Template for Reporting Results of Biomarker Testing of Specimens From Patients With Non-Small Cell Carcinoma of the Lung

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CAP Lung Biomarker Template Revision History

Version Code
The definition of the version code can be found at www.cap.org/cancerprotocols.

Version: LungBiomarkers 1.1.0.0

Summary of Changes
The following changes have been made since the June 28, 2013, early online release article published in Archives of Pathology & Laboratory Medicine (Cagle PT, Sholl LM, Lindeman NI, et al. Template for reporting results of biomarker testing of specimens from patients with non-small cell carcinoma of the lung. Arch Pathol Lab Med. 2013 Jun 28. [Epub ahead of print]):

RESULTS

ALK Rearrangement
Polysomy
The following note was deleted from “Absent”:

#### FISH results are normal. This finding indicates that that tumor is unlikely to respond to therapy with crizotinib. This finding may also be due to the absence of tumor cells in the tested specimen. Clinicopathologic correlation is advised.

METHODS

EGFR Mutational Analysis Testing Method
In the note, “codons” was changed to “exons.”

KRAS Codons Assessed
“146” was deleted.
Biomarker Reporting Template

Completion of the template is the responsibility of the laboratory performing the biomarker testing and/or providing the interpretation. When both testing and interpretation are performed elsewhere (e.g., a reference laboratory), synoptic reporting of the results by the laboratory submitting the tissue for testing is also encouraged to ensure that all information is included in the patient’s medical record and thus readily available to the treating clinical team.

LUNG

Select a single response unless otherwise indicated.

Note: Use of this template is optional.

+ SPECIMEN ADEQUACY

+ Adequacy of Sample for Testing (Note A)
  + ___ Adequate
    + + Estimated tumor cellularity (area used for testing): _____%
  + ___ Suboptimal (explain): __________________________

Note: If “Adequate” not selected, please refer to original laboratory report for explanation.

+ RESULTS

+ EGFR Mutational Analysis (Note B)
  + ___ No mutation detected (wild-type EGFR allele)
  + ___ Mutation identified (select all that apply)
    + + Exon 18 Gly719*
    + + Exon 19 deletion*
    + + Exon 20 insertion**
    + + Exon 20 Thr790Met***
    + + Exon 21 Leu858Arg*
    + + Other (specify)****: __________________________
  + ___ Cannot be determined (explain): __________________________

* This EGFR activation mutation is associated with response to EGFR tyrosine kinase inhibitors.
** This form of EGFR activating mutation is generally associated with resistance to EGFR tyrosine kinase inhibitors although insertions at or before position 768 can be associated with sensitivity.
*** This mutation is typically secondary to other EGFR activating mutations and is associated with acquired resistance to tyrosine kinase inhibitor therapy. If seen in untreated/pretreated patients, may be present in the germline and indicate a hereditary cancer syndrome, in which case genetic counseling is suggested.
**** There is limited data on response to EGFR tyrosine kinase inhibitors for many of the uncommon EGFR activating mutations.

+ ALK Rearrangement (Note C)
  + ___ No rearrangement detected*
  + ___ Rearrangement identified**
  + ___ Cannot be determined (explain): __________________________

+ Data elements preceded by this symbol are not required.
Polysomy
+ ___ Present###
+ ___ Absent

* Absence of ALK rearrangement in cancer cells suggests that this tumor is unlikely to respond to treatment with a targeted inhibitor, such as crizotinib.

** ALK rearrangement predicts response to therapy with a targeted inhibitor, such as crizotinib.

### Polysomy involving the ALK locus confirms that fluorescence in situ hybridization (FISH) scoring was carried out in tumor cells but has no significance with regards to response to therapy with crizotinib.

+ KRAS Mutational Analysis
+ ___ No mutation detected (wild-type KRAS allele)
+ ___ Mutation identified* (select all that apply)
  + Codon 12
    + ___ Gly12Cys (GGT>TGT)
    + ___ Gly12Asp (GGT>GAT)
    + ___ Gly12Val (GGT>GTT)
    + ___ Gly12Ser (GGT>AGT)
    + ___ Gly12Ala (GGT>GCT)
    + ___ Gly12 Arg (GGT>CGT)
    + ___ Specific codon 12 mutation not stated
    + ___ Other codon 12 mutation (specify): __________________________
  + Codon 13
    + ___ Gly13Asp (GGC>GAC)
    + ___ Gly13Arg (GGC>CGC)
    + ___ Gly13Cys (GGC>TGC)
    + ___ Gly13Ala (GGC>GCC)
    + ___ Gly13Val (GGC>GTC)
    + ___ Specific codon 13 mutation not stated
    + ___ Other codon 13 mutation (specify): __________________________
  + Codon 61
    + ___ Gln61Leu (CAA>CTA)
    + ___ Specific codon 61 mutation not stated
    + ___ Other codon 61 mutation (specify): __________________________
  + Other
    + ___ Other codon (specify): __________________________
  + ___ Cannot be determined (explain): __________________________

* No specific tyrosine kinase inhibitors have been approved for lung adenocarcinomas with KRAS mutations. In addition, KRAS mutations are typically mutually exclusive of EGFR and ALK alterations.

+ Other Markers Tested (if applicable) (Note D)
+ Specify marker: __________________________
+ Specify results: __________________________

+ METHODS

+ EGFR Exons Assessed (select all that apply)
  + ___ 18
  + ___ 19
  + ___ 20
  + ___ 21
+ **EGFR** Mutational Analysis Testing Method (select all that apply)
+ ___ Direct (Sanger) sequencing
+ ___ Pyrosequencing
+ ___ High-resolution melting analysis
+ ___ Polymerase chain reaction (PCR), allele-specific hybridization
+ ___ Real-time PCR
+ ___ Next-generation (high-throughput) sequencing
+ ___ Other (specify): ____________________________

Note: Please specify in Comments section if different testing methods were used for different exons.

