Molecular Testing Guideline for Selection of Lung Cancer Patients for EGFR and ALK Tyrosine Kinase Inhibitors

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

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● **Objective.**—To establish evidence-based recommendations for the molecular analysis of lung cancers that are required to guide EGFR- and ALK-directed therapies, addressing which patients and samples should be tested, and when and how testing should be performed.

Participants.—Three cochairs without conflicts of interest were selected, one from each of the 3 sponsoring professional societies: College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. Writing and advisory panels were constituted from additional experts from these societies.

Evidence.—Three unbiased literature searches of electronic databases were performed to capture articles published from January 2004 through February 2012, yielding 1533 articles whose abstracts were screened to identify 521 pertinent articles that were then reviewed in detail for their relevance to the recommendations. Evidence was formally graded for each recommendation.

Consensus Process.—Initial recommendations were formulated by the cochairs and panel members at a public meeting. Each guideline section was assigned to at least 2 panelists. Drafts were circulated to the writing panel (version 1), advisory panel (version 2), and the public (version 3) before submission (version 4).

Conclusions.—The 37 guideline items address 14 subjects, including 15 recommendations (evidence grade A/B). The major recommendations are to use testing for EGFR mutations and ALK fusions to guide patient selection for therapy with an epidermal growth factor receptor (EGFR) or anaplastic lymphoma kinase (ALK) inhibitor, respectively, in all patients with advanced-stage adenocarcinoma, regardless of sex, race, smoking history, or other clinical risk factors, and to prioritize EGFR and ALK testing over other molecular predictive tests. As scientific discoveries and clinical practice outpace the completion of randomized clinical trials, evidence-based guidelines developed by expert practitioners are vital for communicating emerging clinical standards. Already, new treatments targeting genetic alterations in other, less common driver oncogenes are being evaluated in lung cancer, and testing for these may be addressed in future versions of these guidelines.

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BACKGROUND—EGFR MUTATIONS AND ALK FUSIONS

Lung cancer is the leading cause of cancer-related mortality, accounting for approximately 1.4 million deaths per year worldwide and approximately 160 000 deaths per year in the United States, which is approximately

Table 1. Different Outcomes in All Stages of Non–Small Cell Lung Cancer Patients With and Without EGFR Mutations, Treated With Tyrosine Kinase Inhibitor

| Outcome | Mean ± SD | | Percentage | | n (N) | WMD (95% CI) | RR (95% CI) | P Value |
	EGFR Mutation Positive	EGFR Mutation Negative	EGFR Mutation Positive	EGFR Mutation Negative				
Response rate, %[a]			68	11	51 (3644)		5.16 (4.41–6.04)	<.001
Disease control rate, %[b]			86	42	28 (2204)		1.99 (1.73–2.29)	<.001
Time to progression/ progression-free survival, mo[c]	12.0 ± 7.86	3.4 ± 2.59			27 (2347)	8.66 (6.31–11.00)		<.001
Median survival time, mo[d]	23.3 ± 18.4	12.1 ± 13.9			27 (2489)	10.66 (8.36–12.96)		<.001

Abbreviations: CI, confidence interval; n, number of studies; N, number of patients; RR, relative risk; SD, standard deviation; WMD, weighted mean difference.

[a] References 7, 17, 28, 48, 53, 56, 58, 110, 129, 133, 170, 191–198, 202, 211, 250–278.
[b] References 7, 17, 53, 56, 115, 129, 133, 191, 193–195, 202, 250–254, 258, 259, 261, 266, 267, 273–275, 277–279.
[c] References 17, 28, 53, 56, 58, 129, 170, 185, 191, 193, 195, 201, 211, 250–252, 254, 255, 261–263, 266, 267, 275–278.
[d] References 17, 28, 53, 56, 90, 129, 170, 183, 184, 191, 193, 197, 253–255, 258, 259, 261, 262, 266, 267, 270–272, 276–278.

25% to 30% of all US cancer deaths and more than the next 3 cancers (colon, prostate, breast) combined.[1] Fortunately, the past decade has seen major advances in our understanding of the pathogenesis and management of lung cancers, adenocarcinoma in particular. Specifically, the discovery of the biologic and therapeutic importance of acquired genetic alterations in 2 genes that encode pharmacologically targetable tyrosine kinases involved in growth factor receptor signaling, epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK), has changed the way these cancers are diagnosed and treated.

As gefitinib and erlotinib, small-molecule competitive inhibitors of the EGFR tyrosine kinase, were being evaluated in clinical trials of advanced-stage lung cancer in the early part of the last decade, unusual prolonged responses to these medications were recognized in a subset of patients.[2] This unusual clinical behavior, not seen previously with standard chemotherapy, led to investigations that identified a correlation between activating somatic mutation in the EGFR gene and clinical response to gefitinib and erlotinib. This initial exciting observation has led to sustained and continuing laboratory and clinical investigations into the mechanism and clinical consequences of EGFR mutations in lung cancer. In unselected advanced non–small cell lung cancer (NSCLC) patients, gefitinib and erlotinib produce response rates of 8% to 9%, with a median time to progression of 2.2 months to 3.0 months.[3] In contrast, advanced NSCLC patients selected on the basis of activating EGFR mutations in their tumors show response rates (RRs) of 68%, with a mean progression-free survival (PFS) and time to progression of 12 months (Table 1).[4–6]

In 2009, the first randomized clinical trial (the Iressa Pan-Asia Study [IPASS]) showed that, for advanced NSCLC patients with an activating EGFR mutation, initial treatment with an EGFR tyrosine kinase inhibitor (TKI) was superior to standard platinum-based chemotherapy.[7] In this study, which enrolled East Asian patients with stage IIIB/IV lung adenocarcinoma who never smoked tobacco (or only smoked lightly), the patients whose tumors contained an activating EGFR mutation and who received gefitinib had a significantly longer PFS than those receiving chemotherapy (hazard ratio [HR] for progression or death, 0.48; P < .001).[7] Subsequently, 5 additional randomized controlled trials confirmed this association between activating EGFR mutations and objective response to gefitinib and/or erlotinib therapy (Table 2). However, in spite of these impressive differences in PFS, no study has shown an advantage in overall survival for EGFR-mutation–bearing patients treated initially with an EGFR TKI in comparison to chemotherapy. This is likely to be at least partly due to the crossover design of these studies, in that a large fraction of the patients with EGFR-mutated tumors treated initially with chemotherapy crossed over to the EGFR TKI treatment arm, confounding the interpretation of overall survival data.

Three years after the initial discoveries of EGFR mutations in lung cancer, in 2007, Soda and coworkers[8] reported that an inversion on chromosome arm 2p resulted in the creation of an EML4-ALK fusion gene in lung cancer. The fusion gene was identified in 5 of 75 (7%) NSCLC patients examined. Subsequent studies have indicated that the prevalence of this gene fusion event is about 2% to 7% of all NSCLCs seen in the United States, with enrichment in adenocarcinomas in never smokers or light smokers.[9–20] Testing for this ALK gene fusion has been facilitated by the commercial availability of a dual-probe "break-apart"

Table 2. Randomized Clinical Trial Data on Epidermal Growth Factor Receptor (EGFR) Tyrosine Kinase Inhibitor (TKI) Therapy Versus Chemotherapy as First-Line Therapy for Patients With EGFR-Mutated Lung Cancers

Study	No. of Patients With EGFR-Mutated Lung Cancers	Response Rate (EGFR TKI Versus Chemotherapy), %	Progression-Free Survival (EGFR TKI Versus Chemotherapy), mo
EURTAC[280]	173 (86 erlotinib and 87 chemo)	58 versus 15	9.7 versus 5.2 (HR 0.37)
OPTIMAL[281]	154 (82 erlotinib and 72 chemo)	83 versus 36	13.1 versus 4.6 (HR 0.16)
NEJ 002[91]	228 (114 gefitinib and 114 chemo)	74 versus 31	10.8 versus 5.4 (HR 0.30)
WJTOG3405[26]	117 (58 gefitinib and 59 chemo)	62 versus 32	9.2 versus 6.3 (HR 0.49)
IPASS[7,90]	261 (132 gefitinib and 129 chemo)	71 versus 47	9.5 versus 6.3 (HR 0.48)
LUX LUNG3[282]	345 (230 afatinib and 115 chemo)	56 versus 23	11.1 versus 6.9 (HR 0.58)

Abbreviations: Chemo, chemotherapy; HR, hazard ratio.

fluorescence in situ hybridization (FISH) assay for *ALK* rearrangements that was already in clinical use to detect *ALK* fusions in lymphomas and certain sarcomas.[15] A recent report of a large clinical series indicated that *ALK* rearrangements were seen in about 5% of 1500 NSCLC patients screened.[13] Moreover, *ALK* rearrangement-positive patients treated with a novel ALK inhibitor, crizotinib, showed an overall response rate of 57%, with 72% having a PFS of 6 months or greater.[13] The US Food and Drug Administration (FDA) has approved crizotinib for advanced-stage, *ALK*-positive lung cancer as is also recommended by recent guidelines from professional organizations, including the American Society of Clinical Oncology (ASCO), European Society for Medical Oncology, and National Comprehensive Cancer Network (NCCN).

Given the considerable published data on *EGFR*-mutated lung cancer and the rapid pace of work on *ALK*, representatives of 3 professional organizations with interest in the diagnosis and management of lung cancer—the College of American Pathologists (CAP), the International Association for the Study of Lung Cancer (IASLC), and the Association for Molecular Pathology (AMP)—convened to systematically review the published data and develop evidence-based recommendations for the molecular testing of lung cancers for these 2 critical predictive biomarkers in a clinical practice guideline (CPG).

CPGs are systematically developed statements intended to assist practitioners and patients in making decisions about appropriate health care options for specific clinical circumstances. Attributes of good CPGs include validity, reliability, reproducibility, clinical applicability, clinical flexibility, clarity, multidisciplinary process, review of evidence, and documentation. Specifically, utilization of CPG recommendations may provide improvements in outcomes and in medical practice; minimize inappropriate practice variation; provide decision support tools for practitioners, a reference for medical education, criteria for self-evaluation, and indicators and criteria for external quality review; and assist with reimbursement and coverage decisions. Finally, the process of CPG development can also identify areas where further research is needed.

Clinical Practice Guideline Questions

This CPG addresses 5 principal and 14 corollary questions regarding molecular testing in NSCLC:

I. When should molecular testing for NSCLC be performed?

1. *Which patients should be tested for* EGFR *mutations and* ALK *rearrangements?*
2. *When should a patient specimen be tested for* EGFR *mutations or* ALK *rearrangements?*
3. *How rapidly should test results be available?*

II. How should *EGFR* testing be performed?

4. *How should specimens be processed for* EGFR *mutation testing?*
5. *What are the specimen requirements for* EGFR *testing?*
6. *How should* EGFR *testing be performed?*
7. *What is the role of* KRAS *analysis in selecting patients for targeted therapy with EGFR TKIs?*
8. *What additional testing considerations are important in the setting of secondary or acquired EGFR TKI resistance?*

III. How should *ALK* testing be performed?

9. *What methods should be used for* ALK *testing?*

IV. Should other genes be routinely tested in lung adenocarcinoma?

10. *Are other molecular markers suitable for testing in lung cancer?*

V. How should molecular testing of lung adenocarcinomas be implemented and operationalized?

11. *Must all adenocarcinomas be tested for both* EGFR *and* ALK?
12. *How should* EGFR *and* ALK *results be reported?*
13. *How should* EGFR *and* ALK *testing be validated?*
14. *How should quality assurance be maintained?*

Disclaimer

Clinical 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 statements cannot account for individual variation among patients and cannot be considered inclusive of all proper methods of care or exclusive of other treatments. It is the responsibility of the treating physician, 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. 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. 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.

In formulating recommendations for molecular testing in lung cancer, CAP, IASLC, and AMP considered these tenets of guideline development, emphasizing review of data from appropriately conducted and analyzed clinical trials. Practice guidelines are not intended to supplant physician judgment with respect to particular patients or special clinical situations. The literature and expert review process was directed toward evaluating and selecting the best science for the best possible patient care; a cost analysis was not performed for this guideline.

METHODS

A detailed account of the methods used to create this guideline can be found in the supplemental digital content at www.archivesofpathology.org in the June 2013 table of contents.

Panel Composition

The CAP Pathology and Laboratory Quality Center, and representatives from the IASLC and AMP, jointly convened an expert author panel and scientific advisory panel consisting of experts in clinical pathology and oncology, and research and development relevant to molecular testing in NSCLC. A conference with the expert author and advisory panels was held in December 2010, at which representatives from industry, public health policy and regulatory affairs, patient advocacy, and commercial drug and/or diagnostic device manufacturers were invited to participate. Representatives from the FDA, the National Cancer Institute, ASCO, and the NCCN attended the conference. The opinions of panel members associated with official government agencies represent their individual views and not necessarily those of the agency with which they are affiliated. All members of the expert (author) panel were required to disclose financial and personal conflicts of interest (see below).

Systematic Literature Review and Analysis

The literature search strategy involved searching the following electronic databases from January 2004 through February 2012: Ovid MEDLINE, Ovid MEDLINE In-Process & Other Non-indexed Citations, and the Wiley Cochrane Library. The following keywords and MeSH terms were used in the search: *lung neoplasms*; *lung cancer*; *carcinoma, non-small cell lung*; *EGFR*; *Epidermal growth factor receptor*; *ALK*; *KRAS*; *BRAF*; *mutation*; *amplification*; *gene copy number*; *rearrangement*; *fusion*; *translocation*; *inversion*; *immunohisto-chemistry*; *IHC*; and *FISH*. All searches were limited to the English language.

Eligible Study Designs

Systematic reviews with or without meta-analyses, randomized controlled trials, cohort studies, case-control studies, case series, and method comparisons were eligible for this study. Also included were testing guidelines and proficiency testing strategies of various US and international organizations.

Inclusion Criteria

Articles were eligible for inclusion if they met the following criteria:

1. The study compared, prospectively or retrospectively, the sensitivity, specificity, negative predictive value, or positive predictive value of *EGFR* or *ALK* tests for detection of an *EGFR* mutation, *ALK* rearrangement, or response to a targeted EGFR or ALK TKI; the study described technical comparisons across various assay platforms; the study examined potential testing algorithms for NSCLC molecular testing; or the study examined the correlation of *EGFR* or *ALK* status in primary versus metastatic tumors from the same patients.
2. The study population consisted of patients with a diagnosis of NSCLC.
3. The primary outcomes included the sensitivity, specificity, positive predictive value, and negative predictive value of tests to determine *EGFR* or *ALK* status or treatment response, alone and in combination; concordance across platforms; and accuracy in determining *EGFR* or *ALK* status and benefit from EGFR or ALK TKI therapy.

Exclusion Criteria

Letters, commentaries, editorials, reviews, and case reports were excluded.

Tests Examined

Additional test methods considered included *EGFR* copy number by FISH or bright-field chromogenic in situ hybridization, immunohistochemistry for expression of ALK (kinase domain or carboxy-terminal) or mutated EGFR protein, and reverse transcription–polymerase chain reaction (RT-PCR) detection of EML4-ALK

fusion transcript. Alterations in other genes, including *KRAS*, *BRAF*, and *MET*, were also considered.

Outcomes of Interest

The primary outcomes of interest were the correlations between *EGFR* mutation or *ALK* rearrangement and benefit from EGFR or ALK TKI therapies, respectively. Other outcomes of interest included accuracy in determining *EGFR* or *ALK* status, concordance across technical platforms, sensitivity, and specificity of different tests. After careful consideration of each of these, the expert panel and advisory panel agreed that the primary recommendations of this guideline should focus on *EGFR* mutation assays and *ALK* FISH assays.

The panel reviewed the results of randomized controlled trials in lung cancer, evaluating therapies targeting EGFR or ALK, such as gefitinib, erlotinib, and crizotinib. The panel also reviewed unblinded trials comparing various testing methods, describing test characteristics, and defining strategies for quality assurance of testing in the literature.

Environmental Scan

At the December 2010 conference, individuals representing regulatory agencies (FDA) also provided information about the regulatory framework. Individuals involved with quality assurance in the United States (CAP), the Netherlands, and Canada (Province of Ontario) also provided information about programs to measure and improve *EGFR* and *ALK* testing. This information was used to help the panel specify testing requirements and exclusions, and the necessary quality assurance monitoring that will make the testing less variable and more accurate.

