**Supplemental Digital Content\***

**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 the Association for Molecular Pathology**

**Panel Composition**

**Conflict of Interest (COI) Policy**

**Methods Used to Produce Guideline**

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**Panel Composition**

The College of American Pathologists’ Pathology and Laboratory Quality Center, and representatives from the International Association for the Study of Lung Cancer and Association for Molecular Pathology 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 non-small cell lung cancer (NSCLC). Members included representatives from all three organizations. All three organizations utilized their respective organization’s approval processes in formal review and appointment of the project, steering committee, expert panel co-chairs, expert panel members and advisory panel. The steering committee provided guidance for the December conference and oversight for the entire project. Expert panel members reviewed the literature, authored the guideline, and provided input for dissemination and communication plans. The expert panel will also continue to maintain the guideline through periodic review. Advisory panel members provided their expertise throughout the project and were early reviewers of the draft manuscript.

**Conflict of Interest Policy**
Before acceptance on the expert panel, potential members from all guideline partnering organizations completed the CAP conflict of interest process dated April 2010, 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. The CAP Center uses the following criteria:

Nominees who had the following conflicts were excused from the expert panel:

1. Stock or equity interest in a commercial entity that would be affected by the guideline or white paper
2. Royalties or licensing fees from products that would be affected by the guideline or white paper
3. Employee of a commercial entity that would be affected by the guideline or white paper

Manageable conflicts of expert panel are:

1. Patents for products covered by the guideline or white paper
2. Member of an advisory panel of a commercial entity that would be affected by the guideline or white paper
3. Payments to cover costs of clinical trials, including travel expenses associated directly with the trial
4. Reimbursement from commercial entity for travel to scientific or educational meetings

Steering committee and expert panel members were required to disclose new conflicts at each conference call and submitted an updated COI form on a yearly basis. Advisory panel members submitted a conflict of interest form at the start of the project. Regarding members declaring potentially perceived or real conflict, guideline co-chairs 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.

**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, immunohistochemistry, IHC,* and *FISH*. All searches were limited to the English language.

**Eligible Study Designs**

Systematic reviews with or without meta-analyses, randomized controlled trials (RCTs), 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 U.S. 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 anti-EGFR or ALKTKI therapy.

**Exclusion Criteria**

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

**Tests Examined**

Additional test methods considered included *EGFR* copy number by fluorescence *in situ* hybridization (FISH) or bright field chromogenic *in situ* hybridization (CISH), 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 and advisory panels 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 testing anti-EGFR or ALK therapies 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**

Individuals representing regulatory agencies (United States Food and Drug Administration) also provided information about the regulatory framework. Individuals involved with quality assurance in the United States (CAP), the Netherlands, and Canada (Province of Ontario) provided information about programs to measure and improve *EGFR* and *ALK* testing. This information was used to help the panel define the best algorithm for testing, 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.1, 2 For strength of the evidence, we considered the level of evidence based on its hierarchy (Table 1), 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 methodological quality of included studies. The quality of systematic reviews, randomized control trials (RCTs), and cohort studies were assessed by using the AMSTAR (Assessment of Multiple Systematic Reviews) instrument and SIGN (Scottish Intercollegiate Guidelines Network) 50 checklists respectively.3, 4

**Table 1: Hierarchy** **of evidence1**

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| --- | --- |
| **Level** | **Intervention** |
| **I** | A systematic review of level II studies |
| **II** | Randomized Clinical Trial (RCT) (Good Quality) |
| **III-1** | A pseudo- RCT (i.e. alternate allocation or some other method) or RCT (Poor Quality) |
| **III-2** | A comparative study with concurrent controls:▪ Non-randomized, experimental trial ▪Cohort study, Case-control study, Interrupted time series with a control group(Good Quality) |
| **III-3** | A comparative study without concurrent controls:▪ Historical control study▪ Cohort study, Case-control study, Interrupted time series with a control group (Poor quality) ▪ Interrupted time series without a parallel control group ▪ Two or more single arm studies |
| **IV** | Case series with either post-test or pre-test/post-test outcomes |

Both clinical (variation in patients, intervention, comparator, outcome) and statistical heterogeneity (measured as *P* value and I2)were assessed. The scores from evidence base and consistency were obtained based on individual components. An emphasis was given to patient-oriented outcomes rather than disease-oriented outcomes.5 These scores were presented to panel members, who then provided scores for clinical impact, generalizability, and applicability of the evidence (as explained briefly in Table 2); all scores were then collated.

**Table 2: Body of Evidence Matrix Component2**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **A** | **B** | **C** | **D** |
| **Excellent** | **Good** | **Satisfactory** | **Poor** |
| **Evidence base** | several level I or level II studies with low risk of bias  | one or two level II studies with low risk of bias or a systematic review/multiple level III studies with low risk of bias  | level III studies with low risk of bias, or level I or II studies with moderate risk of bias  | level IV studies, or level I to III studies with high risk of bias  |
| **Consistency** | all studies consistent  | most studies consistent and inconsistency may be explained  | some inconsistency reflecting genuine uncertainty around clinical question  | evidence is inconsistent  |
| **Clinical impact**  | very large  | substantial  | moderate  | slight or restricted  |
| **Generalizability**  | population/s studied in body of evidence are the same as the target population for the guideline  | population/s studied in the body of evidence are similar to the target population for the guideline  | population/s studied in body of evidence differ from target population for guideline but it is clinically sensible to apply this evidence to target population | population/s studied in body of evidence differ from target population and hard to judge whether it is sensible to generalise to target population  |
| **Applicability**  | directly applicable to international healthcare context  | applicable to international healthcare context with few caveats  | probably applicable to international healthcare context with some caveats  | not applicable to international healthcare context  |

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 into making appropriate and informed clinical judgments (Table 2). 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.

**Table 3: Definition of grades of recommendations2**

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| **Grade of recommendation**  | **Description**  |
| **A**  | Body of evidence can be trusted to guide practice  |
| **B**  | Body of evidence can be trusted to guide practice in most situations  |
| **C**  | Body of evidence provides some support for recommendation(s) but care should be taken in its application  |
| **D**  | Body of evidence is weak and recommendation must be applied with caution  |

**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.

**OUTCOMES**

**CAP/IASLC/AMP Expert Panel Literature Review and Analysis**

The expert panel co-chairs (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 co-chairs independently reviewed each abstract, and disagreements were resolved by the third co-chair Full-text articles (521) were then reviewed for all selected abstracts by 2 members of the expert author panel; discrepancies were resolved by a co-chair. 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 re-evaluate the recommendation and modify or defend it.

