Advances in Treatment of Lung Cancer With Targeted Therapy

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• Context.—Ongoing preclinical investigations and clinical trials involving new targeted therapies promise to improve survival for patients with lung cancer. Targeted therapeutic agents, based on genetic mutations and signaling pathways altered in lung cancer, have added significantly to our armamentarium for lung cancer treatment while minimizing drug toxicity. To date, 4 targeted therapies have been approved for treatment of lung cancer by the US Food and Drug Administration: gefitinib in 2002, erlotinib in 2003, bevacizumab in 2006, and crizotinib in 2011.

Objective.—To review targeted therapies in lung cancer, the molecular biomarkers that identify patients likely to benefit from these targeted therapies, the basic molecular biology principles, selected molecular diagnostic techniques, and pathologic features correlated with molecular abnormalities in lung cancer. To review new molecular abnormalities described in lung cancer that are predictive for response to novel promising targeted agents in various phases of clinical trials.

Data Sources.—Review of the literature covering the molecular abnormalities of lung cancer with a focus on the molecular diagnostics and targeted therapy. Special emphasis is placed on summarizing evolving technologies useful in the diagnosis and characterization of lung cancer.

Conclusions.—Molecular testing of lung cancer expands the expertise of the pathologist, who will identify the tumor markers that are predictive of sensitivity or resistance to various targeted therapies and allow patients with cancer to be selected for highly effective and less toxic therapies.


Lung cancer was the most commonly diagnosed cancer in the world as well as the leading cause of cancer death in men globally in 2008. Among women, worldwide, it was the fourth most commonly diagnosed cancer and the second leading cause of cancer death. Globally, the overall lifetime risk of lung cancer is about 1 in 13 for men and 1 in 16 for women. The risk is significantly higher for smokers and lower for nonsmokers. Lung cancer is one of the most challenging cancers to treat and the standard therapy includes surgical resection, platinum-based chemotherapy, and radiation therapy alone or in combination. Unfortunately, the prognosis of patients with lung cancer (5-year overall survival rate of 15%) has not changed dramatically in the past 30 years with these conventional therapeutic approaches.

Although therapeutic improvements seen in the clinical trials for lung cancer are modest, the discovery of the first small-molecule inhibitor in lung cancer in the past decade brought hope that an increasing number of agents will be available in the near future to reduce suffering and mortality in patients with this devastating disease. In the last 10 years, 4 targeted therapies have been approved for treatment of lung cancer: gefitinib in 2002, erlotinib in 2003, bevacizumab in 2006, and crizotinib in 2011.

This review focuses on the targeted therapies in lung cancer: the molecular biomarkers that help identify patients likely to benefit from these targeted therapies, the basic molecular biology principles behind these therapies, selected molecular diagnostic techniques, and the pathologic features correlated with molecular abnormalities in lung cancer. Lastly, we discuss predictive biomarkers and their corresponding drugs that are currently under investigation in various phases of clinical trials.

Investigation and analysis of lung cancer for particular abnormalities expands the expertise of the pulmonary oncologic pathologist, who, in addition to conventional pathologic analysis of surgical lung specimens, will determine predictive biomarkers for lung cancer targeted therapies.

MOLECULAR ALTERATIONS AND TARGETED THERAPIES IN LUNG CANCER

In the past years, with the development of new targeted therapies, tremendous efforts have been directed toward identifying potentially druggable molecular alterations, especially against known activating mutations. Although numerous mutations have been described in lung adenocarcinoma, the mutation status remains unknown in more than 50% of cases. To date, we can identify therapeutic targets in only 20% of lung cancers.
Genotype-Phenotype Correlations in Lung Cancer

Adenocarcinoma is the most frequent cell type of lung cancer, accounting for more than 50% of cancers in the most recent series. To date, most validated and investigational predictive biomarkers have been identified in adenocarcinoma, as compared to other cell types, and a new subtype classification of adenocarcinoma has been proposed by the International Association for the Study of Lung Cancer, American Thoracic Society, and the European Respiratory Society, which takes into account the molecular pathology of these tumors. The current classification of lung adenocarcinoma by the World Health Organization recognizes several distinct morphologic subtypes of adenocarcinoma: papillary, acinar, solid, and lepidic.11 Most lung adenocarcinomas exhibit combinations of morphologic patterns.12-14 While the biologic basis for the histologic subtypes remains an area of active investigation,14 there is evidence that some subtypes may be associated with specific molecular alterations14-19 or a better outcome.20-22

Targeted Therapies in Lung Cancers With Epidermal Growth Factor Receptor Abnormalities

Epidermal Growth Factor Receptor.—Recognized mechanisms of epidermal growth factor receptor (EGFR) gain of function in non–small cell lung carcinoma (NSCLC) include somatic activating mutations in the exons encoding the tyrosine kinase domain and EGFR gene amplification.23,24 The EGFR mutation status is best determined by gene sequencing abnormalities of EGFR; status may also be observed with gene copy number determined by fluorescence in situ hybridization (FISH) or chromogenic in situ hybridization and with protein expression determined by immunohistochemistry with mutation-specific antibodies.

