Lung adenocarcinoma: guiding EGFR-targeted therapy and beyond

Marc Ladanyi and William Pao

Departments of Pathology and Medicine, and Human Oncology and Pathogenesis Program, Memorial Sloan-Kettering Cancer Center, New York, NY, USA

Somatic mutations in certain tyrosine kinases have emerged as central ‘drivers’ of specific cancers and these mutant proteins are proving to be excellent substrates for targeted therapies. This is the case for mutant EGFR-dependent lung adenocarcinomas, where *EGFR* mutation testing is already being used to help guide treatment decisions. Here, we provide an overview of the biology of EGFR-targeted therapies and the clinical experience to date, the positive and negative predictors of response, pathologic correlates of EGFR-mutant status, testing methods to establish patient eligibility for these agents, and the basis for primary and secondary resistance. *Modern Pathology* (2008) 21, S16–S22; doi:10.1038/modpathol.3801018

Keywords: EGFR; KRAS; BRAF; non-small cell lung carcinoma

Overview of EGFR biology and clinical correlations

Lung cancer is the leading cause of cancer deaths in both men and women in the United States. Over the past two decades, adenocarcinoma has replaced squamous cell carcinoma as the most common subtype of non-small cell lung cancer (NSCLC) in the United States.

The epidermal growth factor receptor (EGFR, HER-1/ErbB1) is a receptor tyrosine kinase (TK) of the ErbB family, which consists of four closely related receptors: HER-1/ErbB1, HER-2/neu/ErbB2, HER-3/ErbB3 and HER-4/ErbB4. Upon ligand binding and receptor homo- or hetero-dimerization and activation (phosphorylation), activated EGFR signals downstream to the PI3K/AKT and RAS/RAF/MAPK pathways (Figure 1). These intracellular signaling pathways regulate key cellular processes such as proliferation and apoptosis.

The expression of EGFR by some lung cancers, the limited therapeutic options for advanced lung cancer, and the availability of new EGFR-targeted drugs led, in the early part of the decade, to clinical trials of small molecule EGFR TK inhibitors in unselected NSCLC patients. Although tumors in the vast majority of patients failed to respond, a minority showed dramatic tumor shrinkage accompanied by symptomatic improvement. Such responses were

noted to be more common in East Asians, in women, and in patients with adenocarcinomas, especially those with areas of bronchioalveolar carcinoma (BAC).¹ These observations then led to three landmark 2004 studies that showed that the tumors that responded to the EGFR TK inhibitors (TKIs) gefitinib and erlotinib contain somatic mutations in the *EGFR* TK domain.^{2–4} The two most common *EGFR* mutations are short in-frame deletions of exon 19 and a point mutation (CTG to CCG) in exon 21 at nucleotide 2573, that results in substitution of leucine by arginine at codon 858 (L858R). Together, these two types of mutations account for ~90% of all *EGFR* mutations in NSCLC. Other recurrent but far less common *EGFR* mutations known to be associated with sensitivity to EGFR TKIs include the G719 mutations in exon 18 and the L861 mutations in exon 21. Screening for common *EGFR* mutations in patients with lung adenocarcinomas can now be performed in clinical molecular diagnostic laboratories to predict which patients will respond to EGFR TKIs.⁵ It can be performed on archival material as well as on fine-needle biopsies.⁶

Many other rare *EGFR* mutations have been reported in lung cancers, but the majority of such rare mutations are of much lesser clinical significance for three reasons. First, their association with response to EGFR TKIs has not been established. Second, many mutations that have been reported only once (ie, non-recurrent) may be technical artefacts arising from PCR and sequencing of DNA extracted from FFPE samples⁷ (such artefacts can be guarded against by performing the PCR and sequencing in duplicate). Third, rare sequence alterations may actually represent rare germline polymorphisms

Correspondence: Dr M Ladanyi, MD, Department of Pathology, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10065, USA.
 E-mail: ladanyim@mskcc.org
 Received 20 November 2007; accepted 26 November 2007

Figure 1 Schematic of EGFR signaling pathway. Erlotinib and gefitinib block signaling at the level of the kinase domain (K). They are ineffective if the signaling pathway is activated by mutations downstream of this point, such as in *KRAS* or *BRAF*.

because this possibility has not been excluded in all cases (to exclude this possibility, a normal DNA sample from the patient is sequenced).