A total of 278 studies were selected. In studies with duplicate data (companion publications), the original study, or the study reporting detailed or recent data (with a higher number of patients) was included. In cases where different outcomes had been reported, all studies were included. (See Figure 1)

A methodologist performed the original data extraction, and this was verified by CAP technical staff.  All analyses were performed using the Review Manager (RevMan) computer program [Version 5.1. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2011].6  Analyses were performed for main comparisons as well as subgroup-analyses where warranted.  Intention-to-treat data were analyzed where possible; otherwise, per-protocol data were used.  Mean differences (MD) with 95% confidence intervals (CI) were calculated for continuous data and relative risk ratio (RR) with 95% CI were calculated for dichotomous data; values of *P*<.05 was considered to be statistically significant.  Where mean data were not available, median data were used based on the assumption of constant hazard, but were calculated and presented separately.  The random effects model was used for all analyses, as it is the more conservative estimate of treatment effect.  Comparative data were pooled where no clinical heterogeneity was present, as determined by the expert panel co-chairs.  The magnitude of statistical heterogeneity was calculated using the I2 statistic for all pooled comparisons using the methods described by Montori *et al* (1) where a value of 0-25% indicated low, 26-50% indicated moderate, and greater than 51% indicated large heterogeneity.7 For I2 values greater than 75%, sub-group analysis to explore possible sources of heterogeneity would be considered. See Table 4 for a summary of the I2 values obtained.

**Table 4. I2 results by magnitude of statistical heterogeneity**

|  |  |  |
| --- | --- | --- |
| **I2**  | **Comparison** | **I2 value, %** |
| I2=0-25% | *EGFR*+ Asian patients, Age <65 vs. ≥65 years [Table 9] | 0 |
| *EGFR*+ Asian patients, Differentiation two-grades, Well vs. Moderate/Poor [Table 9] | 13 |
| *EGFR*+ Asian patients, Differentiation three-grades, Well vs. Poor [Table 9] | 0 |
| *EGFR*+ Asian patients, Differentiation three-grades, Well vs. Moderate/Poor [Table 9] | 0 |
| EGFR+ Non-Asian patients, Adenocarcinoma vs. Squamous [Table 10] | 0 |
| *ALK*+ NSCLC patients, Adenocarcinoma vs. Squamous [Table 13] | 0 |
| *EGFR*+ vs. *EGFR*- Time to Progression (6 month) [Table 16] | 0 |
| TKI vs. CTx Lung Ca patients, Time to Progression @ 2 years [Table 17] | 0 |
| TKI vs. CTx Lung Ca patients, Overall Survival @ 2 years (Stage III/IV) [Table 17] | 2 |
| *EGFR*+ Concordance, Real-time PCR [Table 20] | 0 |
| *EGFR*+ Concordance, Pyrosequencing [Table 20] | 0 |
| *EGFR*+ Concordance, Peptide nucleic acid/locked nucleic acid clamped amplification [Table 20] | 0 |
| *EGFR*+ Concordance, Smart-amplification process [Table 20] | 0 |
| *EGFR*+ vs. *EGFR*-, Disease Control rate [Table 21] | 12 |
| FISH+ vs. FISH-, Response Rate [Table 22] | 22 |
| FISH+ vs. FISH-, Disease Control rate [Table 22] | 0 |
| *KRAS*+ vs. *KRAS*- Response rate [Table 23] | 0 |
| I2=26-50% | *EGFR* + vs. *EGFR*-, Lung Ca patients, Response rate (Guideline Table 1) | 29 |
| *EGFR*+ Asian patients, Adenocarcinoma vs. Squamous [Table 9] | 34 |
| *EGFR*+ Non-Asian patients, Male vs. Female [Table 10] | 48 |
| *EGFR*+ vs. *EGFR*- Response rates [Table 16] | 35 |
| TKI vs. CTx Disease Control Rates [Table 17] | 37 |
| TKI vs. CTx Lung Ca patients, Time to Progression @ 1 year [Table 17] | 32 |
| I2=51 – 75% | *EGFR*+ vs. *EGFR*-, Lung Ca patients, Median Survival Time (Guideline Table 1) | 54 |
| *EGFR* + vs. *EGFR*-, Lung Ca patients, Disease control rate (Guideline Table 1) | 69 |
| *EGFR*+ - Asian patients, Male vs. Female [Table 9] | 61 |
| *EGFR*+ - Asian patients, Smoking, Ever vs. Never [Table 9] | 64 |
| *ALK*+ vs. *ALK*-, Mean age [Table 11] | 75 |
| *ALK*+ NSCLC patients, Smoking Never vs. Ever [Table 13] | 60 |
| *EGFR*+ vs. *EGFR*- Disease Control Rates [Table 16] | 73 |
| *EGFR*+ vs. *EGFR*- Overall Survival (1 year) [Table 16] | 73 |
| TKI vs. CTx Lung Ca patients, Response rates [Table 17] | 62 |
| TKI vs. CTx Advanced disease patients, Response rates [Table 18] | 72 |
| TKI vs. CTx Advanced disease patients, Median Survival Time (months) [Table 18] | 53 |
| *EGFR*+ Concordance, High resolution melting analysis [Table 20] | 73 |
| *EGFR*+ Concordance, Denaturing HPLC [Table 20] | 75 |
| *EGFR*+ vs *EGFR*-, Response rate [Table 21] | 50 |
| I2=76% or greater | *EGFR* + vs. *EGFR*-, Lung Ca patients, Time to progression (Guideline Table 1) | 93 |
| *EGFR*+ vs. *EGFR*- Median age, Asian patients [Table 6] | 82 |
| *EGFR*+ vs. *EGFR*- Mean age, Asian patients [Table 6] | 98 |
| *EGFR*+ vs. *EGFR*- Median age, non-Asian patients [Table 6] | 86 |
| *EGFR*+ vs. *EGFR*- Mean age, non-Asian patients [Table 6] | 93 |
| *EGFR*+ Non-Asian patients, Smoking Never vs. Ever [Table 10] | 86 |
| *ALK*+ vs. *ALK*-, Median age [Table 11] | 97 |
| *EGFR*+ vs. *EGFR*- Mean Time to Progression [Table 16] | 95 |
| *EGFR*+ vs. *EGFR*- Median Survival [Table 16] | 94 |
| TKI vs. CTx Lung Cancer patients, Time to Progression (months) [Table 17] | 97 |
| TKI vs. CTx Lung Ca patients, Median Survival (months) [Table 17] | 95 |
| TKI vs. CTx Advanced disease patients, Time to Progression (months) [Table 18] | 99 |
| *EGFR*+ vs. *EGFR*-, Time to Progression (months) [Table 21] | 98 |
| *EGFR*+ vs. *EGFR*-, Median Survival (months) [Table 21] | 90 |
| FISH+ vs. FISH-, Time to Progression (months) [Table 22] | 97 |
| FISH+ vs. FISH-, Median Survival (months) [Table 22] | 98 |
| *KRAS*+ vs. *KRAS*- Time to Progression (months) [Table 23] | 78 |
| *KRAS*+ vs. *KRAS*- Median Survival (months) [Table 23] | 78 |

Abbreviations: CTx, Chemotherapy; EGFR, epidermal growth factor receptor; NSCLC, non small cell lung cancer; PCR, Polymerase Chain Reaction; TKI, tyrosine kinase inhibitor

Individual studies were reviewed for each of the pooled estimates and data was visualized as forest plots (figures not shown). For each analysis for which heterogeneity (I2) exceeded 25%, the forest plots were re-examined for outlier studies. For most of these analyses, no outlier studies were apparent. However, 15 potential outlier studies were identified and re-reviewed. Five of these studies were subsequently removed from the analysis due to study design considerations. The remaining 10 studies did not present clinically or methodologically compelling reasons for exclusion from this analysis. The observed heterogeneity was not surprising, given the scope of published literature required for this analysis, with significant variation in study populations, therapeutic strategies, and diagnostic modalities.

