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 al[3] 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 clinical 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 who 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,[4-6] 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 al,[7] 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 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.[16] 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.[17] 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.[18]

Immunohistochemical analysis for EGFR has not been proven to reliably predict response to TKI therapy, nor does it accurately correlate with mutation status.[8,9] 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.[8,9,19]

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.[8,9] 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.[8,9] 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[17] 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.[20,21] 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.[22] However, studies that evaluated patients of East Asian ethnicity have not shown a correlation between survival benefit with TKIs and EGFR positivity by FISH.[23] 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[2] 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 *v* evaluation of 18 through 21), region of delivery, and other laboratory variables. *EGFR* mutation analysis currently costs approximately $650-$1,000.[24] 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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References

1. Miller VA, Riely GJ, Zakowski MF, et al: Molecular characteristics of bronchioloalveolar carcinoma and adenocarcinoma, bronchioloalveolar carcinoma subtype, predict response to erlotinib. J Clin Oncol 26:1472-8, 2008

2. Keedy V, Temin S, Somerfield M, et al: 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. J Clin Oncol doi: 10.1200/JCO.201031.8923

3. Mok TS, Wu YL, Thongprasert S, et al: Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 361:947-57, 2009

4. Mitsudomi T, Morita S, Yatabe Y, et al: Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): An open label, randomised phase 3 trial. Lancet Oncol 11:121-128, 2009

5. Maemondo M, Inoue A, Kobayashi K, et al: Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med 362:2380-2388, 2010

6. Lee JS, Park K, Kim SW: A randomized phase III study of gefitinib versus standard chemotherapy (gemcitabine plus cisplatin) as a first-line treatment for never-smokers with advanced or metastatic adenocarcinoma of the lung. 13th World Conference on Lung Cancer, San Francisco, CA, July 31-August 4, 2009 (abstract PRS. 4)

7. Reddy SK: ERCC, TS, RRM1, EGFR, and KRAS biomarker integration into a community-based medical oncology practice. J Clin Oncol 28:486s, 2010 (suppl; abstract 6156)

8. Dacic S: EGFR assays in lung cancer. Adv Anat Pathol 15:241-247, 2008

9. Sholl LM, Lindeman NI: Molecular diagnostics testing for lung adenocarcinoma: State-of-the-art in 2010. Pathol Case Rev 15:103-110, 2010

10. Smouse JH, Cibas ES, Janne PA, et al: EGFR mutations are detected comparably in cytologic and surgical pathology specimens of nonsmall cell lung cancer. Cancer Cytopathol 117:67-72, 2009

11. Boldrini L, Gisfredi S, Ursino S, et al: Mutational analysis in cytological specimens of advanced lung adenocarcinoma: A sensitive method for molecular diagnosis. J Thorac Oncol 2:1086-1090, 2007

12. Nakajima T, Yasufuku K, Suzuki M, et al: Assessment of epidermal growth factor receptor mutation by endobronchial ultrasound-guided transbronchial needle aspiration. Chest 132:597-602, 2007

13. Dacic S, Shuai Y, Yousem S, et al: Clinicopathological predictors of EGFR/KRAS mutational status in primary lung adenocarcinomas. Mod Pathol 23:159-168, 2009

14. Nakano H, Soda H, Takasu M, et al: Heterogeneity of epidermal growth factor receptor mutations within a mixed adenocarcinoma lung nodule. Lung Cancer 60:136-140, 2008

15. Monaco SE, Nikiforova MN, Cieply K, et al: A comparison of EGFR and KRAS status in primary lung carcinoma and matched metastases. Hum Pathol 41:94-102, 2010

16. Cappuzzo F, Hirsch FR, Rossi E, et al: Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer. J Natl Cancer Inst 97:643-55, 2005

17. Chiosea S, Shuai Y, Cieply K, et al: EGFR fluorescence in situ hybridization-positive lung adenocarcinoma: Incidence of coexisting KRAS and BRAF mutations. Hum Pathol 41:1053-1060, 2010

18. Sholl LM, Xiao Y, Joshi V, et al: EGFR mutation is a better predictor of response to tyrosine kinase inhibitors in non-small cell lung carcinoma than FISH, CISH, and immunohistochemistry Am J Clin Pathol 133:922-934, 2010

19. Jeon YK, Sung SW, Chung JH, et al: Clinicopathologic features and prognostic implications of epidermal growth factor receptor (EGFR) gene copy number and protein expression in non-small cell lung cancer. Lung Cancer 54:387-398, 2006

20. Mitsudomi T, Kosaka T, Endoh H, et al: Mutations of the epidermal growth factor receptor gene predict prolonged survival after gefitinib treatment in patients with non-small-cell lung cancer with postoperative recurrence. J Clin Oncol 23: 2513-2520, 2005

21. Taron M, Ichinose Y, Rosell R, et al: Activating mutations in the tyrosine kinase domain of the epidermal growth factor receptor are associated with improved survival in gefitinib-treated chemorefractory lung adenocarcinomas. Clin Cancer Res 11:5878-585, 2005

22. Fukuoka M, Wu YL, Thongprasert S, et al: Biomarkers analyses from a phase III, randomized, open-label, first-line study of gefitinib (g) versus carboplatin/paclitaxel (C/P) in clinically selected patients (pts) with advanced non-small cell lung cancer (NSCLC) in Asia (IPASS). J Clin Oncol 27, 2009 (suppl; abstract 8006)

23. Sone T, Kasahara K, Kimura H, et al: Comparative analysis of epidermal growth factor receptor mutations and gene amplification as predictors of gefitinib efficacy in Japanese patients with nonsmall cell lung cancer. Cancer 109:1836-1844, 2007

24. Partners Healthcare: Price List & CPT Codes. http://pcpgm.partners.org/lmm/ordering/prices-CPTcodes