Several mutations have been recently described in the tyrosine kinase domain of EGFR.23,25 Epidermal growth factor receptor is expressed in 50% of NSCLCs, and its expression is correlated with poor prognosis.26 These 2 factors make EGFR and its family members prime candidates for the development of targeted therapeutics.27 EGFR kinase domain mutations target 4 exons (18–21), which encode part of the tyrosine kinase domain (the entire kinase domain is encoded by exons 18–24) and are clustered around the adenosine triphosphate–binding pocket of the enzyme.28-32 EGFR gene amplification is detected in some EGFR mutation–positive patients as well, and it is reported to be associated with disease progression.33 A subset of lung adenocarcinomas shows activation of growth factor receptor (EGFR) by mutations and/or amplification but the interaction between them is complex and unclear. Using novel techniques, including EGFR mutation–specific immunohistochemistry, Sholl et al34 studied a unique cohort known to have a high prevalence of EGFR mutations; the authors showed that EGFR-amplified lung adenocarcinomas have distinct genetic alterations, unique clinicopathologic features, and worsened prognosis. Furthermore, EGFR amplification and EGFR mutations are heterogeneously distributed within any given tumor. These are novel and important findings with implications for the efficacy of treatment with tyrosine kinase inhibitors for patients with EGFR-mutant lung adenocarcinoma.35 Recent discoveries have described EGFR mutation–specific antibodies that could help in the rapid screening of lung cancers with EGFR mutations (Figure 1).36

Mutations in the tyrosine kinase domain of the epidermal growth factor receptor have prognostic significance, since patients with EGFR-mutant NSCLC have prolonged disease-free survival, compared with those with wild-type disease, regardless of the treatment received.4,5,6 Although EGFR mutations are predictive of response to EGFR tyrosine kinase inhibitor (TKI) therapy, they do not appear to be predictive of a differential effect on survival.37

Targeted Agents Against Lung Cancer With EGFR Mutations.—The 2 TKI agents currently approved for use in lung cancer, which target lung cancer with EGFR mutations, are gefitinib (2002) and erlotinib (2003). EGFR mutation is a specific target for therapy by TKIs and is a validated biomarker of treatment response. The clinical utility of this biomarker is supported by prospective clinical trials that have demonstrated a progression-free survival benefit of TKI as first-line therapy in EGFR-mutant patients.38 Based on current data, predictive biomarker tests for EGFR should involve mutational analysis. Mutation-specific antibodies for EGFR can be used for screening, but negative immunohistochemistry findings will require mutational analysis to exclude uncommon EGFR mutations that are not detected by these antibodies. EGFR FISH testing is less predictive of TKI response rate than mutation testing in clinical studies, and currently should not be used as a method for EGFR TKI treatment selection.

Resistance to TKI therapy is associated with KRAS mutation and specific acquired EGFR mutations such as T790M.39 These molecular events, as well as other genetic alterations in c-Met (amplification), ERBB3 (overexpression), and epiregulin (autocrine loop activation), account for approximately 50% of cases of TKI resistance.32,36-41

Genotype-Phenotype Correlations.—For patients with lung adenocarcinoma treated with erlotinib and gefitinib, favorable responses were associated with adenocarcinoma with lepidic patterns.42 This finding led to trials of gefitinib and erlotinib that showed that 17% to 22% of patients had a response to gefitinib.43-45 The relationship of EGFR mutation status with adenocarcinoma subtype is a matter of intense debate.44-46 Genetic abnormalities can be seen in different histologic subtypes, although with various frequency. One characteristic correlation is that mucinous adenocarcinoma (Figure 2) may be exclusively thyroid transcription factor 1 (TTF1) negative and EGFR-mutation negative, but it may have Ras mutation and expresses CDX2, possibly because of its presumed derivation from bronchiolar mucinous goblet cells.15