**Methods Used to Produce Guideline**The entire panel met in December 2010 (Chicago, Illinois); additional work on the guideline was completed through electronic mail and monthly teleconferences of the co-chairs 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 non-writing advisory board, and to make writing assignments for the respective sections. All members of the expert panel participated in the preparation of section of the draft guideline. The expert panel then reviewed the guideline in its entirety. The co-chairs met face-to-face in New York City, NY to discuss the grading of the recommendations. Feedback on the draft recommendations was solicited from external reviewers. The open comment period was held from November 21, 2011 to December 20, 2011. An announcement was sent to the following organizations:

* CAP Board of Governors, Councils, Committees and Membership
* International Association for the Study of Lung Cancer (IASLC)
* Association for Molecular Pathology (AMP)
* American Society for Clinical Oncology (ASCO)
* American Society for Clinical Pathology (ASCP)
* US Food and Drug Administration (FDA)
* United States & Canadian Academy of Pathology (USCAP)
* American Cancer Society
* American Lung Association
* National Lung Cancer Partnership
* Lung Cancer Foundation of America
* Uniting Against Lung Cancer
* Addario Lung Cancer Medical Institute (ALCMI)
* European Society for Medical Oncology (ESMO)
* American Association for Clinical Chemistry (AACC)

The website received 528 comments in total. The expert panel co-chairs reviewed all comments and responses (i.e., agreed, disagreed or unsure). The co-chairs documented their action for each recommendation as maintain original recommendation; revise with minor language change, or consider major recommendation change. The expert panel co-chairs then met with the expert panel via WebEx to discuss their decisions to address these comments and finalize the recommendations. The guideline and method supplement were reviewed by a CAP 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.

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| **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.**  |
| **Evidence base: A** |
| **Consistency: A** |
| **Clinical impact: A** |
| **Generalizability: B** |
| **Applicability: B** |
| **Overall Grade: A** |

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| **Table 5:  *EGFR* mutation prevalence in different lung adenocarcinoma patient populationsa**

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| --- | --- | --- | --- | --- | --- |
|  | ***EGFR* mutation prevalence** | ***EGFR* Positive** | ***EGFR* Negative** | **n(N)** |   |
| **Asian/Pacific8-38** | 45% | 1547 | 1905 | 31(3452) |   |
| **White10, 20, 22, 25, 32, 33, 39-42**  | 24% | 853 | 2681 | 10(3534) |   |
| **African American22, 40, 43** | 20% | 19 | 78 | 3(97) |   |
| **Hispanic22, 44-46**  | 17% | 65 | 307 | 4(372) |   |
| **Asian/Indian47** | 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. **Table 6: Mean and Median age in Asian and Non-Asian lung adenocarcinoma patients** **assessed for *EGFR* mutation statusa** |
|  | **Mean + SD** |  |  |  |  |
|  | ***EGFR* Positive** | ***EGFR* Negative** | **n(N)** | **WMD** | ***P*-value** |  |
| **Median Age (Asian patients)10, 12, 15, 27, 31, 34, 35**  | 60.0 + 4.1 | 63.6 + 4.0 | 7(801) | -3.41[-5.12,-1.70] | *P*<.001 |  |
| **Mean Age (Asian patients)36, 37** | 63.3 + 2.4 | 63.4 + 0.9 | 2(701) | -0.04[-2.10,2.02] | *P*=.97 |  |
| **Median Age (non-Asian patients)10, 20, 22, 25, 43, 45, 46, 48**  | 60.4 + 6.7 | 61.7 + 3.8 | 8(3269) | -0.91[-3.05,1.22] | *P*=.40 |  |
| **Mean Age (non-Asian patients)32, 40, 42, 49** | 63.6 + 4.7 | 61.9 + 4.9 | 4(656) | 1.70[-1.90,5.31] | *P*=.35 |  |

Abbreviations: n, Number of studies; N, Number of patients; WMD, Weighted mean difference

a Some studies without detailed ethnic origin data were classified as primarily Asian and primarily non-Asian based on study site