In multiple recent prospective clinical trials of gefitinib or erlotinib, response rates of *EGFR* mutated cases range from 65 to 90%.^{8–13} Survival data from these studies are not yet mature, but in many instances, the median overall survival rates were ‘not reached’ at the time the data were reported. In most retrospective studies, patients with *EGFR* mutations and a response to gefitinib or erlotinib show a longer progression-free survival and a trend toward improved overall survival vs patients whose tumors have wild-type *EGFR*. Whether the longer survival observed in these studies is due to an intrinsically more favorable biology of lung cancers with *EGFR* mutations, or to the treatment with *EGFR* TKIs, is still somewhat controversial, but there is an emerging consensus that both factors are contributing to this longer overall survival. Indeed, a recent study found that *EGFR* mutations are associated with a modest but statistically significant survival benefit in patients with resectable lung adenocarcinoma (not treated with *EGFR* TKIs).¹⁴ Thus, *EGFR* mutations may have both predictive and prognostic value.

Pathologic correlates of *EGFR* mutations

Historically, the practical utility of NSCLC as a clinical entity reflected the lack of distinct treatment regimens for the different histologic types encompassed by this term. With the advent of targeted therapy based on *EGFR* mutations in lung adenocarcinomas, it is evident that the histologic subtype of NSCLC should be explicitly reported. *EGFR* mutations are extremely rare in large cell carcinomas, small cell carcinomas, and pure squamous carcinomas, but have been reported in adenosquamous carcinomas.¹⁵ Indeed, it may be that the very

rare squamous carcinomas reported to harbor mutations were inadequately sampled adenosquamous carcinomas. There have also been two cases reported of *EGFR*-mutant small cell carcinoma preceded by an adenocarcinoma with the same mutation.^{16,17} Incidentally, another recent development that supports the retirement of the term ‘NSCLC’ is that bevacizumab (Avastin) is associated with a high risk of bleeding in squamous lung cancers¹⁸ and therefore, has been approved only for use in ‘non-squamous’ NSCLCs.

Among adenocarcinomas, *EGFR* mutations are more prevalent in cases with BAC features.¹⁹ Interestingly, the observation that BAC features are clinically significant was made before the discovery of *EGFR* mutations, based on the association of adenocarcinomas with BAC features with responses to *EGFR* TKIs.^{1,20} It should be noted that lung adenocarcinomas with a minor or major BAC component are not well delineated in the current WHO classification and therefore strict adherence to WHO categories may reduce the likelihood of making such observations. Microdissection studies have shown that *EGFR* mutations are present in BAC-like areas as well as more solid areas, suggesting that these are early events in these tumors.¹⁹ This notion is reinforced by a recent report describing *EGFR* mutations in a third of lesions diagnosed as atypical adenomatous hyperplasia.²¹

EGFR mutation detection vs *EGFR* FISH/CISH in the determination of *EGFR* status

There has been an ongoing debate regarding the most appropriate testing approach for establishing ‘*EGFR* status’ in lung adenocarcinomas, mutation analysis or gene copy number analysis (by FISH or CISH).^{22–24} The relative importance of these two types of genetic alterations has been difficult to tease out because of their frequent co-occurrence. In our experience, among *EGFR*-mutated cases, approximately 50% also show increased *EGFR* gene copy number, and conversely, approximately 75% of cases with increased *EGFR* gene copy number show mutations.²⁵ These numbers vary in different series due to different scoring systems for gene copy number alterations and mutation detection techniques of different sensitivity (see below). There is now mounting evidence from multiple types of analyses supporting *EGFR* mutation status as a more rational and biologically relevant marker for treatment selection.

- (1) Mutant *EGFR* is biologically linked to ligand-independent increased downstream signaling, unlike simple overexpression of native *EGFR*.
- (2) When both alterations are present, it is the mutated *EGFR* allele that is amplified preferentially.^{25,26} This suggests that in cases with both mutation and amplification, it is the biological advantage provided by the mutation that drives selection for copy number gains.

- (3) *EGFR* mutations are more closely linked to known risk factors (negative association with smoking) and other biological features (female sex, Asian origin) than is *EGFR* amplification.²⁵
- (4) Response rates to *EGFR* TKIs are high in *EGFR*-mutated cases regardless of *EGFR* copy number. In contrast, *EGFR*-amplified cases that lack mutations have very low response rates, in the order of 8%.²⁷
- (5) In several recent studies, it has been shown that *EGFR* mutation status is a better predictor of outcome in patients treated with *EGFR* TKIs than *EGFR* copy number.^{28–30} The failure of some earlier studies to detect survival benefits may have been due to the fact that they were statistically underpowered with respect to *EGFR*-mutant cases.

