Commentary: ASCO Provisional Clinical Opinion—Epidermal Growth Factor Receptor Mutation Testing In Practice

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Introduction
Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) are commonly used in the management of non–small-cell lung cancer (NSCLC). Clinical predictors of response to these agents were identified years ago and include histologic subtype, smoking history, sex, and enrollment in trials in East Asian countries.1 More recently, molecular predictors of response have taken a more prominent role. The major impetus for ASCO’s Provisional Clinical Opinion (PCO)2 on molecular testing in NSCLC was the publication by Mok et al3 that reported the results of the Iressa Pan-Asia Study, or IPASS. ASCO asked the National Cancer Institute’s Physician Data Query Adult Cancer Editorial Board to conduct an assessment of this trial to inform the PCO. In addition, ASCO considered data from four other trials. This companion piece to the ASCO EGFR PCO is intended to summarize recent findings for the community oncologist and will focus on the specific role of EGFR mutation testing in the management of NSCLC. It should be noted that although the PCO is directed toward NSCLC as a whole, the vast majority of tumors harboring EGFR mutations are adenocarcinomas.2

The IPASS study highlighted the importance of EGFR mutation status in treatment-naive patients with advanced adenocarcinoma of the lung.3 The participants were selected for clinical characteristics suggestive of EGFR mutation–positive NSCLC. This study, described in the PCO, demonstrated that patients who tested positive for EGFR mutations who received gefitinib had superior progression-free survival (PFS), response rate, and a trend toward superior overall survival compared with similar patients who received carboplatin/paclitaxel. In contrast, patients who tested negative for EGFR mutations, but nevertheless had favorable clinical characteristics, had inferior PFS and response rates when treated with gefitinib compared with patients who tested negative for EGFR mutations who were treated with chemotherapy. On the basis of the IPASS data and supported by similar observations in other studies,4 the PCO states that “patients with advanced non–small-cell lung cancer who are being considered for first-line therapy with an EGFR-TKI (patients who have not previously received chemotherapy or an EGFR-TKI) should have their tumor tested for EGFR mutations to determine which is an appropriate therapy: an EGFR-TKI or chemotherapy.”2

Testing in the Community Setting
In the community setting, testing to determine patients’ EGFR mutation status has only recently become readily available. In sharp contrast to the reflex testing of breast cancer specimens for molecular markers such as estrogen receptor and human epidermal growth factor receptor 2 status, relevant information on EGFR mutation status is absent from the typical pathology report for pulmonary adenocarcinomas. Such testing is, however, now available separately and, at most community cancer centers, requires a separate order. Once ordered (provided sufficient tumor tissue exists), test results are often available within 2 weeks, as reported by Reddy et al7 and as consistent with clinical experience.

As described in the following paragraph, direct DNA mutation analysis has correlated with TKI response more consistently than other methods.8-9 In practice, such assays can be performed on fresh, frozen, or formalin-fixed paraffin-embedded tissue, and, although larger tissue samples are preferred, these tests may be done on tissue biopsy samples or cytology cell block preparations.10-12 Regardless of whether a specimen is a tissue biopsy or cytology cell block, the specimen will involve small amounts of material, and it is important for the pathologist to communicate with the clinician in order to maximize material for molecular testing. The ability to perform testing and the reliability of the results may be limited by the amount of material available; in addition, other preanalytic elements such as tumor viability, fixation, and variation in processing methods can be factors. It should be noted that formalin fixation is preferred, and fixatives containing heavy metal ions such as Bouin’s, Zenker’s, and B-5, as well as decalcified tissues should not be used because of the nucleic acid degradation caused by these fixatives. Time to fixation (cold ischemia time) should ideally be as short as possible. Further, it has been shown that the best results are obtained when fixation time in 10% neutral buffered formalin is between 6 to 12 hours for small biopsies and 8 to 18 hours for larger specimens. Further, lung adenocarcinomas are known to be histologically heterogeneous, and studies have demonstrated that different molecular profiles may not be obtained from morphologically different areas of the same tumor.8-13,14 Heterogeneity has also been observed between primary tumor and metastasis.15 Although a standardized, universally accepted approach to analysis is still lacking, it is important to be aware of these limitations, especially when dealing with small samples.

Activating mutations in EGFR exons 18 through 21 are the most reliable predictors of response to TKIs.8,9 Deletions in exon 19 and single L858R point mutations in exon 21 are the most common. D790M point mutations in exon 20 of EGFR, KRAS mutations, and MET amplification are negative predictors of response.8,9 Evaluation of EGFR status has been per-
formed by a variety of methods, which has unfortunately led to conflicting results in the literature.

