Molecular Testing in Lung Carcinoma

Quo vadis?

Sanja Dacic and Samuel A. Yousem

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The development of molecular targeted therapies with small-molecule inhibitors of the epidermal growth factor receptor (EGFR) has resulted in new therapeutic options for patients with advanced stage lung cancer. Certain clinical characteristics have been associated with an increased likelihood of response to EGFR tyrosine kinase inhibitors (TKIs; women, never smokers, East Asian ethnicity, and adenocarcinoma histology). Furthermore, several molecular events can also affect the efficacy of these therapies. Activating mutations in \( EGFR \) exons 18 through 21 (insert deletions in exon 19 and the single L858R point mutation in exon 21 being the most common) seem to be more reliable predictors of response than clinical features and are associated with increased response and survival after TKI therapy.\(^1\)-\(^3\) In contrast, \( K-ras \) mutations, insertion mutations or T790M point mutations in exon 20 of \( EGFR \), and \( MET \) amplification are negative predictors of response.\(^4\)-\(^6\) These observations have resulted in numerous retrospective studies that correlate patient response to the molecular profile of lung adenocarcinomas. Unfortunately, different methodological approaches have been used for EGFR assessment, including DNA mutational analysis, fluorescence in situ hybridization (FISH) or chromogenic in situ hybridization (CISH) for gene copy number changes, and immunohistochemical analysis for protein expression. Not surprisingly, reported results are inconsistent, reflecting the lack of standardization of the method and interpretation criteria. In general, \( EGFR \) FISH and DNA mutation analyses are the 2 most extensively studied methods for selection of candidates for EGFR TKI therapy.\(^1\)-\(^3\)

There is a large body of literature addressing the issue of usefulness of \( EGFR \) FISH or CISH in the selection of patients for EGFR TKI therapy.\(^7\)-\(^9\) Most of the confusion about the best choice between \( EGFR \) FISH or mutation arose from the observation that \( EGFR \) mutations are frequently associated with increased \( EGFR \) gene copy numbers. Cappuzzo et al\(^7\) demonstrated that 33% of cases interpreted as FISH+ had a higher response rate to gefitinib (36%) than FISH– cases (3%) and that there was a longer median survival (18.7 vs 7.0 months). Within the same cohort, 17% of cases were positive for the \( EGFR \) mutation, which was associated with a response rate of 53%, compared with 5% in wild-type cases.\(^7\) In contrast, in FISH studies in East Asian patients with non–small cell lung cancer (NSCLC) treated with EGFR TKIs, 41% of patients were FISH+, but FISH positivity was not associated with survival benefit, suggesting a difference between Asian and Caucasian ethnicity.\(^10\),\(^11\)

A recent study also demonstrated that \( EGFR \)-FISH positivity is not restricted to \( EGFR \)-mutated tumors, but can also occur in tumors with \( K-ras \) and other mutations that are well-known predictors of primary resistance to EGFR TKI therapy.\(^12\) These observations certainly reduce the significance of \( EGFR \)-FISH testing in patient selection. Immunohistochemical analysis for EGFR is suboptimal in determining a patient’s eligibility to receive EGFR TKI therapy. The most important variables precluding the use of immunohistochemical analysis for EGFR in clinical practice include the type and source of antibody and the scoring system used. Recent development of EGFR mutation–specific antibodies holds a promise for immunohistochemical studies, but large-scale studies should be done before this assay is considered for clinical practice.\(^13\),\(^14\)

There is a great interest in standardization of molecular testing in patients with lung carcinoma. However, several practical obstacles exist, including the availability of
appropriate tissue samples, technical and human resources, and the time required for analysis. Diagnostic lung cancer material is usually obtained by bronchoscopy or computed tomography–guided needle biopsy, frequently resulting in a very limited tissue material to be used for diagnostic and molecular assays. Therefore, the choice of an optimal method that will result in a high yield of prognostic and predictive information on limited yet sufficient material should be made. It seems that mutational analysis should be the “gold standard,” but despite growing evidence of its significance, mutational profiling has not yet been widely accepted or implemented in practice. Future mutational profiling across a wide range of genes is anticipated and will be integrated with expression and proteomic profiling to determine optimal therapeutic interventions.

In the June issue of the Journal, Sholl et al.\(^4\) reported the results of their retrospective study of the assessment of EGFR status by DNA sequencing, FISH, CISH, and immunohistochemical studies in 40 patients with advanced stage disease treated with erlotinib or gefitinib following failure of conventional chemotherapy as part of phase 1 and 2 clinical trials at the Dana Farber Cancer Institute, Boston, MA. The aim of the study was to determine which method of EGFR analysis best predicts response to TKI therapy in patients with advanced NSCLC. Statistically significant differences in response rates were observed between tumors with EGFR mutations (12/19) and EGFR wild-type tumors (1/20). There was no difference in response rates between FISH+ (7/19) and CISH+ (5/16) and FISH− (4/17) and CISH− (6/21) tumors. No differences were observed with respect to EGFR protein expression determined by immunohistochemical studies. Sholl et al.\(^4\) then looked into clinical outcome and confirmed that EGFR mutations were associated with improved progression-free survival, consistent with results from prior studies. Increased gene copy number (FISH or CISH) and EGFR protein expression (immunohistochemical studies) did not independently predict outcome.

Sholl and colleagues\(^4\) have performed a timely study that presents supporting evidence that mutational profiling of lung carcinoma can be of clinical significance. Notable limitations of the study are the small number of study subjects and single gene analysis, especially because the EGFR-nomutated group may contain mutations that predict a negative response. However, similar observations were reported in a large prospective randomized clinical trial. Recently published results of the large international trial, the Iressa NSCLC Trial Evaluating Response and Survival vs Taxotere demonstrated that EGFR mutations identify good responders to gefitinib and longer progression-free survival compared with docetaxel.\(^8\) A high EGFR copy number assessed by FISH also identified patients with a greater response to gefitinib compared with docetaxel, but unlike patients with EGFR mutations, there was no benefit in progression-free survival. Similar to other large trials, a lack of overall survival benefit was observed.\(^19,20\) Taken together, large- and small-scale studies provide supporting evidence that the presence of EGFR mutations best identifies patients who would benefit from EGFR TKI therapies, as measured by response rate and progression-free survival, especially when paired with other informative genes. It is certain that molecular targeted therapies are not effective in unselected groups of patients with NSCLC. One would hope that we have learned a lesson from our experience with breast carcinoma. It took us more than a decade to standardize methods and interpretation criteria for the assessment of HER2 status in patients with breast cancer and to focus on early biomarker identification, which resulted in significant improvement in cure rates after the use of trastuzumab in the adjuvant setting. Lung cancer is still the leading cause of death among all cancers, and we cannot afford not to offer a standard method of EGFR or other genetic testing to patients with lung cancer. It is time to admit that EGFR mutational analysis of lung carcinomas should be the standard of care.

From the Department of Pathology, University of Pittsburgh Medical Center Health System, Pittsburgh, PA.

References


