Selecting Lung Cancer Patients for Treatment with Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors by Immunohistochemistry and Fluorescence *In situ* Hybridization—Why, When, and How?

Rafal Dziadziuszko,[1,2] Fred R. Hirsch,[1] Marileila Varella-Garcia,[1] and Paul A. Bunn, Jr.[1]

Abstract Recent evidence indicates that high *epidermal growth factor receptor* (*EGFR*) gene copy number evaluated by fluorescence *in situ* hybridization is an excellent predictive biomarker for response and survival benefit in patients with non–small cell lung cancer who receive epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors. Data on EGFR protein expression by immunohistochemistry as a selection marker are conflicting, although several studies showed that the treatment benefit was confined to EGFR-positive patients. Our studies and others showed that fluorescence *in situ* hybridization and immunohistochemistry were associated with the best predictive value. Expeditious validation of this information in prospective clinical trials with patient selection to first-line treatment is currently being done or planned by several cancer research groups worldwide.

Therapeutic strategies of blocking the epidermal growth factor receptor (EGFR)–mediated signal transduction in non–small cell lung cancer (NSCLC) include small-molecule tyrosine kinase inhibitors (TKI; e.g., gefitinib and erlotinib) and monoclonal antibodies (e.g., cetuximab). Several new compounds targeting the EGFR pathway are in development, many of which interfere with multiple receptors of the EGFR family or other target tyrosine kinases (1). Current data indicate that patients with advanced NSCLC whose tumors are characterized by high *EGFR* gene copy numbers, as assessed by fluorescence *in situ* hybridization (FISH), derive clear response and survival benefit from treatment with EGFR TKIs (2–4). The role of EGFR immunohistochemistry for predicting sensitivity to EGFR TKIs might be less clear. There is now compelling evidence to use FISH to select patients for EGFR-based targeted therapy of NSCLC. This article focuses on clinical trials that provided support for the predictive role of immunohistochemistry and FISH *EGFR* analysis in NSCLC, provides discussion on the combination of immunohistochemistry and FISH data with other predictive factors, and highlights possibilities for future clinical studies exploiting immunohistochemistry and FISH as biomarkers of treatment benefit. There are other articles that focus on the prognostic and predictive value of mutations in the *EGFR* gene, and the reader is referred to them for more details.

Clinical Markers for Treatment Benefit

Several patient characteristics associate with increased responsiveness to EGFR TKIs, as shown in clinical studies with gefitinib and erlotinib: "never smoking" history, Asian ethnicity, female gender, and adenocarcinoma histology (5–7). The response rates of these patient categories to single-agent EGFR TKI therapy in large clinical trials in refractory advanced NSCLC vary between 13% and 25%. However, all above characteristics, except Asian ethnicity, are linked to favorable clinical prognosis regardless of the use of EGFR TKIs (8). In a subgroup analysis of the BR.21 placebo-controlled trial, which evaluated erlotinib in patients who failed first-line or second-line chemotherapy and led to the registration of erlotinib, survival benefit was observed regardless of gender [hazard ratio (HR) of death, 0.8 in both females and males], cancer histology (HR, 0.7 in patients with adenocarcinoma versus 0.8 in other histologic types), and ethnicity [HR, 0.6 in Asians versus 0.8 in non-Asians (5)]. Negative smoking history was the only factor that predicted greater survival benefit in this trial (HR, 0.4 in never smokers versus 0.9 in ever smokers). The overall result of Iressa Survival Evaluation in Lung Cancer (ISEL) Study, a clinical trial comparing gefitinib to placebo in chemotherapy-pretreated NSCLC, did not show significant survival benefit with gefitinib (HR, 0.89). However, subsets of patients who never smoked and Asians derived significant benefit (9). In view of the above data, some investigators are testing the efficacy of EGFR TKIs in patient populations selected by clinical characteristics (e.g., never smokers), although this represents relatively small proportion of total population of patients.

Authors' Affiliations: [1]University of Colorado Health Sciences Center and University of Colorado Cancer Center, Aurora, Colorado and [2]Medical University of Gdansk, Gdansk, Poland

Received 1/13/06; revised 4/21/06; accepted 5/5/06.

Grant support: National Cancer Institute grants, Cancer Center Core grant 2P30-CA46934, Specialized Program of Research Excellence grant P01-CA58187, and National Cancer Institute International Union Against Cancer Translational Cancer Research Fellowship (R. Dziadziuszko).

Presented at the Third Cambridge Conference on Novel Agents in the Treatment of Lung Cancer: Advances in EGFR-Targeted Agents, September 23-24, 2005 in Cambridge, Massachusetts.

