Updated Molecular Testing Guideline for the Selection of Lung Cancer Patients for Treatment With Targeted Tyrosine Kinase Inhibitors

Guideline From the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology

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• ***Context.***—**In 2013, an evidence-based guideline was published by the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology to set standards for the molecular analysis of lung cancers to guide treatment decisions with targeted inhibitors. New evidence has prompted an evaluation of additional laboratory technologies, targetable genes, patient populations, and tumor types for testing.**

Objective.—**To systematically review and update the 2013 guideline to affirm its validity; to assess the evidence of new genetic discoveries, technologies, and therapies; and to issue an evidence-based update.**

Design.—**The College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology convened an expert panel to develop an evidence-based guideline to help define the key questions and literature search terms, review abstracts and full articles, and draft recommendations.**

Results.—**Eighteen new recommendations were drafted. The panel also updated 3 recommendations from the 2013 guideline.**

Conclusions.—**The 2013 guideline was largely reaffirmed with updated recommendations to allow testing of cytology samples, require improved assay sensitivity, and recommend against the use of immunohistochemistry for EGFR testing. Key new recommendations include *ROS1* testing for all adenocarcinoma patients; the inclusion of additional genes (*ERBB2, MET, BRAF, KRAS*, and *RET*) for laboratories that perform next-generation sequencing panels; immunohistochemistry as an alternative to fluorescence in situ hybridization for ALK and/or ROS1 testing; use of 5% sensitivity assays for *EGFR* T790M mutations in patients with secondary resistance to EGFR inhibitors; and the use of cell-free DNA to "rule in" targetable mutations when tissue is limited or hard to obtain.**

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Patients with advanced lung cancer have a poor prognosis, with a median survival of 1 year. However, for many patients whose tumors harbor certain specific molecular alterations (eg, activating alterations in the *EGFR, ALK,* and *ROS1* genes), particularly in lung adenocarcinoma, targeted tyrosine kinase inhibitor (TKI) therapy provides significant improvement in survival and quality. Accordingly, patients with the types of advanced lung cancer in which these targetable molecular alterations typically occur should receive the molecular testing required to identify them, and thereby receive appropriate targeted treatments. Importantly, this testing should extend beyond those molecular alterations for which targeted therapies are approved by regulatory agencies such as the US Food and Drug Administration (FDA) to include molecular alterations for which there is compelling evidence of effective investigational targeted therapies (and, more recently, immunotherapies) from published clinical trials.

In 2010, 3 professional societies—the College of American Pathologists (CAP), the International Association for the Study of Lung Cancer (IASLC), and the Association for Molecular Pathology (AMP)—recruited specialists in the biology, diagnosis, and treatment of lung cancer to form a joint working group to systematically assess the evidence supporting the clinical utility of molecular analysis of lung cancer samples. In 2013, this working group published an evidence-based guideline[1–3] for standard-of-care clinical practice concerning which lung cancer patients and samples should be tested, which genes should be tested, and how these tests should be designed, validated, and executed. This guideline was subsequently endorsed by the American Society of Clinical Oncology,[4] and has been cited in guidelines developed by numerous professional societies around the world.[5–26] However, the field has continued to advance rapidly, with the emergence of new genetic discoveries, new therapies, and new technologies, such that these same 3 organizations convened a second working group to systematically assess new evidence and to issue an evidence-based revision of the lung cancer molecular pathology practice guideline.

The revision focuses on new recommendations in 5 specific content areas: (1) Which new genes should routinely be tested for alterations in lung cancers? (2) What methods are appropriate for lung cancer testing, with particular emphases on the use of immunohistochemistry (IHC) and next-generation sequencing (NGS)? (3) Is there a need to test patients with squamous cell, small cell, or other nonadenocarcinoma lung cancers? (4) What testing should be performed for patients with a targetable alteration who have progressed following initial response to appropriately targeted therapy? (5) What is the role of testing circulating cell-free DNA (cfDNA) in lung cancer patient management? In addition, new evidence supporting the original 2013 guideline was reviewed and used to either modify the strength of those recommendations or change them entirely. Finally, a sixth question, regarding diagnostic support for the role of immunomodulatory therapies (eg, programmed death ligand-1 or PD-L1), emerged during the revision process. Although this topic was not subject to the systematic review of evidence, the expert panel decided to issue an opinion statement addressing this question, aware that separate efforts are currently underway to develop evidence-based recommendations regarding the use of biomarkers to select patients for immunomodulatory therapies.

One particular challenge for this evidence-based guideline revision was the rapid pace of discovery in this field. During the time between literature review and guideline drafting, major new discoveries were published and treatment advanced for *BRAF*-mutant lung cancers and for the use of immunotherapies. We expect that many additional advances will emerge in the fields of targeted therapy, cfDNA diagnostics, and immunotherapies in the near term. Although we make strong recommendations for the molecular biomarkers for which there was good evidence at the time we conducted our analysis, we also fully recognize the importance of emerging biomarkers to enable lung cancer patients to be eligible for clinical trials of investigational therapies. Accordingly, we have stratified the biomarkers in this guideline into 3 categories, rather than 2. The first are "must-test" biomarkers, which are standard of care for all patients with advanced lung cancer with an adenocarcinoma component who are being considered for an approved targeted therapy. Second are "should-test" biomarkers, which are used to direct patients to clinical trials and which should be included in any large sequencing panel that is performed for lung cancer patients, but which are not required for laboratories that perform only single-gene assays. All remaining candidate biomarkers are investigational and are not appropriate for clinical use at this time.

PANEL COMPOSITION

The CAP, IASLC, and AMP convened an expert panel consisting of practicing pathologists and oncologists with expertise and experience in lung carcinoma. The CAP, IASLC, and AMP approved the appointment of the project cochairs and expert panel members. In addition, a methodologist experienced in systematic review and guideline development consulted with the panel throughout the project.

CONFLICT OF INTEREST POLICY

Prior to acceptance on the expert panel, potential members completed a joint conflict of interest disclosure process, whose policy and form require disclosure of material financial interest in, or potential for benefit of significant value from, the guideline's development or its recommendations. The potential members completed the conflict of interest disclosure form, listing any relationship that could be interpreted as constituting an actual, potential, or apparent conflict. Potential conflicts were managed by the cochairs. All expert and advisory panel members were required to disclose conflicts prior to beginning and continuously throughout the project's timeline. Disclosed conflicts of the expert panel members are listed in the Appendix. The CAP, IASLC, and AMP provided funding for the administration of the project; no industry funds were used in the development of the guideline. All panel members volunteered their time and were not compensated for their involvement. Please see the supplemental digital content (SDC) at www.archivesofpathology.org in the March 2018 table of contents for full details on the conflict of interest policy.

OBJECTIVE

The expert panel was charged with the review and update of the CAP-IASLC-AMP molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors. The panel reviewed any new studies that

would change or refute the statements from the 2013 guideline. In addition, the panel also addressed additional key questions:

1. Which new genes should be tested for lung cancer patients?
2. What methods should be used to perform molecular testing?
3. Is molecular testing appropriate for lung cancers that do not have an adenocarcinoma component?
4. What testing is indicated for patients with targetable mutations who have relapsed on targeted therapy?
5. What is the role of testing for circulating cell-free DNA for lung cancer patients?

Key questions 1 through 3 relate to patients diagnosed with nonsquamous non–small cell lung cancer (NSCLC) of all stages. The key questions are included in full detail in the SDC.

METHODS

A detailed account of the methods used to create this guideline can be found in the SDC, including additional scope questions.

Systematic Literature Review and Analysis

A systematic literature review was completed with 2 comprehensive searches. The first search was designed to assess the 2013 guideline statements and was based on the original search strategy. It included medical subject headings and keywords to address the concepts *lung cancer*, *tumor biomarkers*, and *laboratory testing* and was run in Ovid MEDLINE (Ovid Technologies, Inc, New York City, New York) on May 17, 2015, to locate studies published in English with publication dates from January 1, 2012 through May 17, 2015. Publication filters were applied to identify guidelines, systematic reviews, meta-analyses (MAs), and randomized clinical trials. The search was rerun on June 27, 2016, to identify relevant new literature published since May 17, 2015.

The second search was based on new key questions that focused on additional biomarkers not included in the 2013 guideline, with specific search strategies designed for each key question. All searches were performed in Ovid MEDLINE and PubMed (US National Library of Medicine, Bethesda, Maryland) (June 28, 2015) and were limited to English-language studies. Supplemental searches were run in Scopus (Amsterdam, Netherlands) (June 25, 2015) to identify relevant publications not indexed in MEDLINE. A search for relevant clinical trials was completed using the clinicaltrials.gov Web site, and focused searches on guideline repository sites (eg, guideline.gov, g-i-n.net) and organizations' Web sites were undertaken to identify relevant publications. Further detail about the systematic literature search, including the Ovid search strings, can be found in the SDC.

Eligible Study Designs

Studies were not limited to randomized controlled trials but also included other study types, including cohort designs, case series, evaluation studies, and comparative studies. Letters, commentaries, editorials, narrative reviews, case reports, studies in mouse models, in vitro studies, consensus documents, abstracts, and non-English articles were excluded a priori.

Inclusion Criteria

Published studies were selected for inclusion in the systematic review of evidence if they were peer-reviewed full-text articles that met the following criteria:

1. The study population consisted of patients with nonsquamous, non–small cell lung adenocarcinoma, small cell lung carcinoma, or squamous cell lung cancer of any stage.
2. The study evaluated, prospectively or retrospectively, sensitivity, specificity, negative predictive value, or positive predictive value of *EGFR*, *ALK*, *KRAS*, *ROS1*, *RET*, *MET*, *BRAF*, or *ERBB2* (*HER2*) tests for detection of gene-specific mutation, rearrangement, translocation, amplification, overexpression, or response to a targeted gene-specific therapy.
3. The study examined potential testing algorithms for NSCLC molecular testing.
4. The study examined the correlation of *EGFR*, *ALK*, *KRAS*, *ROS1*, *RET*, *MET*, *BRAF*, or *ERBB2* (*HER2*) status in primary or metastatic tumors from the same patients.
5. The study included primary outcomes such as accuracy, sensitivity, specificity, positive predictive value, and negative predictive value of tests and concordance across platforms to determine *EGFR*, *ALK*, *KRAS*, *ROS1*, *RET*, *MET*, *BRAF*, or *ERBB2* (*HER2*) status or treatment response, alone or in combination.

Quality Assessment

An assessment of the quality of the evidence was performed for all retained studies following application of the inclusion and exclusion criteria. Using this method, studies deemed low quality would not be excluded from the systematic review, but would be retained and their methodologic strengths and weaknesses discussed where relevant. Each guideline statement includes a rating of the strength of the evidence as described in Table 1 (also in SDC Table 1). The process used to assess the quality of the evidence base is fully detailed in the SDC.

Assessing the Strength of Recommendations

In order to articulate recommendation statements that were clearly written and easy to implement, the expert panel used GLIDES (Guidelines Into Decision Support) methodology and accompanying BridgeWiz software (Yale University, New Haven, Connecticut).[27] This methodology prioritizes the use of active language; however, in some situations, the person responsible for ensuring guidance is implemented is dependent on the organization of the clinic and/or laboratory. To ensure clarity of guidance in these situations, the expert panel used passive-voice language to emphasize the recommended action. Development of recommendations required that the panel review the identified evidence and make a series of key judgments (using procedures described in the SDC). This guideline uses a 3-tier system to rate the strength of recommendations, as well as a "no recommendation" category when there is insufficient evidence to support a recommendation. Table 2 (also in SDC Table 2) summarizes the strength of evidence and net benefits and harms, as well as obligatory language that was used for each of the recommendation types.

Guideline Revision

This guideline will be reviewed every 4 years or earlier in the event of publication of substantive and high-quality evidence that could potentially alter the original guideline statements. If necessary, the entire panel will reconvene to discuss potential changes and, if indicated, recommend revision of the guideline to CAP, IASLC, and AMP.

Disclaimer

Practice guidelines and consensus statements reflect the best available evidence and expert consensus supported in practice. They are intended to assist physicians and patients in clinical decision making and to identify questions and settings for further research. With the rapid flow of scientific information, new evidence may emerge between the time a practice guideline or consensus statement is developed and when it is published or read. Guidelines and statements are not continually updated and may not reflect the most recent evidence. Guidelines and statements address only the topics specifically identified therein and are not applicable to other interventions, diseases, or stages of diseases. Furthermore, guidelines and consensus statements cannot account for individual variation among patients and cannot be considered

Table 1. Grades for Strength of Evidence[a]		
Designation	**Description**	**Quality of Evidence**
Convincing	High confidence that available evidence reflects true effect. Further research is very unlikely to change the confidence in the estimate of effect.	High/intermediate quality of evidence.
Adequate	Moderate confidence that available evidence reflects true effect. Further research is likely to have an important impact on the confidence in estimate of effect and may change the estimate.	Intermediate/low quality of evidence.
Inadequate	Little confidence that available evidence reflects true effect. Further research is very likely to have an important impact on the confidence in the estimate of effect and is likely to change the estimate.	Low/insufficient evidence and expert panel uses formal consensus process to reach recommendation.
Insufficient	Evidence is insufficient to discern net effect. Any estimate of effect is very uncertain.	Insufficient evidence and expert panel uses formal consensus process to reach recommendation.