Part of the explanation for the survival benefit seen in *EGFR* amplified tumors apparently lacking *EGFR* mutations may be the following. Because FISH or CISH for *EGFR* amplification is a morphologic method (ie, non-neoplastic cells are not counted) whereas sequencing for mutation analysis is prone to false negatives due to excess non-neoplastic cells, it is likely that at least some *EGFR*-amplified cases reported to be mutation-negative based on direct sequencing have harbored mutations that would have been detected by more sensitive methods. Indeed, direct sequencing is prone to miss *EGFR* mutations (see below). Nonetheless, the situation remains complex because the subset of patients with tumors that lack detectable *EGFR* mutations but show increased *EGFR* copy number do show improved survival on *EGFR* TKIs despite the lack of radiologic response, compared to *EGFR* non-mutant tumors without *EGFR* amplification.^{27,29,31}

Clinical heterogeneity of *EGFR* mutations

As mentioned above, some mutations are clearly associated with favorable responses to *EGFR* TKIs, whereas others are associated with poor responses or outright resistance to these same drugs. Thus, the relatively uncommon in-frame insertions in exon 20 are associated with primary resistance to *EGFR* TKIs. Interestingly, there may be differences even between the two main sensitivity mutations. Two retrospective studies have compared the outcome of *EGFR* TKI-treated patients based on the type of mutation present, namely exon 19 deletions or exon 21 L858R point mutation, and both found that the former group had a significantly better overall survival.^{32,33}

Criteria for selecting cases for *EGFR* testing and the pathologist's role

On the basis of the above considerations, the surgical pathologist's role in *EGFR* testing is to ensure that the

testing is performed only in lung adenocarcinomas (or adenosquamous carcinomas), unless specifically requested by the oncologist. Among adenocarcinomas, some would suggest to exclude the mucinous subtype of BAC, as this special subtype is associated with *KRAS* mutations and not *EGFR* mutations, unlike non-mucinous BAC.^{34,35} Blocks or unstained sections submitted for DNA extraction and mutation analysis should be the most cellular available, preferably restricted to areas with >50% tumor cells. To ensure this, in many cases, further microdissection of the sections needs to be performed in the molecular diagnostic laboratory.

Importantly, *EGFR* IHC plays little or no role in selecting patients for *EGFR* mutation testing as it is only weakly correlated with the presence of mutations, if at all. Given this, it is not surprising that *EGFR* IHC positivity has generally been found of limited use in predicting response to *EGFR* TKIs.³⁶ Ironically, despite multiple publications questioning the significance of *EGFR* IHC, the Tarceva (erlotinib) product insert still emphasizes its importance.

Common methods for *EGFR* and *KRAS* mutation analysis

Initially, most large series used direct sequencing to detect these mutations. It is known that direct sequencing is likely to miss mutations when the tumor cells make up less than 25% of the sample. In some cases with *EGFR* mutations, this limit is somewhat lower because the mutant allele may be present in multiple copies per tumor cell (concurrent amplification). More recently, a number of studies of *EGFR* in lung cancer have employed more sensitive mutation detection techniques, including mutation-specific PCR assays,^{37,38} PCR with hybridization in real-time with mutation-specific fluorescent probes,^{39,40} single-strand conformational polymorphism,³⁵ denaturing high-performance liquid chromatography,^{41,42} peptide nucleic acid-locked nucleic acid PCR clamp¹¹ and more experimental techniques such as mass spectrometry and single molecule sequencing.⁴³ These have been recently reviewed.⁴⁴ Almost all such studies find a few cases with mutations that were missed by direct sequencing. The technical concern of detection sensitivity may be heightened for the secondary resistance mutations (see below).

Mutations in *KRAS*, *BRAF* and *ERBB2* as predictors of primary resistance to *EGFR* inhibitors

It is now clear that mutations in *EGFR*, *KRAS*, *BRAF* and *ERBB2* are essentially mutually exclusive in lung adenocarcinoma^{19,45–49} (Figure 2). This reflects the fact that they represent alternative ways of oncogenically activating some of the same downstream pathways, including the PI3K–AKT pathway

Figure 2 Pie chart of known activating mutations in signaling molecules in lung adenocarcinoma. The dates the mutations were discovered in human lung cancer are indicated. These mutations are mutually exclusive with the exception of *PIK3CA*. Data on *EML4-ALK* prevalence and relationship to other mutations are preliminary.⁷³

and the MAPK pathway (Figure 1). *ERBB2* mutations (in-frame insertions in exon 20; not amplification) occur in about 1–2% of adenocarcinomas and interestingly these, like *EGFR*-mutant tumors, often show BAC features, and occur preferentially in never smokers and women.^{46,47,50}