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| **Table 7: Clinicopathologic characteristics in relation to *EGFR* mutation status in studies containing primarily Asian patientsa** |   |
|  | ***EGFR* Mutation prevalence** | ***EGFR* Positive** | ***EGFR* Negative** | **n(N)** |   |
| **Age w/ cut-off** |   |   |   |   |   |
| **<6518, 21, 29, 31, 36, 38** | 46% | 370 | 433 | 6(803) |   |
| **>6518, 29, 31, 36, 38** | 38% | 432 | 709 | 5(1141) |   |
| **Sex** |  |  |  |  |   |
| **Female8-19, 21, 23, 24, 26-31, 34-38, 50** | 58% | 1027 | 733 | 27(1760) |   |
| **Male8-19, 23, 24, 26-31, 34-38, 50** | 32% | 456 | 962 | 26(1418) |   |
| **Smoking** |  |  |  |  |   |
| **Never8-12, 15-19, 23, 24, 26, 28-30, 34-38, 50** | 58% | 843 | 599 | 22(1442) |   |
| **Ever8-12, 15-19, 23, 24, 26, 28-30, 34-38, 50** | 26% | 265 | 767 | 22(1032) |   |
| **History of Smoking (pack-years)** |  |  |  |  |   |
| **0-1029** | 67% | 10 | 5 | 1(15) |   |
| **11-4029** | 45% | 5 | 6 | 1(11) |   |
| **>4029** | 23% | 5 | 17 | 1(22) |   |
| **>2031** | 25% | 13 | 40 | 1(53) |   |
| **Histology** |  |  |  |  |   |
| **Adenocarcinoma8-14, 16-19, 23, 24, 26-31, 34-38, 50** | 50% | 1278 | 1256 | 25(2534) |   |
| **Squamous12, 17, 28, 31, 34-36, 50** | 5% | 8 | 160 | 8(168) |   |
| **Adenosquamous28, 31** | 67% | 4 | 2 | 2(6) |   |
| **Large cell13, 28, 31, 35** | 7% | 1 | 14 | 4(15) |   |
| **Differentiation-Two grades** |  |  |  |  |   |
| **Well19, 51, 52** | 37% | 62 | 107 | 3(169) |   |
| **Moderate to Poor19, 51, 52** | 14% | 27 | 162 | 3(189) |   |
| **Differentiation-Three grades** |  |  |  |  |   |
| **Well18, 27** | 65% | 28 | 15 | 2(43) |   |
| **Moderate18, 27** | 48% | 59 | 63 | 2(122) |   |
| **Poor18, 27** | 34% | 17 | 33 | 2(50) |   |
| Abbreviations: n, Number of studies; N, Number of patients a Note: Most studies contained primarily patients with adenocarcinoma |  |
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| **Table 8: Clinicopathologic characteristics in relation to *EGFR* mutation status in studies containing** **primarily Non-Asian patientsa** |
|  | ***EGFR* mutation prevalence** | ***EGFR* Positive** | ***EGFR* Negative** | **n(N)** |  |  |
| **Sex** |   |   |   |   |  |  |
| **Female10, 20, 22, 25, 32, 33, 39-47, 49, 53-55** | 28% | 859 | 2239 | 19(3098) |  |  |
| **Male10, 20, 22, 25, 32, 33, 39-47, 49, 53-55** | 18% | 397 | 1768 | 19(2165) |  |  |
| **Smoking** |  |  |  |  |  |  |
| **Never10, 20, 22, 25, 32, 33, 39-47, 49, 53, 54** | 45% | 666 | 805 | 18(1471) |  |  |
| **Ever10, 20, 22, 25, 32, 33, 39-47, 49, 53, 54** | 15% | 569 | 3154 | 18(3723) |  |  |
| **History of Smoking (pack-years)** |  |  |  |  |  |  |
| **0-1022** | 39% | 18 | 28 | 1(46) |  |  |
| **11-5022** | 8% | 7 | 86 | 1(93) |  |  |
| **>5022** | 5% | 3 | 56 | 1(59) |  |  |
| **Histology** |  |  |  |  |  |  |
| **Adenocarcinoma10, 20, 22, 25, 32, 33, 39-46, 48, 49, 53-55** | 24% | 1266 | 3918 | 19(5184) |  |  |
| **Squamous20, 22, 25, 33, 43, 44, 46, 48, 54** | 5% | 6 | 104 | 9(110) |  |  |
| **Adenosquamous22, 25** | 13% | 1 | 7 | 2(8) |  |  |
| **Large cell25, 33, 44, 46, 48, 54** | 5% | 2 | 37 | 6(39) |  |  |
| Abbreviations: n, Number of studies; N, Number of patients a Most studies contained primarily adenocarcinoma patients**.**  |  |  |   |

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| **Table 9: Pooled analyses of factors associated with *EGFR* mutation status in studies containing primarily Asian patients** |
|  | **n(N)** | ***EGFR* mutation** **RR [95%CI]a** | ***P*-value** |
| **Age cut-off** |  |  |  |
| **<6518, 29, 31, 36, 38** | 5(477) |  |  |
| **>6518, 29, 31, 36, 38** | 5(1141) | 1.14 [0.94, 1.38] | *P*=.19 |
| **Sex** |  |  |  |
| **Female8-19, 23, 24, 26-31 , 34-38, 50** | 26(1425) | 1.81 [1.54, 2.13] | ***P*<.001** |
| **Male8-19, 23, 24, 26-31, 34-38, 50** | 26(1418) |  |  |
| **Smoking** |  |  |  |
| **Never8-12, 15-19, 23, 24, 26, 28-30, 34-38, 50** | 22(1442) | 2.27 [1.84, 2.79] | ***P*<.001** |
| **Ever8-12, 15-19, 23, 24, 26, 28-30, 34-38, 50** | 22(1032) |  |  |
| **Histology** |  |  |  |
| **Adenocarcinoma12, 17, 28, 31, 34-36, 50** | 8(469) | 4.73 [2.21, 10.09] | ***P*<.001** |
| **Squamous12, 17, 28, 31, 34-36, 50** | 8(168) |  |  |
| **Differentiation-Two grades** |  |  |  |
| **Well19, 51, 52** | 3(169) | 2.50 [1.60, 3.90] | ***P*<.001** |
| **Moderate to Poor19, 51, 52** | 3(189) |  |  |
| **Differentiation-Three grades** |  |  |  |
| **Well18, 27** | 2(43) | 1.99 [1.28, 3.09] | ***P*=.002b** |
| **Moderate18, 27** | 2(122) | 1.76 [1.17, 2.66] |  ***P*=.007c** |
| **Poor18, 27** | 2(50) |  |  |
| Abbreviations: n, Number of studies; N, Number of patients; CI, Confidence interval; RR,Relative Risk, Mantel-Haenszel, Random Effects model, [95% CI]a RR of *EGFR* mutation is indicated for the factor more strongly associated with *EGFR* mutationb Comparing Well to Poorc Comparing Well + Moderate to Poor**Table 10:  Pooled analyses of factors associated with *EGFR* mutation status in studies containing primarily Non-Asian patients** |
|   | **n(N)** | ***EGFR* mutation** **RR [ 95%CI]a** | ***P*-value** |
| **Sex** |  |  |  |
| **Female10, 20, 22, 25, 32, 33, 39-47, 49, 53-55** | 19(3098) | 1.67 [1.38, 2.01] | ***P*<.001** |
| **Male10, 20, 22, 25, 32, 33, 39-47, 49, 53-55** | 19(2165) |  |  |
| **Smoking** |  |  |  |
| **Never10, 20, 22, 25, 32, 33, 39-47, 49, 53, 54** | 18(1471) | 3.11 [2.28, 4.25] | ***P*<.001** |
| **Ever10, 20, 22, 25, 32, 33, 39-47, 49, 53, 54** | 18(3723) |  |  |
| **Histology** |  |  |  |
| **Adenocarcinoma20, 22, 25, 33, 43, 44, 46, 48, 54** | 9(761) | 2.17 [1.12, 4.23] | ***P*=.02** |
| **Squamous20, 22, 25, 33, 43, 44, 46, 48, 54** | 9(110) |  |  |

Abbreviations: n, Number of studies; N, Number of patients; CI, Confidence interval; RR,Relative Risk, Mantel-Haenszel, Random Effects model, [95% CI]a RR of *EGFR* mutation is indicated for the factor more strongly associated with *EGFR* mutation |
| **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 base: B** |
| **Consistency: B** |
| **Clinical impact: A** |
| **Generalizability: B** |
| **Applicability: B** |
| **Overall Grade: B** |

