**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

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<table>
<thead>
<tr>
<th>Gene</th>
<th>Testing method</th>
<th>Test result</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALK</td>
<td>IHC</td>
<td>Positive – cytoplasmic staining in tumor cells&lt;br&gt;Equivocal – very weak cytoplasmic staining visible only on higher power by microscopy&lt;br&gt;Negative – no cytoplasmic staining in tumor cells</td>
<td>May predict response to TKI therapy</td>
</tr>
<tr>
<td></td>
<td>FISH</td>
<td>Positive – ALK gene rearrangements detected in ≥15% of nuclei&lt;br&gt;• Does not identify translocation partner</td>
<td>May predict response to TKI therapy</td>
</tr>
<tr>
<td></td>
<td>ALK FISH</td>
<td>Positive – uniform membranous staining in tumor cells&lt;br&gt;Equivocal – any degree of cytoplasmic staining or focal/weak membranous staining in tumor cells&lt;br&gt;• Reflexes to FISH for confirmation&lt;br&gt;○ Does not identify translocation partner</td>
<td>May predict response to TKI therapy</td>
</tr>
<tr>
<td>EGFR</td>
<td>PCR/pyrosequencing</td>
<td>Positive – mutation detected</td>
<td>May predict response to TKI therapy</td>
</tr>
<tr>
<td></td>
<td>EGFR T790M (serum) Digital droplet PCR</td>
<td>Positive – mutation detected&lt;br&gt;• Expressed as percentage</td>
<td>Predicts resistance to TKI therapy</td>
</tr>
<tr>
<td>KRAS</td>
<td>PCR/pyrosequencing</td>
<td>Positive – mutation detected</td>
<td>May predict response to TKI therapy</td>
</tr>
<tr>
<td>MET</td>
<td>FISH</td>
<td>Positive – detects gene amplification</td>
<td>• May predict response to crizotinib TKI therapy&lt;br&gt;• Associated with acquired resistance to EGFR inhibitors in 5-20% of patients with EGFR-mutated tumors</td>
</tr>
<tr>
<td>RET</td>
<td>FISH</td>
<td>Positive – gene rearrangements detected&lt;br&gt;• Does not identify translocation partner</td>
<td>May predict response to TKI therapy</td>
</tr>
<tr>
<td>ROS1</td>
<td>IHC FISH reflex</td>
<td>Positive – uniform membranous staining in tumor cells&lt;br&gt;Equivocal – any degree of cytoplasmic staining or focal/weak membranous staining in tumor cells&lt;br&gt;• Reflexes to FISH for confirmation&lt;br&gt;○ Does not identify translocation partner</td>
<td>May predict response to TKI therapy</td>
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