The presence of a *KRAS* mutation is both an adverse prognostic factor¹⁴ and a predictor of failure to benefit from *EGFR* TKI therapy.^{51–53} Indeed, in one study this went beyond a simple lack of response in that tumors with *KRAS* mutations did significantly worse when an *EGFR* TKI was added to their standard chemotherapy.⁵² Most *KRAS* mutations, in contrast to *EGFR* mutations, appear smoking-related.⁵⁴

From these considerations, it should be evident that obtaining a comprehensive mutational profile of will eventually become clinically highly desirable, as each of these mutations has some impact on treatment selection. Aside from the fact that *KRAS* mutations are known, and *BRAF* and *ERBB2* mutations are likely, to be associated with lack of response to *EGFR* TKIs, *BRAF* and *ERBB2* mutations may also predict sensitivity to other targeted therapies. For instance, *BRAF*-mutant tumors may be especially sensitive to MEK inhibitors.⁵⁵ Finally, it should be noted that some rare primary *EGFR* mutations, such as exon 20 insertions, show poor responses to *EGFR* TKIs.

Secondary (acquired) resistance to *EGFR* inhibitors

In spite of dramatic tumor shrinkage in many patients with *EGFR* mutations, durable complete responses to *EGFR* TKIs remain uncommon. Acquired resistance to *EGFR* TKIs has been associated in at least 50% of cases with selection for a second *EGFR* mutation, most often T790M in exon 20 (rarely D761Y in exon 19).^{13,56–59} The T790M secondary mutation may be underestimated for

technical reasons, especially in cases with amplification of the *EGFR* allele with the first mutation.^{23,60,61} A minority of patients whose tumors contain a T790M have not been exposed to *EGFR* TKIs, so this mutation may also be detectable at diagnosis. A family with multiple cases of NSCLC has been associated with germline transmission of this mutation,⁶² but a subsequent screen of 237 lung cancer families for germline T790M mutations failed to find any additional cases.⁶³

In about 20% of patients with acquired resistance to gefitinib or erlotinib, tumor cells display amplification (thus far without mutations) of the *MET* oncogene at chromosome position 7q31.^{64,65} *MET* encodes a receptor TK implicated in the development, maintenance and progression of cancers in both animals and humans (reviewed in Birchmeier *et al*⁶⁶). Interestingly, *MET* amplification appears to occur with or without T790M mutations.⁶⁵ As *MET* inhibitors and ‘second-generation’ *EGFR* inhibitors that can target T790M-bearing tumors enter into clinical use, pathologists may also need to optimize rapid testing methods for the T790M *EGFR* mutation and for *MET* amplification.

Prospects for IHC markers of *EGFR* and *KRAS* status

As discussed above, *EGFR* expression alone correlates poorly with the presence of activating *EGFR* mutations. In spite of this, the Tarceva package only mentions *EGFR* IHC for determining treatment eligibility. There has been intense interest in developing a better IHC-based marker for *EGFR* TKI treatment selection, that is, a marker that would have both good sensitivity and specificity for *EGFR* mutations. However, such a marker has so far proven elusive. The following have been investigated: phosphorylated *EGFR* or phosphorylated downstream signaling proteins such as phospho-AKT, phospho-ERK/MAPK and phospho-S6.^{29,67–70} In some of these studies, expression of phospho-AKT appeared to correlate with response to *EGFR* TKIs. Adenocarcinomas without evidence of epithelial-to-mesenchymal transition (ie, better differentiated ones) have also been reported to be more likely to respond and they can be identified by staining for E-cadherin.^{71,72}

Conclusion

The clinical development of *EGFR* TKIs in lung cancer and the subsequent discovery of activating mutations in *EGFR* as the molecular basis for increased tumor sensitivity to these drugs have revolutionized lung cancer management. However, the field continues to rapidly evolve. At present, *EGFR* mutation analysis represents the most accurate available test for prediction of response to *EGFR* TKIs, and many studies support its usefulness in

Table 1 Comparison of targeted therapies in breast and lung cancers

Target	HER2 (ERBB2)	EGFR
Cancer	Breast carcinoma	Lung adenocarcinoma
Targeted agent	Trastuzumab	Gefitinib/erlotinib
Approximate prevalence of target	15% have <i>HER2</i> amplification	20% have <i>EGFR</i> mutations
Typical response rate to targeted agent	~15%	>75%
Approximate cost (in USA)	>\$10k per month	\$2.0–2.5k per month

predicting survival benefit as well. Combining mutation analysis with EGFR FISH or CISH may provide additional clinically useful information. Some key areas of further work in the practical molecular pathology of *EGFR*-mutant tumors include defining optimal mutation detection techniques, identifying the key mutations that need to be tested for selection of first or second generation EGFR TKIs, developing cost-effective approaches to testing for a growing number of clinically relevant mutations in EGFR and associated genes, and identifying robust surrogate IHC markers of EGFR status. If other subtypes of adenocarcinoma prove to have molecular aberrations that could direct targeted therapy, the current paradigm of *EGFR*-mutant lung cancers will be extended. Finally, it should be noted that, in several respects, the targeted therapy of lung adenocarcinoma compares favorably with that of breast carcinoma (Table 1).