[a] Adapted from *J Clin Epidemiol*. 2011;64(4):401–406, Balshem H, Helfand M, Schunemann HJ, et al. GRADE guidelines: 3. Rating the quality of evidence, copyright 2011, with permission from Elsevier.[262]

inclusive of all proper methods of care or exclusive of other treatments. It is the responsibility of the treating physician or other health care provider, relying on independent experience and knowledge, to determine the best course of treatment for the patient. Accordingly, adherence to any practice guideline or consensus statement is voluntary, with the ultimate determination regarding its application to be made by the physician in light of each patient's individual circumstances and preferences. The CAP, IASLC, and AMP make no warranty, express or implied, regarding guidelines and statements and specifically exclude any warranties of merchantability and fitness for a particular use or purpose. The CAP, IASLC, and AMP assume no responsibility for any injury or damage to persons or property arising out of or related to any use of this statement or for any errors or omissions.

RESULTS

For the reaffirmation of the 2013 guideline recommendations, a total of 610 studies met the search term requirements. Following a review of the 610 abstracts, the full texts of 77 studies that met the inclusion criteria and were likely to refute or change the 2013 recommendations were reviewed. A total of 21 articles were included for data extraction. Excluded articles were available as discussion or background references.

For the new key questions, 1654 articles met the search term requirements. Based on review of these abstracts, 488 articles met the inclusion criteria and continued to full-text review. Articles that addressed any of the new key questions were moved to a second-level full-text–review phase. A total of 118 articles were included for data extraction. Excluded articles were available as discussion or background references.

The panel convened 5 times (3 times by teleconference and 2 face-to-face meetings) to develop the scope, draft recommendations, review and respond to solicited feedback, and assess the quality of evidence that supports the final recommendations. A nominal group technique was used by the panel for consensus decision making to encourage unique input with balanced participation among group members. An open comment period was held from June 28 to August 2, 2016, during which the 2013 guideline statements and new draft recommendations and statements were posted for public comment. The public comment period was posted on the AMP Web site at www.amp.org. All 2013 recommendations received strong agreement (95%–99%) from the open comment period participants. There were 20 new draft statements with strong agreement, ranging from 86% to 97%, from the open comment period participants (refer to Outcomes in the SDC for full details). The expert panel members were assigned to review the public comments in small groups. The panel modified the draft statements and recommended the deletion of 1 expert consensus opinion and a no recommendation statement based on the feedback during the considered judgment process. The final recommendations were approved by the expert panel with a vote. The panel considered benefits and harms, required resources, feasibility, and acceptability throughout the entire process, although neither cost nor

Table 2. Strength of Recommendations[a]		
Designation	**Recommendation**	**Rationale**
Strong recommendation	Recommend for or against a particular molecular testing practice in lung cancer (can include must or should).	Supported by convincing (high) or adequate (intermediate) quality of evidence and clear benefit that outweighs any harms.
Recommendation	Recommend for or against a particular molecular testing practice in lung cancer (can include should or may).	Some limitations in quality of evidence (adequate [intermediate] or inadequate [low]), balance of benefits and harms, values, or costs, but panel concludes that there is sufficient evidence to inform a recommendation.
Expert consensus opinion	Recommend for or against a particular molecular testing practice in lung cancer (can include should or may).	Serious limitations in quality of evidence (inadequate [low, very low] or insufficient), balance of benefits and harms, values, or costs, but panel consensus is that a statement is necessary.
No recommendation	No recommendation for or against a particular molecular testing practice in lung cancer.	Insufficient evidence, confidence, or agreement to provide a recommendation.

[a] Data derived from Andrews et al.[263]

Table 3. Summary of the Updated Statements With Strength of Recommendations[a]	
2013 Statement	**2018 Statement**
Expert consensus opinion: Cytologic samples are also suitable for EGFR and ALK testing, with cell blocks being preferred over smear preparations.	Recommendation: Pathologists may use either cell blocks or other cytologic preparations as suitable specimens for lung cancer biomarker molecular testing.
Expert consensus opinion: Laboratories should use EGFR test methods that are able to detect mutations in specimens with at least 50% cancer cell content, although laboratories are strongly encouraged to use (or have available at an external reference laboratory) more sensitive tests that are able to detect mutations in specimens with as little as 10% cancer cells.	Expert consensus opinion: Laboratories should use, or have available at an external reference laboratory, clinical lung cancer biomarker molecular testing assays that are able to detect molecular alterations in specimens with as little as 20% cancer cells.
Recommendation: Immunohistochemistry for total EGFR is not recommended for selection of EGFR TKI therapy.	Strong recommendation: Laboratories should not use total EGFR expression by IHC testing to select patients for EGFR-targeted TKI therapy.

Abbreviations: IHC, immunohistochemistry; TKI, tyrosine kinase inhibitor.

[a] Supplemental Table 4b includes a list of the 2013 reaffirmed statements.

cost-effectiveness analyses were performed. A description of the benefits and harms of implementing the guideline statements is included in the SDC (SDC Table 3).

Each organization instituted a review process to approve the guideline. For the CAP, an independent review panel representing the Council on Scientific Affairs was assembled to review and approve the guideline. The independent review panel was masked to the expert panel and vetted through the conflict of interest process. The IASLC approval process required review and approval by the IASLC Board of Directors. The AMP approval process required content review by an independent subject matter expert panel, led by the Publications & Communications chair, with representation from the Clinical Practice Committee and Solid Tumors Subdivision leadership, and organizational approval by the AMP Executive Committee.

GUIDELINE STATEMENTS

Reaffirmation of 2013 Recommendations

The 2013 guideline recommended universal testing of lung cancer patients with advanced-stage cancers with an adenocarcinoma component, using molecular diagnosis for activating "hot-spot" mutations in *EGFR* exons 18 to 21 with at least 1% prevalence (ie, codons 709 and 719, exon 19 deletion 768, and exon 20 insertions 790, 858, and 861), and using fluorescence in situ hybridization (FISH) for rearrangements involving *ALK*. Any methodology or testing algorithm with suitable analytic sensitivity (ability to detect mutations in formalin-fixed samples with 50% or more malignant cells) and turnaround time (10 days between sample receipt and reporting of all results), with appropriate validation and deployment under the Clinical Laboratory Improvement Act of 1988, was acceptable.

The 2013 guideline recommended against applying clinical parameters (eg, tobacco exposure, age, sex, ethnicity) to select patients for testing, testing pure squamous carcinomas, using *KRAS* negativity as a determinant of anti-EGFR therapy, using IHC for EGFR or ALK testing, and using FISH for *EGFR* testing.

The 2013 guideline left several decisions open to each institution to set policy, such as whether or not to test early-stage patients, whether or not to use clinical predictors to select patients with minimally sampled squamous carcinoma biopsies such that a mixed adenosquamous carcinoma could not be excluded, and whether or not to use a simultaneous or sequential testing approach. Of these, the question concerning testing early-stage disease remains open, and awaits data from more clinical trials before an evidence-based recommendation can be made. Although the American Society of Clinical Oncology Clinical Practice Guidelines Committee highlighted consideration of molecular testing for early-stage lung cancer patients,[4] our opinion remains that each institution should set its own policy regarding testing patients with early-stage disease, balancing the benefit of having results on record from testing a high-quality resection sample for alterations that are likely to become necessary at a time of future progression when a high-quality sample could be hard to obtain against the cost of testing patients for whom a subset will be surgically cured and never need the test result. Accordingly, the testing recommended below applies to patients with advanced-stage (stages IIIB and IV) lung cancer.

Following review of literature published since 2013, the original recommendations are largely reaffirmed. Several statements have gained strength with the publication of additional supporting evidence (SDC Tables 4a, 4b, and 5). Some warranted a complete reevaluation in this revision, and will appear subsequently (Table 3); these include the use of IHC for ALK, the use of multigene NGS panels, and the question of testing nonadenocarcinoma samples.

Of the remaining 2013 recommendations, the following changes are made:

1. Any Cytology Sample With Adequate Cellularity and Preservation May Be Tested.—The original recommendation preferred cell blocks over smears. A recent systematic review[28] identified by the literature search has indicated that numerous studies have been published showing excellent performance of smear preparations, such that this preference is no longer appropriate. It is incumbent upon any laboratory that tests cytopathology specimens to perform appropriate validation studies of these as separate sample types, distinct from tissue and blood samples.

2. Analytic Methods Must Be Able to Detect Mutation in a Sample With 20% or More Malignant Cell Content.—Although the original studies demonstrating response of *EGFR*-mutated lung cancers to treatment with EGFR inhibitors used unmodified Sanger sequencing with a sensitivity limit of 50% tumor cellularity, this is insufficient in practice because many lung cancer samples are small and comprise a majority of benign stromal cells, and most of the larger phase III clinical trials that confirmed the clinical

Table 4. Summary of 2018 Guideline Statements

Guideline Statements	Strength of Recommendation
Key Question 1: Which new genes should be tested for lung cancer patients?	
1. *ROS1* testing must be performed on all lung adenocarcinoma patients, irrespective of clinical characteristics.	Strong recommendation
2. ROS1 IHC may be used as a screening test in lung adenocarcinoma patients; however, positive ROS1 IHC results should be confirmed by a molecular or cytogenetic method.	Expert consensus opinion
3. *BRAF* molecular testing is currently not indicated as a routine stand-alone assay outside the context of a clinical trial. It is appropriate to include *BRAF* as part of larger testing panels performed either initially or when routine *EGFR, ALK*, and *ROS1* testing are negative.	Expert consensus opinion
4. *RET* molecular testing is not recommended as a routine stand-alone assay outside the context of a clinical trial. It is appropriate to include *RET* as part of larger testing panels performed either initially or when routine *EGFR, ALK*, and *ROS1* testing are negative.	Expert consensus opinion
5. *ERBB2* (*HER2*) molecular testing is not indicated as a routine stand-alone assay outside the context of a clinical trial. It is appropriate to include *ERBB2* (*HER2*) mutation analysis as part of a larger testing panel performed either initially or when routine *EGFR, ALK,* and *ROS1* testing are negative.	Expert consensus opinion
6. *KRAS* molecular testing is not indicated as a routine stand-alone assay as a sole determinant of targeted therapy. It is appropriate to include *KRAS* as part of larger testing panels performed either initially or when routine *EGFR, ALK*, and *ROS1* testing are negative.	Expert consensus opinion
7. *MET* molecular testing is not indicated as a routine stand-alone assay outside the context of a clinical trial. It is appropriate to include *MET* as part of larger testing panels performed either initially or when routine *EGFR, ALK,* and *ROS1* testing are negative.	Expert consensus opinion
Key Question 2: What methods should be used to perform molecular testing?	
8. IHC is an equivalent alternative to FISH for ALK testing.	Recommendation
9. Multiplexed genetic sequencing panels are preferred over multiple single-gene tests to identify other treatment options beyond *EGFR, ALK,* and *ROS1*.	Expert consensus opinion
10. Laboratories should ensure test results that are unexpected, discordant, equivocal, or otherwise of low confidence are confirmed or resolved using an alternative method or sample.	Expert consensus opinion
Key Question 3: Is molecular testing appropriate for lung cancers that do not have an adenocarcinoma component?	
11. Physicians may use molecular biomarker testing in tumors with histologies other than adenocarcinoma when clinical features indicate a higher probability of an oncogenic driver.	Expert consensus opinion
Key Question 4: What testing is indicated for patients with targetable mutations who have relapsed on targeted therapy?	
12. In lung adenocarcinoma patients who harbor sensitizing *EGFR* mutations and have progressed after treatment with an EGFR-targeted tyrosine kinase inhibitor, physicians must use *EGFR* T790M mutational testing when selecting patients for third-generation EGFR-targeted therapy.	Strong recommendation
13. Laboratories testing for *EGFR* T790M mutation in patients with secondary clinical resistance to EGFR-targeted kinase inhibitors should deploy assays capable of detecting *EGFR* T790M mutations in as little as 5% of viable cells.	Recommendation
14. There is currently insufficient evidence to support a recommendation for or against routine testing for *ALK* mutational status for lung adenocarcinoma patients with sensitizing *ALK* mutations who have progressed after treatment with an ALK-targeted tyrosine kinase inhibitor.	No recommendation
Key Question 5: What is the role of testing for circulating cell-free DNA for lung cancer patients?	
15. There is currently insufficient evidence to support the use of circulating cell-free plasma DNA molecular methods for the diagnosis of primary lung adenocarcinoma.	No recommendation
16. In some clinical settings in which tissue is limited and/or insufficient for molecular testing, physicians may use a cell-free plasma DNA assay to identify *EGFR* mutations.	Recommendation
17. Physicians may use cell-free plasma DNA methods to identify *EGFR* T790M mutations in lung adenocarcinoma patients with progression or secondary clinical resistance to EGFR-targeted tyrosine kinase inhibitors; testing of the tumor sample is recommended if the plasma result is negative.	Expert consensus opinion
18. There is currently insufficient evidence to support the use of circulating tumor cell molecular analysis for the diagnosis of primary lung adenocarcinoma, the identification of EGFR or other mutations, or the identification of *EGFR* T790M mutations at the time of EGFR TKI resistance.	No recommendation

Abbreviations: FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; TKI, tyrosine kinase inhibitor.

utility of *EGFR* mutation testing used polymerase chain reaction (PCR)–based methods that were more sensitive than unmodified Sanger sequencing. Given the widespread availability of technology capable of reliably detecting lower-frequency mutational events in small samples, it is no longer appropriate to offer a low-sensitivity test that cannot test tumors with 20% to 50% tumor content and requires patients to undergo more procedures, and potentially more invasive procedures, solely to procure a tissue sample with high tumor content.

