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 version control and an explanation of version codes can be found at www.cap.org (search: cancer protocol terms).

Version: LungBiomarkers 1.2.0.0

Summary of Changes
The following changes have been made since the October 2013 version.

RESULTS
The results section has been updated to include the option to report EGFR mutation specific immunohistochemistry results under the EGFR mutational analysis section. The following new biomarkers have been added to the template: BRAF, ERBB2, MET, ROS1, RET.

METHODS
The relevant methods sections have been expanded to include additional techniques that may be used to detect a variety of DNA, RNA, and protein-based alterations.
Lung Biomarker Reporting Template

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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.

+ SPECIMEN TYPE

+ ___ Untreated diagnostic specimen
+ ___ Relapse specimen (after treatment; specify: ___________________)

* When data is available, specify treatment type. This is most relevant to targeted inhibitors associated with specific genomic changes conferring treatment resistance (especially erlotinib, gefitinib, and other EGFR tyrosine kinase inhibitors).

+ 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): _______________________________

* EGFR activation mutation associated with response to EGFR tyrosine kinase inhibitors.
** Exon 20 EGFR activating mutations are generally associated with resistance to EGFR tyrosine kinase inhibitors such as erlotinib, afatinib, and gefitinib, although insertions at or before position 768 can be associated with sensitivity.
*** The T790M 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.

* Data elements preceded by this symbol are not required.
+ **EGFR by Mutation-Specific Immunohistochemistry**
+ **EGFR L858R** (clone 43B2)
+ ___ Negative#
+ ___ Positive##
+ ___ Indeterminate### (explain): __________________________

+ **EGFR Exon 19 Deletion** (E746_A750del) (clone 6B6)
+ ___ Negative#
+ ___ Positive##
+ ___ Indeterminate### (explain): __________________________

# A negative result does not exclude the possibility of EGFR mutations, and testing with molecular methods should be performed if a suitable sample is available.

## Diffuse mutation-specific protein expression in tumor cells is highly correlated with EGFR mutation and as such predicts response to EGFR tyrosine kinase inhibitors.

### Tumors with faint cytoplasmic labeling should be designated as indeterminate. This result can rarely occur both with and without mutation.¹

+ **ALK Rearrangement by Molecular Methods (Note C)**
+ ___ No rearrangement detected#
+ ___ Rearrangement identified (type of FISH pattern, when relevant)##:_________________
+ ___ Cannot be determined (explain): __________________________

+ Polysomy:
  + ___ Present###
  + ___ Absent

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

## ALK rearrangement predicts response to therapy with a targeted ALK inhibitor, such as crizotinib or ceritinib. Some evidence suggests the type of FISH pattern (breakapart versus 5' probe deletion) may have implications for treatment response and outcomes.

### Polysomy involving the ALK locus confirms that fluorescence in situ hybridization (FISH) scoring was carried out in tumor cells but does not predict response to therapy with targeted ALK inhibitors.

+ **ALK by Immunohistochemistry**
+ ___ Negative#
+ ___ Positive##
+ ___ Indeterminate### (explain):

# Absence of ALK protein expression in cancer cells suggests that this tumor is unlikely to harbor ALK rearrangement and to respond to treatment with a targeted inhibitor, such as crizotinib and ceritinib.

## ALK protein expression in cancer cells (based on platform criteria) predicts the presence of ALK rearrangement and response to therapy with a targeted inhibitor, such as crizotinib and ceritinib.

+ **ROS1 Rearrangement by Molecular Methods (Note C)**
+ ___ No rearrangement detected#
+ ___ Rearrangement identified##
+ ___ Cannot be determined (explain): __________________________

+ Polysomy:
  + ___ Present###
  + ___ Absent

# Absence of ROS1 rearrangement in cancer cells suggests that this tumor is unlikely to respond to treatment with a targeted ROS1 inhibitor, such as crizotinib.

## ROS1 rearrangement predicts a high response rate to therapy with a targeted inhibitor, such as crizotinib.
Polysomy involving the ROS1 locus confirms that fluorescence in situ hybridization (FISH) scoring was carried out in tumor cells but does not predict response to therapy with targeted inhibitors.

+ **ROS1 by Immunohistochemistry**
  + ___ Negative
  + ___ Positive
  + ___ Indeterminate (explain): __________________________

  # Absence of ROS1 protein expression in cancer cells suggests that this tumor is unlikely to harbor ROS1 rearrangement and to respond to treatment with a targeted inhibitor, such as crizotinib.