Web resources

The Sanger Institute (UK) maintains the COSMIC database (Catalogue of Somatic Mutations in Cancer) that includes a listing of all reported *EGFR* mutations, as well as mutations in *KRAS* and other kinases: <http://www.sanger.ac.uk/genetics/CGP/cosmic/>

Notable recent reviews

Papadopoulos N, Kinzler KW, Vogelstein B. The role of companion diagnostics in the development and use of mutation-targeted cancer therapies. *Nat Biotechnol* 2006;24:985–95.
 Riely GJ, Politi KA, Miller VA, Pao W. Update on epidermal growth factor receptor mutations in non-small cell lung cancer. *Clin Cancer Res* 2006;12:7232–41.
 Sharma SV, Bell DW, Settleman J, Haber DA. Epidermal growth factor receptor mutations in lung cancer. *Nat Rev Cancer* 2007;7:169–81
 Sequist LV, Bell DW, Lynch TJ, Haber DA. Molecular predictors of response to epidermal growth factor

receptor antagonists in non-small-cell lung cancer. *J Clin Oncol* 2007;25:587–95

References

- 1 Miller VA, Kris MG, Shah N, *et al.* Bronchioloalveolar pathologic subtype and smoking history predict sensitivity to gefitinib in advanced non-small-cell lung cancer. *J Clin Oncol* 2004;22:1103–1109.
- 2 Lynch TJ, Bell DW, Sordella R, *et al.* Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2004;350:2129–2139.
- 3 Paez JG, Janne PA, Lee JC, *et al.* EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 2004;304:1497–1500.
- 4 Pao W, Miller V, Zakowski MF, *et al.* EGF receptor gene mutations are common in lung cancers from ‘never smokers’ and correlate with sensitivity of tumors to gefitinib (Iressa) and erlotinib (Tarceva). *Proc Natl Acad Sci USA* 2004;101:13306–13311.
- 5 Sequist LV, Joshi VA, Janne PA, *et al.* Epidermal growth factor receptor mutation testing in the care of lung cancer patients. *Clin Cancer Res* 2006;12:4403s–4408s.
- 6 Shih JY, Gow CH, Yu CJ, *et al.* Epidermal growth factor receptor mutations in needle biopsy/aspiration samples predict response to gefitinib therapy and survival of patients with advanced nonsmall cell lung cancer. *Int J Cancer* 2006;118:963–969.
- 7 Marchetti A, Felicioni L, Buttitta F. Assessing EGFR mutations. *N Engl J Med* 2006;354:526–528.
- 8 Morikawa N, Inoue A, Suzuki T, *et al.* Prospective analysis of the epidermal growth factor receptor gene mutations in non-small cell lung cancer in Japan. *J Clin Oncol (Meeting Abstracts)* 2006;24:7077.
- 9 Paz-Ares L, Sanchez JM, Garcia-Velasco A, Spanish Lung Cancer Group, *et al.* A prospective phase II trial of erlotinib in advanced non-small cell lung cancer (NSCLC) patients (p) with mutations in the tyrosine kinase (TK) domain of the epidermal growth factor receptor (EGFR). *J Clin Oncol (Meeting Abstracts)* 2006;24:7020.
- 10 Sunaga N, Yanagitani N, Kaira K, *et al.* Phase II study of the efficacy of gefitinib in patients with non-small cell lung cancer with the EGFR mutations. *J Clin Oncol (Meeting Abstracts)* 2006;24:7183.
- 11 Sutani A, Nagai Y, Udagawa K, *et al.* Gefitinib for non-small-cell lung cancer patients with epidermal growth factor receptor gene mutations screened by peptide nucleic acid-locked nucleic acid PCR clamp. *Br J Cancer* 2006;95:1483–1489.
- 12 Inoue A, Suzuki T, Fukuhara T, *et al.* Prospective phase II study of gefitinib for chemotherapy-naïve patients with advanced non-small-cell lung cancer with epidermal growth factor receptor gene mutations. *J Clin Oncol* 2006;24:3340–3346.
- 13 Kris MG, Pao W, Zakowski MF, *et al.* Prospective trial with preoperative gefitinib to correlate lung cancer response with EGFR exon 19 and 21 mutations and to select patients for adjuvant therapy. ASCO (Meeting Abstracts). 2006.
- 14 Marks JL, Broderick S, Zhou Q, *et al.* Prognostic implications of EGFR and KRAS mutations in resected lung adenocarcinoma. *J Thorac Oncol* (in press).