+ **ALK** Rearrangement Testing Method (select all that apply)
+ ___ In situ hybridization (fluorescence [FISH] or chromogenic [CISH])
+ ___ Reverse transcriptase polymerase chain reaction (RT-PCR)
  + Fusions identified (specify): ____________________________
+ ___ Immunohistochemistry
  + ___ 5A4 clone
  + ___ D5F3 clone
  + ___ Ventana ALK (D5F3) immunohistochemistry (IHC) assay
+ ___ Next-generation (high-throughput) sequencing
+ ___ Other (specify): ____________________________

+ **KRAS** Codons Assessed (select all that apply)
+ ___ 12
+ ___ 13
+ ___ 61

+ **KRAS** Mutational Analysis Testing Method (select all that apply)
+ ___ Direct (Sanger) sequencing
+ ___ Pyrosequencing
+ ___ High-resolution melting analysis
+ ___ PCR, allele-specific hybridization
+ ___ Real-time PCR
+ ___ Next-generation (high-throughput) sequencing
+ ___ Other (specify): ____________________________

Note: Please specify in Comments section if different testing methods were used for different codons.

+ **Testing Method for Other Markers (Note E)**
+ ___ (specify): ____________________________

+ **COMMENT(S)**
  ___________________________________________________________________
  ___________________________________________________________________

Note: Fixative type, time to fixation (cold ischemia time), and time of fixation should be reported if applicable in this template or in the original pathology report (Note F).
Explanatory Notes

Background

As of 2013, over half of lung adenocarcinomas contain one of a number of identifiable genetic alterations; some of these can be targeted by a specific therapeutic inhibitor that is either approved by the Food and Drug Administration or in clinical trials. The National Comprehensive Cancer Network (NCCN) recommends testing for EGFR mutations and ALK rearrangements in all patients with recurrent or metastatic lung adenocarcinomas in order to guide therapy.\(^1\) The College of American Pathologists (CAP), International Association for the Study of Lung Cancer (IASLC), and Association for Molecular Pathology (AMP) have prepared a joint guideline that provides a detailed description of the patient and specimen requirements and acceptable testing designs and strategies for the detection of these alterations\(^2\); the reader is referred to this guideline for details that are beyond the scope of this document.

Currently, no targeted tyrosine kinase inhibitor therapies are specifically approved for KRAS mutations. However, KRAS testing is often performed in lung adenocarcinomas because (1) KRAS mutations are typically mutually exclusive with EGFR and ALK alterations, (2) KRAS mutations are the most common oncogenic alteration in lung adenocarcinoma (~20% to 30% of tumors), and (3) KRAS mutation testing is typically quicker, easier, and less costly than testing for EGFR and ALK.\(^13,14\) Therefore, KRAS mutation analysis may be used in a molecular testing algorithm to eliminate the need for other more costly and time-intensive testing.

Lung adenocarcinomas contain a number of other less common alterations that may lead to treatment with targeted inhibitors but have not yet been studied in large controlled trials nor emerged as standard of care. These include chromosomal rearrangements involving ROS1 (~2% of lung adenocarcinomas, may respond to treatment with crizotinib) and RET (~2% of lung adenocarcinomas),\(^11\) increased copies of MET,\(^15\) and sequence altering mutations in ERBB2, BRAF, and PIK3CA.\(^16\)

A. Suboptimal Specimen Definition

Suboptimal specimens may be defined as those with:

- Improper fixation (see fixation guidelines below).
- Low tumor content, as defined by the molecular diagnostics laboratory. The cutoff for acceptable tumor content depends on the method used by the laboratory. Samples with tumor content below the recommended cutoff may be falsely negative and should be reported as indeterminate if no mutations are detected.

B. Other Mutations

“Other” mutations include uncommon variants including exon 19 insertions or other missense variants in the kinase domain of EGFR (exons 18-21) that are not listed above. Silent mutations that are known, common, single nucleotide polymorphisms in the general population do not need to be included here.
C. Polysomy
Polysomy (multiple copies) at the ALK locus is common in lung adenocarcinoma and when present confirms that FISH has been performed in a tumor cell population. Current evidence suggests that it does not, however, predict response/resistance to targeted therapies.

D. Other Markers Tested
“Other Markers Tested” should be used to report results from molecular assays not included here that may be relevant to lung cancer therapy. These assays may include but are not limited to detection of mutations in genes such as BRAF, ERBB2, and PIK3CA; rearrangements involving ROS1 and RET genes; and MET copy number changes (see “Background” section above).

E. Testing Method for Other Markers
This section should be completed if the “Other Markers Tested” section is filled out and should describe the type of analyses performed for alterations in genes other than EGFR, ALK, and KRAS, as detailed in note D.

F. Fixation
Improper fixation can lead to failure to obtain results with PCR/sequencing-based assays or FISH. Common problems include:

- Procedures or fixation involving acid (eg, decalcification, Bouin’s) may degrade DNA.
- Fixation with heavy metals (eg, Zenker’s, B5, B+, zinc formalin) inhibit the enzymes used in PCR.
- Underfixation or overfixation. Fixation for at least 8 hours and less than 72 hours in buffered formalin is recommended; prolonged fixation, particularly in unbuffered formalin, degrades DNA.

References