3. It Is Not Appropriate to Use IHC for *EGFR* Mutation Testing.—There is no role whatsoever for IHC against total EGFR protein as a determinant of treatment with an EGFR kinase inhibitor. The targetable mutations lead to activation of the cytoplasmic kinase of this transmembrane protein, but that has no bearing on the

Table 5. Emerging Markers for Molecular Testing in Lung Cancer
Mitogen-activated protein kinase kinase 1 (*MEK1/MAP2K1*)
Fibroblast growth factor receptor 1–4 (*FGFR 1–4*)
Neurotrophic tyrosine kinase, receptor, type 1–3 (*NTRK1-3*)
Neuregulin 1 (*NRG1*)
Ras-like without CAAX 1 (*RIT1*)
Neurofibromin 1 (*NF1*)
Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*)
AKT serine/threonine kinase 1 (*AKT1*)
NRAS proto-oncogene, GTPase (*NRAS*)
Mechanistic target of rapamycin (*MTOR*)
Tuberous sclerosis 1 (*TSC1*)
Tuberous sclerosis 2 (*TSC2*)
KIT proto-oncogene receptor tyrosine kinase (*KIT*)
Platelet-derived growth factor receptor alpha (*PDGFRA*)
Discoidin domain receptor tyrosine kinase 2 (*DDR2*)

extent of expression at the cell surface, which is what is detected by the total EGFR immunostain. Although EGFR expression by IHC was performed for some of the very early studies of EGFR kinase inhibitors in the start of this century, clinical responses were seen in patients with mutations but absent/weak IHC expression, and poor responses were seen in patients with strong IHC expression but no mutations.

Following the discovery of *EGFR* mutations, antibodies were developed for IHC directed at the most common mutated forms of the protein, most notably the L858R substitution and the 746 to 750 ELREA deletion. The original guideline allowed for the use of the mutant-specific EGFR antibodies by IHC in a setting with extremely limited material. Although published evidence for these antibodies shows good accuracy for the L858R activating mutation and for some of the exon 19 deletions, these antibodies have poor sensitivity for other exon 19 deletions, insensitivity to less common mutations (eg, codon 719 mutations), and false-positive results with exon 20 insertions.[29] Overall, the performance is suboptimal for reliable detection of *EGFR* mutations. Given that advances in molecular diagnostic technology now enable analysis of very limited samples as well as circulating tumor DNA (see below), at this time there is no role for routine use of mutant-specific IHC in selecting anti-EGFR treatment for lung cancer patients.

New Recommendations

Question 1: Which New Genes Should Be Tested for Lung Cancer Patients?—In the 2013 guideline, genes fell into 1 of 2 categories: testing is necessary (*EGFR, ALK*), or testing is investigational. One gene, *KRAS*, was considered conditionally necessary in the context of sequential testing algorithms because of its ease of analysis and mutual exclusivity with *EGFR* and *ALK*. By 2018, however, we believe that there are now 3 categories into which genes should be placed. One set of genes must be offered by all laboratories that test lung cancers, as an absolute minimum: *EGFR*, *ALK*, and *ROS1*. A second group of genes should be included in any expanded panel that is offered for lung cancer patients: *BRAF*, *MET*, *RET*, *ERBB2* (*HER2*), and *KRAS*, if adequate material is available. *KRAS* testing may also be offered as a single-gene test to exclude patients from expanded panel testing. All other genes are considered investigational at the time of publication.

In this context, institutions providing care for lung cancer patients have a choice: (1) offer a comprehensive cancer panel that includes all of the genes in the first 2 categories (*EGFR*, *ALK*, *ROS1*, *BRAF*, *MET*, *ERBB2* [*HER2*], *KRAS*, *RET*) for all appropriate patients, or (2) offer targeted testing for the genes in the must-test category (*EGFR*, *ALK*, *ROS1*) for all appropriate patients and offer as a second test an expanded panel containing the second-category genes (*BRAF*, *MET*, *ERBB2* [*HER2*], and *RET*) for patients who are suitable candidates for clinical trials, possibly after performing a single-gene *KRAS* test to exclude patients with *KRAS*-mutant cancers from expanded panel testing. Table 4 includes a list of the recommendation statements with the strength of recommendations.

1. Strong Recommendation.—ROS1 testing must be performed on all lung advanced-stage adenocarcinoma patients, irrespective of clinical characteristics.

The strength of evidence was convincing to support the use of *ROS1* molecular (ie, reverse transcription PCR [RT-PCR] or sequencing) or cytogenetic (ie, FISH or other in situ hybridization) testing to identify *ROS1* rearrangements. The strength of evidence supporting the use of any clinical characteristic to identify patients who should receive *ROS1* testing was adequate. This recommendation is evidence-based and supported by 9 studies,[30–38] 6 of which informed on the association between *ROS1* rearrangement and patient or tumor characteristics[30,31,34–37] and consisted of 1 prospective cohort study (PCS),[35] 1 prospective-retrospective cohort study (PRCS),[31] and 4 retrospective cohort studies (RCSs).[30,34,36,37] The 3 remaining studies assessed clinical outcomes of patients treated with the ROS1-targeted therapy crizotinib[32,33,38] and included 1 nonrandomized clinical trial[33] and 3 RCSs.[32,38] All included studies were assessed for quality and none were found to have methodologic flaws that would raise concerns about the studies' findings (SDC Table 6). Refer to SDC Table 7 for a summary of findings from studies supporting the use of *ROS1* molecular or cytogenetic testing to enable selection of patients for ROS1-targeted therapy.

Although relatively rare, accounting for less than 2% of non–small cell lung carcinomas[30,31,34] and 2% to 3% of lung adenocarcinomas,[30,34,35] structural rearrangements involving the *ROS1* gene generate an oncogenic fusion that can be treated successfully with targeted inhibitors. A single phase I clinical trial of 50 NSCLC patients demonstrated that the presence of a *ROS1* rearrangement by FISH or RT-PCR predicts response to targeted inhibition using crizotinib, with a response rate of 72% and median progression-free survival of 19.2 months.[33] Based on this trial, the FDA approved the expanded use of crizotinib in patients with *ROS1*-rearranged NSCLC in 2016. A European multi-institutional retrospective study of 32 patients with *ROS1*-rearranged NSCLC treated with crizotinib demonstrated an 80% response rate and 9.1-month progression-free survival.[32] Overall survival for patients with *ROS1*-rearranged tumors irrespective of use of targeted therapy appears longer than that for patients with other molecular alterations undergoing targeted therapy.[38,39]

As with *ALK*, *ROS1* activation is driven by structural variants, with multiple different partners fusing to the C-terminal portion of *ROS1* containing the cytoplasmic tyrosine kinase and driving downstream signaling through *MAPK*, *JAK/STAT*, and *PI3K* pathways. Common fusion

partners include *SLC34A2, CD74*, and *TPM3*, among others. The role of wild-type *ROS1* is still being elucidated, but it shares similar structure with *ALK*, albeit with significant differences, notably absence of a dimerization domain, an extracellular domain with some resemblance to cell adhesion molecules, and no clear ligand.

As with *EGFR* mutations and *ALK* rearrangements, light to never smoking history has been associated with an increased incidence of *ROS1* rearrangements in patients with lung adenocarcinoma.[30,37] However, this association has not been consistently observed across studies.[34] Other clinical characteristics, such as younger age, female sex, and non-Asian ethnicity, have been associated with *ROS1* rearrangement in isolated studies only.[30,31,35] Therefore, clinical characteristics should not be used to either select or exclude patients from testing for *ROS1* rearrangements. *ROS1* rearrangements occur in a mutually exclusive fashion with other oncogenic driver alterations (such as *EGFR* and *KRAS* mutation and *ALK* rearrangement). In recognition of the rarity of *ROS1* rearrangement, it may be reasonable to perform sequential testing of *EGFR* and *ALK* followed by *ROS1* testing. Indeed, the frequency of *ROS1* rearrangements is enriched to 5% to 10% in otherwise driver (ie, *EGFR, ALK, KRAS, BRAF*)-negative lung adenocarcinomas.[31,37]

Notably, in the United States in 2016, crizotinib therapy in *ROS1*-rearranged tumors does not require the use of an FDA-approved companion diagnostic. Published methods that have established clinical utility of testing *ROS1* in order to choose ROS1-targeted therapy have relied primarily upon FISH and RT-PCR. Outside the United States, a diagnostic test using RT-PCR was used for an international phase II clinical trial,[40] involving mainly East Asian countries, for selection of tumors with *ROS1* rearrangement. This assay has been approved as an in vitro diagnostic in Europe and China, and may be recognized as a companion diagnostic test in some countries. Although targeted RT-PCR assays may be challenging because of variation in *ROS1* break points (typically introns 31–35) and partner genes, capture-based sequencing strategies for RNA or DNA may be used, provided they are properly validated on known positive samples. Within the United States, FISH methods have been published more frequently. Fluorescence in situ hybridization testing should be performed with a break-apart probe design given the multiple fusion partners, and should show rearrangement, defined as signals split by at least 1 probe diameter, in 15% or more of tumor cells.[41]

2. Expert Consensus Opinion.—ROS1 IHC may be used as a screening test in advanced-stage lung adenocarcinoma patients; however, positive ROS1 IHC results should be confirmed by a molecular or cytogenetic method.

The strength of evidence is inadequate supporting the use of IHC as a screening assay for *ROS1* molecular testing. This statement is evidence-based and supported by 6 studies,[42–47] consisting of 2 PCSs,[43,44] 1 PRCS,[42] and 3 RCSs.[45–47] Five studies compared ROS1 IHC with a FISH reference test[42–45,47] and 1 study compared ROS1 IHC with an RT-PCR reference test.[46] Using reported true-positive, false-positive, true-negative, and false-negative data from studies comparing IHC with FISH, an MA was conducted to determine a pooled estimate of sensitivity and specificity for ROS1 IHC (Figure 1). All included studies were assessed for quality and none were found to have methodologic flaws that would raise concerns about the studies' findings (SDC Table 8). Refer to SDC Table 9 for a summary of findings from studies supporting the use of IHC as a screening assay for *ROS1* molecular testing.

In light of the relative rarity of *ROS1* rearrangement events in NSCLC, screening by IHC may be preferable to FISH or molecular techniques in some settings. Interpretation of ROS1 IHC is challenging, however, as expression can be seen in a patchy pattern, typically at weak intensity, in up to a third of tumors that do not have an underlying rearrangement.[44,45,48] Although some studies suggest that ROS1 expression in the absence of a rearrangement may have prognostic significance,[48] focal or patchy expression in tumor cells is rarely associated with a *ROS1* rearrangement and therefore is unlikely to predict response to ROS1-targeted therapy. Moreover, the pattern of staining can vary among fusion types, including granular to globular staining in *CD74-ROS1* fusions, weak membranous staining in *EZR-ROS1* fusions, and vesicular localization staining in *GOPC-ROS1* fusions.[45]

A single commercially available antibody clone (D4D6, Cell Signaling Technology, Danvers, Massachusetts) has been used in studies published to date. Most retrospective studies of ROS1 IHC using the D4D6 antibody demonstrate a sensitivity of 100% relative to FISH or RT-PCR.[35,42–47] Tumors lacking ROS1 expression can be safely interpreted as lacking a *ROS1* fusion. However, the specificity of ROS1 IHC is more variable, ranging from 92% to 100% using different methods and interpretive cutoffs.[35,42–47] Meta-analysis of 5 studies identified by the literature search determined a pooled sensitivity of 96% (95% CI, 71%–99%) and specificity of 94% (95% CI, 89%–96%) for IHC compared with FISH when the D4D6 antibody with a staining intensity of at least 2+ (as defined within the study) was used (Figure 1). Several cutoffs have been proposed using intensity alone or H score (intensity × percentage of tumor cells staining). In most studies, FISH- or molecularly confirmed *ROS1*-rearranged tumors have at least moderate-intensity ROS1 protein expression, but published evidence is insufficient to recommend one specific cutoff or scoring system,[42,45] and each laboratory must validate its own interpretive cutoff from known positive and negative samples.

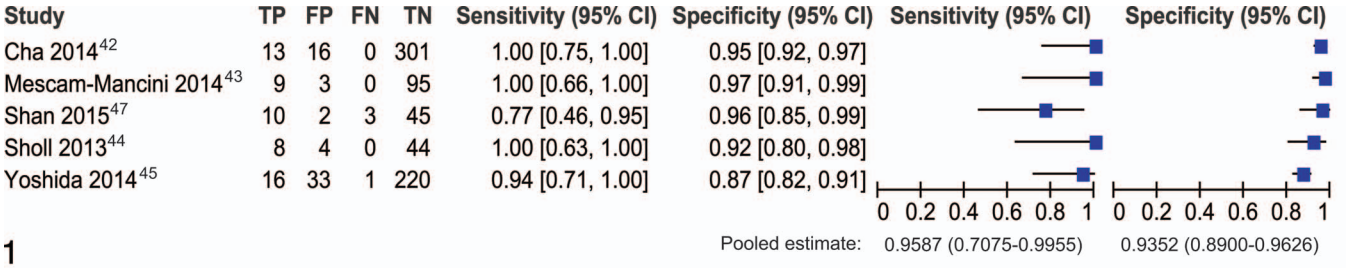
Because of imperfect specificity and challenges related to interpretation of nonspecific expression, we recommend that all ROS1 IHC positive results undergo confirmation by FISH or a molecular method (ie, RT-PCR, NGS) prior to considering a patient for ROS1-targeted therapy. Given the high sensitivity of IHC, however, tumors that clearly lack ROS1 staining can be interpreted as negative for *ROS1* fusion.

Additional Genes

Of the genes newly included in this guideline, only *ROS1* testing must be offered to all appropriate lung cancer patients. Testing for the following genes should be included with any expanded multigene panel testing performed for lung cancer patients, whether or not the panel is offered for all lung cancer patients, or if the panel is reserved as a second-line test for *EGFR/ALK/ROS1* wild-type patients seeking clinical trials.

3. Expert Consensus Opinion.—*BRAF* molecular testing is currently not indicated as a routine stand-alone assay outside the context of a clinical trial. It is appropriate to include *BRAF* as part of larger testing panels performed either initially or when routine *EGFR, ALK*, and *ROS1* testing is negative.