  ## ROS1 protein expression in cancer cells is highly sensitive for a rearrangement involving ROS1 but is not entirely specific. Therefore, confirmatory molecular methods should be used when ROS1 protein expression is detected.

+ **RET Rearrangement by Molecular Methods (Note C)**
  + ___ No rearrangement detected
  + ___ Rearrangement identified
  + ___ Cannot be determined (explain): __________________________

  # Absence of RET rearrangement in cancer cells suggests that this tumor is unlikely to respond to treatment with a targeted RET inhibitor.

  ## RET rearrangement is associated with response to targeted RET inhibitor therapies, such as cabozantinib and vandetinib.

+ **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>GTG)
      + ___ Gly12Ala (GGT>GCT)
      + ___ Gly12Arg (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, ALK, and other driver molecular alterations.
+ **BRAF Mutational Analysis (Note A)**
+ ___ No mutations detected
+ ___ **BRAF** V600E (c.1799T>A) mutation
+ ___ Other **BRAF** V600 mutation (specify): _______________________
+ ___ Cannot be determined (explain): __________________________

+ **ERBB2 Mutational Analysis (Note A)**
+ ___ No mutations detected
+ ___ **ERBB2** 774_775insAYVM insertion mutation
+ ___ **ERBB2** 776_776G>VC insertion mutation
+ ___ Other **ERBB2** exon 20 mutation (specify):
+ ___ Other **ERBB2** mutation (non-exon 20) (specify): ______________
+ ___ Cannot be determined (explain): __________________________

+ **MET Mutational Analysis (Note B)**
+ ___ No mutation detected#
+ ___ **MET** D963_splice mutation detected#
+ ___ **MET** D1010N mutation detected#
+ ___ **MET** D1010_splice mutation detected#
+ ___ Other **MET** intron 13 mutation (specify):
+ ___ Other **MET** intron 14 mutation (specify):
+ ___ Other **MET** exon 14 mutation (specify):
+ ___ **MET** exon 14 deletion detected##
+ ___ Cannot be determined (explain): __________________________

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# **MET** mutation detection is typically based on DNA-based sequencing methods and may include coding (exon) or non-coding (intron) variants, most commonly located at or near the intron-exon junctions around exon 14.

## RNA-based reverse-transcriptase polymerase chain reaction or RNA sequencing may detect deletion of **MET** exon 14 without necessarily indicating the mechanism (DNA-based mutation) leading to the deletion.

+ **MET** Copy Number Analysis
+ ___ No amplification detected#
+ ___ Amplification identified (specify copy number and/or ratio to centromere 7): _____ (copies); _____ (ratio)
+ ___ Cannot be determined (explain): __________________________

+ **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

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* Data elements preceded by this symbol are not required.
+ ___ Mutation-specific immunohistochemistry
  + ___ 43B2 (L858R) clone
  + ___ 6B6 (E746_A750del) clone
  + ___ Other (specify): __________________________
+ ___ 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): __________________________

+ **ROS1** 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
  + ___ D4D6 clone
+ ___ Next-generation (high-throughput) sequencing
+ ___ Other (specify): __________________________

+ **RET** 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): __________________________
+ ___ 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
+ ___ 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 codons.

+ **BRAF** Exons Assessed (select all that apply)
+ ___ 11
+ ___ 15

+ Data elements preceded by this symbol are not required.
+ **BRAF** 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): __________________________

+ **ERBB2** Exons Assessed (select all that apply)
+ ___ 8
+ ___ 16
+ ___ 20

+ **ERBB2** 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): __________________________

+ **MET** Exons Assessed (select all that apply)
+ ___ 14

+ **MET** Mutational Analysis Testing Method (select all that apply)
+ ___ Direct (Sanger) sequencing
+ ___ Next-generation (high-throughput) sequencing
+ ___ Reverse transcriptase-PCR (RT-PCR)
+ ___ Other (specify): __________________________

+ **MET** Copy Number Testing Method (select all that apply)
+ ___ In situ hybridization (fluorescence [FISH] or chromogenic [CISH])
+ ___ Next-generation (high-throughput) sequencing
+ ___ Array comparative genome hybridization (aCGH)
+ ___ Other (specify): __________________________

+ 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).


All reported gene sequence variations should be identified following the recommendations of the Human Genome Variation Society (www.hgvs.org/mutnomen/; accessed February 10, 2015).
Explanatory Notes

Background
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 (FDA) 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. 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; the reader is referred to this guideline for details that are beyond the scope of this document.