- 15 Toyooka S, Yatabe Y, Tokumo M, *et al.* Mutations of epidermal growth factor receptor and K-ras genes in adenocarcinoma of the lung. *Int J Cancer* 2006;118:1588–1590.
- 16 Zakowski MF, Ladanyi M, Kris MG. EGFR mutations in small-cell lung cancers in patients who have never smoked. *N Engl J Med* 2006;355:213–215.
- 17 Morinaga R, Okamoto I, Furuta K, *et al.* Sequential occurrence of non-small cell and small cell lung cancer with the same EGFR mutation. *Lung Cancer* 2007;58:411–413.
- 18 Johnson DH, Fehrenbacher L, Novotny WF, *et al.* Randomized phase II trial comparing bevacizumab plus carboplatin and paclitaxel with carboplatin and paclitaxel alone in previously untreated locally advanced or metastatic non-small-cell lung cancer. *J Clin Oncol* 2004;22:2184–2191.
- 19 Blons H, Cote JF, Le CD, *et al.* Epidermal growth factor receptor mutation in lung cancer are linked to bronchioloalveolar differentiation. *Am J Surg Pathol* 2006;30:1309–1315.
- 20 Ebright MI, Zakowski MF, Martin J, *et al.* Clinical pattern and pathologic stage but not histologic features predict outcome for bronchioloalveolar carcinoma. *Ann Thorac Surg* 2002;74:1640–1646.
- 21 Sakuma Y, Matsukuma S, Yoshihara M, *et al.* Epidermal growth factor receptor gene mutations in atypical adenomatous hyperplasias of the lung. *Mod Pathol* 2007;20:967–973.
- 22 Johnson BE, Janne PA. Selecting patients for epidermal growth factor receptor inhibitor treatment: a FISH story or a tale of mutations? *J Clin Oncol* 2005;23:6813–6816.
- 23 Kaye FJ. A curious link between epidermal growth factor receptor amplification and survival: effect of 'allele dilution' on gefitinib sensitivity? *J Natl Cancer Inst* 2005;97:621–623.
- 24 Bunn Jr PA, Dziadziuszko R, Varella-Garcia M, *et al.* Biological markers for non-small cell lung cancer patient selection for epidermal growth factor receptor tyrosine kinase inhibitor therapy. *Clin Cancer Res* 2006;12:3652–3656.
- 25 Li AR, Chitale DA, Riely GJ, *et al.* EGFR mutations in lung adenocarcinomas: clinical testing experience and relationship to EGFR gene copy number and immunohistochemical expression. *J Mol Diagn* (in press).
- 26 Takano T, Ohe Y, Sakamoto H, *et al.* Epidermal growth factor receptor gene mutations and increased copy numbers predict gefitinib sensitivity in patients with recurrent non-small-cell lung cancer. *J Clin Oncol* 2005;23:6829–6837.
- 27 Miller V, Riely GJ, Zakowski M, *et al.* Molecular characteristics of bronchioalveolar carcinoma and adenocarcinoma BAC subtype predict response to erlotinib. *J Clin Oncol* (in press).
- 28 Endo K, Sasaki H, Yano M, *et al.* Evaluation of the epidermal growth factor receptor gene mutation and copy number in non-small cell lung cancer with gefitinib therapy. *Oncol Rep* 2006;16:533–541.
- 29 Han SW, Kim TY, Jeon YK, *et al.* Optimization of patient selection for gefitinib in non-small cell lung cancer by combined analysis of epidermal growth factor receptor mutation, K-ras mutation, and Akt phosphorylation. *Clin Cancer Res* 2006;12:2538–2544.