The strength of evidence was inadequate to support the use of *BRAF* molecular testing. This statement was



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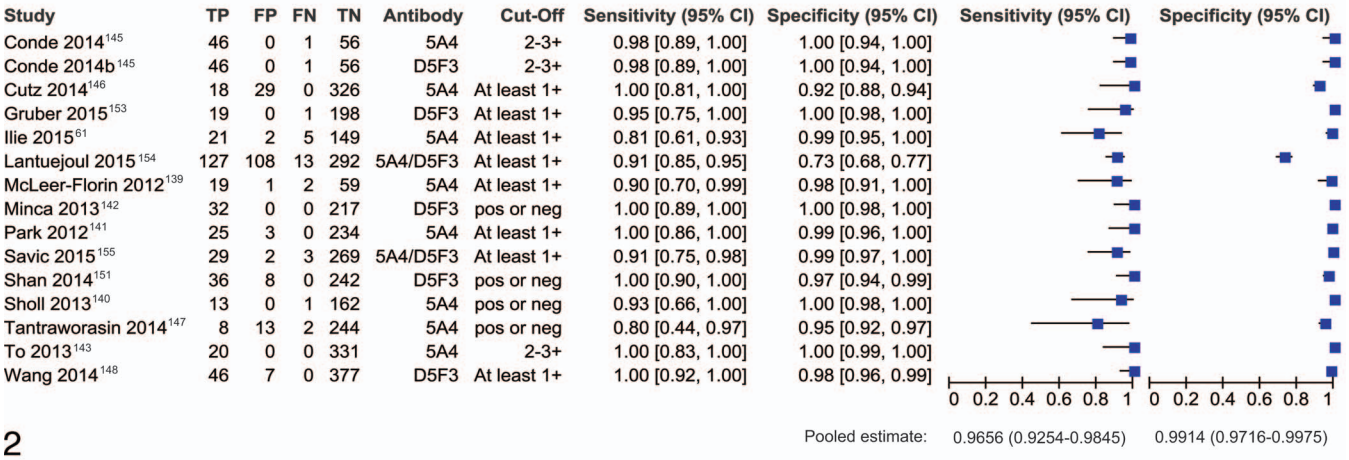

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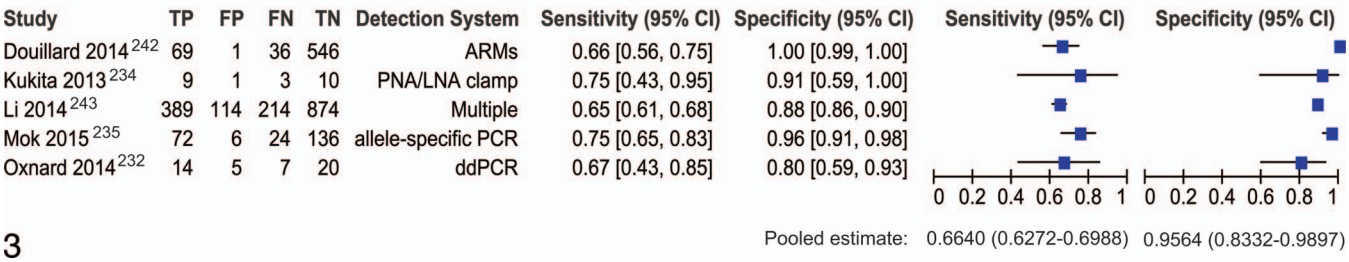

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Figure 1. *Forest plot of sensitivity and specificity for immunohistochemistry (IHC)-based determination of ROS1 rearrangement positivity compared with fluorescence in situ hybridization. Pooled estimate of sensitivity and specificity based on bivariate analysis of included studies. All included studies used an IHC staining intensity of at least 2+ with a D4D6 antibody to define ROS1 rearrangement positivity. Abbreviations: FN, false-negative; FP, false-positive; TN, true-negative; TP, true-positive.*

Figure 2. *Forest plot of sensitivity and specificity for immunohistochemistry-based determination of ALK translocation positivity compared with fluorescence in situ hybridization. Pooled estimate of sensitivity and specificity based on bivariate analysis of included studies. Included studies assessed 5A4, D5F3, or either 5A4 or D5F3 antibodies with positivity cutoffs based on either presence of any staining or staining intensity. Abbreviations: FN, false-negative; FP, false-positive; neg, negative; pos, positive; TN, true-negative; TP, true-positive.*

Figure 3. *Forest plot of sensitivity and specificity for various assays determining* EGFR *mutation positivity with cell-free DNA compared with tumor tissue. Pooled estimate of sensitivity and specificity based on bivariate analysis of included studies. Four included studies compared tumor tissue samples with plasma samples using the same detection system,[234,235,242,243] and a fifth study[232] obtained plasma samples from patients with known* EGFR *and* KRAS *tumor mutation status. Abbreviations: ARMs, amplification refractory mutation system; ddPCR, droplet digital PCR; FN, false-negative; FP, false-positive; PCR, polymerase chain reaction; PNA/LNA, peptide nucleic acid–locked nucleic acid; TN, true-negative; TP, true-positive.*

evidence-based and supported by 9 studies: 4 PCSs[49–52] and 3 RCSs,[53–55] all of which informed on the association between *BRAF* mutation and patient or tumor characteristics,[49–55] and 2 additional nonrandomized clinical trials that assessed the activity of a *BRAF* inhibitor in p.V600E mutant patients.[56,57] All included studies were assessed for quality and none were found to have methodologic flaws that would raise concerns about the studies' findings (SDC Table 10). Refer to SDC Table 11 for a summary of findings from studies supporting the use of *BRAF* molecular testing.

Activating mutations in *BRAF*, especially p.V600E, lead to oncogenic signaling through MAPK, and are rare recurrent alterations in lung adenocarcinoma, seen in 0.5% to 4.9% of tumors.[49–52,54] In lung cancer, data from a 2016 phase II single-arm clinical trial[56] showed that (1) single-agent dabrafenib given in second line to stage IV *BRAF* p.V600E mutant NSCLC had a partial response rate of 33% and disease control rate of 58% and (2) combination dabrafenib-trametinib therapy given in second line to stage IV *BRAF* p.V600E mutant lung adenocarcinoma had a partial response rate of 63% and disease control rate of 75%.

Based on these data, the FDA conferred a breakthrough therapy designation for the combination treatment in *BRAF* p.V600E mutation–positive NSCLC, and FDA approval was granted in 2017. Hence, this was the most controversial of all recommendations among the working panel. Although

there was a strong opinion in the working group that *BRAF* mutation analysis should be performed at the time of initial molecular testing in lung adenocarcinoma, the published evidence available at the time of publication lacked controlled prospective trials, and therefore lacked the strength to warrant an international recommendation for single-gene testing for *BRAF* for all lung adenocarcinoma patients. We anticipate the publication of stronger evidence supporting the utility of BRAF inhibition in *BRAF*-mutant lung cancer, and our opinion is that *BRAF* testing will be proven necessary. We expect that the next revision of this guideline will include a recommendation to include single-gene testing for *BRAF* alongside *EGFR*, *ALK*, and *ROS1*, but we are unable to make that recommendation in the spring of 2017. Although stand-alone single-gene testing for *BRAF* is not currently recommended, if a panel strategy is used, either initially or for patients who are known wild type for *EGFR*, *ALK*, and *ROS1*, then *BRAF* should be included.

As with *EGFR* and *KRAS* mutations, selected hot-spot mutations in *BRAF* exert an oncogenic effect. The V-raf murine sarcoma homolog b (*BRAF*) gene encodes for a nonreceptor serine-threonine kinase in the MAPK kinase signaling pathway, between RAS and MEK. The most common *BRAF* mutation in NSCLC is the c.1799T>A (p.V600E) point mutation that is the predominant mutation in many other cancers, including melanoma, papillary thyroid cancer, colorectal cancer, hairy cell leukemia, and ganglioglioma. However, in contrast to other cancers with *BRAF* mutations, lung cancers frequently have non-p.V600E *BRAF* mutations, including other mutations at codon 600 (eg, p.V600K) and nearby codons in exon 15, and substitutions at codons 466 and 469 in exon 11.

Like many other targetable oncogenes in lung cancer, *BRAF* mutations are more frequent in adenocarcinomas than in squamous cell carcinomas. *BRAF* p.V600E mutation is more frequent in females[52,54] and never smokers[54] in some studies, but several studies failed to show these associations.[49,50,53,58] One distinction between *BRAF* mutations and other targetable oncogenes is that non-p.V600E *BRAF* mutations (particularly the exon 11 mutations) may coexist with mutations in *KRAS*,[49,52,53,59] whereas the p.V600E mutations are mutually exclusive of *KRAS*, *EGFR*, or *ALK* alterations.

Single-gene assays for *BRAF* are in wide use for other cancer types, particularly for melanoma patients being considered for targeted therapy, but most of these methods cannot detect the exon 11 mutations that are seen in lung cancer. Although the evidence supporting utility of *BRAF* testing was restricted to the p.V600E mutations, our opinion is that testing for *BRAF*, done as part of a large panel or for clinical trial enrollment, should use a method that evaluates at a minimum exons 11 and 15.

A similar challenge arises concerning the use of mutation-specific IHC using antibodies against the p.V600E mutant protein (VE1), which have been widely used in melanoma diagnosis. Reported data on small numbers of lung cancer cases[58,60] demonstrate the VE1 clone can stain between 90% and 100% of p.V600E-mutant adenocarcinomas. In 1 of these studies, all non-p.V600E cases were negative on IHC testing,[61] whereas in another, a single non-p.V600–mutated case out of 21, with a unique 599 insertion T mutation, showed positive staining. There is currently insufficient evidence to support a recommendation either for or against *BRAF* p.V600E IHC (VE1) testing in NSCLC.

4. Expert Consensus Opinion.—RET molecular testing is not recommended as a routine stand-alone assay outside the context of a clinical trial. It is appropriate to include *RET* as part of larger testing panels performed either initially or when routine *EGFR, ALK,* and *ROS1* testing is negative.

The strength of evidence to support the use of *RET* molecular testing was inadequate. This statement is evidence-based and supported by 3 studies,[37,62,63] consisting of 1 PCS[62] and 2 RCSs.[37,63] All included studies were assessed for quality and none were found to have methodologic flaws that would raise concerns about the studies' findings (SDC Table 12). Refer to SDC Table 13 for a summary of findings from studies supporting the use of *RET* molecular testing.

Structural variants causing *RET* fusions are rare, being found in 0.6% to 0.9% of NSCLCs and in 1.2% to 2% of adenocarcinomas.[62,64–67] The potential to treat *RET*-positive lung cancers with inhibitors of the *RET* kinase is being explored in phase II clinical trials,[68,69] although small series and case reports have shown promise.[70,71] Given the rarity of *RET* rearrangements and limited evidence of therapeutic benefit, testing for *RET* alterations is not recommended as a stand-alone test for all lung adenocarcinoma patients. However, any large multigene panel test developed for lung cancer patients, either for initial workup or for patients who are wild type for *EGFR, ALK,* and *ROS1*, should include *RET*.

As with *ALK* and *ROS1* rearrangements, *RET* is activated by rearrangements that fuse the tyrosine kinase domain of *RET* with coiled-coil dimerization domains of one of a variety of recurring partner genes, including *KIF5B* (the most common, at 90%),[64,72,73] *CCDC6*,[65,74] *NCOA4*,[62] and *TRIM33*.[72] *RET* rearrangement is mutually exclusive with aberrations in *EGFR, KRAS, ALK, HER2,* and *BRAF* in lung cancer.[62,64,65]

RET fusion occurs more frequently in never smokers than ever smokers.[37,62,64,66,72] Patients with *RET* fusion harboring tumors are usually younger than patients with an *EGFR* mutation and have an equal sex distribution.[67] *RET* fusion proteins have been detected in adenocarcinoma[37,62,64] and in adenosquamous carcinoma.[62] Histologic subtypes in adenocarcinomas include those with mucinous/signet ring cells and those with a cribriform[37,62,65] or solid growth pattern.[37,62] However, no clinical or histologic features (other than excluding from testing pure squamous histology cases) should be used to select a patient for *RET* testing.

Multiple methods have been applied for *RET* analysis, including break-apart FISH analysis,[75] IHC,[37] RT-PCR,[75] and NGS.[37] *RET* FISH is particularly challenging, however, because of the narrow spacing between the split probe signals seen in the common fusion types, and a pattern of split *RET* signals separated by as little as 1 signal diameter distance is interpreted as positive.[37] Similar to *ALK* rearrangement testing by FISH, the threshold for *RET* FISH positivity for rearrangement is 15% of cells with split signals or single 3′ probe signals. In another study, a 4-colored *RET* FISH assay was used[62]; samples were positive for *RET* rearrangement or *KIF5B-RET* fusion if more than 20% of tumor cells exhibited split red-green signals or touching golden-green signals, respectively.

One recent retrospective study used RET IHC (anti-RET antibody ab134100, Abcam, Cambridge, United Kingdom) showing diffusely granular cytoplasm staining and occasionally membranous or perinuclear staining, with moderate to strong intensity. A sensitivity of 100% and specificity of

88% were reported,[37] although corroborating evidence is not strong enough to warrant a recommendation.

