FISH) analysis on formalin-fixed, paraffin embedded tumor tissue
- DNA probes
  - *MET* (7q31.2)
  - *CEP7* (control probe)
- 40 cells evaluated from regions of tumor identified on histopathologic review of a matching hematoxylin and eosin stained section

Tests to Consider

Primary test

MET Gene Amplification by FISH 2013082

- Screening test for MET gene amplification

Related test

c-MET by Immunohistochemistry 2008652

- Detects overexpression of c-MET protein

Disease Overview

Prognostic/therapeutic issues

- In lung cancer, *MET* gene amplification is associated with acquired resistance to EGFR inhibitors
  - Occurs in 5-20% of patients with *EGFR*-mutated tumors
- Amplification is associated with tumor progression and a poor prognosis in other tumors
  - Breast
  - Clear cell ovarian
  - Colorectal
  - Esophageal
  - Gastric
  - Glioma
  - Head and neck
  - Mesenchymal
  - Renal
  - Thyroid
- Multiple ongoing trials using EGFR inhibitors, with some tumors showing sensitivity to crizotinib

Genetics

Gene - *MET*

Test Interpretation

Results

- Amplified
  - *MET*/*CEP7* ≥2.0 **OR**
  - Average *MET* copy number per cell is ≥6.0
- Non-amplified
  - *MET*/*CEP7* <2.0

Limitations

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

Lung Cancer Molecular Markers

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 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 – 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 o Does not identify translocation partner	May predict response to TKI therapy