Quality Assessment and Grading of the Included Evidence

Grading of recommendations was based on overall ratings of individual components of the evidence, such as strength of evidence, its consistency, clinical impact, generalizability, and applicability to the international health care system.[20–22] For strength of the evidence, we considered the level of evidence based on its hierarchy, number of studies and number of patients, magnitude of effect from the weighted mean difference or risk ratio, statistical precision measured as a point estimate or confidence interval, and methodologic quality of included studies.[22] The quality of systematic reviews, randomized control trials, and cohort studies was assessed by using the AMSTAR (Assessment of Multiple Systematic Reviews) instrument and SIGN (Scottish Intercollegiate Guidelines Network) 50 checklists, respectively.[23,24]

The overall grade of the recommendation was obtained by rating all components of the evidence. The overall grade indicates the strength of the body of evidence to assist the users of clinical practice guidelines in making appropriate and informed clinical judgments.[23] Grade A or B evidence supports "recommendations," which are generally based on a body of evidence that can be trusted to guide clinical practice in all or in most situations. Grade C evidence is insufficient for a "recommendation" and provides support for "suggestions," for which care should be taken in application. Grade D evidence is weak and does not provide support for "recommendations" or "suggestions." Expert consensus opinion was used where grade C or above evidence was lacking.

Revision Dates

This guideline will be reviewed regularly, as mandated by publication of substantive and high-quality medical evidence that could potentially alter the original guideline recommendations. If necessary, the entire panel will reconvene to discuss potential changes. When appropriate, panel members will recommend revision of the guideline to their respective organizations for review and approval.

Conflict of Interest Policy

Before acceptance on the expert panel, potential member authors from all guideline partnering organizations completed the CAP

conflict of interest process, whose policy and form requires disclosure of material financial interest in, or potential for benefit of significant value from, the guideline's development or its recommendations beginning 12 months prior and ending when the guideline was submitted for publication (see "Appendix"). The potential members completed the conflict of interest disclosure form conservatively, listing any relationship that could be interpreted as constituting an actual, potential, or apparent conflict. Regarding members declaring potentially perceived or real conflict, guideline cochairs agreed that these individuals would best serve as advisory panel members for the guideline, but not authors on the expert panel. CAP, IASLC, and AMP provided funding for this project; no industry funds were used in the development of the guideline.

OUTCOMES

CAP/IASLC/AMP Expert Panel Literature Review and Analysis

The expert author panel cochairs (N.I.L., P.T.C., M.L.) reviewed 1533 potentially relevant abstracts identified in the original literature searches to select studies pertinent to the guideline: 2 cochairs independently reviewed each abstract, and disagreements were resolved by the third cochair. Full-text articles (521) were then reviewed for all selected abstracts by 2 members of the expert author panel; discrepancies were resolved by a cochair. Evidence tables were developed from selected studies that met the criteria for inclusion. A third literature review was performed by the authors of each section of the guideline, to verify that the highest levels of evidence supported each of their recommendations and, if not, to reevaluate the recommendation and modify or defend it.

Consensus Development Based on Evidence

The entire panel met in December 2010 (Chicago, Illinois); additional work on the guideline was completed through electronic mail and monthly teleconferences of the cochairs and/or expert panel. The purposes of the panel meeting were to refine the questions addressed by the guideline, solicit input and testimony from the nonwriting advisory panel, and make writing assignments for the respective sections. All members of the expert panel participated in the preparation of the draft guideline, which was then disseminated for review by the entire panel. Feedback from external reviewers was also solicited. The content of the guideline and the manuscript were reviewed by an independent review panel and approved by the CAP Transformation Program Office Steering Committee, by the IASLC Board of Directors, and by the AMP Clinical Practice Committee and Executive Council. The recommendations are summarized in Table 3.

SECTION I: WHEN SHOULD MOLECULAR TESTING FOR NSCLC BE PERFORMED?

Question 1: Which Patients Should Be Tested for *EGFR* Mutations and *ALK* Rearrangements?

1.1a: Recommendation.—*EGFR* molecular testing should be used to select patients for EGFR-targeted TKI therapy, and patients with lung adenocarcinoma should not be excluded from testing on the basis of clinical characteristics.

1.1b: Recommendation.—*ALK* molecular testing should be used to select patients for ALK-targeted TKI therapy, and patients with lung adenocarcinoma should not be excluded from testing on the basis of clinical characteristics.

Evidence Grade: EGFR: A; *ALK*: B.—Clinical characteristics (eg, age, sex, ethnicity, smoking history) are not sufficiently sensitive or specific to be used to select or exclude patients for treatment or testing. Ethnicity, smoking history, and sex have all been associated with the presence of *EGFR* mutations in NSCLC. Multiple studies have established that *EGFR* mutations are more common in women than men, in patients who have never smoked tobacco than in patients who have smoked tobacco, and in East Asians than in other ethnic groups.[7,25–29] In contrast to *EGFR*-mutated lung cancer, *ALK* gene fusions do not show sharp differences in prevalence according to sex and ethnic origin, but do show a similar strong association with patients who have never smoked tobacco and younger age.[11,18,30,31] However, while these clinical characteristics may have value for population studies, they are insufficiently specific to be used to select individual patients for treatment with a targeted inhibitor. Similarly, these characteristics are insufficiently sensitive to be used as prerequisites for testing an individual patient for *EGFR* mutation or *ALK* fusion, as significant numbers of patients who might benefit from EGFR- or ALK-targeted therapy might be inappropriately excluded (Tables 4 through 7). Prediction models combining several of these variables to define patients who have a very low probability of *EGFR* mutations (eg, <1%) have been developed but will require further evaluation.[32,33]

1.2: Recommendation.—In the setting of lung cancer resection specimens, *EGFR* and *ALK* testing is recommended for adenocarcinomas and mixed lung cancers with an adenocarcinoma component, regardless of histologic grade. In the setting of fully excised lung cancer specimens, *EGFR* and *ALK* testing is not recommended in lung cancers that lack any adenocarcinoma component, such as "pure" squamous cell carcinomas, "pure" small cell carcinomas, or large cell carcinomas lacking any immunohistochemistry (IHC) evidence of adenocarcinoma differentiation.

Evidence Grade: EGFR and *ALK*: A.—As a preamble to the discussion of this recommendation, we note that "non–small cell" lung carcinoma is no longer considered appropriate as a pathologic diagnosis for resection specimens or as an operational category for clinical management. This evolution is also reflected in the version 3.2012 NCCN guidelines that recommend avoiding use of the generic term *non–small cell lung carcinoma* (NSCLC) as a single diagnostic term for complete resection specimens.[34] The distinction between squamous carcinoma, small cell carcinoma, neuroendocrine carcinoma, and adenocarcinomas has become critical for determining subsequent molecular characterization of tumors and patient management.

EGFR mutations have been detected in several histologic lung cancer types, but most tumors with *EGFR* mutations are adenocarcinomas or mixed carcinomas with an adenocarcinoma component, including adenosquamous carcinomas. While there is some evidence that *EGFR* mutations are more likely in low-grade adenocarcinomas with lepidic, papillary, or acinar histology than in poorly differentiated, mucinous, or solid adenocarcinomas, *EGFR* mutations are found at significant frequencies in adenocarcinomas of all grades. Therefore, adenocarcinoma subtype should not be used as a determinant of which samples should, or should not, be tested.[35–38]

ALK rearrangements are also associated with adenocarcinoma histology, without any single subtype being strongly predictive. Studies in Western populations have shown that *ALK* rearrangements are more frequent in adenocarcinomas

Table 3. Summary of Guideline Recommendations

Section I: When Should Molecular Testing of Lung Cancers Be Performed?

Question 1: Which Patients Should Be Tested for EGFR Mutations and ALK Rearrangements?

1.1a: Recommendation: *EGFR* molecular testing should be used to select patients for EGFR-targeted TKI therapy, and patients with lung adenocarcinoma should not be excluded from testing on the basis of clinical characteristics.

1.1b: Recommendation: *ALK* molecular testing should be used to select patients for ALK-targeted TKI therapy, and patients with lung adenocarcinoma should not be excluded from testing on the basis of clinical characteristics.

1.2: Recommendation: In the setting of lung cancer resection specimens, *EGFR* and *ALK* testing is recommended for adenocarcinomas and mixed lung cancers with an adenocarcinoma component, regardless of histologic grade. In the setting of fully excised lung cancer specimens, *EGFR* and *ALK* testing is not recommended in lung cancers that lack any adenocarcinoma component, such as pure squamous cell carcinomas, pure small cell carcinomas, or large cell carcinomas lacking any immunohistochemistry (IHC) evidence of adenocarcinoma differentiation.

1.3: Recommendation: In the setting of more limited lung cancer specimens (biopsies, cytology) where an adenocarcinoma component cannot be completely excluded, *EGFR* and *ALK* testing may be performed in cases showing squamous or small cell histology but clinical criteria (eg, young age, lack of smoking history) may be useful in selecting a subset of these samples for testing.

1.4: Recommendation: To determine *EGFR* and *ALK* status for initial treatment selection, primary tumors or metastatic lesions are equally suitable for testing.

1.5: Expert consensus opinion: For patients with multiple, apparently separate, primary lung adenocarcinomas, each tumor may be tested but testing of multiple different areas within a single tumor is not necessary.

Question 2: When Should a Patient Specimen Be Tested for EGFR Mutation or ALK Rearrangement?

2.1a: Recommendation: *EGFR* mutation testing should be ordered at the time of diagnosis for patients presenting with advanced-stage disease (stage IV according to the 7th edition TNM staging system) who are suitable for therapy or at time of recurrence or progression in patients who originally presented with lower-stage disease but were not previously tested.

2.1b: Suggestion: *ALK* rearrangement testing should be ordered at the time of diagnosis for patients presenting with advanced-stage disease (stage IV according to the 7th edition TNM staging system) who are suitable for therapy or at time of recurrence or progression in patients who originally presented with lower-stage disease but were not previously tested.

2.2a: Expert consensus opinion: *EGFR* testing of tumors at diagnosis from patients presenting with stage I, II, or III disease is encouraged but the decision to do so should be made locally by each laboratory, in collaboration with its oncology team.

2.2b: Expert consensus opinion: *ALK* testing of tumors at diagnosis from patients presenting with stage I, II, or III disease is encouraged, but the decision to do so should be made locally by each laboratory, in collaboration with its oncology team.

2.3: Recommendation: Tissue should be prioritized for *EGFR* and *ALK* testing.

Question 3: How Rapidly Should Test Results Be Available?

3.1: Expert consensus opinion: *EGFR* and *ALK* results should be available within 2 weeks (10 working days) of receiving the specimen in the testing laboratory.

3.2: Expert consensus opinion: Laboratories with average turnaround times beyond 2 weeks need to make available a more rapid test—either in-house or through a reference laboratory—in instances of clinical urgency.

3.3: Expert consensus opinion: Laboratory departments should establish processes to ensure that specimens that have a final histopathologic diagnosis are sent to outside molecular pathology laboratories within 3 working days of receiving requests and to intramural molecular pathology laboratories within 24 hours.

Section II: How Should EGFR Testing Be Performed?

Question 4: How Should Specimens Be Processed for EGFR Mutation Testing?

4.1: Expert consensus opinion: Pathologists should use formalin-fixed, paraffin-embedded (FFPE) specimens or fresh, frozen, or alcohol-fixed specimens for PCR-based *EGFR* mutation tests. Other tissue treatments (eg, acidic or heavy metal fixatives, or decalcifying solutions) should be avoided in specimens destined for *EGFR* testing.

4.2: Expert consensus opinion: Cytologic samples are also suitable for *EGFR* and *ALK* testing, with cell blocks being preferred over smear preparations.

Question 5: What Are the Specimen Requirements for EGFR Testing?

5.1: Expert consensus opinion: Pathologists should determine the adequacy of specimens for *EGFR* testing by assessing cancer cell content and DNA quantity and quality.

5.2: Expert consensus opinion: Each laboratory should establish the minimum proportion and number of cancer cells needed for mutation detection during validation.

5.3: Expert consensus opinion: A pathologist should assess the tumor content of each specimen and either perform, or guide a trained technologist to perform, microdissection for tumor cell enrichment as needed.

Question 6: How Should EGFR Testing Be Performed?

6.1: Recommendation: Laboratories may use any validated *EGFR* testing method with sufficient performance characteristics.

6.2: 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.

6.3: Expert consensus opinion: Clinical *EGFR* mutation testing should be able to detect all individual mutations that have been reported with a frequency of at least 1% of *EGFR*-mutated lung adenocarcinomas.

6.4: Recommendation: Immunohistochemistry for total EGFR is not recommended for selection of EGFR TKI therapy.

6.5: Recommendation: *EGFR* copy number analysis (ie, FISH or CISH) is not recommended for selection of EGFR TKI therapy.

Question 7: What Is the Role of KRAS Analysis in Selecting Patients for Targeted Therapy With EGFR TKIs?

7.1: Recommendation: *KRAS* mutation testing is not recommended as a sole determinant of EGFR TKI therapy.

Question 8: What Additional Testing Considerations Are Important in the Setting of Secondary or Acquired EGFR TKI Resistance?

8.1: Recommendation: If a laboratory performs testing on specimens from patients with acquired resistance to EGFR kinase inhibitors, such tests should be able to detect the secondary *EGFR* T790M mutation in as few as 5% of cells.

Table 3. Continued

Section III: How Should *ALK* Testing Be Performed?

Question 9: What methods should be used for ALK testing?

9.1: Recommendation: Laboratories should use an *ALK* FISH assay using dual-labeled break-apart probes for selecting patients for ALK TKI therapy; ALK immunohistochemistry, if carefully validated, may be considered as a screening methodology to select specimens for *ALK* FISH testing.

9.2: Recommendation: RT-PCR is not recommended as an alternative to FISH for selecting patients for ALK inhibitor therapy.

9.3: Expert consensus opinion: A pathologist should be involved in the selection of sections for *ALK* FISH testing, by assessing tumor architecture, cytology, and specimen quality.

9.4: Expert consensus opinion: A pathologist should participate in the interpretation of *ALK* FISH slides, either by performing the analysis directly or by reviewing the interpretations of cytogeneticists or technologists with specialized training in solid tumor FISH analysis.

9.5: Expert consensus opinion: Testing for secondary mutations in *ALK* associated with acquired resistance to ALK inhibitors is not currently required for clinical management.

Section IV: Should Other Genes Be Routinely Tested in Lung Adenocarcinoma?

Question 10: Are Other Molecular Markers Suitable for Testing in Lung Cancer?

10.1a: Recommendation: Testing for *EGFR* should be prioritized over other molecular markers in lung adenocarcinoma.

10.1b: Suggestion: After *EGFR* testing, testing for *ALK* should be prioritized over other proposed molecular markers in lung adenocarcinoma, for which published evidence is insufficient to support testing guideline development at the present time.

Section V: How Should Molecular Testing of Lung Adenocarcinomas Be Implemented and Operationalized?

Question 11: Must All Adenocarcinomas Be Tested for Both EGFR *and* ALK?

11.1: Expert consensus opinion: Laboratories may implement testing algorithms to enhance the efficiency of molecular testing of lung adenocarcinomas, provided the overall turnaround time requirements are met.

Question 12: How Should EGFR *and* ALK *Results Be Reported?*

12.1: Expert consensus opinion: *EGFR* mutation testing reports and *ALK* FISH reports should include a results and interpretation section readily understandable by oncologists and by nonspecialist pathologists.

Question 13: How Should EGFR *and* ALK *Testing Be Validated?*

13.1: Expert consensus opinion: *EGFR* and *ALK* testing validation should follow the same guidelines as for other molecular diagnostics and FISH tests.

Question 14: How Should Quality Assurance Be Maintained?

14.1: Expert consensus opinion: Laboratories should follow similar quality control and quality assurance policies and procedures for *EGFR* and *ALK* testing in lung cancers as for other clinical laboratory assays. In particular, laboratories performing *EGFR* and *ALK* testing for TKI therapy should enroll in proficiency testing, if available.

Abbreviations: CISH, chromogenic in situ hybridization; EGFR, epidermal growth factor receptor; FISH, fluorescence in situ hybridization; PCR, polymerase chain reaction; RT-PCR, reverse transcription–polymerase chain reaction; TKI, tyrosine kinase inhibitor; TNM, tumor, node, metastasis.

with largely solid histology and/or signet ring cells, but this has not been observed in East Asian populations.[17,39,40] *ALK* fusions appear very infrequent in squamous cell carcinomas lacking any adenocarcinoma component (Table 8) but have been reported in adenosquamous carcinomas.[41]

Likewise, *EGFR* mutations are very infrequent in well-characterized fully excised specimens of squamous cell carcinoma lacking any adenocarcinoma component.[35,38,42–51] A few studies[52–60] have reported *EGFR* mutations in squamous cell carcinoma at a low frequency (Table 9). Most of these latter studies have focused on molecular or clinical data from patients with advanced disease diagnosed by small biopsies, raising concerns that many, if not most, of these

EGFR-mutated "squamous" cancers may have been small biopsies in which the diagnosis of adenosquamous or poorly differentiated adenocarcinomas can be challenging.[35,40] As this histologic distinction is becoming critical in the selection of cases for mutation analysis, in cases where the distinction between poorly differentiated squamous cell carcinoma and poorly differentiated adenocarcinoma is especially difficult, appropriate IHC should be performed for TTF-1, p63, or p40, and other relevant markers.[61–63] Likewise, undifferentiated or large cell carcinomas with histochemical or immunohisto-chemical evidence of adenocarcinoma lineage (eg, TTF-1 or mucin positive) or lacking IHC evidence of squamous carcinoma lineage (eg, p63 or p40 negative) are also

Table 4. *EGFR* Mutation Prevalence in Different Lung Adenocarcinoma Patient Populations[a]				
	EGFR Mutation Prevalence, %	*EGFR* Mutation Positive	*EGFR* Mutation Negative	n (N)
Asian/Pacific[b]	45	1547	1905	31 (3452)
White[c]	24	853	2681	10 (3534)
African American[d]	20	19	78	3 (97)
Hispanic[e]	17	65	307	4 (372)
Asian/Indian[f]	52	114	106	1 (220)

Abbreviations: n, number of studies; N, number of patients.