Although multiplex RT-PCR may be successful for common fusions involving *KIF5B-RET* and *CCDC6-RET*,[75] as with *ALK* and *ROS1*, targeted RT-PCR alone is usually insufficient to detect new partners or isoforms. However, although the diversity of treatable rearrangements in *ALK* and *ROS1* has matured sufficiently through years of testing and clinical trials, such that targeted RT-PCR assays for these genes can be designed with adequate clinical sensitivity, the diversity of treatable *RET* rearrangements is earlier in evolution. A capture-based sequencing approach, involving DNA or RNA, may be more sensitive and more readily integrated into a large multigene panel.[76]

5. Expert Consensus Opinion.—ERBB2 (*HER2*) molecular testing is not indicated as a routine stand-alone assay outside the context of a clinical trial. It is appropriate to include *ERBB2* (*HER2*) mutation analysis as part of a larger testing panel performed either initially or when routine *EGFR*, *ALK*, and *ROS1* testing is negative.

The strength of evidence was inadequate to support the use of *ERBB2* (*HER2*) molecular testing. This recommendation was evidence-based and supported by 10 studies, 9 that reported on the association between *ERBB2* (*HER2*) and patient or tumor characteristics[49,77–84] and 1 that assessed the use of ERBB2-targeted therapy (dacomitinib)[85] in patients with *ERBB2* (*HER2*) mutations and amplifications. All included studies were assessed for quality and none were found to have methodologic flaws that would raise concerns about the studies' findings (SDC Table 14). Refer to SDC Table 15 for a summary of findings from studies supporting the use of *ERBB2* (*HER2*) molecular testing.

Alterations in the human epidermal growth factor receptor 2 gene (*HER2*, *ERBB2*) have emerged as oncogenic drivers and as potential therapeutic targets in lung cancer.[81,83,84,86] Sequence alterations and gene amplification occur in this setting and constitute approximately 2% to 3% and 2% to 5% of reported recurrent alterations, respectively. Therapeutic targeting of HER2 (the protein product of the *ERBB2* gene) remains an area of active investigation at this time. Earlier clinical trials selecting patients based on protein expression by IHC or *ERBB2* amplification by FISH did not demonstrate a clear benefit.[87,88] An additional phase II trial using *ERBB2* mutation and *ERBB2* amplification for patient selection demonstrated durable responses to dacomitinib, but only in patients with specific *HER2* mutations.[85]

In-frame insertions in exon 20 and substitutions at S310 are the most common mutations seen, and are typically mutually exclusive with other recurrent alterations, including mutations in *EGFR*, *KRAS*, and *BRAF*, as well as rearrangements involving *ALK* and *ROS1*. Insertions in exon 20 are variable, with most being a 12–base pair duplication of codons 775–778 encoding amino acids YVMA,[81] and are more commonly observed in younger patients and patients with no smoking history. De novo *ERBB2* amplification may occur with or without *ERBB2* mutation,[82,84,86] with highly variable reported rates of co-occurrence from 0% to 87%.[81,84,86] Although differences in methods and criteria defining amplification levels may be responsible for these observed discrepancies and require standardization, the higher prevalence of *ERBB2* amplification independent of *ERBB2* mutation suggests that mutation and amplification could represent distinct markers and therapeutic targets in lung cancer.[89] *ERBB2* amplification has also been reported rarely as a secondary event in patients with sensitizing *EGFR* mutations and as a potential mechanism of resistance following treatment with EGFR inhibitors.[90]

In this context and with current evidence, routine stand-alone testing for *ERBB2* mutations is not indicated outside a clinical trial. Nevertheless, when broader testing is performed through a multiplex assay or NGS, it is appropriate to include *ERBB2* as part of the testing, as it may identify patients to be directed to clinical trials—in this context, testing for sequence alterations in *ERBB2*, particularly insertions/duplications in exon 20, which have been associated with response to treatment with targeted inhibitors of ERBB2 in case reports and small series.[85,91]

6. Expert Consensus Opinion.—KRAS molecular testing is not indicated as a routine stand-alone assay as a sole determinant of targeted therapy. It is appropriate to include *KRAS* molecular testing as part of larger testing panels performed either initially or when routine *EGFR, ALK*, and *ROS1* testing is negative.

The strength of evidence was adequate to support the use of *KRAS* molecular testing when selecting patients for targeted therapy. The strength of evidence supporting the use of any clinical characteristic to identify patients who should receive *KRAS* testing was inadequate. This statement is evidence-based and supported by 7 studies,[49,51,52,92–95] comprising 2 MAs,[93,94] 4 PCSs,[49,51,52,92] and 1 RCS.[95] Five studies attempted to identify associations between patient or tumor characteristics and *KRAS* mutational status.[49,52,92,93,95] Two MAs[93,94] reported on overall survival and EGFR-TKI response rates when *KRAS* mutation–positive patients were treated with standard care. All included studies were assessed for quality and none were found to have methodologic flaws that would raise concerns about the studies' findings (SDC Table 16). Refer to SDC Table 17 for a summary of findings from studies supporting the use of *KRAS* molecular testing.

KRAS mutations are reported in 20% to 30% of lung adenocarcinomas. *KRAS* mutations are encountered more frequently in people with tobacco exposure, but have been reported in approximately 5% of lung cancer patients who have never used tobacco. Most studies indicate an increased incidence in males and those of white or African ancestry, in comparison with females and those of Asian ancestry. *KRAS* mutations occur most frequently in codon 12 and 13, much less commonly in codon 61, and rarely in codon 146, and can readily be detected by quick targeted hot-spot assays (ie, real-time PCR, droplet digital PCR, or pyrosequencing) interrogating these codons, as well as incorporated into larger panel tests. They are typically mutually exclusive with other driver mutations such as *EGFR* mutations and *ALK* rearrangements.[49,51,92,93,95–101]

Therapies directed against mutated *KRAS* have not been proven clinically effective. For example, although promising results (37% objective response rate) were obtained in a phase II study of selumetinib, an inhibitor of MEK1 (downstream of *KRAS*), plus docetaxel[102] in *KRAS*-mutant advanced lung cancer, this combination failed to demonstrate an outcome benefit in the Selumetinib Evaluation as Combination Therapy-1 (SELECT-1) phase III trial,[103] and a phase II study of selumetinib + erlotinib in *KRAS*-mutant lung cancers failed to show response to selumetinib independent of erlotinib.[104] Hence, intense research investigation into therapeutic strategies against this common mutation continues, and it is appropriate to include *KRAS* in

a larger testing panel used for directing patients to investigational therapies.

Another application of *KRAS* mutation testing is in a sequential testing algorithm, with a positive result greatly diminishing the likelihood of another, targetable oncogenic alteration. If the *KRAS* test is performed prior to *EGFR, ALK*, or *ROS1* testing, however, the laboratory must ensure that sufficient tumor is available for *EGFR, ALK*, and *ROS1* testing within the recommended time frame, particularly in the event of a negative *KRAS* result. Similarly, the presence of a *KRAS* mutation renders unlikely the other oncogenes recommended for larger panels, such as *RET, ERBB2* (*HER2*), and *BRAF*. In this context, a rapid, targeted assay for *KRAS* may have value in helping to determine whether or not an *EGFR/ALK/ROS1* wild-type patient would benefit from expanded panel testing, in that panel testing would be less likely to benefit *KRAS*-mutant cancer patients. This model may, however, change as technology evolves, as newer ultrasensitive methods have shown co-occurrence of driver oncogenes, including *KRAS*, in subpopulations within tumors that previously had not been detected by less sensitive methods.[105,106]

7. Expert Consensus Opinion.—MET molecular testing is not indicated as a routine stand-alone assay outside the context of a clinical trial. It is appropriate to include *MET* as part of larger testing panels performed either initially or when routine *EGFR, ALK*, and *ROS1* testing is negative.

The strength of evidence is inadequate supporting the use of *MET* molecular testing. This statement was evidence-based and supported by 7 studies,[107–113] comprising 1 MA,[107] 1 phase II randomized controlled trial,[109] 1 PCS,[110] and 4 RCSs.[108,111–113] All included studies were assessed for quality and none were found to have methodologic flaws that would raise concerns about the studies' finding (SDC Table 18). Refer to SDC Table 19 for a summary of findings from studies supporting the use of *MET* molecular testing.

Initially reported as a mechanism of secondary resistance to EGFR TKI therapy in *EGFR*-mutant lung cancer,[114,115] both the understanding of the mechanism of activation of *MET* and the utility of MET testing in lung cancer have gone through several phases. *MET* copy gain was initially recognized in association with secondary resistance to EGFR inhibitors,[114] prompting the development of targeted therapies that showed disappointing results.[116] More recently, interest in targeting *MET* has been rekindled by the discovery of activating mutations that may respond to targeted inhibition.

The *MET* gene encodes for the receptor for hepatocyte growth factor (HGFR), and its activation has pleotropic functions in promoting cell survival, proliferation, motility, invasion, and epithelial-mesenchymal transition.[117–120] HGFR can become activated and drive oncogenesis through several different mechanisms, including (1) amplification resulting in high expression of the receptor,[121,122] (2) tyrosine kinase domain mutations resulting in constitutive activation of the receptor,[123] and (3) splicing mutations resulting in skipping of exon 14 and loss of Y1003, the Casitas B-lineage lymphoma proto-oncogene (CBL) binding site required for the ubiquitin-mediated degradation of the protein.[124] Although most of the exon-skipping mutations involve canonical splice sites, some are located further into the intronic sequence, and thus can be difficult to interpret or may be missed by assays examining only exons and the immediately adjacent 5′ and 3′ splice acceptor and donor sites.

Activating *MET* alterations are inhibited by crizotinib, a treatment for *ALK*- and *ROS1*-rearranged lung cancers. Despite the reported association of the *MET* gene amplification and high protein expression as a poor prognostic marker[107,125] and recent reports that patients with *MET* amplification or *MET* exon 14 mutation are sensitive to crizotinib in some cases, there is as yet no approved targeted therapy to treat patients whose tumor harbor these *MET* genomic aberrations.[126–130] In this context, a routine stand-alone testing for these *MET* genomic aberrations or HGFR protein level is not indicated outside a clinical trial. Nevertheless, when multiplex testing for putative oncogenic driver mutations is applied to lung cancer patients, either initially when testing for *EGFR/ALK/ROS1* or after they are found to be negative, these *MET* gene aberrations should be included in the test panels.

To date, more than 100 somatic splice site alterations resulting in *MET* exon 14 skipping have been described. Mutations exhibit a highly diverse sequence composition, encompassing small insertions, deletions, complex indels, and single-nucleotide variants, which are primarily located in splice donor and acceptor sites. Point mutations deeper in the introns, up to 25 base pairs into the intronic noncoding regions, adjacent to the splice acceptor sites, have also been reported at a lower frequency, although many assays do not interrogate this region. In general, the overall incidence and the effect of these less common mutations in exon splicing have not been defined.

Given the wide variability and complexity of mutations affecting *MET* exon 14, comprehensive diagnostic testing could prove challenging depending on the method used. Targeted NGS-based assays interrogating *MET* as part of a wider gene panel are preferred for screening purposes. For DNA-based testing, assay design should be such as to allow accurate and full sequencing of exon 14 and its flanking introns. Novel mutations, particularly those alterations affecting regions adjacent to splice sites but deeper into the introns, may require confirmation of exon 14 skipping using an RNA-based assay. Alternatively, up-front RNA-based testing interrogating *MET* as part of a wider gene panel designed for comprehensive assessment of structural variants or gene expression may also be used.

Fluorescence in situ hybridization has been traditionally used for detection of gene amplification in clinical practice. Currently, there is no guideline for cutoff of *MET* positivity in lung cancer specimens. *MET* amplification has been classified by using *MET*:CEP7 ratio as low (≥1.8 to ≤2.2), intermediate (>2.2 to <5), and high (≥5).[131] Other examples of *MET* FISH-positive criteria include 5 or more *MET* signals per cell[132] and a *MET*:CEP7 ratio of 2 or higher (PathVysion, Abbott Park, Illinois). Low and intermediate levels of *MET* amplification can occur synchronously with other oncogenic mutations and gene rearrangements (*KRAS, EGFR, BRAF, ERBB2* [*HER2*], *ALK, ROS1, RET*) in up to 63% of lung carcinomas.[133] However, this overlap has not been observed in high–*MET*-amplification tumors (*MET*:CEP7 ratio ≥5), suggesting that *MET* amplification is probably a true oncogenic driver.[133] This group of tumors, with high-level amplification, showed a response to crizotinib, whereas no response was seen in tumors with low- or intermediate-level amplification. Importantly, about 20% of lung adenocarcinomas with *MET* exon 14–skipping mutations have concurrent high-level *MET* amplification, confounding the interpretation of each.[125,126,129] Regarding

the significance of amplification alone, case reports have shown response to crizotinib.[134,135]

The same challenges defining a clinically valid cutoff of *MET* amplification positivity exist in the setting of acquired resistance to EGFR TKI. A recent phase II study showed 40% response rate in patients with acquired EGFR TKI resistance and a *MET* copy number of 5 or higher when treated with a combination of gefitinib and capmatinib; no response was observed in a group with a *MET* copy number less than 5.[136]

Immunohistochemistry for MET protein expression performed on formalin-fixed, paraffin-embedded tissue samples has been the most frequently used method in lung cancer specimens. A number of commercially available monoclonal and polyclonal antibodies are directed against various epitopes of MET, with different sensitivities and specificities for both total and phosphorylated MET. Immunohistochemistry procedures and scoring methods for MET assessment have not been standardized. As a result, MET protein overexpression in unselected NSCLC cases has been reported to range from 20% to 70%.[137,138] An MA has found that MET expression by IHC in NSCLC is a negative prognostic factor in patients with surgically resected NSCLC.[107] A frequently used commercially available antibody, particularly in clinical trials, is the CONFIRM anti-total MET (SP44) rabbit monoclonal primary antibody (Ventana Medical Systems, Tucson, Arizona) directed against a membranous and cytoplasmic epitope of MET.[109] At the time of publication, it remains unclear whether total MET or phosphoMET protein overexpression represents a reliable indicator of MET activation. Both MET IHC and FISH are not predictive of efficacy of onartuzumab combined with erlotinib in advanced NSCLC patients.[116]

Other Genes.—The spectrum of recurring alterations in lung cancer continues to evolve, and several promising alterations have been reported that were not included in this recommendation. This includes fusions involving genes in the *NTRK* and *FGFR* families, both of which have experimental targeted inhibitors with supporting in vitro data and case reports. No guideline can be completely up to date, and practitioners of lung cancer care are advised to keep abreast of these and other developments. Table 5 includes a list of emerging biomarkers for molecular testing in lung cancer.

