

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

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### 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](#)

- 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), urothelial, and cervical 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)

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## 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
- NCCN Clinical Practice Guidelines in Oncology, Lung Cancer Screening. National Comprehensive Cancer Network. Fort Washington: Pennsylvania [Accessed: February 2018]

Single Gene			
Gene	Testing method	Test result	
<i>ALK</i>	IHC	Positive – cytoplasmic staining in tumor cells Equivocal – very weak cytoplasmic staining visible only on higher power by microscopy Negative – no cytoplasmic staining in tumor cells	May predict response to TKI therapy
	FISH	Positive – <i>ALK</i> gene rearrangements detected in ≥15% of nuclei <ul style="list-style-type: none"><li>• Does not identify translocation partner</li></ul>	May predict response to TKI therapy
<i>EGFR</i>	PCR/pyrosequencing	Positive – mutation detected	May predict response to TKI therapy
	<i>EGFR</i> T790M (serum) Digital droplet PCR	Positive – mutation detected <ul style="list-style-type: none"><li>• Expressed as percentage</li></ul>	Predicts resistance to TKI therapy
<i>KRAS</i>	PCR/pyrosequencing	Positive – mutation detected	May predict response to TKI therapy

Gene	Testing method	Test result
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<i>MET</i>	FISH	Positive – detects gene amplification	<ul style="list-style-type: none"> <li>• May predict response to crizotinib TKI therapy</li> <li>• Associated with acquired resistance to EGFR inhibitors in 5-20% of patients with <i>EGFR</i>-mutated tumors</li> </ul>
<i>RET</i>	FISH	Positive – gene rearrangements detected <ul style="list-style-type: none"> <li>• Does not identify translocation partner</li> </ul>	May predict response to TKI therapy
<i>ROS1</i>	IHC FISH reflex	Positive – uniform membranous staining in tumor cells Equivocal – any degree of cytoplasmic staining or focal/weak membranous staining in tumor cells <ul style="list-style-type: none"> <li>• Reflexes to FISH for confirmation <ul style="list-style-type: none"> <li>○ Does not identify translocation partner</li> </ul> </li> </ul>	May predict response to TKI therapy