See below for Additional Technical Information topics

**EGFR T790M Mutation in Circulating Tumor DNA**

**Lung Cancer Molecular Markers**

### 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 nonsmall cell lung cancer (NSCLC)
- Response to therapy and disease progression in patients receiving *EGFR* T790M-specific TKIs

### 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 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

### 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

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**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**

- 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 IHC for NSCLC with Interpretation, pembrolizumab (KEYTRUDA) 2013284**

- Aid in prediction of response to pembrolizumab (KEYTRUDA) as first- or second-line therapy for patients with nonsmall cell lung cancer (NSCLC)
- Use for NSCLC specimens only
- Can be performed in conjunction with or instead of PD-L1 28-8
- For gastroesophageal junction (GEJ) 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**

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

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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 44 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 (e.g., *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

**Single Gene Testing**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Testing method</th>
<th>Test result</th>
<th>Test result</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALK</td>
<td>IHC</td>
<td>Positive – cytoplasmic staining in tumor cells</td>
<td>May predict response to TKI therapy</td>
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<tr>
<td></td>
<td></td>
<td>Equivocal – very weak cytoplasmic staining visible only on higher power by microscopy</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative – no cytoplasmic staining in tumor cells</td>
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<tr>
<td>FISH</td>
<td></td>
<td>Positive – <em>ALK</em> gene rearrangements detected in ≥15% of nuclei</td>
<td>May predict response to TKI therapy</td>
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<tr>
<td></td>
<td></td>
<td>• Does not identify translocation partner</td>
<td></td>
</tr>
<tr>
<td><em>EGFR</em></td>
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<tr>
<td><em>EGFR T790M (serum)</em></td>
<td>Digital droplet PCR</td>
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<tr>
<td><em>KRAS</em></td>
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<tr>
<td>Gene</td>
<td>Testing method</td>
<td>Test result</td>
<td>Additional notes</td>
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</table>
| 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  
○ Does not identify translocation partner | May predict response to TKI therapy |