Question 2: What Methods Should Be Used to Perform Molecular Testing?

8. Recommendation.—Immunohistochemistry is an equivalent alternative to FISH for ALK testing.

The strength of evidence supporting the use of IHC for ALK testing was adequate. This recommendation is evidence-based and was supported by 20 studies,[61,111,139–156] comprising 6 PCSs,[61,139,141–143,154] 3 PRCSs,[140,146,155] and 11 RCSs.* Of the 20 studies, 19 used FISH as the reference standard when assessing the diagnostic potential of IHC.[111,145,147–153,156] The remaining study used IHC as the reference standard and FISH as the index test.[144] Using reported true-positive, false-positive, true-negative, and false-negative data from 14 studies using FISH as the reference standard, an MA was conducted to determine a pooled estimate of sensitivity and specificity for ALK IHC (Figure 2). All included studies were assessed for quality and none were found to have methodologic flaws that would raise concerns about the studies' findings (SDC Table 20). Refer to SDC Table 21 for a summary of findings from studies supporting the use of the IHC assay for ALK testing.

At the time of the original guideline, the only assay that had evidence of clinical utility from prospective studies to select patients for crizotinib therapy was an *ALK* FISH break-apart assay, which is interpreted as positive if at least 15% of tumor cells show signals separated by at least 2 probe diameters or a single 3′ signal (deleted 5′). The *ALK* FISH assay can be technically challenging, particularly with tumors showing positive signals near the cutoff of 15%. FISH assays in general are limited by high cost, need for specialized personnel for interpretation, and limited availability of equipment, space, and testing personnel. However, ALK IHC can also show variation in staining among antibodies, protocols, and interpretation.

For all of these reasons, different assay approaches have been proposed for identification of *ALK* rearrangements in lung carcinoma. Many studies have focused on IHC as a widely available and cost-effective screening assay. In 2013, IHC had been shown comparable with FISH in some studies, but significant variations among antibodies and methods, and limited market availability of some of the more accurate antibodies, precluded making an evidence-based recommendation at that time.

By 2016, however, numerous publications[61,111,139–156] had established the technical performance of several ALK IHC assays and their correlation with *ALK* FISH results. An important early observation was that the amount of ALK fusion protein expression in the *ALK* rearranged non–small cell carcinomas tends to be lower than is found in anaplastic large cell lymphoma, from which the gene gets its name and for which the first IHC antibodies were developed.[157] The ALK1 antibody (mouse monoclonal anti-human CD246, clone ALK1) typically used to diagnose anaplastic large cell lymphoma failed to identify a significant number of the *ALK* rearranged NSCLCs using standard techniques.[140,158–161] To overcome this problem, several technical steps have been applied, such as tyramide amplification and enhanced polymer-based detection systems. Despite these advances, although the ALK1 antibody has good specificity (91%–99%), sensitivity is still poor, ranging from 67% to 100%, and therefore the ALK1 antibody is not recommended for *ALK* rearrangement screening in lung carcinoma.

Subsequently, 2 commercially available clones, mouse monoclonal 5A4 (Novocastra, Leica Biosystems, Buffalo Grove, Illinois)– and rabbit monoclonal D5F3 (Ventana)–based assays, showed clinically acceptable sensitivities and specificities, ranging from 95% to 100%, when compared with *ALK* FISH results.[61,111,139,141–156] Studies also showed that IHC-positive ALK protein expression correlates with tumor response to ALK inhibitors even in *ALK* FISH–negative cases.[162] In the United States, an assay using the D5F3 antibody (Ventana) is now approved by the FDA for selection of lung cancer patients to receive treatment with crizotinib.

Based on published evidence with 5A4 and D5F3 monoclonal antibodies, properly validated IHC assays are an equivalent alternative to *ALK* FISH. A meta-analysis that pooled 14 studies using FISH as a reference standard determined a pooled sensitivity of 97% (95% CI, 93%–98%) and a pooled specificity of 99% (95% CI, 97%–100%) for ALK IHC for both the 5A4 or D5F3 assays (Figure 2). The laboratory may choose which antibody to use based on analytic precision, clinical sensitivity, and clinical specificity

* *References 111, 144, 145, 147–153, 156.*

in accordance with published standards. ALK IHC is an acceptable alternative to FISH and treatment decisions can be made when IHC results are clearly positive, as manifested by strong granular cytoplasmic staining with/without membrane accentuation, or negative; however, weak staining can be challenging to interpret, and the specificity of weak staining relative to FISH should be determined in each laboratory during validation. Occasional cases may be difficult to interpret because of heterogeneous fixation/preservation and/or nonspecific staining artifacts, such as light cytoplasmic staining in alveolar macrophages, neural cells, extracellular mucin, necrosis, and glandular epithelium. In these settings, these cases should also be tested by a validated method (eg, *ALK* FISH, RT-PCR, NGS).[141,146]

Discordant results between ALK FISH and IHC assays have been described in rare cases. ALK IHC–negative cases were reported in association with *ALK* FISH–positive assays that showed a lower percentage of tumor cells with rearrangement (15%–20%). Technical errors cannot be reliably excluded in a case with a lower percentage of nuclei positive for rearrangement. Recent studies suggest that a 5′ deletion FISH pattern may more commonly represent a false-positive result with discrepant IHC results than cases with a split FISH signal.[163–165] Importantly, however, clinical outcomes of patients with discrepant FISH and IHC results have not shown a consistent pattern of superiority of one method over the other.[156,166]

Although at the time of writing RT-PCR and NGS are not approved by the FDA in the United States as first-line methods for determining ALK status in selection of patients for ALK inhibitor therapy, these approaches have shown comparable performance with IHC[163–165] when designed to detect the majority of fusions, and are standard practice in many other countries.[163–165,167] These methods are highly specific for most fusions,[97,168,169] and patients with positive results should be treated with an ALK inhibitor, although patients with negative results may benefit from a more sensitive method to exclude the possibility of a variant fusion. Similarly, amplicon-based NGS assays of DNA may likewise fail to detect all fusion variants, and therefore a capture-based DNA or RNA approach is preferred for NGS testing for *ALK* fusions. Current data are still too limited to develop a specific recommendation either for or against the use of NGS for *ALK* fusions as a sole determinant of ALK-TKI therapy.

Next-Generation Sequencing.

9. Expert Consensus Opinion.—Multiplexed genetic sequencing panels are preferred over multiple single-gene tests to identify other treatment options beyond *EGFR, ALK,* and *ROS1*.

The strength of evidence is inadequate supporting the use of multiplexed genetic sequencing panels compared with single-gene tests. The statement is evidence-based and supported by 5 studies,[169–173] comprising 1 PCS,[172] 2 PRCSs,[171,173] and 2 RCSs.[169,170] All included studies were assessed for quality and none were found to have methodologic flaws that would raise concerns about the studies' findings (SDC Table 22). Refer to SDC Table 23 for a summary of findings from studies supporting the use of multiplexed genetic sequencing panels.

The rapid recent emergence of so-called NGS, or massively parallel sequencing, has changed the practice of molecular diagnostics considerably, in lung cancer and in other contexts. This technology involves spatial separation of individual DNA molecules from a sample, PCR amplification (either with or without a preceding hybridization capture step) of predetermined regions of the genome, and parallel sequencing by synthesis of each of the amplified single DNA molecules on a massive scale, followed by computational data processing to recombine and identify the sequences and provide a digital display of each sample's genomic features. This technology enables sensitive and specific assessment of multiple genomic regions at once, up to and including the entire genome. The resources necessary for clinical NGS implementation are substantial and the assays are complex in design, performance, and interpretation. Consequently, this technology is not universally deployed. Nonetheless, multiple academic institutions and private companies have used this technology at scale, providing a relatively DNA- and cost-efficient method for assessment of cancer gene mutations as compared with performing multiple single-gene analyses. In the first guideline, this was a new and unproven technology, but it is now well established. In early 2017, professional practice guidelines were published regarding the validation[174] and interpretation/reporting[17] of NGS assays for cancer.

NGS enables the simultaneous assessment of all 3 of the "must-test" genes in lung cancer—*EGFR, ALK, ROS1*—as well as each of the genes suggested for inclusion in larger panels—*BRAF, RET, ERBB2* (*HER2*), *KRAS, MET*—and hundreds to thousands of other genes that may have potential roles in cancer development. In addition to small mutations, NGS assays are able to detect fusions/rearrangements and copy number changes in the targeted genes, if designed with these alterations in mind.

Numerous studies[169–173] have demonstrated the excellent sensitivity of NGS methods relative to single-gene targeted assays, particularly for single-nucleotide–substitution mutations. Next-generation sequencing methods typically require less input DNA and can accommodate smaller samples with lower concentrations of malignant cells, and, although typically slower than 1 single-gene assay, can often be performed more rapidly than sequential multiple single-gene assays.[175,176] A reduced need for repeat biopsy is an additional benefit of panel testing.

Two basic designs of NGS assays are used in molecular oncology: amplicon based and hybrid capture based. Both of these designs generate a "library" of amplified DNA that is sequenced as single molecules, using one of several sequencing platforms. Amplicon sequencing uses multiple PCR reactions to generate the library, which is generally easier, faster, and able to detect mutations at lower allele frequencies (ie, subclonal populations). However, it is more suitable for simpler assays of fewer genes, and typically used for analysis of oncogene hot spots or small, selected regions of selected genes of interest. In its basic form, it cannot reliably detect fusions or copy number variations. The greater analytic sensitivity of this method makes it suitable for very small or heterogeneous samples. Capture-based methods, by contrast, use hybridization to generate the library and are more complex and involve more steps, resulting in a longer turnaround time, but are better for larger set of genes or genomic regions to be analyzed; however, these methods typically are less sensitive in highly heterogeneous or small samples. In general, capture-based methods may be preferable for initial testing of lung cancer samples in order to detect rearrangements, such as in *ALK* and *ROS1*, as well as a broader range of potential genetic markers. For monitoring during secondary clinical resistance

(ie, *EGFR*), where a more narrow range of possible mutations is needed and smaller or more heterogeneous samples may be desired, amplicon sequencing may be preferred; however, either method may be designed and deployed, successfully, for either application.

10. Expert Consensus Opinion.—Laboratories should ensure test results that are unexpected, discordant, equivocal, or otherwise of low confidence are confirmed or resolved using an alternative method or sample.

The strength of evidence supporting the routine use of orthogonal methods to confirm results for any of the molecular markers is insufficient. However, our opinion is that good laboratory practice for somatic alterations is to perform confirmatory testing for results that are unusual, suboptimal, or inconsistent with other laboratory findings or clinical information.

All assays should be appropriately validated before being offered for clinical use. This typically includes an assessment of sensitivity, specificity, and reproducibility, in addition to other performance characteristics, as required by most laboratory-certifying authorities. The performance characteristics of sequencing-based assays can be readily determined for the more common alterations. The reliability of infrequently encountered mutations or specific categories of chromosomal alterations may be more challenging to document, and laboratories using such technologies should have procedures in place to verify any results that are unexpected, discordant with other results, equivocal, or of compromised confidence in order to provide an optimal result for patient care and to better understand any intrinsic assay limitations. Corroboration of such questionable results might be sought by evaluating a separate specimen from the same lesion, confirmatory testing in another laboratory, or evaluation using an orthogonal methodology.

Question 3: Is Molecular Testing Appropriate for Lung Cancers That Do Not Have an Adenocarcinoma Component?

11. Expert Consensus Opinion.—Physicians may use molecular biomarker testing in tumors with histologies other than adenocarcinoma when clinical features indicate a higher probability of an oncogenic driver.

The strength of evidence supporting the use of molecular biomarker testing in lung cancers that lack an adenocarcinoma component is insufficient. This statement is based on expert consensus opinion.

Upon systematic review, no new evidence established the routine molecular testing of any genes for typical squamous cell carcinoma, small cell carcinoma, or other neuroendocrine lung tumors. Although small studies have reported rare *EGFR* mutations in squamous cell carcinoma biopsies, these may have represented partial sampling of adenosquamous cancers and have not been borne out in fully resected samples with confirmed squamous histology.[177] Evidence from controlled and well-powered studies, supporting the clinical utility of molecular testing of lung cancers for selection of targeted therapies, remains confined to nonsquamous non–small cell lung carcinomas, predominantly adenocarcinomas or mixed cancers with an adenocarcinoma component. However, strict reliance upon adenocarcinoma histology may occasionally exclude some patients who do not have a definitive diagnosis of adenocarcinoma (eg, non–small cell lung carcinoma, not otherwise specified), and might benefit from targeted therapy, particularly for small biopsies that partially sample a larger tumor. Although actionable mutations have been reported in biopsies with nonadenocarcinoma non–small cell types, the frequency of such findings is low enough that it is not recommended to test all small biopsy samples with nonadenocarcinoma histology. In this context, molecular testing is appropriate to perform in lung cancers with non–small cell histologies other than adenocarcinoma when clinical features are atypical and/or consistent with a higher likelihood of a targetable mutation.