[a] Data for other populations were absent or too limited for analysis.

[b] References 7, 17, 32, 35, 48, 49, 55, 133, 184, 185, 191, 257, 264, 269, 270, 272–276, 278, 283–292.

[c] References 17, 32, 191, 192, 253, 273, 288, 292–294.

[d] References 256, 288, 293.

[e] References 128, 254, 267, 288.

[f] Reference 295.

Table 5. Clinicopathologic Characteristics in Relation to *EGFR* Mutation Status in Studies Containing Primarily Asian Patients[a]

	EGFR Mutation Prevalence, %	*EGFR* Mutation Positive	*EGFR* Mutation Negative	n (N)
Age with cutoff, y				
<65[b]	46	370	433	6 (803)
≥65[c]	38	432	709	5 (1141)
Sex				
Female[d]	58	1027	733	27 (1760)
Male[e]	32	456	962	26 (1418)
Smoking				
Never[f]	58	843	599	22 (1442)
Ever[f]	26	265	767	22 (1032)
History of smoking, pack-years				
0–10[g]	67	10	5	1 (15)
11–40[g]	45	5	6	1 (11)
>40[g]	23	5	17	1 (22)
>20[h]	25	13	40	1 (53)
Histology				
Adenocarcinoma[i]	50	1278	1256	25 (2534)
Squamous[j]	5	8	160	8 (168)
Adenosquamous[k]	67	4	2	2 (6)
Large cell[l]	7	1	14	4 (15)
Differentiation				
Two grades				
Well[m]	37	62	107	3 (169)
Moderate to poor[m]	14	27	162	3 (189)
Three grades				
Well[n]	65	28	15	2 (43)
Moderate[n]	48	59	63	2 (122)
Poor[n]	34	17	33	2 (50)

Abbreviations: n, number of studies; N, number of patients.

[a] Most studies contained primarily patients with adenocarcinoma.
[b] References 7, 55, 184, 272, 276, 290.
[c] References 55, 184, 272, 276, 290.
[d] References 7, 32, 35, 48, 49, 55, 133, 184, 185, 257, 264, 269, 270, 272, 274–276, 278, 283–287, 289–291, 296.
[e] References 32, 35, 48, 49, 55, 133, 184, 185, 257, 264, 269, 270, 272, 274–276, 278, 283–287, 289–291, 296.
[f] References 32, 35, 55, 133, 184, 185, 257, 264, 269, 270, 274–276, 278, 283–285, 287, 289–291, 296.
[g] Reference 290.
[h] Reference 272.
[i] References 32, 35, 48, 49, 55, 133, 184, 185, 257, 264, 269, 270, 272, 274–276, 278, 283–286, 289–291, 296.
[j] References 55, 257, 264, 272, 274, 275, 289, 296.
[k] References 272, 289.
[l] References 48, 272, 275, 289.
[m] References 119, 184, 202.
[n] References 49, 184.

appropriate for *EGFR* and *ALK* testing. The use of IHC and other specialized histochemical techniques to establish lineage in lung adenocarcinoma is an evolving field (and the selection of stains/antibodies and their associated interpretive criteria) and is outside the scope of this guideline. The interested reader is referred to published IASLC guidelines for an introduction to this topic.[63]

EGFR mutations have not been detected in small cell lung cancer (SCLC), except in rare cases of combined SCLC with an adenocarcinoma component; in some of these cases a response to EGFR TKI was reported.[64–69] In addition, adenocarcinomas with *EGFR* mutations that were initially treated successfully with an EGFR TKI may show transformation to small cell histology at relapse, as a mechanism of resistance in these patients, the recurrence of the lung cancer as a small cell carcinoma retains the *EGFR* mutation from the antecedent adenocarcinoma.[69–71]

Among other subtypes of lung cancer, rare *EGFR* mutations have been reported in pulmonary salivary gland–type tumors, large cell carcinomas, sarcomatoid carcinomas, and large cell neuroendocrine carcinoma.[34,72–77] This guideline does not make specific recommendations for

these less common tumors, although testing of large cell carcinomas showing IHC evidence of adenocarcinoma differentiation should be considered.[77] *EGFR* mutations have not been found in carcinoids.[78]

1.3: Recommendation.—In the setting of more limited lung cancer specimens (biopsies, cytology) where an adenocarcinoma component cannot be completely excluded, *EGFR* and *ALK* testing may be performed in cases showing squamous or small cell histology but clinical criteria (eg, young age, lack of smoking history) may be useful in selecting a subset of these samples for testing.

Evidence Grade: EGFR and *ALK*: A.—Lung cancers with mixed histology (eg, adenosquamous, mixed adeno/small cell) can have mutations in *EGFR* or rearrangements in *ALK* and, if so, respond to treatment.[79] Therefore, in tissues with incomplete sampling in which the possibility of an adenocarcinoma component cannot be excluded, testing may be indicated. In this context, clinical criteria, such as young age, lack of smoking history, or a documented preceding adenocarcinoma, may be used to select patients for testing.

Table 6. Clinicopathologic Characteristics in Relation to *EGFR* Mutation Status in Studies Containing Primarily Non-Asian Patients[a]

	EGFR Mutation Prevalence, %	*EGFR* Mutation Positive	*EGFR* Mutation Negative	n (N)
Sex				
Female[b]	28	859	2239	19 (3098)
Male[b]	18	397	1768	19 (2165)
Smoking				
Never[c]	45	666	805	18 (1471)
Ever[c]	15	569	3154	18 (3723)
History of smoking, pack-years				
0–10[d]	39	18	28	1 (46)
11–50[d]	8	7	86	1 (93)
>50[d]	5	3	56	1 (59)
Histology				
Adenocarcinoma[e]	24	1266	3918	19 (5184)
Squamous[f]	5	6	104	9 (110)
Adenosquamous[g]	13	1	7	2 (8)
Large cell[h]	5	2	37	6 (39)

Abbreviations: n, number of studies; N, number of patients.

[a] Most studies contained primarily patients with adenocarcinoma.
[b] References 17, 32, 87, 115, 128, 191–193, 253, 254, 256, 267, 273, 288, 292–295, 297.
[c] References 17, 32, 87, 128, 191–193, 253, 254, 256, 267, 273, 288, 292–295, 297.
[d] Reference 288.
[e] References 17, 32, 87, 115, 128, 191–193, 253, 254, 256, 267, 273, 288, 292–294, 297, 298.
[f] References 17, 191, 193, 254, 256, 267, 273, 288, 298.
[g] References 17, 288.
[h] References 17, 193, 254, 267, 273, 298.

Table 7. Clinicopathologic Characteristics in Relation to *ALK* Rearrangement Status[a]

	ALK Rearrangement Prevalence, %	*ALK* Rearrangement Positive	*ALK* Rearrangement Negative	n (N)
Age with cutoff, y				
≤65[20]	6	13	210	1 (223)
>65[20]	3	6	224	1 (230)
Sex				
Females[11,12,15,20]	5	28	579	4 (607)
Males[11,12,15,20]	4	30	738	4 (768)
Smoking				
Never[11,15,20]	8	30	331	3 (361)
Ever[11,15,20]	3	20	652	3 (672)
Ethnicity				
Asian/Pacific[11,12,14]	5	35	654	3 (689)
White (Caucasian)[12,15]	4	22	474	2 (496)
Histology				
Adenocarcinoma[9,11,12,15,16,19,20]	5	67	1319	7 (1386)
Squamous[9,11,12,16,19,20]	0.2	1	522	6 (523)
Adenosquamous[11,12,19]	0	0	19	3 (19)
Differentiation				
Well[11]	1	1	97	1 (98)
Not well[11]	6	10	145	1 (155)

Abbreviations: n, number of studies; N, number of patients.

[a] Most studies contained primarily patients with adenocarcinoma.

1.4: Recommendation.—To determine *EGFR* and *ALK* status for initial treatment selection, primary tumors or metastatic lesions are equally suitable for testing.

Evidence Grade: EGFR and *ALK*: B.—Discordances in *EGFR* mutation status between primary tumors and corresponding metastases appear rare[80] (Table 10). The clinical significance of these relatively uncommon discrepancies remains uncertain but it should be noted that some discrepancies may reflect the higher risk of false negatives when analyzing lung cancer metastatic to lymph nodes due to the admixture of lymphoid cells. Accordingly, the choice of which sample to test should be based primarily on the specimen qualities

Table 8. Studies Specifically Reporting Outcome of *ALK* Rearrangement Studies in Squamous Cell Carcinomas

Source, y	n	*ALK* Rearrangement Positive, %
Takeuchi et al,[299] 2008	71	0
Takahashi et al,[39] 2010	75	0
Inamura et al,[10] 2008	48	0

Abbreviation: n, number of squamous cell carcinoma samples tested.

Table 9. Major Studies Specifically Reporting *EGFR* Mutation Analysis in Surgically Resected Squamous Cell Carcinomas as Compared to Adenocarcinomas

Source, y	Predominant Ethnic Origin of Study Population	*EGFR* Mutations in Resected Adenocarcinomas, No. (%)	*EGFR* Mutations in Resected Squamous Cell Carcinomas, No. (%)
Marchetti et al,[45] 2005	European	39/375 (10.4)	0/454
Sugio et al,[43] 2006	Asian	136/322 (42.2)	0/102
Tsao et al,[44] 2006	North American	14/96 (14.6)	0/63
Tsao et al,[60] 2011	North American	32/231 (13.9)	8/162 (4.9)
Bae et al,[284] 2007	Asian	20/55 (36.4)	0/60
Lee et al,[300] 2010	Asian	36/117 (30.8)	0/56
Miyamae et al,[301] 2011	Asian	...	3/87[a] (3.4)
Rekhtman et al,[36] 2012	North American	...	0/95
TCGA,[302,b] 2012	North American	...	2/178[c] (1.1)

[a] These numbers do not include 2 *EGFR*-mutated cases reclassified as adenocarcinoma and adenosquamous carcinoma on the basis of immunohistochemistry (IHC) for thyroid transcription factor 1 (TTF-1)/p63; the remaining 3 *EGFR*-mutated cases were reported as IHC-confirmed squamous cell carcinoma.
[b] The Cancer Genome Atlas Research Network.
[c] Both *EGFR* mutations were L861R.

themselves (tumor content and preservation), rather than whether they are primary or metastatic lesions. All things being equal, the most recent available tissue is preferred, but no evidence supports subjecting a patient to a procedure to procure tissue specifically to obtain testing of a metastasis before initiation of TKI therapy when an earlier primary lesion is available and suitable for analysis, unless there is a strong clinical suspicion of its origin from a separate primary tumor.

This is in contrast to patients with metastasis or relapse after initially successful response to TKI treatment (ie, acquired resistance), in which case repeated biopsies are performed to confirm a clonal relationship to the treated primary tumor, permit analysis of the mechanism of resistance and, potentially, direct the patient to targeted, protocol-based experimental therapies that may differ according to the mechanism of acquired resistance.

1.5: Expert Consensus Opinion.—For patients with multiple, apparently separate, primary lung adenocarcinomas, each tumor may be tested but testing of multiple different areas within a single tumor is not necessary.

Separate primary tumors that harbor different mutations are not uncommon.[81] If an *EGFR* mutation is discovered in any tumor, the patient may benefit from an EGFR TKI. Therefore, if a patient presents with apparently separate primary tumors (based on location and nonoverlapping histologic features), each primary tumor may be tested. However, the decision whether or not to test each of a patient's multiple tumors depends on each patient's clinical context and requires communication between the laboratory and the clinical care team.

Adequate evidence has not been published to support mutation analysis of different regions of a tumor, neither with respect to mutation sensitivity in otherwise sufficiently cellular regions, nor with respect to clinical outcomes. Although some data suggest that variation in *EGFR* copy number within a tumor may impact mutation detection rate in samples from different zones of the tumor, performing FISH to select an area for mutation testing is impractical, and any area with sufficient tumor content to enable detection of a mutation may be selected for analysis.[80,82–84] Recent detailed genomic studies in other cancers have provided further support for the concept that key driver mutations are well preserved but secondary mutations are not.[85] As key driver mutations, *EGFR* mutations and *ALK*

fusions appear to follow this pattern and this is further supported by the extreme rarity of the *EGFR* wild-type recurrent tumors in patients with *EGFR*-mutated lung adenocarcinomas treated with EGFR TKIs.[70]

Question 2: When Should a Patient Specimen Be Tested for *EGFR* Mutation or *ALK* Rearrangement?

2.1a: Recommendation.—*EGFR* mutation testing should be ordered at the time of diagnosis for patients presenting with advanced-stage disease (stage IV according to the 7th edition tumor node metastasis [TNM] staging system) who are suitable for therapy, or at time of recurrence or progression in patients who originally presented with lower-stage disease but were not previously tested.

2.1b: Suggestion.—*ALK* rearrangement testing should be ordered at the time of diagnosis for patients presenting with advanced-stage disease (stage IV according to the 7th edition TNM staging system) who are suitable for therapy, or at time of recurrence or progression in patients who originally presented with lower-stage disease but were not previously tested.

Evidence Grade: EGFR: A; ALK: C.—Patients with advanced-stage disease have short life expectancies, on the order of 4 to 5 months in the absence of treatment. Although patients may derive some benefit from first-line chemotherapy with use of targeted TKIs as second-line agents, patients with *EGFR* mutations/*ALK* rearrangements show superior outcomes when the targeted TKI therapies are administered as first-line agents.[34,86] For these patients, timely diagnosis is critical and molecular testing should be initiated as soon as a diagnosis of adenocarcinoma is established.

Reflex testing, a testing policy that does not require a separate clinician order for each case, is appropriate if agreed upon by the lung cancer care team and may help to

Table 10. Summary of Studies of the Concordance of *EGFR* Mutations in Primary Versus Metastatic Tumors in the Same Patient[a]

Metastatic Lesions	Primary Tumor	
	EGFR+	*EGFR*−
EGFR+	108	6
EGFR−	11	183

[a] Data derived from Park et al[57]; Yatabe et al[80]; and Sun et al.[303]

ensure expedited and consistent routing of specimens for molecular testing. However, some patients may not be candidates for targeted TKI therapy for clinical reasons, and good communication between the clinical care team and the testing laboratory is needed to ensure testing is performed for patients whose management will be impacted by the test result. Specifically, testing is not necessary for patients with stage IV disease who are being considered for palliative or hospice care only. Similarly, in settings in which reflex testing is the practice, a mechanism should be provided for the clinical care team to communicate to the pathologist examining a small biopsy or cytology sample when a more suitable diagnostic specimen (eg, a resection) is expected to be obtained, and the molecular testing should be deferred to the subsequent, more generous sample.

2.2a: Expert Consensus Opinion.—*EGFR* testing of tumors at diagnosis from patients presenting with stage I, II, or III disease is encouraged but the decision to do so should be made locally by each laboratory, in collaboration with its oncology team.

2.2b: Expert Consensus Opinion.—*ALK* testing of tumors at diagnosis from patients presenting with stage I, II, or III disease is encouraged, but the decision to do so should be made locally by each laboratory, in collaboration with its oncology team.

Patients with localized disease have a potential for surgical cure and may never need targeted therapies. Testing the initial surgical specimen at the time of resection affords the benefits of having recent tissue to test as well as, in many instances, larger resection specimens with ample material. By contrast, excluding patients with early-stage disease from testing and waiting until progression may necessitate testing of a smaller biopsy sample of borderline quality or quantity and/or subjecting patients to invasive procedures in order to obtain adequate samples, or trying to retrieve the earlier resection specimen from long-term storage or from another institution, which can be challenging and time-consuming. Furthermore, *EGFR* testing may have use as a favorable prognostic factor, beyond its use as a predictor of response to targeted inhibitors.[86,87]

These benefits of testing all early-stage disease patients must be balanced against the cost of performing testing that may not be used to select therapy for the patients who never relapse. The portability of the initial testing results should also be considered, in the case of a patient with a delayed relapse who may present for targeted therapy years later at another institution.

