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%
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)

Screening for immune checkpoint inhibitor therapy FDA-approved PD-L1 companion tests
PD-L1 22C3 IHC for NSCLC with Interpretation, pembrolizumab (KEYTRUDA) 2013284
- Companion diagnostic test to aid in prediction of response to pembrolizumab (KEYTRUDA) as first- or second-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) and urothelial 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
- FDA-approved complementary codiagnostic test to aid in prediction of response to nivolumab (OPDIVO) for patients with nonsquamous NSCLC, melanoma, urothelial carcinoma, and head and neck squamous cell carcinoma (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 44 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

<table>
<thead>
<tr>
<th>Gene</th>
<th>Testing method</th>
<th>Test result</th>
<th>May predict response to TKI therapy</th>
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<tbody>
<tr>
<td>ALK</td>
<td>IHC</td>
<td>Positive – cytoplasmic staining in tumor cells</td>
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<tr>
<td></td>
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<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>
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<td></td>
<td>FISH</td>
<td>Positive – ALK gene rearrangements detected in ≥15% of nuclei</td>
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<td></td>
<td></td>
<td>• Does not identify translocation partner</td>
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<tr>
<td>EGFR</td>
<td>PCR/pyrosequencing</td>
<td>Positive – mutation detected</td>
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<td></td>
<td>EGFR T790M (serum)</td>
<td>Positive – mutation detected</td>
<td>Predicts resistance to TKI therapy</td>
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<td></td>
<td>Digital droplet PCR</td>
<td>• Expressed as percentage</td>
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<tr>
<td>KRAS</td>
<td>PCR/pyrosequencing</td>
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| **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 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  
  o Does not identify translocation partner | May predict response to TKI therapy |