Chief among these clinical factors that may indicate a higher probability of a targetable oncogenic driver in the setting of a nonadenocarcinoma histology are young age and absence of tobacco exposure. In nonadenocarcinoma non–small cell histologies, the finding of *EGFR*, *ALK*, or *ROS1* alterations has been most commonly reported in situations in which patients had a minimal (1–10 packs per year) or no history of tobacco exposure.[30,159–161,178–214] Thus, across the spectrum of lung carcinomas, light or absent tobacco exposure should be sufficient rationale to prompt testing, regardless of sampling methodology or completeness of exclusion of adenocarcinoma component.

Similarly, some studies have suggested associations between the presence of *ALK* or *ROS1* alterations and younger patient age.[30,35,181,215,216] Although other studies have indicated that these findings may reflect testing bias,[217] the documentation of an association between younger patient age and an actionable biomarker is another consideration in selecting patients for testing. The boundary between young and not young is not well defined, however, and a clear evidence-based cutoff for this guideline cannot be established. Systematic review from the original (2013) guideline[1] demonstrated that adenocarcinoma patients with *EGFR* mutations had a significantly lower mean age than patients without mutations (56 versus 63, $P = .03$), although the difference in mean ages for patients with and without *ALK* fusions was not significant (60 versus 66), nor is the difference in mean ages for patients with and without *ROS1* fusions from this review (65 versus 62), and the difference in means does not completely capture the distribution of ages and, accordingly, the sensitivity and specificity of any given age cutoff. In the absence of published evidence, our opinion is that a reasonable strategy would be to test patients younger than 50 years with nonadenocarcinoma histology.

Of note, reflex testing algorithms initiated and/or managed by pathologists need to accommodate intricacies of clinical management, which can be challenging as sufficient clinical information is often not available for pathologists to incorporate into their evaluation. Establishing a program for reflex molecular testing of lung cancer samples should be an institutional decision, and should include an open dialogue between pathologists and oncology teams, in order to put in place an optimal strategy. Once those practices are established by the team, however, reflex testing initiated by the pathologist is reasonable.

Lastly, in the context of increasing use of panel-based/NGS-based testing, it may become unnecessary to identify specific analytes of interest in specific clinical situations, instead identifying clinical situations in which panel-based testing may be beneficial.

Question 4: What Testing Is Indicated for Patients With Targetable Mutations Who Have Relapsed on Targeted Therapy?

12. Strong Recommendation.—In lung adenocarcinoma patients who harbor sensitizing *EGFR* mutations and have progressed after treatment with an EGFR-targeted TKI,

EGFR T790M mutational testing should be used to guide selection of treatment with third-generation EGFR inhibitors.

The strength of evidence was adequate to support the use of *EGFR* T790M mutation testing when selecting patients for third-generation EGFR-targeted therapy. This recommendation is evidence-based and supported by 5 studies,[218–222] including 1 MA,[222] 2 single-arm phase I nonrandomized clinical trials,[220,221] 1 PCS,[219] and 1 RCS.[218] All studies were assessed for quality and none were found to have methodologic flaws that would raise concerns about the studies' findings (SDC Table 24). Refer to SDC Table 25 for a summary of findings from the studies supporting the use of *EGFR* T790M mutation testing when selecting patients for third-generation EGFR-targeted therapy.

13. Recommendation.—Laboratories testing for *EGFR* T790M mutation in patients with secondary clinical resistance to EGFR-targeted kinase inhibitors should deploy assays capable of detecting *EGFR* T790M mutations in as little as 5% of *EGFR* alleles.

The strength of evidence supporting this recommendation is insufficient. This recommendation is based on the analytical sensitivity of the allele-specific real-time PCR assay used in clinical trials that established the utility of third-generation EGFR inhibitors directed against the T790M mutant protein.

The major mechanism of secondary clinical resistance to the first-generation EGFR TKIs erlotinib and gefitinib is the development of T790M mutation on the same *EGFR* allele that harbors the original sensitizing mutation, blocking inhibition of the mutant protein by these TKIs. The detection of the *EGFR* T790M mutation in this setting has become clinically necessary because of the development of third-generation EGFR TKIs, such as osimertinib, which are active in the presence of this mutation.[223,224] However, although rare responses have been reported to third-generation inhibitors in *EGFR* T790M–negative disease,[225] such cases may harbor other resistance mechanisms such as *MET* or *ERBB2* amplification[90,114,226] that may be more effectively targeted by other agents. Therefore, determining appropriate therapy in the setting of secondary clinical resistance to an EGFR inhibitor requires knowledge of the presence or absence of the T790M mutation.

Importantly, genetic mechanisms of secondary clinical resistance arise in a subclonal fashion because they confer resistance to the subpopulation of cells in which they are present, and this subclone expands gradually under the selective pressure of the EGFR TKI. Experimental studies have shown that the presence of an *EGFR* T790M mutation in small proportion of a bulk tumor cell population (as little as 5%), often undetectable by Sanger sequencing, can lead to increased growth in spite of treatment with EGFR TKI.[225,227] Detection is made even more challenging when biopsies contain a high proportion of nontumor cells. These considerations lead us to recommend that laboratories should have a high sensitivity assay available for the detection of the *EGFR* T790M mutation in posttreatment biopsies from patients who demonstrate progression or relapse after an initial response to EGFR TKI.

The clinical trials[221,228–230] that established the clinical utility of T790M testing in predicting response to osimertinib used a commercial allele-specific real-time PCR assay with a reported lower limit of detection of 5% mutant allele fraction.[231] Several studies[232,233] have shown comparable, if not superior, analytical sensitivity with droplet digital PCR, and NGS methods[234,235] can also provide this level of sensitivity, given the appropriate assay design. It is important, however, to recognize that unmodified Sanger sequencing, which was an acceptable method in the original 2013 guideline,[1] does not provide adequate sensitivity for this application. Regardless of the method chosen, careful validation must be performed to establish appropriate sensitivity.

Finally, it should be noted that trials are currently underway to assess the value of these third-generation EGFR inhibitors as first-line treatment of *EGFR*-mutant lung adenocarcinoma.[220,221,236] Data are emerging[237] that a second acquired resistance mutation, C797S, can arise in tumors that have progressed after osimertinib treatment for T790M disease, but these cases are so far rare and this mutation is poorly studied and not currently treatable, so testing for C797S is not recommended for routine management at this time.

14. No Recommendation.—There is currently insufficient evidence to support a recommendation for or against routine testing for *ALK* mutational status for lung adenocarcinoma patients with sensitizing *ALK* mutations who have progressed after treatment with an ALK-targeted TKI.

The evidence was insufficient to inform a recommendation on the association between pretreatment or rebiopsy discovery of ALK secondary resistance and clinical outcomes.

Several groups have reported a diverse set of secondary mutations in *ALK* that confer acquired resistance to crizotinib (eg, L1152R, C1156Y, F1174L, L1196M, L1198P, D1203N, and G1269A).[238] For second-line ALK inhibitors, other acquired mutations have been reported (G1202R, G1202del, V1180L, S1206Y, E1201K) as well.

However, although some studies[238] have suggested that different secondary *ALK* mutations may show sensitivity or resistance to different ALK inhibitors, these data are still limited and insufficient to guide selection of treatment in the setting of acquired resistance. Moreover, second-generation ALK inhibitors also show activity in NSCLC without *ALK* resistance mutations, suggesting that a significant proportion of ALK-rearranged lung carcinomas become resistant to crizotinib because of inadequate suppression of *ALK*. Accordingly, current practice is to administer one of several second-generation ALK inhibitors (ceritinib, brigatinib, lorlatinib, and alectinib) that have received FDA approval for the treatment of crizotinib-refractory, *ALK*-rearranged NSCLC, without testing for secondary *ALK* mutations. As more patients experience resistance and receive second-generation inhibitors, we anticipate maturation of data to strengthen the association between secondary mutation and sensitivity/resistance to different inhibitors.

For now, however, we believe there is insufficient clinical utility to warrant routine testing for secondary *ALK* mutations in patients who have relapsed after initial response to an ALK inhibitor.

Question 5: What Is the Role of Testing for Circulating cfDNA for Lung Cancer Patients?—Numerous recent studies[99,100,239] have demonstrated that lung cancer cells shed their DNA into the circulation at levels that are detectable with several modern technologies, such as droplet digital PCR, allele-specific PCR, and NGS. This event enables testing of plasma cfDNA obtained from peripheral blood samples, at least in some instances, as an alternative to a biopsy sample, to identify mutations

occurring in lung cancer both at diagnosis and during the course of disease.

A theoretical advantage of these assays is the derivation of circulating tumor DNA from multiple disease sites, and therefore it may represent an integrative measure of all sites of disease. Although not formally proven, this potential advantage of cfDNA is particularly important in the setting of secondary clinical resistance,[236] to enable broad sampling of different tumor subclones.[232,240,241]

Analytical methods for cfDNA have high analytical specificity, with very low (<5%–20%) false-positive rates,[232,234,235,242,243] such that demonstration of a mutation, in the proper clinical context, can be used to guide treatment with a targeted inhibitor. However, sensitivity of cfDNA analysis is lower (60%–70%),[232,234,235,242,243] such that the absence of mutation finding does not exclude the possibility of a mutation.

It is also important to understand that, despite the promise afforded by this technology, much is still unknown about the dynamics of release of DNA from cancer cells. Factors that increase or decrease the release of DNA from cells, and its half-life in circulation and mechanisms of elimination, are poorly understood.[244–246] There is, however, an overall correlation between burden of disease (both volume and number of metastatic sites) and prevalence of mutations in cfDNA.

Finally, other methods of analysis can be applied to blood samples. Circulating tumor cells can be isolated from the blood, as can exosomes bearing DNA that have been released by cancer cells. Analysis of these latter 2 samples is more challenging technically, and has not been sufficiently studied in lung cancer to warrant consideration in this guideline. Similarly, data are emerging regarding the analysis of cfDNA in other body fluids, particularly urine, but are similarly insufficient to warrant a recommendation at this time. Most of the data to date, and the subject of the comments that follow, apply to plasma cfDNA.

15. No Recommendation.—There is currently insufficient evidence to support the use of circulating plasma cfDNA molecular methods for establishing a primary diagnosis of lung adenocarcinoma.

The evidence was insufficient to inform a recommendation on the use of cfDNA for diagnosis of primary lung adenocarcinoma.

Theoretically, because sensitizing mutations in *EGFR* are characteristic and specific alterations in lung cancers, one may question whether the combination of a cfDNA result showing such a mutation in an appropriate clinical context, with radiographic evidence of a lung lesion, could enable a diagnosis of *EGFR*-mutant lung cancer without requiring an anatomic pathology diagnosis. However, no studies in the medical literature have rigorously evaluated this approach in a prospective manner.

16. Recommendation.—In some clinical settings in which tissue is limited and/or insufficient for molecular testing, physicians may use a cfDNA assay to identify *EGFR* mutations.

The strength of evidence supporting the use of cfDNA to determine *EGFR* mutation status in situations where tissue is limited or insufficient is adequate. This recommendation is evidence-based and supported by 6 studies,[†] comprising 2 MAs,[243,247] 2 PCSs,[235,242] and 2 PRCSs.[232,234] The identified studies used various *EGFR* detection methods, but all verified the results from cfDNA with results from tumor tissue. Using reported true-positive, false-positive, true-negative, and false-negative data from 4 studies, a meta-analysis was conducted to determine a pooled estimate of sensitivity and specificity for cfDNA detection of *EGFR* mutation (Figure 3). All included studies were assessed for quality and none were found to have methodologic flaws that would raise concerns about the studies' findings (SDC Table 26). Refer to SDC Table 27 for a summary of findings from studies supporting the use of cfDNA to determine *EGFR* mutational status in situations where tissue is limited or insufficient.

Analysis of cfDNA for *EGFR* mutations has intermediate sensitivity (66.4%; 95% CI, 62.7%–69.9%) and high specificity (95.6%; 95% CI, 83.3%–99.0%) in lung adenocarcinoma (Figure 3). In some clinical settings in which tissue biopsy material is unavailable or insufficient and tissue rebiopsy is not feasible, so that a tissue-based *EGFR* analysis cannot be performed, then a cfDNA assay for an activating *EGFR* mutation may be conducted as an alternative molecular diagnostic procedure. Because the sensitivity of this assay is less than 80% in all reports, it should be recognized that not all lung adenocarcinoma patients with *EGFR* mutation–positive disease will have the mutation detected in their cfDNA, so that a negative result by cfDNA analysis is not reliable evidence that there is not an *EGFR* mutation in a given patient's cancer. In this context, physicians should renew efforts to obtain an adequate tissue sample for analysis.

17. Expert Consensus Opinion.—Physicians may use plasma cfDNA methods to identify *EGFR* T790M mutations in lung adenocarcinoma patients with progression or secondary clinical resistance to EGFR-targeted TKIs; testing of the tumor sample is recommended if the plasma result is negative.

The strength of evidence supporting the use of cfDNA methods to identify *EGFR* T790M mutation is inadequate. This statement is evidence-based and supported by 4 studies,[233,236,248,249] comprising 2 PCSs[233,236] and 2 RCSs.[248,249] All included studies were assessed for quality and none were found to have methodologic flaws that would raise concerns about the studies' findings (SDC Table 28). Refer to SDC Table 29 for a summary of findings from studies supporting the use of cfDNA methods to identify *EGFR* T790M mutation.