Briefly, approximately 20% of lung adenocarcinomas contain an EGFR activating mutation that predicts response to therapy with EGFR tyrosine kinase inhibitors such as erlotinib. Up to 90% of EGFR mutations occur in two hot spots within the kinase domain, as small deletions in the LREA motif of exon 19 or as a leucine to arginine substitution at amino acid 858 (exon 21). Molecular testing (sequencing or PCR-based approaches) is recommended for EGFR mutation detection whenever possible. Immunohistochemistry (IHC) with EGFR mutation-specific antibodies has high specificity, but suboptimal sensitivity: 76% for EGFR L858R and 60% or less for EGFR exon 19 deletions. In addition, these antibodies do not detect less common types of sensitizing EGFR mutations. In particular, EGFR exon 19 deletion antibody recognizes primarily a 15-base-pair deletion (E746_A750del), which comprises ~50% of exon 19 deletions, but inconsistently detects other types of exon 19 deletions. Because of suboptimal sensitivity, IHC for EGFR mutations may only have a role in situations in which molecular testing is not available or a sample is insufficient or technically unsuitable for testing, such as due to decalcification. If performed, a note should be included that a negative result does not exclude the possibility of EGFR mutations, and testing with molecular methods should be performed if a suitable sample becomes available.

The vast majority of patients with EGFR-mutated lung adenocarcinoma treated with EGFR tyrosine kinase inhibitors will relapse as a result of resistance to these drugs. The most common resistance mechanism is acquisition of the EGFR exon 20 T790M mutation. However, new covalent inhibitors have shown efficacy against relapsed lung adenocarcinomas with EGFR T790M mutations in large phase 2 clinical trials but appear less effective in tumors with alternative resistance mechanisms. Therefore, biopsy at the time of relapse may be required to determine the best course of therapy.

Approximately 5% of lung adenocarcinomas have a chromosomal rearrangement involving the ALK gene, most commonly in the form of an intrachromosomal inversion leading to the EML4-ALK fusion product associated with ALK protein overexpression. Patients with this tumor type are responsive to therapy with ALK tyrosine kinase inhibitors, such as crizotinib. Fluorescence in situ hybridization (FISH) using break-apart probes is currently considered the “gold standard” for detection of ALK rearrangement, which may manifest as separation of the 5’ and 3’ FISH probes or as deletion of 5’ probe. Although both types of alterations are associated with response to ALK-targeted therapies, some studies suggest that 5’ probe deletion can rarely represent a false positive FISH result. Detection of ALK protein overexpression by IHC using the 5A4 or D5F3 clones is highly sensitive and specific for an ALK gene rearrangement in lung adenocarcinoma, and the FDA has approved the use of the Ventana ALK (D5F3) CDx Assay as a companion diagnostic for crizotinib. According to the current guidelines, other carefully-validated, but non-FDA approved, ALK immunohistochemistry assays may also be used to screen for ALK rearrangement with confirmation by FISH before initiating ALK-targeted therapy.

Patients receiving ALK-targeted inhibitors inevitably acquire resistance to these drugs, often in the form of ALK kinase domain mutations. The more potent ALK inhibitor ceritinib has shown efficacy in these relapsed patients and has been approved for those who developed resistance to, or could not tolerate, crizotinib. The mechanism of resistance does not clearly predict response to this next line therapy; therefore, the role for rebiopsy in patients with ALK-rearranged tumors is not clear.
ROS1 rearrangement occurs in 1% to 2% of non-small cell lung carcinomas and predicts response to crizotinib therapy.\textsuperscript{15,22,23} ROS1 fusion partners include SLC34A2, CD74, TPM3, GOPC (FIG), SDC4, EZR, LRIG3, KDELR2, and CCDC6. Several methods, including FISH, IHC, anchored multiplex or reverse-transcriptase PCR, and next-generation sequencing, may be considered for ROS1 rearrangement detection\textsuperscript{21,23}; no "gold standard" method has been defined. Notably, IHC using the available D4D6 antibody appears to be a robust screening tool, but due to suboptimal specificity, positive results should be confirmed by another technique.\textsuperscript{24,25}

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.\textsuperscript{26} Therefore, KRAS mutation analysis may be used in a molecular testing algorithm to eliminate the need for other more costly and time-intensive testing.