Currently, the question of whether or not to test a diagnostic specimen in early-stage disease is a local decision that must be made in conjunction with each institution's oncology care team, as insufficient published evidence supports a universal recommendation. However, evidence to support this treatment practice may be forthcoming. Traditionally, most novel therapeutic agents are studied initially in patients with advanced disease that is refractory to standard therapy. If activity is noted in this setting, the agents are typically next studied in the first-line setting for advanced-stage disease. If activity is noted in this setting, then the agent may be studied in earlier-stage disease. This is where EGFR inhibitors are today—given the success in first-line treatment of patients with advanced-stage disease and *EGFR* mutations, a next wave of clinical trials will test whether these agents may be beneficial as adjuvant therapy in early-stage disease, a notion already supported by retrospective analyses.[88] ALK therapy is one step removed,

as trials testing its value as first-line therapy in advanced disease are ongoing.

2.3: Recommendation.—Tissue should be prioritized for *EGFR* and *ALK* testing.

Evidence Grade: EGFR: A; *ALK:* B.—*EGFR* and *ALK* testing are the most important uses of the diagnostic sample after a diagnosis of adenocarcinoma is established. If specimens are insufficient for molecular testing, patients may need to undergo another invasive diagnostic procedure before they can be treated. Therefore, it is critical to retain sufficient material for molecular analysis and to be judicious in the use of sections for IHC studies, histochemical stains, or deeper levels that may not be essential to establish a histopathologic diagnosis. This is particularly critical for specimens that have a limited amount of cancer cells. This concept has also now been incorporated into the new IASLC classification.[63] In instances where there is not enough tissue for *EGFR* and *ALK* testing, pathologists should alert the oncologist and include a note in the surgical pathology report to this effect. Because substantial tissue can be lost when blocks are refaced in histology laboratories while cutting unstained slides after initial sectioning, consideration should be given to cutting multiple additional unstained sections "up front" when the sample is first processed in histology. These unstained sections could then be used for deeper levels or additional histochemical/immunohistochemical stains as needed to establish the diagnosis, and for ensuing molecular testing, without having to reface and recut the blocks. The benefit of such a sample-sparing protocol must be balanced against the cost of cutting additional sections, some of which might not be used.

Question 3: How Rapidly Should Test Results Be Available?

3.1: Expert Consensus Opinion.—*EGFR* and *ALK* results should be available within 2 weeks (10 working days) of receiving the specimen in the testing laboratory.

Clinical response to EGFR TKI in *EGFR*-mutated tumors is typically seen rapidly, and the drugs themselves have relatively modest side effects when compared to platinum-based chemotherapy.[89] For these reasons, some oncologists are willing to initiate EGFR TKI therapy before the *EGFR* test result is available. However, randomized controlled trials have demonstrated that *EGFR* wild-type tumors respond better to conventional chemotherapy than to EGFR TKIs.[7,26,90,91] Thus, in most clinical circumstances, EGFR TKI therapy should not be administered as first-line therapy without evidence of a sensitizing *EGFR* mutation. Therefore, the turnaround time (TAT) for *EGFR* testing has become increasingly important, especially for patients with advanced-stage disease.

To that end, and in the absence of published data establishing an evidence-based recommendation, it is our expert consensus opinion that a TAT goal of 1 week (5 working days) should be established for *EGFR* and *ALK* testing, up to a maximum TAT of 2 weeks (10 working days). This TAT refers to the period from the receipt of suitable material by the molecular pathology laboratory where the testing is performed to the reporting of the final results to the clinical care team, and is not related to the period of time between when a patient undergoes a diagnostic procedure and when a specimen is submitted to the laboratory for testing. We consider achieving this TAT goal to be most critical for patients with advanced-stage disease, although we believe it is a reasonable goal for all testing.

3.2: Expert Consensus Opinion.—Laboratories with average TATs beyond 2 weeks need to make available a more rapid test—either in-house or through a reference laboratory—in instances of clinical urgency.

Patients with stage IV lung cancer have median untreated life expectancy of approximately 16 weeks; 20% of this time should not be spent waiting for test results. If laboratories cannot provide results for the sickest of patients within 2 weeks, then they need to make available a method or another laboratory that can. This is particularly germane for large-scale multiplexed assays that afford the capability of testing many genes at once but may take several weeks to complete and analyze. While this technology has great scientific promise and platform consolidation is logistically appealing, the ability to generate large amounts of data of unproven significance should not take precedence over the timely generation of clinically useful data. This recommendation is based upon expert consensus, given the lack of published experimental data addressing this topic.

3.3: Expert Consensus Opinion.—Laboratory departments should establish processes to ensure that specimens that have a final histopathologic diagnosis are sent to outside molecular pathology laboratories within 3 working days of receiving requests and to intramural molecular pathology laboratories within 24 hours.

Another critical component of the overall testing time is the delivery of tissues necessary to perform these tests, both between institutions and within pathology departments. It is our expert consensus opinion that pathology departments should have established processes in place to retrieve and send out materials (blocks or unstained sections) within 3 working days of receiving such requests, once a final histopathologic diagnosis has been established. In local cases, where tumor material must be transferred from surgical pathology or cytology to the molecular diagnostic laboratory, intradepartmental delivery of materials should occur within 1 working day for finalized current or recent (eg, not in long-term storage) cases. As stated above, reflex testing and/or preparation of unstained recut sections at the time the sample is first processed in the histology laboratory may help to ensure expedited and consistent intradepartmental routing of specimens for molecular testing.

Molecular pathology laboratories with high demand for these tests in otherwise busy pathology departments may benefit from having a specialized histologic service dedicated to expeditious processing of samples for molecular testing within the pathology department or having a dedicated histology technician and equipment within the molecular pathology laboratory.

SECTION II: HOW SHOULD *EGFR* TESTING BE PERFORMED?

Question 4: How Should Specimens Be Processed for *EGFR* Mutation Testing?

4.1: Expert Consensus Opinion.—Pathologists should use formalin-fixed, paraffin-embedded (FFPE) specimens or fresh, frozen, or alcohol-fixed specimens for polymerase chain reaction (PCR)–based *EGFR* mutation tests. Other tissue treatments (eg, acidic or heavy metal fixatives, or decalcifying solutions) should be avoided in specimens destined for *EGFR* testing.

The effects of fixation and preanalytic specimen processing are no different for *EGFR* mutation analysis than for other molecular diagnostics applications that rely upon PCR

amplification, and *EGFR* testing can be performed on fresh, frozen, FFPE, or alcohol-fixed specimens. Accordingly, molecular tests should be validated for each of the specimen types likely to be encountered (ie, FFPE, fresh, frozen, alcohol fixed), and testing should be performed and reported only on validated specimen types. Other specimen types should be rejected as inadequate or tested at the discretion of the laboratory director, with clear communication that the test was performed on a nonvalidated specimen type.

Fresh or frozen specimens are optimal for analysis of long (ie, >1000 bp) DNA segments.[92–94] However, this is not typically necessary for *EGFR* testing. One drawback to testing fresh or frozen tissues is the need for correlative histologic examination, which may require cutting and staining frozen sections flanking the portion of the specimen submitted for testing. In contrast, the use of FFPE material for DNA extraction allows a more convenient and accurate assessment of tumor content.

Different tissue fixatives, processing protocols, and storage conditions, with associated variations in chemical and physical conditions, including time to fixation, mechanism of fixation, processing temperature, pressure, and pH, storage time and conditions, all can affect the quality and quantity of DNA, RNA, and proteins in the specimens, and their analyses. Selection of a proper section for analysis, and correct interpretation of negative results, requires that the molecular pathology laboratory have access to this information.

Ten percent neutral-buffered formalin is the fixative that is most widely used, and most molecular assays have been optimized and validated on such tissues. Formalin fixation leads to chemical cross-links to proteins, RNA, and other DNA molecules, with concomitant fragmentation of DNA, which inhibits molecular analysis in a length-dependent fashion. In general, molecular analyses of formalin-fixed DNA that require DNA segments shorter than 300 bp are usually successful, while those requiring a length between 300 and 1000 bp succeed inconsistently, and those requiring a length of greater than 1000 bp are often unsuccessful.[92] Formalin fixation also causes random nucleotide base changes, which can lead to false-positive results, typically in samples with low DNA concentration or with ultrasensitive assays.[95–97]

Alcohol (70% ethanol) has been shown to be comparable to, if not better than, 10% neutral-buffered formalin for molecular assays requiring nucleic acid extraction, but alcohol is not a favored fixative in routine histology laboratories for a variety of reasons, including tradition, safety, cost, and compatibility with some other FDA-approved procedures (eg, *ERBB2* [*HER2/neu*] FISH, estrogen receptor IHC).[93,98] Most cytology fixatives are alcohol based and, therefore, cytology specimens are typically suitable for DNA-based molecular assays.[99–101]

Most molecular analyses are inhibited by heavy metal fixatives (eg, Zenker, B5, B plus, acid zinc formalin) owing to competition between the metals in the fixative and the magnesium needed as a cofactor for most DNA polymerases and other enzymes involved in molecular methods.[93,102,103] Specimens processed with heavy metal fixatives should not be used for *EGFR* testing.

Acidic solutions (eg, Bouin solution, bone-decalcifying solutions) fragment DNA extensively.[104,105] Tissues treated with acidic solutions should not be used for *EGFR* testing. This is particularly problematic for analysis of bone

metastases, which are usually decalcified in acidic solutions. The use of nonacidic chelating decalcifying solutions may better preserve DNA for molecular testing.[105] Moreover, unbuffered formalin spontaneously oxidizes to formic acid over time.[106]

The relatively broad time range of specimen fixation found in pathology practice usually has no effect on morphologic details, but longer durations of fixation adversely affect the quality of nucleic acid.[92] Fixation times of 6 to 12 hours for small biopsy samples and 8 to 18 hours for larger surgical specimens generally give best results, although our expert consensus opinion is that fixation times of 6 to 48 hours should give acceptable results, in accordance with CAP Laboratory Accreditation Program Checklist MOL.39358 for *HER2/neu* in situ hybridization.[98,107] This is a generalization, however, and the effect of extreme fixation times should be assessed by each laboratory during validation. This knowledge should be incorporated into the interpretation and reporting of molecular pathology results when fixation times are extreme.

4.2: Expert Consensus Opinion.—Cytologic samples are also suitable for *EGFR* and *ALK* testing, with cell blocks being preferred over smear preparations.

Although it has been shown that smears can be used effectively for DNA extraction for *EGFR* mutation assays, it is our expert consensus opinion that, for cytology specimens such as malignant pleural effusions, use of a cell block is recommended over smear preparations because of the ability to correlate with malignant cell content, the preservation of the original diagnostic specimen, and the possible retention of more material for additional diagnostic studies.[62,101,108,109] In addition, analysis of FISH requires nonoverlapping tumor cells, which can be a challenge to identify on 4′,6-diamidino-2-phenyl-indole (DAPI)–stained smears. As with surgical pathology specimens, the cell pellet should be fixed in 10% neutral-buffered formalin for 6 to 48 hours before processing. Fixation in 70% ethanol is also acceptable; in which case validation of the molecular assays on DNA extracted from alcohol-fixed tissues should be done.

Question 5: What Are the Specimen Requirements for *EGFR* Testing?

5.1: Expert Consensus Opinion.—Pathologists should determine the adequacy of specimens for *EGFR* testing by assessing cancer cell content and DNA quantity and quality.

EGFR mutation testing can be performed on specimens procured by almost any procedure: surgical resection, open biopsy, endoscopy, transthoracic needle biopsy, fine-needle aspiration, or thoracentesis. In general, larger tumor specimens (eg, resections) are generally preferred for mutation assays because of greater amount of material and greater capacity to enrich the malignant content by dissection. However, most advanced-stage lung tumor specimens are small biopsy or cytology specimens such as pleural fluids or fine-needle aspirates. *EGFR* mutation assays have been shown to be able to detect mutations from cytologic specimens, particularly if cell blocks are available.[109–114] Ultimately, any specimen that meets the laboratory's requirements for tumor content, fixation, and quality, as established during validation, may be chosen for analysis. Specimens that fail to meet these requirements may be analyzed at the laboratory director's discretion, but these specimens present some additional challenges that might necessitate additional testing procedures or communication in the report.

One of the issues with small specimens is the possibility of false-negative results.[45,115–117] The number of tumor cells in comparison with normal tissue, such as inflammatory and stromal cells, is an important factor that influences the reliability of mutational analysis.[115] This is particularly important for less sensitive methods, such as unmodified direct DNA sequencing. In such cases, it may be appropriate to add to the report of a negative result a recommendation for repeated testing on additional material if it becomes available.

Although PCR-based methods can in principle detect mutations from a single cell, a low copy number DNA template can generate sequence artifacts, mainly guanine to adenine transitions, due to stochastic occurrence of polymerase errors early in the PCR in the setting of low template.[96] In addition, the DNA damage caused by formalin fixation can also lead to sequence artifacts.[95] As much as 1 artificial mutation per 500 bases may be observed in the analysis of formalin-fixed tissue with low DNA content.[97] The frequency of errors reflects both *Taq* DNA polymerase's normal error frequency and the degree of damage and cross-linking of DNA by formalin. For this reason, some laboratories perform duplicate amplifications of FFPE samples to ensure accurate results. For direct DNA sequencing, mutational artifacts should be distinguished from true mutations by bidirectional sequencing and by confirmatory sequencing of independent PCR products.[107] Whole genome amplification is a method to increase the amount of template DNA in insufficient specimens. It is prone to the same types of artifacts as PCR amplification of low templates and it is therefore prudent to analyze specimens in duplicate, beginning with a duplicate whole-genome-amplification step.[118]

Laboratories offering *EGFR* testing should determine the requirements for each type of specimen they may encounter (eg, fluids, fresh tissues, fixed tissues). All specimens from a patient should be considered for testing, and the choice of which specimen to test should be made by a pathologist familiar with the molecular testing to be performed, based primarily upon tumor content and likely DNA quality as influenced by fixatives and other solutions used in histology.

If no adequate specimen is available and a new specimen needs to be procured, then the decision regarding which specimen to obtain is a complex one. Factors in this decision include the patient's overall health, accessibility of lesions, radiographic appearance of lesions, and ability to schedule procedures promptly. The pathology factors that contribute to this decision include the minimum specimen requirements for each sample type, as well as the relative likelihood of each sample type meeting these requirements. As such, it is important for the molecular pathologist to monitor the percentage of specimens that are being rejected as inadequate for each specimen type and to provide consultation to the oncology care team to assist in obtaining the specimen type that is most likely to yield a diagnostic result. Although not a guideline recommendation, some pathology departments may send a cytopathologist or cytotechnologist to provide immediate consultation in the clinic or interventional radiology suite regarding adequacy of specimens obtained by needle biopsy procedures.

5.2: Expert Consensus Opinion.—Each laboratory should establish the minimum proportion and number of cancer cells needed for mutation detection during validation.

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The minimum percentage tumor cellularity requirements will depend on the methodology being used for analysis. In general, a minimum mutated allele frequency of 25% (50% cancer cell frequency, assuming heterozygosity and disomy) is required for unmodified Sanger sequencing, although this is a generalization and each laboratory must determine its sensitivity cutoff for each assay.[119] Several more sensitive mutation detection techniques have sensitivities in the range of 10% down to 1% or even lower.[119–122] These are general approximations, however, and each laboratory must determine the minimum amount of tumor cells needed for analysis (analytic sensitivity) during validation, for each specimen type that will be accepted by the laboratory (fixed tissue, fresh/frozen tissue, fluid) and for each analyte measured. Ideally, these studies are performed with patient specimens, although cell lines may be necessary if appropriate patient specimens are not available. The use of plasmids is not recommended owing to an increased risk for intralaboratory contamination similar to that of PCR products.

Importantly, the association between mutated allele content and cancer cell content is significantly affected by genomic copy number changes. *EGFR*-mutated alleles are often amplified.[123,124] While it might be ideal to determine the *EGFR* copy numbers for the validation specimens (ie, by FISH) and use disomic tumors for sensitivity assessment, this may not be practical for most laboratories, in which case the sensitivity studies should be performed with more than 1 specimen, to control for the variation in *EGFR* copies between tumors or cell lines.

In addition to establishing the analytic sensitivity of the *EGFR* testing method during validation, the performance of the assay also needs to be monitored in an ongoing fashion once clinical testing is initiated. Accordingly, a low-positive control specimen (near the lower limit of tumor content of specimens accepted by the laboratory) should be tested in each clinical assay run.

5.3: Expert Consensus Opinion.—A pathologist should assess the tumor content of each specimen and either perform, or guide a trained technologist to perform, microdissection for tumor cell enrichment as needed.

Appropriate assessment of the specimen is critical for accurate results and preventing false-negative results and assay failures. A representative stained slide must be reviewed by a pathologist to determine and document the cellular content and tumor purity in the focus of tissue to be tested. An ideal specimen would have not only a very high proportion of malignant cells relative to the admixed nonneoplastic cells, but also a minimal amount of substances that may inhibit amplification, such as mucin and necrotic tissue. In general, assessment of tumor content and purity is more accurate in fixed sections than in frozen sections, and this has prompted some laboratories to designate FFPE tumor material as the preferred specimen type.