Various methodologies have been utilized for EGFR assessment, including immunohistochemical staining, fluorescent in situ hybridization (FISH), chromogenic in situ hybridization (CISH), and DNA mutation analysis. FISH and CISH methodology measure EGFR gene copy number. An increase in EGFR gene copy number is frequently associated with EGFR mutation, and some studies evaluating EGFR copy number by FISH have shown a higher rate of TKI response in cases interpreted as FISH positive. Variances in scoring methodology, however, may lead to greater subjectivity in the reporting of FISH results. In addition, EGFR positivity by FISH does not appear to be restricted to tumors with EGFR mutations, but can also be seen in tumors with KRAS mutations or other mutations associated with resistance to TKI therapy. CISH has not been as extensively evaluated. It appears to have some advantages and disadvantages relative to FISH, although the significance of these differences is uncertain.

Immunohistochemical analysis for EGFR has not been proven to reliably predict response to TKI therapy, nor does it accurately correlate with mutation status. EGFR immunohistochemistry (IHC) results depend on a variety of factors and are influenced by the type of antibody used, producer used, and method of interpretation, among other factors. More recently, EGFR mutation–specific antibodies for IHC have been developed that appear promising; however, large-scale studies are still needed to determine their applicability to clinical practice.

Mutation analysis has most consistently correlated with response to TKI therapy. There is no consensus on which methodology is preferable. Mutation analysis can be evaluated by several methods, including direct sequencing, amplification refractory mutations systems (ARMS), length analysis, and denaturing high-performance liquid chromatography. All of the methods have advantages and disadvantages. Direct sequencing is widely used and detects all mutations, in contrast to ARMS, which is more sensitive but detects fewer mutations. Briefly, with sequencing techniques, an area of tumor is selected and genomic DNA is extracted, followed by amplification of the exons of interest by the polymerase chain reaction technique. Bidirectional direct sequencing of the amplified polymerase chain reaction products is then performed. Some laboratories perform sequencing only for exons 19 and 21, the most common locations for mutations, whereas others perform sequencing on exons 18 to 21. It should be noted that direct sequencing is most optimal when viable tumor cells constitute at least 25% or more of the sample. The recent European classification and other studies recommend at least 50% of the sample be composed of tumor; however, it should be noted that tumor enrichment by manual or laser capture microdissection may improve results in smaller samples, and more sensitive methods may detect mutations in specimens with as few as 10% tumor cells. It should also be noted that extremely sensitive methodologies may have more issues with contamination and false positives.

A recent study by Sholl et al retrospectively evaluated response to TKI therapy and correlated the response with EGFR status by DNA sequencing, FISH, CISH, and IHC methodologies. Of these methods, a statistically significant difference in response rate was observed only for those in which EGFR mutations were detected by mutation analysis, but not with the other methodologies. Similarly, this study also demonstrated an improved PFS correlated only with EGFR mutations detected by DNA analysis. These results are similar to those reported in previous studies. The results of the IPASS study also demonstrated improved response and PFS in patients with EGFR-mutated tumors as identified by DNA analysis. This study also demonstrated no correlation between PFS or response outcomes with protein expression detected by IHC, although patients with IHC-positive tumors did exhibit a greater PFS with gefitinib than patients with IHC-negative tumors. FISH-positive tumors were correlated with PFS and overall response rate. However, studies that evaluated patients of East Asian ethnicity have not shown a correlation between survival benefit with TKIs and EGFR positivity by FISH. Overall, the predictive significance of FISH has not yet been confirmed in a randomized phase III study.

On the basis of the evidence available thus far, mutation analysis has proven to be the most reliable methodology to evaluate for EGFR mutations that correlate with response to TKI sensitivity or PFS. Therefore, ASCO’s PCO recommends the use of EGFR mutation status testing in this setting and has recommended that assessment of EGFR gene copy number and EGFR expression not be routinely incorporated in management decisions.

Cost and Reimbursement Issues
Mutation testing is unquestionably expensive, and cost varies depending on the number of probes used (eg, evaluation of exon 19 and 21 only vs evaluation of 18 through 21), region of delivery, and other laboratory variables. EGFR mutation analysis currently costs approximately $650-$1,000. Although it is covered by many insurers and Medicare, it is not clear whether all insurers cover this testing. If there is any question in regard to coverage, confirmation should be made with the laboratory performing the testing. If EGFR mutation analysis is not covered in a region, it would be appropriate for oncologists and their state professional society to advocate for their patients with insurance companies on this matter, as EGFR mutation analysis may enable better care and avoidance of potentially ineffective therapies.

Additional Resources
To read the American Society of Clinical Oncology Provisional Clinical Opinion: Epidermal Growth Factor Receptor (EGFR) Mutation Testing for Patients with Advanced Non-Small Cell Lung Cancer Considering First-Line EGFR Tyrosine-Kinase (TKI) Inhibitor Therapy in its entirety, please visit www.jco.org/EGFRPCO
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Authors’ Disclosures of Potential Conflicts of Interest

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