Molecular testing for *EGFR* T790M mutation should be performed in lung adenocarcinoma patients with sensitizing *EGFR* mutations whose disease progresses on, or shows secondary clinical resistance to, EGFR TKI. Such testing is particularly appropriate because third-generation EGFR inhibitors (eg, osimertinib) are proven to have significant benefit for T790M mutant cancers[221] and have been approved for this indication by health authorities around the world. Analysis of cfDNA or analysis of a new tissue biopsy is appropriate for *EGFR* T790M detection. Cell-free DNA may be preferred for patients unwilling or unable to undergo a biopsy at the time of progression; moreover, as progression may represent subclonal processes, cfDNA testing may represent a more global sampling of disease as compared with a tissue biopsy. However, cfDNA analysis for *EGFR* T790M has intermediate sensitivity (0.40–0.78) and high specificity.[233,236,248,249] Hence, a negative result does not rule out the possibility that *EGFR* T790M mutation is the mechanism of resistance to TKI therapy, and a new tissue

[†] *References 232, 234, 235, 242, 243, 247.*

biopsy from a site of progressive disease should be considered if the cfDNA result is wild type.

Note that acquired T790M mutations are often subclonal, and a cfDNA sample could show the original sensitizing *EGFR* mutation (eg, L858R) and still have a false-negative result for T790M, requiring a tissue or cytology sample for confirmation; implicit in this is the benefit of including the original sensitizing mutation (eg, exon 19 deletion, L858R) in the assay to confirm that tumor DNA is being shed into the circulation, although this may not be practical for less common sensitizing mutations. On the other hand, because of the high specificity of cfDNA, a positive T790M finding in cfDNA is equivalent to a tissue biopsy finding of T790M,[236] and can be used to guide therapy with osimertinib.

18. No Recommendation.—There is currently insufficient evidence to support the use of circulating tumor cell molecular analysis for the diagnosis of primary lung adenocarcinoma, the identification of *EGFR* or other mutations, or the identification of *EGFR* T790M mutations at the time of EGFR TKI resistance.

The evidence was insufficient to inform a recommendation on the use of circulating tumor cells for diagnosis of primary lung adenocarcinoma.

What Is the Role of Testing to Select Patients for Treatment With Immunomodulatory Therapies?—*Opinion.*—Tissue should be preserved to enable testing for immunomodulatory therapies.

Evidence.—Subject of upcoming guideline.

Since publication of the last guideline, immunotherapy in lung cancer has rapidly evolved to become a part of standard of care for many patients with advanced NSCLC. Recent studies[250–254] have shown significant benefits in a subset of advanced lung cancer patients when treated with these agents. Government regulatory agencies have approved immunomodulatory therapies as second-line agents for advanced lung cancer patients,[255–257] as well as first-line therapy for patients with high level of PD-L1 expression and absence of sensitizing *EGFR* mutations or *ALK* rearrangements. For some of these agents, selection of patients with a companion diagnostic is required,[258] for others a complementary diagnostic is recommended,[259–261] and for some no biomarker selection is indicated. Putative biomarkers to predict response to these agents, and the methods used to assess them, are varied and not yet standardized.

The principle of the immunomodulatory therapies is their ability to disrupt inhibitory signaling between tumor cells and immune cells (typically T cells), which occurs when tumor cells express proteins that induce immunologic tolerance and prevent the immune system from attacking the tumor. Normally this mechanism is used to control the immune response and prevent autoimmune disease. Several such inhibitory signaling processes exist, although the greatest progress in clinical therapy in lung cancer involves the interaction between PD-L1 on tumor cells and programmed death receptor-1 (PD-1) on T cells. This interaction effectively silences the T-cell response to a tumor. By blocking PD-1 with so-called immune checkpoint inhibitors, T cells become enabled to recognize and respond to foreign antigens presented on the cancer cells.

Because most lung cancer cells contain many mutations beyond their oncogenic drivers, they typically express a large number of mutant proteins, some of which can be displayed on the cell surface by human leukocyte antigen molecules as "foreign" neoantigens. The more mutations in a cell, the more neoantigens are probably expressed, and the more likely the immune system is to destroy the cells, provided that the tolerance mechanisms, such as PD-L1/PD-1, are not activated. Expression of PD-L1 by tumor cells (or local macrophages), expression of PD-1 by tumor-infiltrating lymphocytes, number of mutations and neoantigens, and evidence of an immune infiltrate ("inflamed tumor") are all candidates to predict response to these treatments. Other inhibitory signaling pathways may also be involved, such as the interaction between cytotoxic T-lymphocyte–associated protein 4 (CTLA-4) and CD80/86, which is targeted by the immunomodulatory agent ipilimumab, typically given to patients with melanoma.

The results of treatment with these agents have been impressive,[250–254] with some patients experiencing durable responses that have lasted years. However, unlike the targeted therapies, the frequency with which patients respond, even in biomarker-selected populations, is closer to 20% to 30% in the second line and 50% in the first line (as opposed to 80% for targeted therapies),[252–254] although radiographic response may not be the best indicator of effectiveness of an immunomodulatory therapy because of the impact of active inflammation on the size of a tumor as assessed by standard imaging. In this regard, better biomarkers are needed, as the companion diagnostic biomarkers do not fully address the clinical need to determine, with sensitivity and specificity, who should receive these drugs. Complicating this further are the multitude of different biomarkers, with different methodologies developed in parallel by competing companies for competing drugs, such that no clear message emerges, with evidentiary basis, regarding which biomarkers should be tested or how to test for them. Studies are currently underway to compare a variety of candidate markers and to attempt to harmonize different assays targeting each biomarker (eg, different IHC methods for PD-L1).

Because of the lack of firm evidence supporting specific methodology or agents, we cannot make evidence-based recommendations regarding testing for these drugs in this guideline. A subsequent practice guideline is being planned to focus specifically on evidence-based assessment of methods for selecting patients to receive immunomodulatory therapies.

Despite the exclusion of this question from our systematic literature review, it is our opinion that samples should be preserved for assessment of biomarkers that predict response to immunomodulatory therapies, in accordance with the labeling requirements of the drugs under consideration. Importantly, often the ideal section of a tumor for this application is different from the ideal section of tumor required for molecular testing. For molecular testing, especially sequencing, an ideal sample of cancer has nearly "pure cancer," with little intervening or adjacent stroma or inflammation. Assessment of biomarkers for immunomodulatory therapies, theoretically, should be performed on a section of tumor containing intervening and adjacent stroma, particularly if rich in infiltrating T cells; however, there are no guideline recommendations and this has not been prospectively studied. Response to some drugs[250] has been shown to associate with the nature of the inflammatory cells within a tumor more than with the tumor cells themselves. This distinction, with the operational implications of needing, potentially, to identify and recut 2 sections from each cancer (1 for molecular testing, 1 for the IHC assessment of immune-regulatory molecules) is essential for

surgical pathologists and histology laboratories to understand.

For most applications, the necessary analysis is IHC for PD-L1, although the exact antibody and staining protocol used and the interpretive criteria vary for different treatments. Other candidate IHC biomarkers may be required, however, as well as characterization of reactive cell populations within a tumor. The potential validity and utility of mutational burden calculations (mutations/base pair of total genomic sequence) as assessed by NGS panels are being explored as an investigational biomarker, as are neoantigen prediction algorithms derived from whole-exome sequencing data.

CONCLUSIONS

The importance of molecular diagnostics for the care of lung cancer patients continues to develop at a rapid pace. Many of the guideline recommendations for clinical practice that were published in 2013 were subject for reconsideration by 2015. Similarly, this guideline will likely need revision within a relatively short period of time. Relying upon published prospective studies to serve as a basis for practice recommendations will always lag behind the latest discoveries and advancing edge of care as presented in meetings of professional societies. Practitioners today, and those of us making recommendations, are faced with the daunting challenge of balancing precision oncology, the notion that each patient has a unique combination of factors that should be incorporated into determining individual treatment plans, with evidence-based medicine, the notion that appropriate treatment decisions should be based upon large interventional studies of otherwise identical patients. Compounding this is the increasingly small sizes of the populations being defined by large-scale genomic analyses today, which makes designing effective large controlled interventional studies exceptionally difficult. When a dramatic clinical response is seen in 0.5% of patients with a condition—even a common condition such as lung cancer—how do we recruit enough patients to study to prove that the dramatic response is a general truth that should change practice, and how do we decide that everyone in the world should get the test that can determine if they are in that 0.5%?

We have updated the 2013 recommendations to recognize the changes that have passed this threshold—the importance of *ROS1* testing, the value of IHC for ALK, and the importance of testing for T790M mutations in patients who progress on anti-EGFR therapy. In addition, we have laid out the emerging and promising molecular alterations that are "1 step lower"—alterations in *BRAF, MET, ERBB2* (*HER2*), and *RET*—which we anticipate will pass this bar in a short time, and which we believe should be included in the expanded analyses that are possible because of the emergence of NGS technology. We also see the promise and results obtained with immunomodulatory therapies and await the systematic review that will be conducted to identify and recommend best practices to select patients for these therapies. We look forward to the continuing evolution in diagnostics and care for lung cancer patients as technology, scientific understanding, and clinical practice evolve.

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APPENDIX. Disclosed Interests and Activities January 2015–June 2017[a]		
Name	**Interest/Activity Type**	**Entity**
Dara L. Aisner, MD, PhD	Consulting/advisory fees	Oxford Oncology
		Casdin Capital
	Speaker fees/honoraria	Clovis Oncology
		AstraZeneca
Maria E. Arcila, MD	Speaker fees/honoraria	Raindance Technologies
Mary Beth Beasley, MD	Consulting/advisory fees	Roche/Genentech
Eric H. Bernicker, MD	Speaker fees/honoraria	Myriad Genetics
	Consulting/advisory fees	Foundation Medicine
Philip T. Cagle, MD	Position of Influence	Editor in chief, *Archives of Pathology & Laboratory Medicine*
	Research funding	Roche
Fred R. Hirsch, MD, PhD	Advisory board	Pfizer
		AstraZeneca
	Consulting/advisory fees	Novartis
		Bristol Meyers Squibb
		Merck Sharp Dohme
		HTG Molecular
		Pfizer
		Eli Lilly
		Roche
		AstraZeneca
		Boehringer Ingelheim
	Research grants	Lilly/Imclone
		Celgene
	Research grants, advisory board	Bristol Meyers Squibb
	Research grants, consulting/advisory fees	Amgen
		Roche/Genentech
	Position of Influence	Chief executive officer, IASLC, 2013–current
Keith Kerr, MB ChB	Advisory board, consulting/advisory fees	Roche/Genentech
	Speaker fees/honoraria	Boehringer Ingelheim
		Pfizer
		Eli Lilly
	Consulting/advisory fees, speakers fees/honoraria	Pfizer
		Boehringer Ingelheim
		Roche
		AstraZeneca
		Novartis
	Position of Influence	Board member, IASLC
		Member, Scottish NHS Molecular Pathology Evaluation Panel
David J. Kwiatkowski, MD, PhD	Consulting/advisory fees	AstraZeneca
		Novartis
Marc Ladanyi, MD	Consulting/advisory fees	NCCN/Boehringer-Ingelheim Afatinib Targeted Therapy Advisory Committee
	Stock options/bonds	Foundation Medicine
	Research grants	LOXO Pharmaceuticals
Jan A. Nowak, MD, PhD	Employment	Chief medical officer, OmniSeq, LLC
	Position of Influence	CAP Center Committee, Pathology and Laboratory Quality Center, 2009–2015
		Council on Governmental and Professional Affairs–PHC Working Group, 2012–2016, CAP PHC
		Archives of Pathology & Laboratory Medicine, associate editor for Clinical Pathology, 2012–current
		CAP Guideline Metrics Expert Panel, member, 2014–current
		AMA CPT Editorial Panel member (American Hospital Association) 2015–current
		AMA CPT MPAG 2015–current
		AMP Economic Affairs Committee 2009–current; (co-chair 2013–2014)
		Pathology Coding Caucus–AMP representative 2005–2008; 2013–2015

APPENDIX. Continued		
Name	**Interest/Activity Type**	**Entity**
Lynette Sholl, MD	Research grants, consulting/ advisory fees	Roche/Genentech
	Consulting/advisory fees	Eli Lilly
Benjamin Solomon, MBBS, PhD	Advisory board	Bristol Meyers Squibb
		Roche/Genentech
	Royalties	University of Colorado for (Veristrat) Biodesix
	Consulting/advisory fees, speakers fees/honoraria	Bristol Meyers Squibb
		AstraZeneca
		Pfizer
Erik Thunnissen, MD, PhD	Research grants	Pfizer
	Speaker fees/honoraria	Pfizer
	Consulting/advisory fees	Merck Sharp Dohme
		Clovis Oncology
		Bristol Meyers Squibb
Ming S. Tsao, MD	Research grants, consulting/ advisory fees	Pfizer Canada
		AstraZeneca
		Merck Canada
	Consulting/advisory fees	Ventana/Hoffmann La Roche
		Boehringer Ingelheim Canada
		Bristol Meyers Squibb
	Position of Influence	Member, Advisory Committee on Research, Canadian Cancer Society Research Institute
		Co-chair, Correlative Science and Tumor Biology Committee, Canadian Cancer Trials Group
Yasushi Yatabe, MD, PhD	Speaker fees/honoraria	AstraZeneca
		Pfizer
		Chugai-pharm
		Yakuruto-pharm
		Novartis
		Roche
		Merck Sharp Dohme

Abbreviations: AMA, American Medical Association; AMP, Association for Molecular Pathology; CAP, College of American Pathologists; CPT, Current Procedural Terminology; IASLC, International Association for the Study of Lung Cancer; MPAG, Molecular Pathology Advisory Group; PHC, Personalized Healthcare Committee.

[a] Carol Colasacco, MLIS, SCT(ASCP), Sanja Dacic, MD, PhD, Neal I. Lindeman, MD, Robyn Temple-Smolkin, PhD, Lesley H. Souter PhD, Christina B. Ventura, MPH, MT(ASCP), and Murry W. Wynes, PhD, have no reported conflicts of interest to disclose.