- 30 Ichihara S, Toyooka S, Fujiwara Y, *et al.* The impact of epidermal growth factor receptor gene status on gefitinib-treated Japanese patients with non-small-cell lung cancer. *Int J Cancer* 2007;120:1239–1247.
- 31 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* 2005;97:643–655.
- 32 Riely GJ, Pao W, Pham D, *et al.* Clinical course of patients with non-small cell lung cancer and epidermal growth factor receptor exon 19 and exon 21 mutations treated with gefitinib or erlotinib. *Clin Cancer Res* 2006;12:839–844.
- 33 Jackman DM, Yeap BY, Sequist LV, *et al.* Exon 19 deletion mutations of epidermal growth factor receptor are associated with prolonged survival in non-small cell lung cancer patients treated with gefitinib or erlotinib. *Clin Cancer Res* 2006;12:3908–3914.
- 34 Marchetti A, Buttitta F, Pellegrini S, *et al.* Bronchioalveolar lung carcinomas: K-ras mutations are constant events in the mucinous subtype. *J Pathol* 1996;179:254–259.
- 35 Marchetti A, Martella C, Felicioni L, *et al.* EGFR mutations in non-small-cell lung cancer: analysis of a large series of cases and development of a rapid and sensitive method for diagnostic screening with potential implications on pharmacologic treatment. *J Clin Oncol* 2005;23:857–865.
- 36 Parra HS, Cavina R, Latteri F, *et al.* Analysis of epidermal growth factor receptor expression as a predictive factor for response to gefitinib ('Iressa', ZD1839) in non-small-cell lung cancer. *Br J Cancer* 2004;91:208–212.
- 37 Pan Q, Pao W, Ladanyi M. Rapid PCR-based detection of epidermal growth factor receptor gene mutations in lung adenocarcinomas. *J Mol Diagn* 2005;7:396–403.
- 38 Asano H, Toyooka S, Tokumo M, *et al.* Detection of EGFR gene mutation in lung cancer by mutant-enriched polymerase chain reaction assay. *Clin Cancer Res* 2006;12:43–48.
- 39 Sasaki H, Endo K, Konishi A, *et al.* EGFR mutation status in Japanese lung cancer patients: genotyping analysis using LightCycler. *Clin Cancer Res* 2005;11:2924–2929.
- 40 Zhou C, Ni J, Zhao Y, *et al.* Rapid detection of epidermal growth factor receptor mutations in non-small cell lung cancer using real-time polymerase chain reaction with TaqMan-MGB probes. *Cancer* 2006;12:33–39.
- 41 Janne PA, Borras AM, Kuang Y, *et al.* A rapid and sensitive enzymatic method for epidermal growth factor receptor mutation screening. *Clin Cancer Res* 2006;12:751–758.
- 42 Cohen V, Agulnik JS, Jarry J, *et al.* Evaluation of denaturing high-performance liquid chromatography as a rapid detection method for identification of epidermal growth factor receptor mutations in nonsmall-cell lung cancer. *Cancer* 2006;107:2858–2865.
- 43 Thomas RK, Nickerson E, Simons JF, *et al.* Sensitive mutation detection in heterogeneous cancer specimens by massively parallel picoliter reactor sequencing. *Nat Med* 2006;12:852–855.
- 44 Pao W, Ladanyi M. Epidermal growth factor receptor mutation testing in lung cancer: searching for the ideal method. *Clin Cancer Res* 2007;13:4954–4955.
- 45 Soung YH, Lee JW, Kim SY, *et al.* Mutational analysis of EGFR and K-RAS genes in lung adenocarcinomas. *Virchows Arch* 2005;446:483–488.