Requests for reprints: Paul A. Bunn, Jr., University of Colorado Cancer Center, P.O. Box 6511, Mail Stop 8111, Aurora, CO 80045. E-mail: Paul.Bunn@UCHSC.edu.

doi:10.1158/1078-0432.CCR-06-0087

EGFR Immunohistochemistry

High EGFR protein expression determined by immunohistochemistry is observed in the majority of squamous cell carcinomas and a smaller majority of large cell and adenocarcinomas and is also often seen in bronchial preneoplastic lesions, implicating its significance in lung carcinogenesis (10, 11). Systematic review of several investigations on the prognostic significance of EGFR expression revealed no association with survival, which was in accordance with our findings (10, 12). However, other studies have reported high EGFR expression to be an adverse prognostic factor in NSCLC (13, 14).

Early studies evaluating the predictive role of immunohistochemistry for response and survival benefit to EGFR TKIs showed no value of EGFR immunohistochemistry. The biomarker analysis of two phase II gefitinib Iressa Dose Evaluation in Lung Cancer (IDEAL) studies was done with the DAKO Cytomation EGFR PharmDx (DAKO Corp., Glostrup, Denmark) assay and reported as quantitative assessment, taking into account both percentage of positive cells and staining intensity (15). No association was found between staining intensity and response or symptom improvement, but survival data were not reported. EGFR immunohistochemistry analysis from 516 patients participating in two phase III trials evaluating addition of gefitinib to first-line chemotherapy (INTACT 1 and 2, Iressa NSCLC Trials Assessing Combination Treatment) also did not reveal any predictive value of EGFR immunostaining for treatment benefit (16). In contrast to the above, Cappuzzo et al. reported on 102 gefitinib-treated patients evaluated for EGFR immunostaining by Zymed antibody (Zymed Laboratories, San Francisco, CA) using the "Colorado scoring system," based on the fraction of positive cells times staining intensity (Table 1; ref. 3). The cutoff level for positive immunostaining was 200 on a scale of 0 to 400, indicating that immunohistochemistry-positive tumors show strong EGFR expression, whereas immunohistochemistry-negative tumors have low or no expression. Positive EGFR immunostaining, observed in 59% of patients, was associated with a significantly increased response rate (21% versus 5%), median progression-free survival (5.2 versus 2.3 months), and overall survival (11.5 versus 5.0 months). In multivariate analysis, EGFR protein expression remained associated with overall survival [HR, 0.60; 95% confidence interval (95% CI), 0.36-1.01]. An evaluation of a subgroup of 325 patients participating in the BR.21 study confirmed the value of EGFR immunohistochemistry to predict treatment outcome (2). Positive EGFR immunostaining with DAKO antibody could not well discriminate responding from nonresponding patients (11% response rate in EGFR-positive versus 4% in EGFR-negative patients) but associated with better survival (HR, 0.68 versus 0.93, respectively). Discordant results among the reported studies may be due to different methodologies, different study populations, and different efficacies of gefitinib versus erlotinib. The cutoff value for EGFR-positive immunostaining in the Colorado studies is set on a continuous scale to discriminate between strong or moderate versus weak or no staining using Zymed antibody (3, 10), whereas many other studies with DAKO antibody select different cut points (2, 15, 16). The optimal cut point remains to be established and validated in larger series of patients participating in prospective studies.

Low EGFR protein expression may also be considered as valuable information on whom we should not treat with EGFR TKIs. In two large clinical monotherapy studies with erlotinib and gefitinib, patients with negative EGFR immunohistochemistry did not derive survival benefit from EGFR TKIs (HR, 0.93; 95% CI, 0.63-1.36 for erlotinib versus placebo in a subset of EGFR immunohistochemistry-negative patients (2); similar results were presented for gefitinib-treated patients in the ISEL trial; ref. 17). Both studies used DAKO EGFR PharmDx and classified tumors as positive when >10% of cells showed membranous staining of any intensity.

EGFR, HER-2, and *HER-3* Gene Copy Numbers by FISH

High *EGFR* gene copy number or gene amplification is noted by FISH in 22% to 45% of NSCLC patients (2–4, 10, 17). A positive correlation between EGFR protein expression and high *EGFR* gene copy number was observed in a study of 183 surgically treated patients (10). Both abnormalities carried no significant prognostic information in this report, but a tendency of shorter survival was noted for patients with high *EGFR* copy number. The study of Cappuzzo et al. (3) was the first to show that high *EGFR* copy number (high polysomy and gene amplification evaluated by FISH) correlated significantly with improved survival in univariate and multivariate analysis of patients treated with gefitinib (Table 1). FISH results were classified according to six categories with ascending number of gene copies per cell: disomy, low trisomy, high trisomy, low polysomy, high polysomy, and gene amplification. The two latter categories were considered as FISH positive, whereas all the other categories were classified as FISH negative.

