See below for Additional Technical Information topics

***EGFR* T790M Mutation in Circulating Tumor DNA**

Lung Cancer Molecular Markers

EGFR T790M Mutation in Circulating Tumor DNA

Indications for Ordering

Monitor blood plasma or cerebrospinal fluid (CSF) for

- Development of *EGFR* T790M drug-resistant mutation in patients administered tyrosine kinase inhibitor (TKI) therapy for *EGFR*-mutant non-small cell lung cancer (NSCLC)
- Response to therapy and disease progression in patients receiving *EGFR* T790M-specific TKIs

Test Description

Digital droplet polymerase chain reaction

Tests to Consider

Typical testing strategy

In patients with *EGFR* gene mutations who are treated with early generation TKIs, consider monitoring for development of an acquired *EGFR* T790M mutation

- Blood plasma
  - Serial quantitative testing
    - Guides clinical treatment decisions about acquired resistance to early generation TKIs and decision to switch to *EGFR* T790M-specific TKIs
    - Monitors response to therapy in patients taking *EGFR* T790M-specific TKIs
- CSF
  - Serial quantitative testing for patients with isolated NSCLC brain metastases and undetectable blood plasma levels of *EGFR* T790M mutation
  - Guides clinical treatment decisions about switching to *EGFR* T790M-specific TKIs
  - Monitors response to therapy

Primary test

EGFR T790M Mutation Detection in Circulating Tumor DNA by Digital Droplet PCR 2012868

- Detect and quantify circulating *EGFR* T790M point mutation from blood plasma or CSF

Disease Overview

Prevalence and/or incidence

- NSCLC – 84% of lung cancer in the U.S.
- *EGFR* gene mutations are initially detected in 10-15% of NSCLC patients who will be treated with TKIs targeting these mutations
  - ~100% of treated patients' cancers will progress on these initial TKIs
    - >60% are due to development of an acquired *EGFR* T790M point mutation

Treatment issues

- *EGFR* T790M gene mutation in circulating cell-free DNA from NSCLC causes resistance to early generation *EGFR* TKI drugs
  - Detection of this mutation is an indication to switch therapy to an *EGFR* T790M-specific TKI
  - Serial quantification of mutation levels by this assay guides treatment response to T790M-specific TKIs

Genetics

Gene – *EGFR*

Mutations

- Common activating mutations confer sensitivity to TKI therapy
  - L858R point mutation in exon 21
  - Insertions/deletions in exon 19
- *EGFR* T790M point mutation in exon 20 confers resistance to early generation TKIs in >60% of patients

Test Interpretation

Sensitivity/specificity

- Clinical sensitivity – 94%
- Clinical specificity – 100%
- Analytical sensitivity/specificity – 100%

Results

- Positive
  - *EGFR* T790M circulating mutation detected
    - T790M quantitative allele frequency reported as percentage
      - Predictive of resistance to *EGFR*-targeted early generation TKI therapy
      - Possibly predictive of response to *EGFR* T790M mutant-specific TKI therapy
- Negative
  - No *EGFR* T790M circulating mutation detected
    - Disease progression not related to *EGFR* T790M mutation

Limitations

- Limit of detection – ranges from 0.5% to 0.02% mutant alleles, based upon amplifiable DNA
- Optimal clinical testing intervals are unknown
- Mutations other than *EGFR* T790M are not detected
- Presence or absence of *EGFR* T790M does not guarantee a response to *EGFR* T790M-specific drug therapy

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 – 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