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| --- |
| **Table 11: Mean and Median age in lung adenocarcinoma patients** **assessed for *ALK* rearrangement status** |
|  | **Mean + SD** |  |  |  |  |
|  | ***ALK* Pos** | ***ALK* Neg** | **n(N)** | **WMD** | ***P*-value** |  |
| **Median Age56, 57**  | 53.5 + 3.5 | 64.0 + 2.8 | 2(663) | -10.55 [-19.37, -1.73] | *P*=.02 |  |
| **Mean Age58-60**  | 58.9 + 9.7 | 65.2 + 3.9 | 3(967) | -7.23 [-13.03, -1.43] | *P*=.01 |  |

Abbreviations: n, Number of studies; N, Number of patients; WMD, Weighted mean difference

|  |  |
| --- | --- |
| **Table 12: Clinicopathologic characteristics in relation to *ALK* rearrangement statusa** |  |
|  | ***ALK* rearrangement prevalence** | ***ALK* Positive** | ***ALK* Negative** | **n (N)** |
| **Age with cut-off** |   |   |   |   |
| **≤ 65 years61**  | 6% | 13 | 210 | 1(223) |
| **> 65 years61**  | 3% | 6 | 224 | 1(230) |
| **Sex** |   |   |   |   |
| **Females56, 57, 59, 61**  | 5% | 28 | 579 | 4(607) |
| **Males56, 57, 59, 61**  | 4% | 30 | 738 | 4(768) |
| **Smoking** |   |   |   |   |
| **Never57, 59, 61**  | 8% | 30 | 331 | 3(361) |
| **Ever57, 59, 61**  | 3% | 20 | 652 | 3(672) |
| **Ethnicity** |  |  |  |  |
| **Asian/Pacific56, 59, 63** | 5% | 35 | 654 | 3(689) |
| **White (Caucasian)56, 57** | 4% | 22 | 474 | 2(496) |
| **Histology**  |   |   |   |   |
| **Adenocarcinoma56-59, 61, 64, 65** | 5% | 67 | 1319 | 7(1386) |
| **Squamous56, 58, 59, 61, 64, 65**  | 0.2% | 1 | 522 | 6(523) |
| **Adenosquamous56, 59, 65** | 0% | 0 | 19 | 3(19) |
| **Differentiation** |   |   |   |   |
| **Well59** | 1% | 1 | 97 | 1(98) |
| **Not well59**  | 6% | 10 | 145 | 1(155) |

Abbreviations: n, Number of studies; N, Number of patients

a Most studies contained primarily adenocarcinoma patients

|  |
| --- |
| **Table 13:  Pooled analyses of factors associated with *ALK* rearrangement status in NSCLC patients** |
|  | **n (N)** | ***ALK* rearrangement RR [95%CI]** | ***P*-value** |
| **Smoking** |  |  |  |
| **Never57, 59, 61**  | 3(361) | 2.81 [1.14, 6.89] | *P*=.02 |
| **Ever57, 59, 61**  | 3(672) |  |  |
| **Histology**  |  |  |  |
| **Adenocarcinoma56, 58, 59, 61, 64, 65** | 6(1028) | 5.47 [1.82, 16.47] | *P*=.002 |
| **Squamous56, 58, 59, 61, 64, 65**  | 6(523) |  |  |

Abbreviations: n, Number of studies; N, Number of patients; CI, Confidence interval; RR,Relative Risk, Mantel-Haenszel,

Random Effects model, [95% CI]

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| **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 base: A** |
| **Consistency: A** |
| **Clinical impact: A** |
| **Generalizability: A** |
| **Applicability: A** |
| **Overall Grade: A** |

|  |
| --- |
| **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 base: A** |
| **Consistency: A** |
| **Clinical impact: A** |
| **Generalizability: A** |
| **Applicability: A** |
| **Overall Grade: A** |

**Table 14: Histology in relation to *EGFR* mutation and *ALK* rearrangement status**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | ***EGFR* Mutation Prevalence** | ***EGFR* Positive** | ***EGFR* Negative** | **n (N)** |
| ***EGFR* mutations**  |
| Primarily Asian patientsAdenocarcinoma**8-14, 16-19, 23, 24, 26-31, 34-38, 50**Squamous**12, 17, 28, 31, 34-36, 50** Adenosquamous**28, 31**Large cell carcinoma**13, 28, 31, 35**Primarily Non-Asian patientsAdenocarcinoma**10, 20, 22, 25, 32, 33, 39-46, 48, 49, 53-55**Squamous**20, 22, 25, 33, 43, 44, 46, 48, 54** Adenosquamous**22, 25**Large cell carcinoma**25, 33, 44, 46, 48, 55** | 50%5%67%7%24%5%13%5% | 12788411266612 | 12561602143918104737 | 25(2534)8(168)2(6)4(15)19(5184)9(110)2(8)6(39) |
| ***ALK* rearrangements**  | ***ALK* Rearrangement Prevalence** | ***ALK* Positive** | ***ALK* Negative** | **n (N)** |
| Adenocarcinoma57-60, 62, 65, 66Squamous56, 58, 59, 61, 64, 65 Adenosquamous57, 60, 66 | 5%0.19%0% | 6710 | 131952219 | 7(1386)6(523)3(19) |

Abbreviations: n, Number of studies; N, Number of patients

|  |
| --- |
| **1.4: Recommendation:** **To determine *EGFR* and *ALK* status for initial treatment selection, primary tumors or metastatic lesions are equally suitable for testing.** |
| **Evidence base: B** |
| **Consistency: B** |
| **Clinical impact: A** |
| **Generalizability: B** |
| **Applicability: B** |
| **Overall Grade: B** |

**Table 15: Detection of *EGFR* mutations in Primary tumors versus Metastatic Lesions in the Same Patient**

|  |  |  |
| --- | --- | --- |
|  |  |    **Primary tumor** |
| **Metastatic lesions** |  | *EGFR* + | *EGFR*- |
| *EGFR* + | 108 | 6 |
| *EGFR* - | 11 | 183 |

Data derived from references66-68

|  |
| --- |
| **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.** |

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| **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.**  |
| **Evidence base: A** |
| **Consistency: A** |
| **Clinical impact: A** |
| **Generalizability: A** |
| **Applicability: A** |
| **Overall Grade: A** |