Estimates of tumor content from hematoxylin-eosin–stained sections vary considerably between pathologists.[125] Establishing gold standard calibration specimens through formal cell counting of selected areas can be used to train pathologists and assess their accuracy and precision. In the absence of a true standard, consensus opinion can be used to assess tumor content in a specimen near the threshold for acceptability.

Often, the entire tissue section may have a suboptimal proportion of tumor for analysis, but a subregion within the section may be more suitable, and an enrichment strategy must be used to isolate cells from the more concentrated area only. In this event, the tumor enrichment can be performed directly by the pathologist or by a trained technologist under guidance by the pathologist, in which case, the ideal region should be demarcated on the slide by a pathologist during their hematoxylin-eosin review of the case, with documentation of tumor content, and used as a reference for the tumor enrichment procedure. Tumor enrichment procedures should also be assessed during test validation.

Dissection of areas rich in tumor cells from surrounding normal tissue is the typical method for enriching a specimen for malignant DNA content. The main benefit of dissection is the production of relatively pure specimens of a morphologically confirmed cell population. Dissection methods include gross macrodissection or coring of an area out of tissue or a paraffin block, microdissection from glass slides, with or without visualization under a microscope, and flow cytometric sorting or laser capture microdissection (LCM) techniques to enrich the specimen cell by cell. Manual dissection is quick, relatively simple and requires inexpensive equipment such as a standard or inverted microscope. As different laboratories will have different resources available for this step, any method can be adopted for tumor enrichment, as long as the cellularity requirements for the assay are established accordingly.

Laser capture microdissection is a time-consuming and labor-intensive method that requires expensive equipment. Although the actual microdissection is relatively simple, personnel must be specially trained to use the equipment. Another disadvantage of LCM is that DNA yield is often low. The main advantage of this technique is that a pure cell population with minimal normal tissue contaminants can be acquired. Small specimens, such as endobronchial or needle biopsies requiring LCM to isolate tumor cells, showed a high failure rate (53%–66%) by DNA direct sequencing in contrast to larger resection or excisional biopsy specimens (24%).[126] In addition, LCM specimens typically have low DNA content, which can lead to assay imprecision and PCR artifacts that can be misinterpreted as unusual or novel mutations[96,127] (see "Question 6" discussion below).

Question 6: How Should *EGFR* Testing Be Performed?

6.1: Recommendation.—Laboratories may use any validated *EGFR* testing method with sufficient performance characteristics.

Evidence Grade: B.—Multiple test platforms are acceptable for *EGFR* mutation testing, and this guideline does not recommend any individual method(s) to the exclusion of others. Rather, we recommend that tests offered for clinical care meet specified minimal performance characteristics, and require that laboratories establish acceptable performance during validation and maintain acceptable quality during production. The performance characteristics that are most relevant for this discussion are sensitivity and specificity. Other test characteristics of particular importance are TAT and spectrum of mutations detected.

A variety of methods are used to detect *EGFR* mutations, including Sanger sequencing with and without mutated allele enrichment, the amplification refractory mutation system, length analysis, restriction fragment length polymorphism, real-time PCR, high-resolution melting curve analysis, single-base extension genotyping (including mass spectrometry–based genotyping), and denaturing high-

Table 11. Comparison of Sanger Sequencing to Other Methods for the Detection of *EGFR* Mutations[a]								
					Incidence of *EGFR* Mutations, %			
Sanger Sequencing Versus Other Methods	Concordance of Methods, %	n (N)	Seq−/ Other+	Seq+/ Other−	Sanger Sequencing	Other Method	RR (95% CI)	P Value
PCR-based mutation detection								
Allele-specific PCR/ARMS[130]	73	1 (83)	18	4	16	33	0.48 (0.27–0.87)	.01
Real-time PCR[119,304]	97	2 (102)	2	1	26	27	0.94 (0.60–1.46)	.78
Cycleave PCR[131,b]	95	1 (195)	1	8	40	36	1.10 (0.85–1.41)	.47
Post-PCR mutation detection								
Capillary electrophoresis[296,304]	98	2 (61)	1	0	16	18	0.91 (0.42–2.01)	.82
Restriction fragment length polymorphism[133]	99	1 (109)	1	0	33	34	0.97 (0.67–1.41)	.89
INVADER[305,c]	86	1 (42)	5	1	43	52	0.82 (0.52–1.29)	.39
Pyrosequencing[159,306]	96	3 (140)	6	0	16	20	0.78 (0.49–1.25)	.30
Mutation scanning								
Denaturing HPLC[139,307]	94	2 (196)	12	0	20	27	0.66 (0.27–1.63)	.37
Single-stranded conformational polymorphism[45]	98	1 (375)	8	0	8	10	0.79 (0.51–1.25)	.32
High-resolution melting analysis[121,135,308]	83	3 (321)	54	0	36	53	0.70 (0.46–1.06)	.09
Loop-hybrid mobility shift assay[309]	100	1 (43)	0	0	26	26	1.00 (0.49–2.06)	>.99
Mutant enrichment								
Peptide nucleic acid/locked nucleic acid amplification[301,310]	96	2 (150)	4	2	11	12	0.91 (0.49–1.67)	.75
COLD PCR[138]	100	1 (126)	0	0	10	10	1.00 (0.48–2.07)	>.99
Smart Amplification Process[120,132,301]	86	4 (220)	30	0	20	34	0.58 (0.44–0.77)	<.001

Abbreviations: ARMS, amplification refractory mutation system; CI, confidence interval; COLD, coamplification at low denaturation temperature; HPLC, high-performance liquid chromatography; n, number of studies; N, number of patients; PCR, polymerase chain reaction; RR, relative risk, Mantel-Haenszel random effects model, (95% CI); Seq−, negative by Sanger sequencing; Seq+, positive by Sanger sequencing.

[a] The variation in *EGFR* mutation rate between rows may reflect studies performed in different patient populations (Asian versus non-Asian). No statistical comparisons were performed between rows.

[b] Cycleave (Takara Bio, Otsu, Shiga, Japan).

[c] Invader (Hologic, Madison, Wisconsin).

performance liquid chromatography, each having different advantages and disadvantages[27,36,57,115,120,121,128–156] (Table 11). At the time of writing, published data on the application of massively parallel sequencing to the detection of *EGFR* mutations were still quite limited but this technology is expected to soon become more widespread.[157–159]

6.2: 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.

The historical reference method, bidirectional sequence determination by the Sanger method with fluorescence-tagged dideoxy terminators, is usually able to precisely detect mutated sequences when they constitute approximately 25% of the total DNA (potentially even lower for specific nucleotide changes), which corresponds to a minimum tumor content of approximately 50% for a heterozygous mutation with no polysomy or amplification.[160] Although this method, with this level of performance, was the initial method used to establish the value of the test for predicting response of tumors to erlotinib and gefitinib, many specimens fall short of this tumor content.[47,161,162] As a consequence, laboratories exclusively using this method would have to reject a significant proportion of specimens as inadequate for testing.

All laboratories must establish the lower sensitivity of their methodology during test validation. Any tumor enrichment procedures, such as manual microdissection, must be included as part of the validation. The sensitivity limit (also called *analytic sensitivity*) should be defined as the lowest concentration of tumor cells in which a mutation is detected with 100% precision in replicates repeated both within run and between run.[107] As stated earlier, because of possible variation in the genomic copy number of mutated *EGFR* alleles between tumors, this cutoff should be validated on more than 1 tumor specimen. Wild-type results above this limit can be reported confidently as negative.

Although the published evidence supports a recommendation that any method must be at least as sensitive as the Sanger sequencing technique that first established the clinical value of mutation detection, our expert consensus opinion is that more sensitive methods should be available, because of the many patients who only have had samples with low tumor content.[115,163] In our opinion, an ideal test should be able to detect mutations in specimens with as little as 10% cancer cells. In particular, laboratories that use Sanger sequencing are strongly encouraged to use a mutated allele–enriching strategy, such as locked nucleic acid or peptide nucleic acid clamps, coamplification at lower denaturation temperature PCR, or enzymatic digestion of wild-type sequences, to enhance the sensitivity for the common critical mutations in exon 19 (747_750 LREA deletions), 20 (T790M), and 21 (L858R).[70,156,164] If a laboratory cannot offer a more sensitive method than

unmodified Sanger sequencing, then that laboratory must communicate its limitation clearly to its clinicians and make available referral to another laboratory for more sensitive testing for specimens with lower tumor content.

Although analytic sensitivity is very important for expanding testing to patient specimens with low tumor content or purity, ultrasensitive molecular assays (defined here as an analytic sensitivity of below 1%) can be problematic. In specimens with high tumor content, if an ultrasensitive molecular assay finding is positive while an assay finding of conventional sensitivity is negative, the result is either interpreted as a possible false positive due to mispriming or low cross-contamination, or as a true positive reflecting a very small mutated subclone. Thus, there is a risk of losing specificity with regard to predicting response to targeted therapy. Some studies using such methods have found novel, possibly artifactual mutations, or failed to show a relationship between classic *EGFR* mutations and treatment response.[165] Finally, technical artifacts may be seen with ultrasensitive methods that require experience and caution in interpretation.

Accordingly, specificity of ultrasensitive methods must receive additional attention during validation. Multiple negative lung cancer specimens should be tested, as should no-template controls. Given the huge collective testing experience accumulated and reflected in online databases such as the Catalogue of Somatic Mutations in Cancer (COSMIC), novel *EGFR* mutations are rare today, and the discovery of novel mutations is cause for careful scrutiny and reevaluation of methodology. Artifacts should be presented in the laboratory's standard test operating procedure manual to assist in interpretation, and laboratories should clearly communicate with clinicians when results are unclear or uncertain.

Although not a guideline recommendation, a suggested approach is to offer a 2-tiered testing strategy, in which both standard-sensitivity and high-sensitivity testing is performed and reported. An example would be Sanger sequencing with, and without, mutated enrichment by locked nucleic acid/peptide nucleic acid. The report would then indicate whether a mutation was detected only with the ultrasensitive method or also by the less sensitive method, which could then be correlated with the morphologic estimate of tumor content.

Each laboratory should have a dialogue with its clinicians to understand the TAT needs within its health care setting. If results are needed within a few days, then multistep testing such as Sanger sequencing may be less desirable than a 1-step procedure such as allele-specific PCR. Similarly, if a sample has borderline tumor content for the method in question and TAT is critical, it may be better to go directly to a more sensitive method or have the patient undergo another sampling procedure, rather than attempt an analysis that may end up with an inconclusive interpretation.

6.3: Expert Consensus Opinion.—Clinical *EGFR* mutation testing should be able to detect all individual mutations that have been reported with a frequency of at least 1% of *EGFR*-mutated lung adenocarcinomas.

Another critical issue in method selection is the spectrum of mutations tested. This is not a concern for Sanger sequencing, which provides information on mutations throughout the exons sequenced (usually exons 18 to 21), but it is an important consideration in the selection or design of mutation-specific assays. The 2 most common mutations in *EGFR*, the short in-frame deletions in exon 19

and the L858R point mutation in exon 21, account for about 90% of all *EGFR* mutations, and these are the mutations with by far the most extensive data on EGFR TKI response rates (Table 12). Nonetheless, response data are accumulating for other less common mutations and therefore, our consensus opinion is that limiting testing to the 2 major mutations is no longer considered acceptable.

Routine *EGFR* assays for *EGFR* exon 19 deletions should be designed to detect not just the common 15-bp and 18-bp deletions, but also the less common 9-, 12-, 24-, and 27-bp deletions, as well as the uncommon 15-bp and 18-bp insertions.[166] *EGFR* exon 18 should be analyzed for E709 and G719 mutations; exon 20 for S768, T790M, and insertions; and exon 21 for L858R, T854, and L861Q mutations. Pretreatment T790M mutations and most exon 20 insertions are associated with lack of response to first-generation EGFR TKIs, and this should be communicated in the report.[137, 167–169]

Given the accumulated experience with *EGFR* mutations, the detection of "novel" mutations or mutations only reported very rarely should be viewed with great caution and should prompt replicate assays on new DNA extracts to rule out artifactual mutations due to formalin fixation, PCR errors, or whole-genome-amplification errors (if used). Nevertheless, rare variants and rare mutations will occur, and such findings should not be automatically discarded as errors.

The recommendation for broad mutation detection may conflict with the recommendation for TAT. In these instances, laboratories may consider offering 2 assays: a rapid assay for the most common mutations, which can be reported within a few days in cases of clinical urgency, and a more comprehensive follow-up assay to detect the remaining mutations, which may take longer to report.

6.4: Recommendation.—Immunohistochemistry for total EGFR is not recommended for selection of EGFR TKI therapy.

Evidence Grade: A.—Interest in IHC-based testing is driven by the fact that it is a technology available to essentially all pathology departments, and it can be performed for specimens where the number or proportion of tumor cells poses challenges for molecular tests based on bulk DNA extraction from tissue. There are 3 main types of EGFR IHC: IHC for total EGFR, IHC for phosphorylated EGFR, and IHC for mutated forms of EGFR.

Immunohistochemistry for total EGFR is not an acceptable test for EGFR TKI treatment selection because it has been shown to correlate poorly or not at all with the presence of *EGFR* mutations.[123,170,171] However, in other settings, the role of IHC for total EGFR may need to be reassessed in the future if the anti-EGFR monoclonal antibody cetuximab becomes a therapeutic option in mutation-negative, EGFR-overexpressing patients.[172]

Experience with IHC for phosphorylated EGFR is still limited and concerns remain regarding the stability of phosphorylation status in routinely handled pathology material. Use of such IHC assays for EGFR TKI treatment selection would be premature at this point.

The third type of IHC assay that has been evaluated uses commercially available mutation-specific rabbit monoclonal antibodies directed against the most common mutated forms of EGFR: the 15-bp/5-amino-acid deletion (E746_A750del) in exon 19 and the L858R point mutation in exon 21.[173] In several independent studies,[173–175] IHC with the EGFR L858R mutated antibody has confirmed excellent

Table 12. *EGFR* mutations accounting individually for at least 1% of all *EGFR* mutations				
***EGFR* exon**	***EGFR* codon**	**Mutations[a]** **(amino acid)**	**Nucleotide** **substitutions**	**Approximate % of all** ***EGFR* mutations**
18	E709	E709K	c.2125G>A	1%
		E709A	c.2126A>C	
		E709G	c.2126A>G	
		E709V	c.2126A>T	
		E709D	c.2127A>C, c.2127A>T	
		E709Q	c.2125G>C	
	G719	G719S	c.2155G>A	2–5%
		G719A	c.2156G>C	
		G719C	c.2155G>T	
		G719D	c.2156G>A	
19	K739, I740, P741, V742, A743, I744	Insertions 18 bp ins		1%
	E746, L747, R748, E749, A750, T751, S752, P753	Deletions 15bp del 18bp del 9 bp del 24bp del 12bp del		45%
20	A763, A767, S768, V769, D770, N771, P772, H773, V774	Insertions 3 bp ins 6 bp ins 9 bp ins 12 bp ins		5–10%
	S768	S768I	c.2303G>T	1–2%
	T790	T790M	c.2369C>T	2%[b]
21	L858	L858R	c.2573T>G	40%
		L858M	c.2572C>A (rare)	
	L861	L861Q	c.2582T>A,	2–5%
		L861R	c.2582T>G	

Abbreviations: del, deletion; ins, insertion.

[a] For each codon or region, missense mutations are listed in decreasing order of frequency; for less common missense mutations, this order is approximate. For insertions or deletions, the codons most commonly involved are simply listed by cDNA order only.

[b] Up to 50% of T790M mutations detected in pre-treatment tumor samples may be germline mutations.

Notes: All mutations listed are generally associated with sensitivity to EGFR TKIs except T790M and some exon 20 insertions. G719 and L861 mutations are considered sensitive but somewhat less so than the major exon 19 and 21 mutations. Exon 20 insertions are variable in exact position and structure and EGFR TKI response data remain very limited for some types of insertions. Mutations at E709 and S768 often occur in combination with another of the listed mutations. Data derived from Chen et al[110]; He et al[166]; Oxnard et al[210]; Wu et al[278]; Bamford et al[311]; De Pas et al[312]; and Murray et al.[313]

sensitivity and specificity relative to mutation testing. The EGFR exon 19 mutated–specific antibody showed excellent sensitivity and specificity for cases with the 15-bp deletion in exon 19 but reduced sensitivity for exon 19 deletions of other sizes.[174,176–178] If scoring cutoffs are set stringently to ensure a high positive predictive value, IHC with *EGFR* mutation–specific antibodies could be used as an initial screen to identify most patients who are candidates for EGFR inhibitors; however, for all specimens negative with these 2 mutation-specific monoclonal antibodies, that is, most samples overall, molecular testing is still needed. In the absence of an algorithm that includes molecular testing of negative cases, mutated EGFR allele-specific IHC is currently too insensitive to be used as a stand-alone assay for EGFR TKI treatment selection. However, for patients with only a low cellularity specimen deemed inadequate for DNA analysis, this IHC may be the best option available, but there is still no prospective clinical experience with this special situation.[179] Overall, the body of published data is insufficient to make an evidence-based recommendation regarding the use of *EGFR* mutation–specific IHC at this time. Laboratories that plan to use these antibodies clinically should validate their use against a valid molecular assay, communicate their clinical performance characteristics (eg, sensitivity, specificity) to their clinicians, and make available

referral to another molecular laboratory for molecular testing to exclude mutations in IHC-negative tumors (if not available in-house).

