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 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 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%
**Lung Cancer Molecular Markers**

**Indications for Ordering**
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 with KRAS 2008895**
- Screening panel detects
  - EGFR 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 3001302**
- 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**

**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

**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 3001313**
- Aids in prognostication and therapeutic decisions for neoplasms where amplification has been demonstrated
- Screening test for MET gene amplification

**RET Gene Rearrangements by FISH 3001312**
- Detects RET gene rearrangements in solid tumors
- Does not identify the translocation partner or variant

**ROS1 by FISH 3001308**
- 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
For more information about PD-L1 testing, refer to the ARUP Consult PD-L1 Testing topic and algorithm.

**PD-L1 22C3 IHC with Tumor Proportion Score (TPS) Interpretation, pembrolizumab (KEYTRUDA) 2013284**
- Companion diagnostic test to aid in prediction of response to pembrolizumab (KEYTRUDA) as first-line monotherapy for patients with non-small cell lung cancer (NSCLC)
- Can be performed in conjunction with or instead of PD-L1 28-8
- For NSCLC specimens only
  - For gastroesophageal junction (GEJ), urothelial, cervical, or head and neck squamous cell carcinoma (HNSCC) specimens, see PD-L1 22C3 IHC with Combined Positive Score (CPS) Interpretation, pembrolizumab (KEYTRUDA) 3000197
FDA-approved complementary coddagnostic test to aid in prediction of response to nivolumab (OPDIVO) for patients with nonsquamous NSCLC, melanoma, urothelial carcinoma, and HNSCC

**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

**Disease Overview**

- Lung cancer is the second most common cancer in U.S.

**Incidence**

**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>
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<th>Testing method</th>
<th>Test result</th>
<th>Limitations</th>
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<tr>
<td>ALK</td>
<td>IHC</td>
<td>Positive – uniform membranous 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>
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<tr>
<td></td>
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<td>Negative – no cytoplasmic staining in tumor cells</td>
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<td></td>
<td>FISH</td>
<td>Positive – ALK gene rearrangements detected in ≥15% of nuclei</td>
<td>May predict response to TKI therapy</td>
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<td></td>
<td></td>
<td>• Does not identify translocation partner</td>
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<table>
<thead>
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<th>Assay</th>
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<tr>
<td>EGFR</td>
<td>PCR/pyrosequencing</td>
<td>Positive – mutation detected</td>
<td>May predict response to TKI therapy</td>
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<tr>
<td></td>
<td>EGFR T790M (serum) Digital droplet PCR</td>
<td>Positive – mutation detected  • Expressed as percentage</td>
<td>Predicts resistance to TKI therapy</td>
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<tr>
<td>KRAS</td>
<td>PCR/pyrosequencing</td>
<td>Positive – mutation detected</td>
<td>May predict response to TKI therapy</td>
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<tr>
<td>MET</td>
<td>FISH</td>
<td>Positive – detects gene amplification</td>
<td>• May predict response to crizotinib TKI therapy  • 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  • 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 – cytoplasmic staining in tumor cells  Equivocal – any degree of cytoplasmic staining or focal/weak membranous staining in tumor cells  • Reflexes to FISH for confirmation</td>
<td>May predict response to TKI therapy</td>
</tr>
</tbody>
</table>