**Table 16: Different outcomes in advanced disease (stage III and IV) lung cancer patients with and without *EGFR* mutations, treated with Tyrosine Kinase Inhibitor**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Outcome** | **Percentage** | **n (N)** | **RR [95% CI] or****WMD [95% CI]** | ***P* value** |
| ***EGFR*****Mutations** | **No Mutations** |
| Response rate (%)12, 16, 20, 21, 23, 24, 31, 33-35, 37, 38, 41, 43, 44, 46, 54, 55, 69-88, 90, 92Advanced disease (Stage III and IV) | 68% | 11% | 40(3093) | 5.17 [4.29, 6.22] | ***P*<.001** |
| Disease control rate (%)16, 20, 21, 33-35, 37, 44, 46, 54, 55, 69, 71, 73, 74, 76, 78-81, 84, 87, 89, 90Advanced disease (Stage III and IV) | 85% | 42% | 24(2003) | 1.97 [1.70, 2.29] | ***P*<.001** |
| Time to Progression/Progression Free Survival (%)Advanced disease (Stage III and IV)* + 6 months12, 78
	+ 1 year21
 | 88%23% | 24%2% | 2(118)1(223) | 3.71 [2.5, 5.49]10.69[2.62, 43.54] | ***P*<.001 *P*<.001** |
| Overall Survival (%)Advanced disease (Stage III and IV)* + 1 year52, 54, 80
 | 59% | 42% | 3(222) | 1.80 [0.94, 3.46] | *P*=.08 |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Outcome** | **Mean ± SD** | **n (N)** | **RR [95% CI] or****WMD [95% CI]** | ***P* value** |
| ***EGFR*****Mutations** | **No Mutations** |
| Time to Progression/Progression Free Survival (months)20, 26, 35, 37, 38, 44, 46, 54, 69-71, 73-75, 77, 78, 81, 83, 84, 87, 88, 90-92Advanced disease (Stage III and IV)  | 10.4+ 4.5 | 2.9 + 1.5 | 24(2238) | 7.48 [5.87, 9.08]] | ***P*<.001** |
| Median Survival time (months)20, 24, 31, 33, 37-39, 44, 46, 54, 69, 71, 74, 75, 77, 79-81, 83-87, 90, 93, 94Advanced disease (Stage III and IV)  | 20.5 + 6.6 | 9.8 + 4.3 | 26(2558) | 10.72 [8.63, 12.8] | ***P*<.001** |

Abbreviations: CI, Confidence interval; n, Number of studies; N, Number of patients; RR, Relative risk; WMD, Weighted mean difference

**Table 17: Different outcomes in advanced disease (stage III and IV) lung cancer patients with *EGFR* mutations, treated with Tyrosine Kinase Inhibitor (TKI) Therapy or Chemotherapy, Data from RCTs**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Outcome** | **Percentage** | **n (N)** | **RR [95% CI] or****WMD [95% CI]** | ***P* value** |
| **TKI** **Therapy** | **Chemo-therapy** |
| Response rate (%)21, 75, 77, 95-98Advanced disease (Stage III and IV)  | 70% | 33% | 7(1013) | 2.17[1.69,2.78] | ***P*<.001** |
| Disease control rate (%)21, 96-98Advanced disease (Stage III and IV) | 90% | 79% | 4(705) | 1.13[1.04,1.22] | *P*=.002 |
| Time to Progression/Progression Free Survival (%)Advanced disease (Stage III and IV)* + 1 year21, 96, 97
	+ 2 years96, 97
 | 30%7% | 5%0% | 3(658)2(397) | 5.72[3.06,10.72]13.74[1.81,104.18] | ***P*<.001***P*=.01 |
| Overall Survival (%)Advanced disease (Stage III and IV)* + 1 year98
	+ 2 years93, 95, 97
 | 80%42% | 83%35% | 1(154)3(662) | 0.97[0.83, 1.12]1.20 [1.00, 1.44] | *P*=.65*P*=.05 |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Outcome** | **Mean ± SD** | **n (N)** | **RR [95% CI] or****WMD [95% CI]** | ***P* value** |
| **TKI Therapy** | **Chemo-therapy** |
| Time to Progression / Progression Free Survival (months)75, 77, 93, 95-97Advanced disease (Stage III and IV) | 9 + 1.24 | 5.6 + 0.88 | 6(910) | 3.38 [2.52, 4.23] | ***P*<.001** |
| Median survival time (Months)75, 77, 93, 95, 97Advanced disease (Stage III and IV)  | 22.6 + 6.44 | 21.4 + 3.51 | 5(744) | 1.19 [-2.3, 4.70] | *P*=.50 |

Abbreviations: CI, Confidence interval; n, Number of studies; N, Number of patients; RCT, Randomized controlled trial; RR, Relative risk; WMD, Weighted mean difference

**Table 18: Different outcomes in advanced disease patients without *EGFR* mutations treated with Tyrosine Kinase Inhibitor Therapy or Chemotherapy, Data from RCTs**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Outcome** | **Percentage** | **n (N)** | **RR [95% CI] or****WMD [95% CI]** | ***P* value** |
| **TKI Therapy** | **Chemotherapy** |
| Response rate (%)21, 75, 77 | 7% | 20% | 3(459) | 0.35 [0.11, 1.12] | *P*=.08 |
| Disease control rate (%)21 | 40% | 84% | 1(176) | 0.47 [0.36, 0.62] | ***P*<.001** |
| Time to Progression/Progression Free Survival (%)* 1 year21
 | 2% | 1% | 1(176) | 1.87[0.17, 20.23] | *P*=.61 |
| Overall Survival (%) * 1 year93
* 2 years93
 | 44%21% | 52%22% | 1(176)1(176) | 0.85 [0.62, 1.16]0.93 [0.53, 1.64] | *P*=.30*P*=.81 |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Outcome** | **Mean ± SD** | **n (N)** | **RR [95% CI] or****WMD [95% CI]** | ***P* value** |
| **TKI Therapy** | **Chemotherapy** |
| Time to Progression/Progression Free Survival (months)75, 77, 93 | 1.8 + 0.31 | 4.8 + 1.99 | 3(459) | -3.05[-5.41, -0.70] | *P*=.01 |
| Median survival time (months)75, 77, 93 | 12 + 6.04 | 13.5 + 7.98 | 3(483) | -1.06[-3.05, 0.95] | *P*=.29 |

Abbreviations: CI, Confidence interval; n, Number of studies; N, Number of patients; RCT, Randomized controlled trial; RR, Relative risk; WMD, Weighted mean difference

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| --- |
| **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 base: C** |
| **Consistency: C** |
| **Clinical impact: A** |
| **Generalizability: A** |
| **Applicability: A** |
| **Overall Grade: C** |

**Table 19: Outcomes in advanced adenocarcinoma patients with *ALK* rearrangements at a mean treatment duration of 6.4 months with crizotinib60**

|  |  |  |
| --- | --- | --- |
| **Outcome** | **Percentage**  | **n (N)** |
| **Overall Response rate (%)** | **57%** | **1 (82)** |
| **Stable Disease** | **33%** | **1 (82)** |
| **Disease control rate (%) at 8 weeks** | **87%** | **1 (82)** |
| **Estimated 6 month probability of Progression free survival**  | **72%** | **1 (82)** |

Abbreviations: n, Number of studies; N, Number of patients

|  |
| --- |
| **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.** |

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| **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.** |