- 46 Shigematsu H, Takahashi T, Nomura M, *et al.* Somatic mutations of the HER2 kinase domain in lung adenocarcinomas. *Cancer Res* 2005;65:1642–1646.
- 47 Buttitta F, Barassi F, Fresu G, *et al.* Mutational analysis of the HER2 gene in lung tumors from Caucasian patients: mutations are mainly present in adenocarcinomas with bronchioloalveolar features. *Int J Cancer* 2006;119:2586–2591.
- 48 Yokoyama T, Kondo M, Goto Y, *et al.* EGFR point mutation in non-small cell lung cancer is occasionally accompanied by a second mutation or amplification. *Cancer Sci* 2006;97:753–759.
- 49 Marks JL, McLellan MD, Zakowski MF, *et al.* Mutational analysis of EGFR and related signaling pathway genes in lung adenocarcinomas identifies a novel somatic kinase domain mutation in FGFR4. *PLoS ONE* 2007;2:e426.
- 50 Sonobe M, Manabe T, Wada H, *et al.* Lung adenocarcinoma harboring mutations in the ERBB2 kinase domain. *J Mol Diagn* 2006;8:351–356.
- 51 Pao W, Wang TY, Riely GJ, *et al.* KRAS mutations and primary resistance of lung adenocarcinomas to gefitinib or erlotinib. *PLoS Med* 2005;2:e17.
- 52 Eberhard DA, Johnson BE, Amler LC, *et al.* Mutations in the epidermal growth factor receptor and in KRAS are predictive and prognostic indicators in patients with non-small-cell lung cancer treated with chemotherapy alone and in combination with erlotinib. *J Clin Oncol* 2005;23:5900–5909.
- 53 Massarelli E, Varella-Garcia M, Tang X, *et al.* KRAS mutation is an important predictor of resistance to therapy with epidermal growth factor receptor tyrosine kinase inhibitors in non-small-cell lung cancer. *Clin Cancer Res* 2007;13:2890–2896.
- 54 Riely GJ, Kris MG, Rosenbaum D, *et al.* Frequency and distinctive spectrum of KRAS mutations in never smokers with lung adenocarcinoma (submitted).
- 55 Solit DB, Garraway LA, Pratils CA, *et al.* BRAF mutation predicts sensitivity to MEK inhibition. *Nature* 2006;439:358–362.
- 56 Pao W, Miller VA, Politi KA, *et al.* Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. *PLoS Med* 2005;2:e73.
- 57 Kobayashi S, Boggon TJ, Dayaram T, *et al.* EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2005;352:786–792.
- 58 Balak MN, Gong Y, Riely GJ, *et al.* Novel D761Y and common secondary T790M mutations in epidermal growth factor receptor-mutant lung adenocarcinomas with acquired resistance to kinase inhibitors. *Clin Cancer Res* 2006;12:6494–6501.
- 59 Kosaka T, Yatabe Y, Endoh H, *et al.* Analysis of epidermal growth factor receptor gene mutation in patients with non-small cell lung cancer and acquired resistance to gefitinib. *Clin Cancer Res* 2006;12:5764–5769.
- 60 Engelman JA, Mukohara T, Zejnullahu K, *et al.* Allelic dilution obscures detection of a biologically significant resistance mutation in EGFR-amplified lung cancer. *J Clin Invest* 2006;116:2695–2706.
- 61 Inukai M, Toyooka S, Ito S, *et al.* Presence of epidermal growth factor receptor gene T790M mutation as a minor clone in non-small cell lung cancer. *Cancer Res* 2006;66:7854–7858.
- 62 Bell DW, Gore I, Okimoto RA, *et al.* Inherited susceptibility to lung cancer may be associated with the T790M drug resistance mutation in EGFR. *Nat Genet* 2005;37:1315–1316.
- 63 Vikis H, Sato M, James M, *et al.* EGFR-T790M is a rare lung cancer susceptibility allele with enhanced kinase activity. *Cancer Res* 2007;67:4665–4670.
- 64 Engelman JA, Zejnullahu K, Mitsudomi T, *et al.* MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. *Science* 2007;316:1039–1043.
- 65 Bean J, Brennan C, Shih JH, *et al.* MET amplification occurs with or without T790M mutations in EGFR mutant lung tumors with acquired resistance to gefitinib and erlotinib. *Proc Natl Acad Sci USA* 2007;104:20932–20937.
- 66 Birchmeier C, Birchmeier W, Gherardi E, *et al.* Met, metastasis, motility and more. *Nat Rev Mol Cell Biol* 2003;4:915–925.
- 67 Cappuzzo F, Magrini E, Ceresoli GL, *et al.* Akt phosphorylation and gefitinib efficacy in patients with advanced non-small-cell lung cancer. *J Natl Cancer Inst* 2004;96:1133–1141.
- 68 Han SW, Hwang PG, Chung DH, *et al.* Epidermal growth factor receptor (EGFR) downstream molecules as response predictive markers for gefitinib (Iressa, ZD1839) in chemotherapy-resistant non-small cell lung cancer. *Int J Cancer* 2005;113:109–115.
- 69 Han SW, Kim TY, Hwang PG, *et al.* Predictive and prognostic impact of epidermal growth factor receptor mutation in non-small-cell lung cancer patients treated with gefitinib. *J Clin Oncol* 2005;23:2493–2501.
- 70 Sonobe M, Nakagawa M, Takenaka K, *et al.* Influence of epidermal growth factor receptor (EGFR) gene mutations on the expression of EGFR, phosphoryl-Akt, and phosphoryl-MAPK, and on the prognosis of patients with non-small cell lung cancer. *J Surg Oncol* 2007;95:63–69.
- 71 Yauch RL, Januario T, Eberhard DA, *et al.* Epithelial versus mesenchymal phenotype determines *in vitro* sensitivity and predicts clinical activity of erlotinib in lung cancer patients. *Clin Cancer Res* 2005;11:8686–8698.
- 72 Coldren CD, Helfrich BA, Witta SE, *et al.* Baseline gene expression predicts sensitivity to gefitinib in non-small cell lung cancer cell lines. *Mol Cancer Res* 2006;4:521–528.
- 73 Soda M, Choi YL, Enomoto M, *et al.* Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. *Nature* 2007;448:561–566.