Targeted Therapies With Angiogenesis Inhibitors in Nonsquamous NSCLC

Recent studies show that NSCLCs with histologic profiles other than squamous cell carcinoma appear to be more strongly associated with response to treatment with bevacizumab. Bevacizumab (Avastin, Genentech/Roche, South San Francisco, California) is a monoclonal antibody with high affinity for vascular endothelial growth factor (VEGF). Despite the potential benefit of bevacizumab for some patients with previously untreated advanced NSCLC,46,47 the appropriate clinical setting for the use of this antiangiogenic agent is stringent, owing to safety issues raised in patients with lung
squamous cell carcinoma (SCC), and requires an accurate diagnosis of the pretreatment biopsy specimens. The clinical activity of bevacizumab in inoperable, locally advanced, metastatic, or recurrent NSCLC was first shown in chemotherapy-naive patients. Patients with nonsquamous NSCLC histology are the only patients who benefit from treatment with bevacizumab in combination with chemotherapy. Bevacizumab is currently contraindicated in patients with SCC on the basis of the results of a recently published phase II trial in which 31% of patients with SCC histology developed a life-threatening or fatal hemoptysis associated with bevacizumab, although it is still not clear whether histology alone is the reason for increased bleeding risk. Excluding patients with SCC appeared to markedly limit the risk of life-threatening bleeding complications associated with bevacizumab.

Targeted Therapies in Lung Cancers With Anaplastic Lymphoma Kinase Abnormalities

On August 26, 2011, the US Food and Drug Administration approved crizotinib for the treatment of patients with locally advanced or metastatic NSCLC that is positive for anaplastic lymphoma kinase (ALK) by FISH (Figure 3). Anaplastic large cell lymphoma kinase gene (ALK) was originally identified through cloning of the t(2;5)(p23;35) translocation found in a subset of anaplastic large cell lymphomas (ALCLs), a tumor of T-cell lineage. ALK encodes a tyrosine kinase receptor that is normally expressed only in select neuronal cell types. In ALK-rearranged ALCLs, the intracytoplasmic portion of ALK is fused to the N-terminal portion of nucleophosmin (NPM), resulting in a chimeric protein with constitutive kinase activity. Several other balanced translocations

Figure 1. Using recently described EGFR exon 19 deletion–specific antibody, one is able to directly visualize the location of tumor cells with EGFR exon 19 deletion mutations and to show heterogeneity in receptor overexpression among different tumor cells (EGFR exon 19 deletion–specific antibody, original magnification ×200). Abbreviation: EGFR, epidermal growth factor receptor.

Figure 2. Mucinous adenocarcinoma is exclusively TTF1 negative and EGFR mutation negative but may have Ras mutation (hematoxylin-eosin, original magnification ×100). Abbreviations: EGFR, epidermal growth factor receptor; TTF1, thyroid transcription factor 1.

Figure 3. Identification of lung cancers with chromosomal translocations involving ALK requires fluorescence in situ hybridization on formalin-fixed, paraffin-embedded tumor tissues with a break-apart probe to the ALK gene (original magnification ×1000). Abbreviation: WT, wild-type.

Figure 4. Most ALK-rearranged adenocarcinomas had a distinct histologic appearance represented by solid tumor growth and frequent signet ring cells with abundant intracellular mucin (hematoxylin-eosin, original magnification ×100).
involving ALK have been discovered in ALCLs; however, the various resulting chimeric proteins all retain the ALK kinase domain. The importance of the kinase activity is exemplified by ALK-rearranged ALCL cell lines, which are dependent upon ALK enzymatic activity for growth and survival.

Recently, ALK rearrangements were identified in rare NSCLC cell lines and in isolated primary adenocarcinomas from Japanese and Chinese populations. Most ALK rearrangements within NSCLCs derive from an interstitial deletion and inversion in chromosome arm 2p and result in the EML4-ALK fusion gene product. Murine tumors, human cell lines, and a recently published clinical trial have shown that lung cancers expressing EML-ALK are sensitive to inhibitors of ALK kinase activity. Thus, it is critical to efficiently and accurately identify those lung adenocarcinomas that harbor ALK rearrangements in routine practice in order to guide the appropriate clinical therapy.

None of the ALK-rearranged adenocarcinomas showed coexistent mutations in EGFR. Recently published studies show that ALK-rearranged adenocarcinomas are more likely to present in younger patients with a history of never-smoking, and at higher stage, relative to those without ALK rearrangements (ALK germline). Most ALK-rearranged adenocarcinomas had a distinct histologic appearance represented by solid tumor growth and frequent signet ring cells with abundant intracellular mucin (Figure 4).

The developing evidence-based guideline recommendations of the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology for the molecular testing of lung cancers will likely conclude that (1) ALK rearrangements should be typically assessed by molecular cytogenetic techniques such as FISH and (2) the currently commercially available ALK monoclonal antibodies may potentially help in screening lung cancers for ALK rearrangements. However, some antibodies have poor performance in identifying ALK-rearranged lung cancers; some antibodies are very good, but not commercially available; and some hold promise, but the published data and the number of cases tested are limited. Therefore, it is premature to make recommendations stating that immunohistochemistry is useful in identifying cases with ALK rearrangements. In the meantime, FISH is the recommended test for ALK.