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| **2.3: Recommendation: Tissue should be prioritized for *EGFR* and *ALK* testing.** |
| See recommendation 1.1a and 1.1b for evidence tables and grading. |

|  |
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| **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.** |

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| **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.** |

|  |
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| **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.**  |

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| --- |
| **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.** |

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

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| **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.**  |

|  |
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| **6.1:**  **Recommendation: Laboratories may use** **any validated *EGFR* testing method with sufficient performance characteristics.** |
| **Evidence base: B** |
| **Consistency: B** |
| **Clinical impact: A** |
| **Generalizability: A** |
| **Applicability: A** |
| **Overall Grade: B** |

**Table 20:**  **Comparison of Sanger Sequencing to other methods for the detection of *EGFR* mutationsa**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Sanger Sequencing vs. Other Methods** | **Concordance of Methods** | **n(N)** | **Seq-/ Other+** | **Seq+/****Other-** | **Incidence of *EGFR* Mutations** | **RR [95% CI]** | ***P* value** |
| **Sanger Sequencing** | **Other Method** |
| **PCR-based mutation detection** |  |  |  |  |  |  |  |  |
| Allele-specific PCR/ARMS99 | 73% | 1(83) | 18 | 4 | 16% | 33% | 0.48[0.27,0.87] | ***P*=.01** |
| Real-time PCR51, 100 | 97% | 2(102) | 2 | 1 | 26% | 27% | 0.94[0.60,1.46] | *P*=.78 |
| Cycleave PCR101, b | 95% | 1(195) | 1 | 8 | 40% | 36% | 1.10[0.85,1.41] | *P*=.47 |
| **Post-PCR Mutation detection** |  |  |  |  |  |  |  |  |
| Capillary electrophoresis50, 100 | 98% | 2(61) | 1 | 0 | 16% | 18% | 0.91[0.42,2.01] | *P*=.82 |
| Restriction fragment length polymorphism16 | 99% | 1(109) | 1 | 0 | 33% | 34% | 0.97[0.67,1.41] | *P*=.89 |
| INVADER102, c | 86% | 1(42) | 5 | 1 | 43% | 52% | 0.82[0.52,1.29] | *P*=.39 |
| Pyrosequencing103, 104 | 96% | 3(140) | 6 | 0 | 16% | 20% | 0.78[0.49,1.25] | *P*=.3 |
| **Mutation scanning** |  |  |  |  |  |  |  |  |
| Denaturing HPLC105, 106 | 94% | 2(196) | 12 | 0 | 20% | 27% | 0.66[0.27,1.63] | *P*=.37 |
| Single stranded conformational polymorphism107 | 98% | 1(375) | 8 | 0 | 8% | 10% | 0.79[0.51,1.25] | *P*=.32 |
| High resolution melting analysis108-110 | 83% | 3(321) | 54 | 0 | 36% | 53% | 0.70[0.46, 1.06] | ***P*=.09** |
| Loop-Hybrid mobility shift assay111 | 100% | 1(43) | 0 | 0 | 26% | 26% | 1.00[0.49,2.06] | *P*>.99 |
| **Mutant enrichment** |  |  |  |  |  |  |  |  |
| Peptide nucleic acid/locked nucleic acid clamped amplification112, 113 | 96% | 2(150) | 4 | 2 | 11% | 12% | 0.91[0.49,1.67] | *P*=.75 |
| COLD PCR114 | 100% | 1(126) | 0 | 0 | 10% | 10% | 1.00[0.48,2.07] | *P*>.99 |
| Smart-Amplification process112, 115, 116 | 86% | 4(220) | 30 | 0 | 20% | 34% | 0.58[0.44,0.77] | ***P*<.001** |

 Abbreviations: ARMS, amplification refractory mutation system; CI, Confidence interval; COLD, co-amplification 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]; PCR, polymerase chain reaction; HPLC, high performance liquid chromatography; 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 vs. non-Asian). No statistical comparisons were performed between rows.

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

c Invader (Hologic, Madison, Wisconsin).

|  |
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| **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.** |

|  |
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|  **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.**  |

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| --- |
| **6.4: Recommendation: Immunohistochemistry for total EGFR is not recommended for selection of EGFR TKI therapy.** |
| **Evidence base: A** |
| **Consistency: A** |
| **Clinical impact: A** |
| **Generalizability: A** |
| **Applicability: A** |
| **Overall Grade: A** |

**Table 21: Different outcomes with the use of Tyrosine Kinase Inhibitors in patients tested by EGFR Immunohistochemistry**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Outcome** | **Percentage** | **n(N)** | **RR[95% CI[ or WMD [95% CI]** | ***P* value** |
| **EGFR** **Expression** | **No Expression** |
| Response rate (%)54, 69, 71, 75, 83, 86, 117, 118 | 21% | 16% | 8(653) | 1.29 [0.75, 2.21] | *P*=.35 (ns) |
| Disease control rate (%)54, 69, 89, 118 | 55% | 34% | 4(341) | 1.57 [1.18, 2.09] | *P*=.002 |
| Overall Survival (%) at 1 year54 | 56% | 38% | 1(200) | 0.71 [0.54, 0.92] | ***P*=.01** |
|  |  |  |  |  |  |
| **Outcome** | **Mean ± SD** | **n (N)** | **RR[95% CI[ or WMD [95% CI]** | ***P* value** |
| **EGFR** **Expression** | **No Expression** |
| Time to Progression/ Progression Free Survival (months)54, 69, 75, 83, 91, 117 | 3. 4+ 1.2 | 3.2 + 0.53 | 6(898) | 0.22 [-.80,1.24] | *P*=.68 (ns) |
| Median Survival time (months)23, 54, 75, 83, 86, 117 | 12.5 + 4.1 | 8.4 + 4.8 | 6(563) | 4.18 [1.37,6.99] | ***P*=.004** |

Abbreviations: CI, Confidence interval; n, Number of studies; N, Number of patients; ns, Not significant; RR, Relative risk; WMD, Weighted mean difference

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| **6.5: Recommendation: *EGFR* copy number analysis (ie, FISH or chromogenic in situ hybridization) is not recommended for selection of EGFR TKI therapy.** |
| **Evidence base: B** |
| **Consistency: B** |
| **Clinical impact: B** |
| **Generalizability: A** |
| **Applicability: A** |
| **Overall Grade: B** |