6.5: Recommendation.—*EGFR* copy number analysis (ie, FISH or chromogenic in situ hybridization) is not recommended for selection of EGFR TKI therapy.

Evidence Grade: B.—Increased *EGFR* gene copy number (polysomy or amplification) is observed in about 40% of cases, with a range of 8% to 66%.[54,129,180–182] Across multiple studies, the EGFR TKI response rates for patients with *EGFR* polysomy/amplification is 30% (Table 13), consistently well below the mean response rate seen for patients with *EGFR* mutations (68%) (Table 1). Amplification of the mutated *EGFR* allele is common and drives a strong statistical association of *EGFR* polysomy/amplification with *EGFR* mutation.[170,171] The correlation of *EGFR* polysomy/amplification with EGFR TKI response is secondary to this strong association with *EGFR* mutation, and in the cases where *EGFR* mutation and copy number are discrepant, the mutation status is better associated with EGFR TKI response.[170,171] In studies with data on both, EGFR TKI response rates for cases with *EGFR* polysomy/amplification and wild-type *EGFR* sequence are very low, essentially in the range of *EGFR*-nonmutated cases without increased *EGFR* copy number.[170,171] In contrast, *EGFR*-mutated cases

Table 13. Different Outcomes of Tyrosine Kinase Inhibitor Therapy for Patients Tested by *EGFR* Fluorescence In Situ Hybridization (FISH)[a]

Outcome	Mean ± SD		Percentage		n (N)	Risk Ratio M-H, Random (95% CI)	P Value
	EGFR FISH+ (High Copy No.)	*EGFR* FISH− (Low Copy No.)	*EGFR* FISH+ (High Copy No.)	*EGFR* FISH− (Low Copy No.)			
Response rate, %[b]			30	9	11 (861)	2.69 (1.86–3.90)	<.001
Disease control rate, %[c]			47	24	5 (438)	2.09 (1.63–2.69)	<.001
Overall survival at 1 y, %[d]			68	37	1 (183)	1.83 (1.37–2.44)	<.001
Time to progression/ progression-free survival, mo[e]	4.4 ± 2.8	2.9 ± 0.7			7 (638)	4.06 (1.97–6.16)	<.001
Median survival time, mo[f]	10.3 ± 3.4	8.7 ± 2.4			8 (778)	1.54 (−1.83–4.91)	.37

Abbreviations: CI, confidence interval; M-H, Mantel-Haenszel; n, number of studies; N, number of patients; Random, random effects model; SD, standard deviation.

[a] FISH scored according to the criteria of Cappuzzo et al.[251]
[b] References 28, 180, 191, 193, 195, 197, 198, 251, 314–316.
[c] References 191, 193, 195, 279, 314.
[d] Reference 193.
[e] References 28, 185, 191, 193, 251, 316, 317.
[f] References 28, 180, 185, 191, 193, 197, 316, 317.

lacking amplification show response rates comparable to *EGFR*-mutated cases overall.[170,171] Finally, comparisons of *EGFR* amplification and *EGFR* mutation as predictors of response and clinical outcome in earlier clinical trials of TKI versus placebo in the second-line (or greater) setting were hampered by the small size of the subsets of patients with available molecular data, in particular mutation data.[4,6,163] The IPASS study, a large phase III randomized clinical trial with data on both *EGFR* mutation and amplification, showed that EGFR TKI treatment selection based on mutation status leads to better clinical outcomes in the first-line setting than selection based on *EGFR* gene copy number, and subsequently published large phase III randomized controlled trials of TKI treatment response in the first-line setting used mutation analysis.[7,26,90,91,183–185] Thus, EGFR copy number testing, whether by FISH or chromogenic in situ hybridization, is less predictive than mutation testing and should not be used as a method for EGFR TKI treatment selection.[170,171] More study is needed to determine if mutated allele copy number is a modifier of benefit in the setting of *EGFR* mutation.

Question 7: What Is the Role of *KRAS* Analysis in Selecting Patients for Targeted Therapy With EGFR TKIs?

7.1: Recommendation.—*KRAS* mutation testing is not recommended as a sole determinant of EGFR TKI therapy.

Evidence Grade: B.—The most common (~30%) oncogene mutated in lung adenocarcinomas is *KRAS*. The frequency of *KRAS* mutations varies between ethnic groups; they are less frequent in Asians (5%–10%) when compared with individuals of white European or white American ancestry (25%–35%) and African ancestry (15%–25%).[25,186–188] Approximately 90% to 95% of patients with *KRAS*-mutated lung adenocarcinomas have a history of tobacco use and therefore, regional differences in the proportions of *KRAS*-mutated lung adenocarcinomas may also reflect variations in smoking prevalence, but *KRAS* mutations can also be observed in approximately 5% of lung cancer patients who never smoked tobacco.[189,190] The substantial cumulative clinical experience and published data have shown that *EGFR* and *KRAS* mutations are mutually exclusive.

Several studies compared response rates, PFS, and overall survival in patients with *KRAS*-mutated lung cancer treated with EGFR TKI, and 2 meta-analyses addressing these questions have been reported.[28,180,191–201] The association between *KRAS* mutations and a lack of response to EGFR TKI was based on retrospective reviews of EGFR TKI in the second- and third-line setting.[28,180,191–193,195–202] Objective response to EGFR TKI can be seen in 0% to 3% of patients with *KRAS* mutations and 26% of patients with *KRAS* wild type.[28,180,191–193,195–202]

In part because of the ease of testing for *KRAS* codon 12 and 13 mutations and its widespread availability owing to indications in metastatic colorectal cancer, testing for *KRAS* mutations as a negative predictor of response to EGFR TKI has become part of molecular diagnostic algorithms for lung adenocarcinoma in many centers. However, with more recent data showing that *EGFR* wild-type tumors have less favorable outcomes if they are treated with EGFR TKI than if they are treated with conventional platinum-based chemotherapy, the decision to treat with an EGFR TKI can no longer be made without an *EGFR* result, and the role of *KRAS* testing in this context has diminished.[26,90,91] The significance of *KRAS* mutational analysis may become increasingly important with the further development of new therapies targeting downstream RAS pathways such as PI3K/AKT/mTOR and RAS/RAF/MEK, but at this time, the absence of a *KRAS* mutation does not add clinically useful information to the *EGFR* mutation result and should not be used as a determinant of EGFR TKI therapy. However, because *KRAS* and *EGFR* mutations are mutually exclusive, a rapid and inexpensive *KRAS* assay may be performed initially to exclude *KRAS*-mutated tumors from *EGFR* mutation testing as part of an algorithm designed to maximize testing efficiency, provided that the sample is sufficient to perform the *KRAS* test without sacrificing *EGFR* and *ALK* testing, and that the totality of clinically relevant molecular results can be obtained within the target TAT.

There are other clinical settings in which *KRAS* mutation has been examined as a biomarker that are not directly related to the present guidelines. In patients without *EGFR* mutations for whom chemotherapy fails, erlotinib may be administered as a second-line agent. In these patients, *KRAS* mutation may presage a poorer outcome, although the evidence for this from the BR21 trial is limited by small sample size and lack of statistical significance.[170] *KRAS* mutation is also not predictive of benefit (or lack of benefit) from cetuximab therapy in lung cancer patients.[172,203]

Question 8: What Additional Testing Considerations Are Important in the Setting of Secondary or Acquired EGFR TKI Resistance?

8.1: Recommendation.—If a laboratory performs testing on specimens from patients with acquired resistance (AR) to EGFR kinase inhibitors, such tests should be able to detect the secondary *EGFR* T790M mutation in as few as 5% of cells.

Evidence Grade: B.—Although clinical and radiographic responses to EGFR TKIs in patients with "sensitizing" *EGFR* mutations are significant in approximately 70% of cases, these patients almost invariably experience recurrence or progression while on treatment after a median of 8 to 16 months, a clinical phenomenon termed *acquired resistance*.[137,204–206] Clinical management implications of AR mechanisms are still evolving without established treatment guidelines; additional tumor material may be procured in this clinical setting in the course of diagnostic or therapeutic procedures, such as biopsies, to confirm recurrence or metastasis, or management of malignant effusions. In the event that tumor specimens from AR patients are tested, the following suggestions are included in this guideline.

The most common mechanism of AR involves the emergence of an additional *EGFR* tyrosine kinase domain mutation, T790M, caused by a single base substitution, C to T, at nucleotide 2369; this mutation is found as a second mutation on the *EGFR* allele harboring the initial "sensitizing" *EGFR* mutation.[137,168,207] Because AR is, within the tumor cell population, a subclonal process, that is, it is driven by the selection and outgrowth of a subclone of tumor cells that harbor T790M that confers a survival advantage in the presence of EGFR TKI, the technical sensitivity of the detection method is even more critical than in the testing for baseline, sensitizing *EGFR* mutations that are present in every tumor cell. Initial reports found this mutation in approximately 50% of tumors at the time of treatment failure.[137,168] However, because T790M is often not present in every tumor cell, conventional Sanger sequencing, even with microdissection, is considered insufficient for this testing.[207] More recent studies based on higher sensitivity approaches place the prevalence of T790M in AR samples in the 60% to 70% range.[208] In vitro studies have shown that cell population–level EGFR TKI resistance becomes detectable in the presence of as little as 5% T790M-bearing cells and, in the absence of further clinical data, our consensus opinion is that assays for T790M should have sensitivity to detect mutations in 5% of cells.[209] This means that unmodified Sanger sequencing alone is insufficient, and laboratories using Sanger sequencing should consider a mutation-enriching strategy such as peptide nucleic acid/locked nucleic acid clamps, or have a more sensitive assay (eg, allele-specific PCR) that targets the T790M mutation in the setting of AR.

Most studies have only rarely detected T790M in pretreatment samples.[210] When it is detected in the pretreatment setting, it should be confirmed as either somatic or germline by testing of normal DNA from the patient. Germline T790M mutation has been associated with familial lung cancer, and therefore its detection should trigger evaluation of the family history and genetic counseling, keeping in mind that risk estimates and screening recommendations for unaffected T790M carriers remain to be determined.[210, 211]

Other rare second-site mutations in the EGFR tyrosine kinase domain have been described in AR specimens, including L747S, D761Y, and T854A, but owing to their relatively low prevalence, there is not much clinical experience with these and insufficient data have been published to formulate an evidence-based recommendation.[212]

A less common mechanism of EGFR TKI resistance is amplification of another receptor tyrosine kinase, most often *MET* or *ERBB2*. In initial reports, *MET* amplification was reported in up to 20% of AR cases, with a portion of these also harboring the *EGFR* T790M mutation, but more recent studies suggest this number is closer to 10%.[71,204,213,214] However, there is currently a lack of a precise definition of clinically significant *MET* amplification in this setting and more research is needed before guidelines can be formulated. More recently, *ERBB2* amplification has been reported in another subset of AR cases.[318]

Several clinical trials aimed at overcoming these distinct mechanisms of AR are underway and it is therefore likely that the further management of these patients will soon require determining T790M status and amplification of other receptor tyrosine kinases. T790M status may also become important in determining whether first-line EGFR TKIs should be continued in patients with AR. Recent data suggest that AR patients with the T790M mutation can derive continued clinical benefit from the first-line EGFR TKI.[209,213]

Interestingly, SCLC histology and associated "SCLC-type" radiosensitivity and chemosensitivity have been observed in some AR cases, further supporting the notion that biopsy of recurrent tumor can be clinically valuable in AR.[213]

SECTION III: HOW SHOULD *ALK* TESTING BE PERFORMED?

Question 9: What methods should be used for *ALK* testing?

9.1: Recommendation.—Laboratories should use an *ALK* FISH assay using dual-labeled break-apart probes for selecting patients for ALK TKI therapy; ALK immunohistochemistry, if carefully validated, may be considered as a screening methodology to select specimens for *ALK* FISH testing.

Evidence Grade: B.—The genetic alteration of *ALK* in lung adenocarcinoma is due to chromosomal rearrangement. The most common of these rearrangements involves a pericentric inversion on the short arm of chromosome 2, inv(2)(p21p23), which creates a fusion gene encoding the amino-terminal portion of *EML4* (2p21) and the intracellular region of *ALK* (2p23), genes that are normally approximately 13 Mb apart.[8,215] Although the *EML4-ALK* fusion is the most common, other less common variant fusions have been reported, including translocations with other chromosomes (*KIF5B-ALK*, *TFG-ALK*).[215,216] The *NPM-ALK* translocation that has been well characterized in anaplastic large cell lymphoma (for which the gene was named) has not been reported in lung cancer.

FISH was the methodology used in the initial studies that demonstrated improved clinical response of patients with *ALK*-rearranged tumors to treatment with crizotinib, a targeted ALK TKI.[15,17,217] Although FISH assays have been developed by using both break-apart and fusion strategies, the break-apart assay design has shown the best association with clinical outcome.[12,218] A commercial assay (Abbott Molecular Probes, Abbott Park, Illinois) is available that contains a SpectrumOrange-labeled 300-kb probe on the
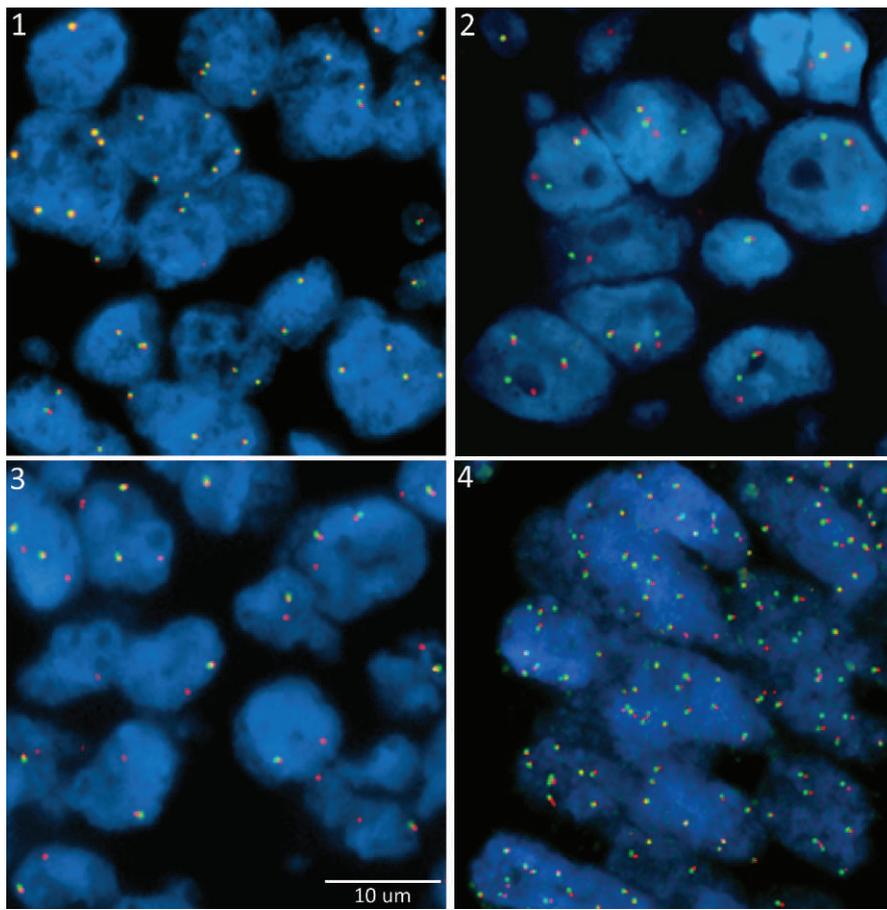
Figure 1. *Negative for* ALK *rearrangement (original magnification* ×1000*).*

Figure 2. *Positive for* ALK *rearrangement (split 3′* ALK-5′ ALK*) (original magnification* ×1000*).*

Figure 3. *Positive for* ALK *rearrangement (single 3′* ALK*) (original magnification* ×1000*).*

Figure 4. *Negative for* ALK *rearrangement with* ALK *high copy number (original magnification* ×1000*).*

telomeric 3′ side of *ALK* and a SpectrumGreen-labeled 442-kb probe on the centromeric 5′ side. With this probe set, the wild-type configuration appears as a fused yellow signal, while *ALK* rearrangement is seen as distinct and separated orange and green signals (Figures 1 through 4). In the USA, FDA has approved this commercial assay as a "companion diagnostic" to select patients to receive an FDA-approved ALK TKI. The published evidence indicates that this FISH assay is an acceptable means of selecting patients for treatment with an ALK TKI.