The results of three other studies confirmed that FISH-positive patients have prolonged survival after treatment with EGFR TKIs. Hirsch et al. analyzed 81 samples from 137 eligible participants in Southwest Oncology Group S0126, a phase II study evaluating the effectiveness of gefitinib in bronchioalveolar carcinoma and adenocarcinoma with bronchioalveolar carcinoma features (4). Objective response and disease control rates were 26% and 63% in the *EGFR* FISH-positive group compared with 11% and 39%, respectively, in the FISH-negative group. The median survival was significantly better in EGFR FISH-positive group compared with negative patients with a HR of 0.50 (95% CI, 0.25-0.97). *HER-2* copy numbers were not associated with response or survival in this study. In the BR.21 phase III clinical trial, data on the subgroup of 125 patients, in whom FISH analysis was successful, showed a survival difference favoring erlotinib confined to the *EGFR* FISH-positive patients (HR, 0.44; 95% CI, 0.23-0.82) as contrasted to *EGFR* FISH-negative patients (HR, 0.85; 95% CI; 0.48-1.51; ref. 2). In a subset analysis of the ISEL trial, FISH-positive gefitinib-treated patients had median survival of 8.3 months compared with 4.3 months for patients receiving placebo (HR, 0.61; 95%CI, 0.36-1.04; ref. 17).

High gene copy number of *HER-2*, as assessed by FISH, might be important for sensitivity to gefitinib in NSCLC. In a report by Cappuzzo et al., high *HER-2* copy number, present in 22% of gefitinib-treated patients, was associated with

Table 1. Predictive value of EGFR immunohistochemistry, *EGFR, HER-2*, and *HER-3* gene copy numbers for response and survival in advanced NSCLC treated with EGFR TKIs

Biomarker	Author (reference)	*N*	Drug (dose, mg/d)	Proportion positive (%)	Response rates; positive vs. negative	Survival hazard ratio (95% CI)
EGFR IHC	Cappuzzo (3)	102	Gefitinib (250)	59	21% vs. 5%	0.60* (0.36-1.01)
EGFR IHC	Tsao (2)	325	Erlotinib (150)	57	11% vs. 4%	0.68† (0.49-0.95)
EGFR copy number by FISH	Cappuzzo (3)	102	Gefitinib (250)	32	36% vs. 3%	0.44* (0.23-0.82)
EGFR copy number by FISH	Hirsch (4)	82	Gefitinib (500)	32	26% vs. 11%	0.50‡ (0.25-0.97)
EGFR copy number by FISH	Tsao (2)	125	Erlotinib (150)	45	20% vs. 2%	0.44§ (0.23-0.82)
EGFR copy number by FISH	Hirsch (17)	352	Gefitinib (250)	32	16% vs. 3%	0.61‖ (0.36-1.04)
HER-2 copy number by FISH	Cappuzzo (18)	102	Gefitinib (250)	22	35% vs. 6%	NR
HER-2 copy number by FISH	Hirsch (4)	56	Gefitinib (500)	30	36% vs. 46%	NR
HER-3 copy number by FISH	Cappuzzo (20)	82	Gefitinib (250)	27	36% vs. 10%	NR
EGFR copy number by qPCR	Bell (21)	453	Gefitinib (250 or 500)	7	56% vs. 53%¶	2.03‖ (0.67-6.13)
EGFR copy number by qPCR	Takano (22)	66	Gefitinib (250)	44	OR, 4.6** (95% CI, 0.84-25)	0.59* (0.26-1.4)
EGFR copy number by qPCR	Dziadziuszko(23)	82	Gefitinib (250)	51	12% vs. 10%	1.04‡ (0.61-1.76)

NOTE: Unpublished studies on EGFR immunohistochemistry of Bailey et al. (15, 16) and from ISEL trial (9) are not included.
Abbreviations: IHC, immunohistochemistry; NR, not reported.
*Comparison between positive and negative patients, multivariate analysis.
†Comparison between erlotinib and placebo in immunohistochemistry-positive subgroup of patients, univariate analysis.
‡Comparison between positive and negative patients, univariate analysis.
§Comparison between erlotinib and placebo in FISH-positive subset of patients.
‖Comparison between gefitinib and placebo in FISH-positive or *EGFR*-amplified subset of patients.
¶Response rates to chemotherapy and gefitinib versus chemotherapy and placebo.
**Odds ratio for response, indicating higher response probability in patients with high *EGFR* copy number.

significantly better response rate, time to progression, and a trend for improved survival (Table 1; ref. 18). This study showed that increased *HER-2* copy number correlates with increased *EGFR* copy number, and patients with both abnormalities have the highest response rates and the longest survival.