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| **Table 22: Different outcomes for Tyrosine Kinase Inhibitor therapy in patients tested by *EGFR* Fluorescence In Situ Hybridization (FISH)a**  |  |  |
|  |  |  |  |  |  |
| **Outcome** | **Percentage** | **n(N)** | **Risk Ratio M-H, Random, 95% CI** |  |
| ***EGFR* FISH Positive (High copy #)** | ***EGFR* FISH Negative****(Low copy #)** | ***P*-value** |
| Response rate (%)20, 54, 71, 75, 78, 86, 119-123 | 30% | 9% | 11(861) | 2.69 [1.86, 3.90] | ***P*<.001** |
| Disease control rate (%)20, 54, 78, 89, 120 | 47% | 24% | 5(438) | 2.09 [1.63, 2.69] | ***P*<.001** |
| Overall Survival (%) at 1 year54 | 68% | 37% | 1(183) | 1.83 [1.37, 2.44] | ***P*<.001** |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  | **Mean + SD** | **n(N)** | **Risk Ratio M-H, Random, 95% CI** |  |
| **Outcome** | ***EGFR* FISH Positive (High copy #)** | ***EGFR* FISH Negative** **(Low copy #)** | ***P*-value** |
| Time to Progression/ Progression Free Survival (months)20, 26, 54, 71, 75, 122, 124 | 4.4 + 2.8 | 2.9 + 0.7 | 7(638) | 4.06 [1.97, 6.16] | ***P<.001*** |
| Median Survival time (Months)20, 26, 54, 75, 86, 122-124 | 10.3 + 3.4 | 8.7 + 2.4 | 8(778) | 1.54 [-1.83, 4.91] | ***P*=.37** |

Abbreviations: CI, Confidence interval; M-H, Mantel-Haenszel; n, Number of studies; N, Number of patients; Random, random effects model; SD, standard deviation; TKI, tyrosine kinase inhibitor

a FISH scored by Cappuzzo, et al.

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| **7.1: Recommendation: *KRAS* mutation testing is not recommended as a sole determinant of EGFR TKI therapy.** |
| **Evidence base: B** |
| **Consistency: B** |
| **Clinical impact: A** |
| **Generalizability: A** |
| **Applicability: A** |
| **Overall Grade: B** |

**Table 23: Different outcomes in patients for treated with Tyrosine Kinase Inhibitor, with or without *KRAS* mutations**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Outcome** | **Percentage** | **n (N)** |  **RR [95% CI]**  | ***P* value** |
| ***KRAS*****Mutations** | **No *KRAS*****Mutations** |
| Response rate (%)20, 41, 54, 75, 76, 78, 86, 119, 123, 125-127 | 3% | 24% | 12(1041) | 0.33 [0.18, 0.60] | ***P*<.001** |

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| --- | --- | --- | --- | --- |
| **Outcome** | **Mean ± SD** | **n (N)** | **WMD [95% CI]** | ***P* value** |
| ***KRAS*****Mutations** | **No *KRAS*****Mutations** |
| Time to Progression/ Progression Free Survival (months)20, 41, 54, 75, 91, 125, 126 | 3.4 + 2.7 | 5 + 3.7 | 7(918) | -1.84 [-2.99, -1.70] | ***P*=.002** |
| Median Overall Survival time (months)20, 41, 54, 75, 86, 125, 126 | 9.2 + 5.6 | 13.2 + 7.1 | 7(737) | -3.69 [-6.32, -1.06] | ***P*=.006** |

Abbreviations: CI, Confidence interval; n, Number of studies; N, Number of patients; WMD, Weighted mean difference Inverse-variance, Random effects [95% CI]; RR, Relative Risk, Mantel-Haenszel, Random Effects model, [95% CI]

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| **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 base: B** |
| **Consistency: B** |
| **Clinical impact: B** |
| **Generalizability: C** |
| **Applicability: B** |
| **Overall Grade: B** |

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| --- | --- |
| **Table 24: TKI Post-treatment detection of T790M** |  |
| **Patients with relapse after initial response to TKI treatment** |
| **Study or Subgroup** | **Events** | **Total** | **Percent** |
| Chen HJ, *et al*., 2009128 | 14 | 29 | 48% |
| Kosaka T, *et al*., 2006129 | 7 | 14 | 50% |
| Onitsuka T, *et al*., 2010130 | 7 | 10 | 70% |
| Oxnard, *et al*., 2011131 | 58 | 93 | 62% |
| **Total** | **86** | **146** | **59%** |

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| **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 base: B** |
| **Consistency: B** |
| **Clinical impact: B** |
| **Generalizability: B** |
| **Applicability: B** |
| **Overall Grade: B** |

**Table 25: Comparing *ALK* FISH with Immunohistochemistry (IHC)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  |  | **Concordance** | **Discordance** |
| **IHC Antibody** | **n(N)** | **FISH+/IHC+** | **FISH-/IHC-** | **FISH+/IHC-** | **FISH-/IHC+** |
|
| IHC - CD24657, 58, 132, 133  | 4(391) | 25 | 344 | 20 | 2 |
| IHC - D5F3/D9E4 60, 64, 132 | 3(148) | 46 | 101 | 1 | 0 |
| IHC - 5A463  | 1(640) | 28 | 602 | 0 | 10 |

Abbreviations: n, Number of studies; N, Number of patients

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| **9.2: Recommendation: RT-PCR is not recommended as an alternative to FISH for selecting patients for ALK inhibitor therapy**  |
| **Evidence base: B** |
| **Consistency: B** |
| **Clinical impact: B** |
| **Generalizability: B** |
| **Applicability: B** |
| **Overall Grade: B** |

**Table 26: Comparing *ALK* RT-PCR with other methods**

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| --- | --- | --- | --- | --- |
|  |  |  | **Concordance** | **Discordance** |
| ***ALK* RT-PCR vs. Other Methods** | **n(N)** | **RT-PCR+/ Other+** | **RT-PCR-/ Other-** | **RT-PCR-/ Other+** | **RT-PCR+/Other-** |
|
| IHC - CD246134  | 1(5) | 5 | 0 | 0 | 0 |
| IHC - D5F3132 | 1(10) | 9 | 0 | 1 | 0 |
| FISH58, 60, 132, 135 | 4(66) | 34 | 19 | 12 | 1 |
| Abbreviations: n, number of studies; N, pairs of specimens tested;  |  |  |  |  |  |
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|  **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.** |

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| **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.** |

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| **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.** |

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| **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.** |
| See recommendation 2.1a and 2.1b for data tables and grading. Only a few studies reported data on biomarkers other than *EGFR* and *ALK*. |

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| **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.** |

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| **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.** |

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

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| **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.**  |

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**Figure 1**

## Identification

## Eligibility

## Screening

Articles excluded, with reasons (n= 151)

Non-comparator studies,

Reviews or duplicate data,

No relevant data

Records after duplicates removed
(n =1533)

Additional records identified through other sources
(n = 165)

Records identified through database searching
(n = 1424)

Studies included for final grading

(n =127)

Studies included in qualitative synthesis
(n = 278)

Full-text articles assessed for eligibility
(n =521)

Records screened
(n = 1533)

## Included

Records excluded
(n =1012)

Full-text articles excluded, with reasons
(n =243)

Outside scope of paper,

Article does not provide primary data