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

Summary of 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 tyrosine kinase inhibitor 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 tyrosine kinase inhibitor 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.

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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 TKI?

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.

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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.

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