Whether or not the FDA-approved commercial assay is used, for accurate and precise results, the probe set used for clinical testing should be characterized in detail and the signal configurations and cutoff values for detecting *ALK* rearrangement should show reproducible performance with normal controls and known abnormal patient specimens and cell lines. If another set of probes or assay design is used, validation studies should demonstrate comparable or superior performance when compared to the commercial probes with regard to signal intensity, magnitude of signal splitting in positive cases, analytic precision, clinical sensitivity, and clinical specificity in accordance with published standards.[219,220] For laboratories that elect to use laboratory-developed probes for *ALK* FISH testing, attention should also be given to batch variability of clones, DNA-labeling enzymes, and other reagents. Moreover, any laboratory-developed tests should retain the ability to detect variant fusions of *ALK* with partners other than *EML4*.

IHC-mediated identification of lung adenocarcinomas with overexpression of ALK has been investigated as a simpler, quicker, and cheaper alternative to FISH-based identification of *ALK* rearrangements. However, IHC studies using the anti-ALK1 antibody typically used for anaplastic large cell lymphoma (mouse monoclonal anti-human CD246, clone ALK1) have been disappointing, likely due to the low expression level of the fusion protein in *ALK*-rearranged lung adenocarcinomas in comparison to anaplastic large cell lymphoma.[11,79,216,221–223] A substantial proportion of *ALK*-rearranged lung adenocarcinomas are not identified by the ALK1 antibody, using standard techniques, and thus it is not reliable for *ALK* rearrangement screening in this setting.

To improve the sensitivity of detection of the *ALK* rearrangement by IHC with the commercially available ALK1 antibody, some groups have developed alternative methods, including an intercalated antibody-enhanced polymer method, tyramide amplification, and an enhanced polymer-based detection system, with subsequent triaging of equivocal cases for *ALK* FISH.[15,216] The current data are still limited and more studies need to be published to recommend this approach with this antibody.

A different antibody to ALK (mouse monoclonal, clone 5A4) has been reported to have excellent sensitivity and specificity relative to *ALK* FISH results, at least for strong IHC staining and for negative or weak IHC staining, while intermediate IHC staining results were poorly predictive of *ALK* rearrangement status.[221] High sensitivity, specificity, and reproducibility, as compared to FISH, have been shown with 2 rabbit monoclonal anti-human ALK antibodies (clones D5F3 and D9E4), and the former, ALK antibody D5F3, has just recently become commercially available.[221]

Based on promising recent results with these newer monoclonal antibodies, IHC assays hold the potential to facilitate the routine identification of *ALK*-rearranged lung adenocarcinoma. Where sensitive IHC assays are available, our consensus opinion is that a properly validated IHC method may be used as a screening modality, and that tumors that fail to demonstrate ALK immunoreactivity with a sensitive IHC method need not be tested for *ALK* rearrangement by FISH. The use of sensitive ALK IHC assays as screening tests has been adopted in some countries. Our opinion is that tumors that are positive for ALK IHC, either weakly or strongly, should still be referred to FISH for confirmation of a rearrangement. At this time, there are insufficient data available to develop a specific recommendation on the use of ALK IHC as a sole determinant of ALK TKI therapy.

9.2: Recommendation.—RT-PCR is not recommended as an alternative to FISH for selecting patients for ALK inhibitor therapy.

Evidence Grade: B.—RT-PCR is not currently recommended as a first-line diagnostic method for determining *ALK* fusion status because of concerns for a higher failure rate of an RNA-based assay in routine FFPE pathology material, and the risk of false negatives, owing to variability in the *EML4-ALK* fusion structure and the existence of other *ALK* fusion partners. To date, there have been at least 13 molecular variants of *EML4-ALK* reported, representing chimeric transcripts fusing *EML4* exons 2, 6, 13, 14, 15, 17, 18, or 20 to *ALK* exon 20 or immediately upstream within intron 19 of *ALK*.[222] Fusion of *ALK* to *TFG* and *KIF5B* has been reported in 1 case each, raising the likelihood that additional variant fusions may exist.[215,216,224] It is possible to design multiplexed RT-PCR assays or to use multiple pairs of primers in separate or sequential reactions to detect the different *EML4-ALK* variants but multiplexed assays can be difficult to optimize and multiple separate assays may require more RNA than can be regularly extracted from small FFPE samples.

9.3: Expert Consensus Opinion.—A pathologist should be involved in the selection of sections for *ALK* FISH testing, by assessing tumor architecture, cytology, and specimen quality.

Because cells are analyzed individually for evidence of *ALK* rearrangement using a fluorescent microscope, tumor percentage is not as critical for *ALK* FISH testing as it is for *EGFR* mutation testing. For *ALK* FISH, it is important to choose slides or regions of slides in which the tumor cells can be readily distinguished from admixed normal cells under fluorescence, typically through a combination of cytologic and architectural features that can be appreciated without stains or visualization of cytoplasm. In addition, areas should be chosen in which tumor cells are not overlapping one another.

Specimen requirements for *ALK* FISH are generally similar to those for *EGFR* mutation testing: formalin fixation is acceptable, specimens should have enough cancer cells to analyze clearly, and DNA-damaging fixatives or acidic decalcifying agents should be avoided, as should specimens with abundant necrosis. Unlike *EGFR* mutation testing, however, FISH testing can be problematic when performed on alcohol-fixed samples. Another important distinction between *ALK* FISH and *EGFR* mutation analysis is that FISH testing should ideally be performed on recently cut sections, although protocols can be adapted to older slides.

Laboratories may follow the standard operating procedures that have proven to be successful for FISH on formalin-fixed, paraffin-embedded tissue sections in their setting. Attention must be paid to particular steps in the protocol that may affect probe penetration and hybridization to target DNA in order to optimize signal intensities.

Particular attention should be paid during validation to the choice of glass slides used for FISH. Several types of slides are manufactured for specialized applications at the expense of suitability for FISH. For example, some slides designed for tissue microarrays have a heavy coating that generates a fluorescent matrix where tumor cells get embedded and cannot be properly treated for FISH-probe penetration. Other slides are designed for microdissection and do not hold the tissue adequately during pretreatment for FISH.

Modifications in the protease digestion protocol may be required, depending on the size of the tissue, duration and type of fixation, nuclear structure and tissue preservation, and time between sectioning and digestion. This may be particularly valuable with difficult sections, including cytology specimens and samples from bone biopsies. Tissue digestion should be standardized to maintain nuclear morphology. Overdigested chromatin may display artifactual "split signals" that may lead to false-positive findings.

Hybridization and washing steps should be standardized by using established protocols. Use of automated tissue processors and standardized commercial tissue digestion kits can improve consistency and should be considered.

9.4: Expert Consensus Opinion.—A pathologist should participate in the interpretation of *ALK* FISH slides, either by performing the analysis directly or by reviewing the interpretations of cytogeneticists or technologists with specialized training in solid tumor FISH analysis.

The selection of nuclei to analyze is a step that is distinct from the scoring of probe signals. When evaluating the results of FISH, several factors should be kept in mind: the architecture of the tissue, including local variations in neoplastic cell content, fixation, and tumor cellularity within the section; the frequent presence of truncated nuclei; and the complex nature of cytogenetic arrangements such as heterogeneous increases in ploidy and aneusomy often seen in lung cancer. The FISH technologist should work closely with a pathologist who can identify tumor-rich areas. Typically, areas selected for FISH evaluation will be marked on a hematoxylin-eosin–stained slide that is directly parallel to the section used for FISH. Areas of the FFPE section selected for signal scoring should pass rigorous quality criteria as being suitable for FISH analysis. Inclusion of macrophages or other nontumor cells in the analysis will dilute positive break-apart scores and can lead to false-negative results. Experienced scorers who have undergone specific training in FISH in solid tumors should analyze the slides. The scorers should also have had training on the morphologic appearance of lung cancer, and should have easy access to assistance from a pathologist with training in FISH. Laboratories with experienced reviewers may use 1 scorer in cases with clearly negative or positive (>50% of cells) cases and a second scorer for less clear cases; otherwise 2 independent reviewers are recommended.

Interpretation should be performed in areas of the slide with good signal, in which at least 50% of all nuclei are easily analyzable, with minimal background or nuclear fluorescent "noise." The FISH signal intensity should be consistently greater than background intensity in the

regions of the slide chosen for analysis. Areas where the borders of individual nuclei are not clearly identifiable and/or high cell density causes excessive nuclear overlap are easy to misinterpret, and should be avoided. At the same time that the quality of the tissue section is reviewed, the FISH signals should be assessed, looking for areas with bright, distinct signals and low background in which individual nuclei are clearly distinguishable. Signals in a nucleus should in general have the same intensity and the DAPI staining should be uniform.

Importantly, the interpretive criteria of FISH assays for *ALK* rearrangement in lung carcinoma are not necessarily identical to those applied in other neoplastic diseases (eg, anaplastic large cell lymphoma, inflammatory myofibroblastic tumor), even if the identical FISH probe set is used. The most common positive result of a break-apart dual-labeled FISH assay in lung cancer will result in 1 separate orange/red and 1 separate green signal (Figure 4). The native unaltered *ALK* region will remain as a yellow fusion signal but also commonly appears as 2 narrowly split orange/red and green signals. The second most common positive result in lung cancer is loss of the green 5′ probe with a remaining unpaired 3′ orange/red probe, indicating an unbalanced rearrangement. Importantly, proper interpretation of the FDA-approved commercial break-apart assay considers only nuclei with loss of a green signal, or orange/red and green signals that are separated by a gap larger than 2 signal diameters, to be indicative of an *ALK* gene rearrangement. Split signals of lesser magnitude separation are seen in the absence of *ALK* rearrangement and should not be interpreted as positive.[15] The identification of a 2-diameter gap by readers requires experience, and inclusion of well-characterized negative and positive control sections is an essential part of validating the assay.

Other observations likely to be encountered when scoring signals include extra isolated 3′ *ALK* signals as well as extra signals (split and/or fusion) arising from polysomies, ploidy changes, and more complex *ALK* rearrangements.[15] Currently, these findings are of uncertain significance. All results should be entered onto score sheets and should be coded.

In the trials demonstrating ability of *ALK* FISH to predict treatment response, a case was considered positive if 15% or more of 50 nuclei assessed in a tumor-rich portion of the section showed the classic split-signal pattern.[15,217] Therefore, this cutoff is suggested and is part of the labeling of the FDA-approved commercial assay. Laboratories should still validate clinically sensitive and specific cutoffs in their own hands and different cutoffs must be reconciled with those from the commercial reference method. In certain situations with low tumor cell content, cutoffs as low as 5% may be considered, especially if IHC or RT-PCR results are available to support it, but this area requires more study.

Limited data exist to recommend cutoff values for the other "nonclassic" patterns of *ALK* rearrangement, such as loss of 5′ signal, and all testing laboratories should establish their own cutoff values for these other patterns.

9.5: Expert Consensus Opinion.—Testing for secondary mutations in *ALK* associated with acquired resistance to ALK inhibitors is not currently required for clinical management.

Several groups have reported a diverse set of secondary mutations in *ALK* that confer acquired resistance to crizotinib, including L1152R, C1156Y, F1174L, L1196M, L1198P, D1203N, and G1269A.[225–230] To date, however, the numbers of such cases are too small to recommend testing

for these mutations for routine clinical management, although we anticipate this indication to grow in the near future as effective second-line therapies become available.

SECTION IV: SHOULD OTHER GENES BE ROUTINELY TESTED IN LUNG ADENOCARCINOMA?

Question 10: Are Other Molecular Markers Suitable for Testing in Lung Cancer?

10.1a: Recommendation.—Testing for *EGFR* should be prioritized over other molecular markers in lung adenocarcinoma.

10.1b: Suggestion.—After *EGFR* testing, testing for *ALK* should be prioritized over other proposed molecular markers in lung adenocarcinoma, for which published evidence is insufficient to support testing guideline development at the present time.

Evidence Grade: EGFR: A; *ALK*: C.—Many additional molecular markers have been proposed as having value in management of lung cancer, in a variety of settings, including exposure to other molecularly targeted therapies, traditional chemotherapy, radiation, or surgery, as well as in other stages of disease and other histologic types of lung cancer. For each of these molecular markers, insufficient data have been published to establish definitive recommendations as to where, when, and how they should be used. However, as discussed in the sections above, clear and compelling published evidence supports the need for *EGFR* and *ALK* testing of advanced-stage lung adenocarcinomas as prerequisites to treatment with targeted TKIs. Precious tumor tissue must be reserved for these analyses, before any other molecular analysis is considered. Other tests may be performed in clinical trials or in clinical-pathologic contexts deemed appropriate by agreement between pathologists and clinicians at each individual treatment center, provided sufficient material remains after the essential *EGFR* and *ALK* tests are completed.

SECTION V: HOW SHOULD MOLECULAR TESTING OF LUNG ADENOCARCINOMAS BE IMPLEMENTED AND OPERATIONALIZED?

Question 11: Must All Adenocarcinomas Be Tested for Both *EGFR* and *ALK*?

11.1: Expert Consensus Opinion.—Laboratories may implement testing algorithms to enhance the efficiency of molecular testing of lung adenocarcinomas, provided the overall TAT requirements are met.

Currently, the higher costs and labor of simultaneous testing make it difficult to implement, in spite of its obvious advantage in TAT. Stepwise-testing algorithms make more efficient use of resources, but pose a challenge for timely delivery of final results. Given this time constraint, we recommend that stepwise-testing algorithms, if used, should nonetheless be completed within 10 working days. These algorithms are based on the observation that *EGFR*, *ALK*, and *KRAS* alterations are mutually exclusive, with very rare reported exceptions.[217,231,232]

The simplest algorithm would be to test for *EGFR* mutations first and proceed to *ALK* FISH if the *EGFR* results are wild type.

An alternative algorithm would involve an initial sensitive and rapid *EGFR* mutation screening test by a method such as denaturing high-performance liquid chromatography, high-resolution melting analysis, or single-stranded conforma-

tional polymorphism. Depending on assay design, these rapid screening methods could detect a mutation but fail to characterize it completely (ie, fail to define the size of an exon 19 deletion or distinguish between L858R and L861Q point mutations) or may be affected by single nucleotide polymorphisms (SNPs). For samples in which a mutation is detected but not adequately characterized by a screening method, a more specific method, such as sequencing, would be performed to establish a definitive diagnosis. If the *EGFR* mutation screening test result is negative, then testing for *ALK* FISH should be performed. This involves an additional step, but would reduce the amount of definitive *EGFR* and *ALK* FISH testing considerably.

A third algorithm, slower still but potentially more cost-effective, would begin with a simple *KRAS* analysis. *KRAS*-mutated tumors, which represent 25% to 30% of lung adenocarcinomas and which do not have either *EGFR* mutations or *ALK* rearrangements, would not proceed to either *EGFR* or *ALK* testing. The tumors without *KRAS* mutations would then enter into one of the algorithms above. If used, such an approach should still meet the above TAT recommendations and should not be undertaken if the *KRAS* testing will exhaust the sample and thereby preclude *EGFR* and *ALK* testing.

Whether or not to use any of these, or other testing algorithms, is a decision that each testing laboratory must make, in conjunction with its clinical care team, to balance its available resources and clinical needs within its individual health care setting.

Question 12: How Should *EGFR* and *ALK* Results Be Reported?

12.1: Expert Consensus Opinion.—*EGFR* mutation testing reports and *ALK* FISH reports should include a results and interpretation section readily understandable by oncologists and by nonspecialist pathologists.

Oncologists should be able to readily glean the information needed to select appropriate therapy, and to explain the result to their patients. Pathologists should be able to learn sufficient details from the reports to help determine whether repeated testing is appropriate, or to help resolve discrepancies between clinical and laboratory results or between tests performed at different laboratories.

The preclinical section of reports should include the standard identifiers of the patient and specimen, as well as an assessment of the specimen's morphologic characteristics: diagnosis and tumor content (percentage of total nuclei that are malignant). In addition, histopathologic characteristics that may affect the interpretation should be mentioned, such as extensive necrosis, atypical specimen processing or fixation, or low total number of tumor cells.

The results section of reports should include, prominently, the names of any clinically significant mutations identified, in formal Human Genome Variation Society (HGVS) nomenclature. Additional, more commonly used terminology may be included, as requested by each institution's clinical care teams. Incidental findings, variants of uncertain significance, and benign polymorphic variants should be clearly presented as such, to leave the reader no doubt as to the lack of evidence supporting their role in clinical management. For multiplexed assays, ideally, results could be presented in a table listing each clinically significant variant that is assessed by the test, with an adjacent result for each. Inconclusive results should be clearly reported as such.