Other Molecular Abnormalities That Show Promise for Targeted Therapies in Lung Cancer

Human Epidermal Growth Factor Receptor 2.—Unlike the other members of the human epidermal growth factor receptor (HER) family, HER2/neu is not strictly a receptor tyrosine kinase because no high-affinity endogenous ligand has been identified. HER2/neu acts as a signaling network coordinator and amplifier when it forms heterodimers with other HER family members. HER2/neu mutations occur in 2% of NSCLCs. These are in-frame insertions in exon 20 that target the corresponding tyrosine kinase domain region, as in EGFR-insertion mutations. These mutations occur in the same subpopulation as that with EGFR mutations (adenocarcinoma, never-smoker, East Asian, and women). Although HER2/neu mutations occur in only 2% of patients, HER2/neu is frequently overexpressed (to some degree) in NSCLC and appears to be associated with drug resistance, increased metastatic potential, increased production of VEGF, and poor prognosis. HER2/neu-mediated resistance to DNA-damaging agents requires the activation of Akt, which phosphorylates murine double minute 2 (MDM2) and therefore enhances MDM2-mediated ubiquitination and degradation of p53. Blocking the Akt pathway mediated by HER2/neu increases the cytotoxic effect of DNA-damaging drugs in tumor cells with wild-type TP53. Furthermore, recent studies have shown that the G/G genotype of the MDM2 polymorphism is associated with worse overall survival among patients with early-stage NSCLC, particularly those whose tumors have squamous cell histology.

Trastuzumab is a chimerized monoclonal antibody against HER2/neu. Combinations of trastuzumab and chemotherapy are well tolerated, with response rates of 21% to 40%. One trial showed that patients whose tumors highly overexpressed HER2/neu (3+) by immunohistochemistry or showed evidence of amplification by FISH had a good response. It appears that highly overexpressing HER2/neu cases of NSCLC (3+ by immunohistochemistry), although relatively infrequent (3%–9%), may show benefit with treatment with trastuzumab.

MET Proto-oncogene.—MET can be activated by mutations, autocrine/paracrine growth, overexpression by gene amplification, or decreased degradation. Germ-line and somatic MET gene mutations have been reported in hereditary and sporadic papillary renal cell cancers. In other cancers, MET gene mutations and amplifications have been reported to be predictors of response to therapy. Expression of MET and phospho-MET has been studied in lung cancer; recently, 40% of lung cancer tissues were shown to overexpress MET. Recent studies have shown that survival for patients with NSCLC who have 5 or more copies/cell is worse than for those who have less than 5 copies/cell; moreover, MET gene amplification leads to EGFR tyrosine kinase resistance in EGFR-mutant patients. Anti-hepatocyte growth factor antibodies, anti-MET antibodies, and small-molecule MET TKI inhibitors are all in various stages of development, and elucidation of predictive biomarkers for MET inhibitors will be important for future trials and treatment decisions.

OTHER TARGETED MOLECULAR THERAPIES

There has been tremendous research and investment in the development of small molecules that target key proteins in cell signaling pathways that are aberrantly altered in disease, particularly in carcinogenesis. For instance, receptor tyrosine kinases (RTKs) serve as potential therapeutic targets in several solid tumors, including lung cancer. The RTK c-KIT is highly expressed in small cell lung carcinomas (although it is not mutated), and this has led to clinical trials with the specific c-KIT inhibitor ST1571 (Gleevec [imatinib], Novartis, East Hanover, New Jersey), alone and in combination therapy. However, these trials have failed to show a meaningful benefit from the imatinib treatment. Antibodies against the angiogenic factor VEGF and small molecules against VEGF receptors, such as SU5416 (an inhibitor of Flk-1 receptor), are being tested in NSCLC and other tumor types. More recently, modification of gene expression with small interfering RNAs has the promise of being the most powerful tool yet.
CONCLUSION

Surgical excision remains the only therapeutic modality that can cure selected patients with lung cancer. Pathologists play an important role in the surgical management of patients with lung cancer, from preoperative diagnosis and staging to intraoperative evaluation and postoperative assessment of tumor genetic alterations. With the design of new targeted therapies, pathologists are required to identify the “targeted” population or the subset of patients who benefit most from these novel therapies.

The clinical application of molecular diagnostic techniques has allowed a more precise and rapid assessment of lung cancer and will help to triage the patient to “personalized” therapies that will have the highest success rates for eradicating the tumor. Our knowledge about lung cancer has changed radically in the past decade, and progress mainly depends on identifying new predictive biomarkers. We need to better understand both the tumor and the host biology that underlies tumor sensitivity and resistance in order to provide a rationale for specific targeted therapy. Since many targets can be evaluated by multiple laboratory methods, such as sequence analysis, in situ hybridization, and immunohistochemistry, it is critical that efforts focus on standardizing methodologies for biomarker testing.

References