BRAF mutations are detected in about 5% of lung adenocarcinomas; V600E mutations comprise approximately half of these and are therefore found in 2% to 3% of tumors.\textsuperscript{27} The role of BRAF as a targetable oncogene was recently established in phase II clinical trials combining the BRAF inhibitor dabrafenib with the MEK inhibitor trametinib, where this drug combination was associated with a 63% overall response rate in patients with BRAF V600E-mutated non-small cell lung carcinoma.\textsuperscript{28}

The utility of BRAF V600E mutation-specific IHC is not well established in lung adenocarcinoma. The variable sensitivity and specificity of this immunohistochemistry assay relative to mutation analysis reported in other tumor types suggests it may not be appropriate as a clinical tool for BRAF V600E detection in lung tumors.\textsuperscript{29}

MET has been recognized as a putative biomarker in lung adenocarcinoma for many years, with amplification implicated as a mechanism of resistance to EGFR inhibitors.\textsuperscript{30} In rare cases, de novo MET amplification has been associated with profound responses to therapy with crizotinib. Recently, splicing variants and insertion-deletion mutations leading to MET exon 14 deletion have been found in about 3% of lung adenocarcinomas; these events lead to MET activation and are associated with response to crizotinib and cabozantinib.\textsuperscript{31,32} MET amplification can be seen in tumors with MET exon 14 deletion. MET protein overexpression appears to correlate with MET amplification, although MET immunohistochemistry is controversial as a biomarker for MET-targeted therapies.\textsuperscript{33}

Oncogenic RET gene rearrangements have been reported in 1% to 2% of lung adenocarcinoma and are more common in never-smokers.\textsuperscript{34,35} Due to the relative rarity of RET-rearranged tumors, phase 3 clinical trials with targeted agents for RET have not been conducted yet, but preliminary data from phase 2 trials of a multi-targeted inhibitor cabozantinib in RET-rearranged lung tumors are promising. RET rearrangements are detectable by FISH; however, the most commonly described rearrangement, KIF5B-RET, is a product of small intrachromosomal inversion leading to only a subtle split in the FISH probe signals. Thus, the rearrangement could be difficult to detect in practice.\textsuperscript{36} Alternative detection methods such as anchored multiplex PCR or next generation sequencing may be useful. The role of RET IHC in predicting RET rearrangements has not been well evaluated.

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. ERBB2 exon 20 insertion mutations are established as oncogenic alterations in lung adenocarcinoma; patients whose tumors harbor these mutations have shown response to HER-tyrosine kinase inhibitors in phase I studies.\textsuperscript{37} Alterations in MAP2K1 (MEK1) are rare (<1% of lung adenocarcinoma) but are highly correlated with a smoking history and may predict sensitivity to MEK inhibitors.\textsuperscript{38} Alterations leading to PI3K pathway activation, including PIK3CA or AKT1 activating mutations or PTEN loss of function may serve as biomarkers for enrollment in PI3K- or AKT-inhibitor trials.\textsuperscript{39} Recently identified fusion events involving NRG1 and NTRK1 (TRKA) appear to be rare but distinctive oncogenic events in a subset of lung adenocarcinomas.\textsuperscript{40,41}

The programmed death-1 receptor (PD-1) and its ligand, PD-L1, are targeted by FDA-approved monoclonal antibody therapeutics such as nivolumab (Opdivo) and pembrolizumab (Keytruda).\textsuperscript{42-44} While the biomarker of PD-L1 expression has been studied by IHC within clinical trials, the detection antibodies used and scoring cutoffs for the tumor cells and immune cells are highly variable. As of October 2015, the FDA granted accelerated
approval of pembroluzimab for treatment of patients with metastatic non-small cell carcinoma following progression on other therapies. This drug was approved together with a companion diagnostic immunohistochemistry assay (PD-L1 IHC 22C3 pharmDx test) for use in patients with PD-L1 expression in at least 50% of tumor cells. Also in October 2015, the FDA expanded approval of nivolumab for non-squamous non-small cell lung carcinomas but did not require a companion diagnostic. However, PD-L1 expression may indicate likely response to nivolumab, and the FDA approved the PD-L1 IHC 28-8 PharmDx assay as a complementary diagnostic for nivolumab for non-squamous non-small cell lung carcinomas. Additional immunotherapeutics and distinct paired diagnostics are expected to receive FDA approval for use in lung cancer in the future. At this point in time, the best approach to PD-L1 assay selection and scoring is unclear and may be subject to the tumor type and available therapy, recognizing that most laboratories will be challenged to introduce, validate, and reproducibly report diverse immunohistochemical assays on diverse platforms.

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 \textit{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 \textit{ALK}, \textit{RET}, and \textit{ROS1} loci may be seen 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 \textit{BRAF}, \textit{ERBB2}, and \textit{PIK3CA}; rearrangements involving \textit{ROS1} and \textit{RET} genes; and \textit{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 \textit{EGFR}, \textit{ALK}, and \textit{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 the following:
- 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

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