Reports should include a histopathologic assessment of tumor content for the tumor section tested and the reported result should include an overall statement of the cancer's likelihood to respond or resist EGFR TKI therapy. If the result is inconclusive, whether due to assay failure or an insufficient specimen, or another reason, the interpretation should state why (as best as is known) and suggest requirements for testing a different specimen that would be more likely to yield a successful result.

The technical section of the report should include enough information for another laboratorian to understand what was done, in the event of a discrepancy between laboratories, or when requested to retest in another laboratory. The basic methodology should be reported, along with the assay sensitivity. For sequencing assays, each exon sequenced should be listed; for targeted mutation assays, each mutation targeted should be listed. Standard language regarding FDA oversight of laboratory-developed tests should be used, as appropriate.

The same overall principles apply to *ALK* reports, with a few distinctions. The results section should also include the number of cells analyzed, and the number and percentage of cells with each finding. Proper International System for Human Cytogenetic Nomenclature (ISCN) nomenclature may be used but, perhaps even more so than for HGVS nomenclature and molecular test results, ISCN nomenclature is difficult for the nonspecialist to understand, and colloquial nomenclature is essential for clear communication of results.

Question 13: How Should *EGFR* and *ALK* Testing Be Validated?

13.1: Expert Consensus Opinion.—*EGFR* and *ALK* testing validation should follow the same guidelines as for other molecular diagnostics and FISH tests.

Technical validation, the set of experiments performed in the clinical laboratory to assure that an assay is safe and reliable for use in patient care, is required in the United States under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). Although the required procedures differ between laboratory-developed tests and FDA-approved commercial assays, all tests must be properly validated before introduction into clinical use.[233] The CAP has published recommendations and examples on validation for a variety of assays.[234–239] This section will focus on specific points in the validation that the panel believes are of particular emphasis for *EGFR* and *ALK* validation.

Validation samples should be of all types that will be subjected to testing—frozen, fresh, and fixed specimens (including each fixative to be used), as appropriate. However, it is not necessary to separately validate identically processed tissues of different origins (ie, not necessary to validate separately FFPE lung, FFPE lymph node, and FFPE brain).

All relevant mutations or rearrangements that are to be reported should be included in the validation set, to whatever extent is possible. While some rare *EGFR* mutations may not be obtainable, the common exon 19 deletions, L858R, T790M, G719, and exon 20 insertions are required. Similarly, *ALK*-positive cases with split signals and with loss of 5′ signals should both be included in validation sets. It is not necessary to separately validate each individual mutation in each specimen-processing type, although it is recommended that each type of mutation (ie, point mutation, deletion, insertion, split signal, loss of signal) be

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assessed in each specimen-processing type, if possible. Cell lines may be used, but not to the exclusion of clinical specimens except for rare mutations.

Precision studies should assess the reproducibility of the entire analytic process, beginning with the pathologist's tumor assessment and enrichment strategies (eg, dissection). Operationally, therefore, when validation specimens undergo repeated testing on different assay runs, those samples should be reexamined by a pathologist and redissected.

Accuracy of results is best assessed by comparison with another laboratory performing a properly validated assay. Similarly, a new method within a laboratory may be validated against a previously validated method in the same laboratory. Comparison with clinical history of treatment response is suboptimal, but may be used as evidence of true positive mutated specimens, in the absence of another accredited laboratory for comparison. This should not be an issue in the United States, where many CLIA-certified laboratories offer these tests.

Analytic sensitivity of *EGFR* testing should be assessed in DNA from mutated specimens with low tumor content, diluted both in water/buffer and in normal DNA, to determine tumor cell content, in terms of both absolute cell count and tumor percentage, at which accuracy and precision (reproducibility) deteriorate. The need for replicate measurements to improve accuracy as tumor content decreases should be determined thus. Because of variation in *EGFR* copy number, sensitivity studies should be done with more than 1 specimen, and the least sensitive result should be stated as the overall test sensitivity. Cell lines are not ideal substitutes for clinical specimens, although FFPE cell pellets may be helpful, especially for mutations that are difficult to obtain. The sensitivity for FFPE specimens may differ from that for specimens fixed in alcohol or frozen.

Analytic specificity studies should establish criteria for distinguishing between true-positive and false-positive results. Specificity of *EGFR* and *ALK* results should be confirmed by clinically validated Sanger sequencing and Abbott Vysis ALK Break Apart FISH Probe Kit, respectively, or by methods traceable to those methods.

No template controls and very-low-concentration wild-type specimens are essential to establish specificity of ultrasensitive *EGFR* mutation detection methods. Artifacts associated with ultrasensitive methods should be recorded in the standard operating procedure manual.

ALK FISH should be performed on clearly benign tissue as well as *ALK* wild-type tumors, to help establish the minimum frequency of split signals that can be reported as true positive, as well as to confirm the 2-probe-diameter minimum distance of signal splitting that can be interpreted as true positive.

Question 14: How Should Quality Assurance Be Maintained?

14.1: Expert Consensus Opinion.—Laboratories should follow similar quality control and quality assurance policies and procedures for *EGFR* and *ALK* testing in lung cancers as for other clinical laboratory assays. In particular, laboratories performing *EGFR* and *ALK* testing for TKI therapy should enroll in proficiency testing, if available.

While specific requirements and approaches may vary somewhat from one country to another, or even between different accrediting organizations within one country (eg., Joint Commission versus CAP in the United States), the fundamental principles are conserved and retained; tests must be be properly validated, undergo regular quality control and instrument maintenance, with monitoring of the laboratory environment and reagent integrity, be performed by competent personnel following clear and informative standard operating procedures, with participation in external proficiency testing procedures and subject to regular inspections by accrediting agencies.

Outside the United States, there are local initiatives for *EGFR* mutation testing in, among others, Germany, Austria, France, Italy, Greece, the Netherlands, Spain, and Japan.[1,125,240–242] In some countries the national pathology society has rules stating that participation in ring trials for external quality assurance in molecular diagnostics is obligatory. The frequency of these assessments varies from once a year (the Netherlands, Greece, Italy) to twice a year (Germany).

In some countries, proficiency testing is performed by sending a similar sample (set) simultaneously to the participating laboratories, which report the results to the organizing body. Subsequently, the results are compared and a concordant result is a sign of adequate performance. Currently, there are no US regulatory requirements for reporting proficiency testing for *EGFR* and *ALK* assays, although proficiency testing must be performed and documented, as with all laboratory tests. The CAP offers an external proficiency testing program for *EGFR* and *KRAS* mutation testing, and is developing a program for *ALK* FISH. CLIA regulations require alternative assessment schemes as substitutes for mandated successful performance on external proficiency testing. When an external proficiency testing program is not available, then laboratories may organize their own proficiency testing program by exchanging specimens with 1 or more other laboratories at least twice per year.

In Europe, an initiative for *EGFR* testing has been started in collaboration with the European Molecular Genetics Quality Network, European Society of Pathology, European Society for Medical Oncology, and European Thoracic Oncology Platform, in which 10 specimens (cell lines, neutral buffered formalin fixed for 24 hours and embedded in paraffin) are validated by 4 laboratories (Greece, United Kingdom, Germany, Italy) and now run in a pilot scheme of 24 laboratories.[125]

In regions of the world where sample exchange is not available, laboratories may confirm accuracy of their results by semiannual clinical chart review of tested patient specimens, with the understanding that accurate determination of *EGFR* and *ALK* status may not be determined exclusively by benefit from EGFR TKI therapy, as erlotinib/gefitinib response rates for *EGFR*-mutated lung cancers can be in the 75% to 90% range, and the crizotinib response rates are comparable for *ALK*-rearranged lung cancers.[243] This may be due to upstream or downstream factors that render the EGFR TKI ineffective.

CONCLUSIONS: GUIDELINE DEVELOPMENT IN THE ERA OF GENOMIC MEDICINE

During the past decade, scientific and technologic progress in cancer genomics research has accelerated the pace of discoveries that can be potentially translated into significant clinical advances for patients with major common cancers. The clinical translation of these discoveries drives an ever-increasing need for tumor genotyping, based on

these newly established relationships between type of targetable gene mutation and response to targeted agents. In the case of the present molecular testing guideline for selection of lung cancer patients for EGFR and ALK TKIs, the pressing need to establish standards and provide recommendations had to be addressed in the context of the limitations of the literature. For many recommendations, especially more technical ones, articles reporting controlled studies were few or absent. For recommendations shaped by survival data, the recent and rapid clinical development of these indications, especially that of crizotinib in *ALK*-rearranged lung cancers, meant that relatively limited published data were available as compared to previous molecular testing guidelines such as the ASCO/CAP Guideline Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer.[98] In such a rapidly evolving area (and era) of medical practice, we expect that testing guidelines will increasingly have to be developed on more limited published data, integrating expert consensus opinion, with an expectation that such guidelines will be updated regularly as more rigorous meta-analyses of controlled studies become possible over time. Indeed, the present guideline does not encompass less prominent but nonetheless important testing areas, for instance, for selection of patients for MET-targeted therapies (*MET* amplification or overexpression)[244,245] and ERBB2-targeted therapies (*ERBB2* mutations),[246,247] and moreover, even as the present guideline was under development, new testing indications in lung cancer emerged, notably for rearrangements of the *ROS* and *RET* genes.* Moreover, recent technical innovations, such as "next generation" or massively parallel sequencing, afford the potential to detect all of these alterations, plus many others, in 1 assay.[157,248,249] Although these technologies are very promising, at the present, there are still insufficient published data on the accuracy, precision, sensitivity, specificity, TAT, and clinical validity of these methods in a clinical laboratory setting. Whereas these next-generation sequencing-based methods are currently not ready for routine widespread clinical implementation, their application to clinical medicine is growing rapidly, and they may form the core technology of the next version of these guidelines. Nonetheless, many aspects of the current guideline are "platform-independent" or "platform-agnostic" and, therefore, they should inform the evaluation and implementation of emerging diagnostic tests for *EGFR* and *ALK* status based on massively parallel sequencing.

Thus, the challenges for guideline development are mounting and include the variety of types of genetic alterations to be tested, the rapidly increasing number of clinically relevant cancer genes, the limited published literature and testing experience associated with the accelerated regulatory approval of targeted drugs, and the special issues created by the coapproval of commercial companion diagnostic tests. Even as models for guideline development evolve to adapt to these new factors and pressures, there remains an ongoing clinical need for such testing guidelines to establish and widely disseminate best practices based on systematic and critical literature review and broad consensus opinion from highly experienced stakeholders.

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APPENDIX: Disclosed Interests and Activities		
Name	Interest/Activity Type	Entity
Mary Beth Beasley, MD	Board or advisory board	Pulmonary Pathology Society Board of Trustees
	Lecture fees paid by entity	International Association for the Study of Lung Cancer
	Expert witness	Willcox and Savage, Asbestos Litigation
		McGuire Woods, Cooch and Taylor, Asbestos Litigation
		Cetrulo and Capone, Asbestos Litigation
		Rassmussen, Dickey, Willis and Moore, Asbestos Litigation
		Informa Health Care
	Royalties	Lippincott, Williams & Wilkins
	Leadership in associations	Pulmonary Pathology Society, Secretary
		College of American Pathologists, Chair, Surgical Pathology Resource Committee
Philip T. Cagle, MD	Lecture fees paid by entity	Emory University
		New York University
		Oklahoma State Association of Pathologists
		University of Michigan
		Massachusetts General Hospital
	Royalties	Springer Publishing
		Lippincott, Williams & Wilkins
	Leadership in associations	*Archives of Pathology & Laboratory Medicine,* Editor-in-Chief[a]
		Texas Society of Pathologists, Chair of Education Council
		Pulmonary Pathology Society, Past President
		Pulmonary Pathology Society, Chair of Nominating Committee
Dhananjay A. Chitale, MD	Grants	Pennsylvania Breast Cancer Coalition
		Department of Defense
		Susan G. Komen for the Cure
		National Institutes of Health
David J. Kwiatkowski, MD, PhD	Consultancy	Novartis
	Lecture fees paid by entity	Millenium
Marc Ladanyi, MD	Consultancy	Arqule/Daiichi Sankyo (April 2010)
		NanoString (September 2012)
	Lecture fees paid by entity	Genzyme (March 2010)
		Infinity (July 2010)
		Sequenom (November 2009)
		Remedica Medical Education (June 2012)
	Family and business partners	Wife: Continuing medical education activities for Abbott
	Institutional financial interest	Memorial Sloan-Kettering Cancer Center licensed patent for EGFR T790M testing to MolecularMD. M.L. is not a patent holder.
	Leadership in associations	Association for Molecular Pathology, Strategic Opportunities Committee
		United States and Canadian Academy of Pathology, Translational Genomics in Anatomic Pathology Task Force
		American Association for Cancer Research, 2012 Annual Meeting Program Committee
Neal I. Lindeman, MD	Institutional financial interest	Partners Health Care has a patent on *EGFR* Mutation Testing. N.I.L. is not a patent holder.
	Leadership in associations	Academy of Clinical Laboratory Physicians and Scientists, Paul E. Strandjord Young Investigator Award Coordinator
		Association for Molecular Pathology, Solid Tumor Representative, Clinical Practices Committee
		Association for Molecular Pathology, Nominating Committee, Solid Tumors Subdivision Representative
		AACC, Nominating Committee, Molecular Division
		AACC, Short Course/Interactive Workshops Director for AACC Annual Meeting Organizing Committee
		College of American Pathologists, Molecular Oncology Resource Committee
Juan-Sebastian Saldivar, MD	Additional employment	Sequenom Center for Molecular Medicine
		Existence Genetics
	Institutional financial interest	City of Hope functions as a reference laboratory, providing somatic testing for NSCLC
Jeremy A. Squire, PhD	Consultancy	CymoGen Dx, LLC
	Board or advisory board	Applied Spectral Imaging
	Patents received or pending	Development of *PTEN* FISH Probe through Queens' University Commercialization Office (Parteq)
	Royalties	Parteq-Queen's Commercialization Office
	Leadership in associations	NCIC (National Cancer Institute of Canada) Clinical Trials Group, Director of Laboratory Translational Research

APPENDIX: Continued		
Name	**Interest/Activity Type**	**Entity**
Erik Thunnissen, MD, PhD	Board or advisory board	Merck
		Eli Lilly
		Pfizer
	Lecture fees paid by entity	Merck
		Pfizer
		Eli Lilly
	Leadership in associations	European Society of Pathology lung external quality assessment
Paul A. Bunn Jr, MD, Steering Committee	Consultancy	Eli Lilly
		OSI/Genentech/Roche
		Astra Zeneca
		Boehringer Ingelheim
		Pfizer
		Amgen
		GlaxoSmithKline
		Bristol-Myers-Squibb
		Sanofi-Aventis
	Board or advisory board	OSI/Genentech/Roche
		Astra Zeneca
		Boehringer Ingelheim
		Amgen
		Bristol-Myers-Squibb
		Sanofi-Aventis
		Eli Lilly
	Patents received or pending	Patent relating to use of EGFR FISH results as predictive biomarkers for EGFR therapy
	Employment	University of Colorado Denver
		International Association for the Study of Lung Cancer
	Leadership in associations	International Association for the Study of Lung Cancer, Executive Director
Jan A. Nowak, MD, Steering Committee	Lecture fees paid by entity	William Beaumont Symposium
	Employment	North Shore University Health System
	Leadership in associations	AMP, Past President 2010
		AMP, Professional Relations Committee Member
		AMP, Economic Affairs Committee Member

Abbreviations: AACC, American Association for Clinical Chemistry; AMP, Association for Molecular Pathology; EGFR, epidermal growth factor receptor; FISH, fluorescence in situ hybridization; NSCLC, non–small cell lung cancer; PTEN, phosphatase and tensin homolog.

[a] Dr. Cagle recused himself from the 3 journals' peer review process for this guideline, a process completed subsequent to the approval of the guideline by the 3 societies.

INSTRUCTIONS FOR AUTHORS

(See January 2013 issue, page 139. Also available at www.archivesofpathology.org.)

Erratum

A recently published article [Lindeman NI, Cagle PT, Beasley MB, et al. Molecular Testing Guideline for Selection of Lung Cancer Patients for EGFR and ALK Tyrosine Kinase Inhibitors: Guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. *Arch Pathol Lab Med*. 2013;137 (6):828-860] contains an error in Table 4, *EGFR* Mutation Prevalence in Different Lung Adenocarcinoma Patient Populations, and in Supplemental Table 5, *EGFR* Mutation Prevalence in Different Lung Adenocarcinoma Patient Populations. These analyses failed to distinguish between European Spanish and Latin American (Hispanic) populations. Re-analysis of the 4 studies originally cited as providing data for Latin American (Hispanic) populations led to the re-categorization of 369 patients, with only 3 remaining Latin American (Hispanic) patients, which was too limited for meaningful analysis of this population. The re-categorization of the remaining 369 patients did not make a significant change in the data for the other patient populations as originally presented in Table 4 and in Supplemental Table 5. doi:10.5858/arpa.2013-0952-CO

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