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

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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)

**KRAS Mutation Detection 0040248**

- Detects overexpression of c-MET protein

**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 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 \textit{EGFR} T790M resistance

\textit{EGFR} T790M Mutation Detection in Circulating Tumor DNA by Digital Droplet PCR 2012868

- Monitor blood plasma or cerebrospinal fluid (CSF) for
  - Development of \textit{EGFR} T790M drug-resistant mutation in patients administered TKI therapy for \textit{EGFR}-mutant NSCLC
  - Response to therapy and disease progression in patients receiving \textit{EGFR} T790M-specific TKIs

Related test

\textit{Solid Tumor Mutation Panel by Next Generation Sequencing} 2007991

- Aids in therapeutic decisions for solid tumor cancers
- Simultaneously evaluates mutations in 44 genes, including \textit{AKT1, ALK, BRAF, EGFR, ERBB2, ERBB4, KRAS, NRAS, and PIK3CA}
- Does not detect translocations

Test Methodology

\textit{ALK}

- Immunohistochemistry (IHC) using ALK clone D5F3
- Fluorescence in situ hybridization (FISH)

\textit{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

\textit{EGFR T790M} (serum) – digital droplet PCR

\textit{KRAS} – PCR and pyrosequencing

- Detects mutations at codons 12, 13 (exon 2), and 61 (exon 3)

\textit{c-MET} – IHC

\textit{MET} – FISH

\textit{PD-L1} – IHC

\textit{RET} – FISH

- Detects all \textit{RET} gene fusions

\textit{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
- \textit{Mutation status}
  - Determines TKI therapy eligibility
  - Confers resistance to TKIs (eg, \textit{EGFR} T790M mutation)
    - Monitoring in serum for \textit{EGFR} T790M mutation may detect TKI resistance sooner and alter treatment plans
  - \textit{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>\textit{ALK}</td>
<td>IHC</td>
<td>Positive – cytoplasmic staining in tumor cells</td>
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<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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<tr>
<td></td>
<td>FISH</td>
<td>Positive – \textit{ALK} gene rearrangements detected in ≥15% of nuclei</td>
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<td></td>
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<td>- Does not identify translocation partner</td>
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<td>\textit{EGFR}</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 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  
  o Does not identify translocation partner | May predict response to TKI therapy |