See below for Additional Technical Information topics

**EGFR T790M Mutation in Circulating Tumor DNA**

**Lung Cancer Molecular Markers**

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**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

**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 2012686*

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

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**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

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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**

- **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**
  - 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 gastric GEJ specimens, see PD-L1 22C3 IHC for Gastric GEJ with 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
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 (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

### Single Gene

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