In vitro studies suggest that high expression of HER-3 also affects gefitinib sensitivity (19). Cappuzzo et al. evaluated the predictive value of *HER-3* gene copy number by FISH in a subset of 82 patients previously characterized for other abnormalities of EGFR pathway (Table 1; ref. 20). *HER-3* FISH-positive patients (27%) had significantly higher response rate (36% versus 10%), time-to-progression (7.7 versus 2.7 months), but not overall survival (10 versus 11 months).

EGFR Gene Copy Number by Quantitative Real-time PCR

Bell et al. reported recently on the predictive value of *EGFR* copy number, evaluated by quantitative real-time PCR (qPCR), and *EGFR* mutations in a series of NSCLC patients treated with single-agent gefitinib in the phase II IDEAL trials and gefitinib in combination with chemotherapy in the INTACT studies (Table 1; ref. 21). *EGFR* amplification (defined as more than four copies of *EGFR* relative to the housekeeping gene) was found in 7 of 90 (8%) patients in the IDEAL and 33 of 453 (7%) patients in the INTACT studies. In the latter trials, subset of patients with amplified *EGFR* did not benefit from the addition of gefitinib to chemotherapy (HR, 2.03; 95% CI, 0.67-6.13). Takano et al. reported that high *EGFR* gene copy number evaluated by qPCR correlated with *EGFR* mutations and predicted for time to progression but not overall survival benefit in a group of 66 gefitinib-treated patients, whereas *EGFR* mutations were associated with improved survival (22). In our study of 82 gefitinib-treated NSCLC patients, *EGFR* gene copy number evaluated by qPCR did not correlate with *EGFR* FISH data and did not predict for response, progression-free nor overall survival (23).

It should be noted that FISH and qPCR methods for *EGFR* gene copy number assessment differ substantially. The former

method allows for direct determination of gene copy number in single tumor cells and is available in many clinical laboratories. Determination of gene copy number by qPCR (referred also as gene dosage) is relatively fast and inexpensive. However, the result is relative to a housekeeping gene, making interstudy comparisons and cutoff determinations difficult. It also remains unknown if reference gene copy number is disomic and not amplified or deleted in tumor cells. Quantification of gene copy number in tumor cells may be affected by the proportion of disomic inflammatory and tumor stroma cells if tumor microdissection is not done.

Combination of Immunohistochemistry, FISH, and Other Predictive Factors

Data on the predictive value of combined EGFR immunohistochemistry and FISH assessment are relatively sparse, due to the limited number of patients analyzed for these abnormalities in currently available studies. Combined analysis of an Italian cohort of gefitinib-treated patients and the Southwest Oncology Group S0126 study was presented by Hirsch et al. at the 11th World Conference on Lung Cancer (24). Patients with both tests positive had excellent response rate and prolonged survival, whereas patients with both tests negative had dismal outcome. This study suggests that patients with negative EGFR immunohistochemistry and FISH results (who represented ~30% of study population) do not derive any clinical benefit from EGFR TKIs. Multivariate analysis of the BR.21 study is unfortunately inconclusive due to the limited number of evaluable patients with available biomarker results (2).

A detailed summary of predictive information carried by *EGFR* mutations is not within the scope of this report. It should be noted, however, that *EGFR* mutations have been linked to increased response rate and prolonged survival of patients treated with EGFR TKIs in several studies, with survival benefit mainly in Asian populations. *EGFR* mutations associate with *EGFR* copy number both *in vitro* and in clinical data sets (3, 22, 25), and long-lasting responses had been reported in *EGFR*-mutant patients (26, 27). In the study of Cappuzzo et al., patients harboring *EGFR* mutations had prolonged survival (21 versus 8 months); the difference was, however, not statistically significant (3). A recent study indicated that patients with exon 19 deletions have longer survival compared with patients with exon 21 point mutations (28). Table 2 shows the available data on predictive significance of EGFR protein expression, *EGFR* copy number by FISH, and *EGFR* mutations in large randomized placebo-controlled trials with a brief explanation of the trial design. The value of *EGFR* mutations as a marker for patient selection to treatment with EGFR TKIs remains unclear. As contrasted to the data indicating significant and clinically meaningful improvement in survival for FISH-positive patients, no randomized trial has shown the predictive value of *EGFR* mutations for survival benefit in patients treated with EGFR TKIs. There might be several explanations why *EGFR* mutations did not predict survival benefit from EGFR TKIs compared with placebo in these studies. The prevalence of *EGFR*

Table 2. Clinical use of predicting survival benefit according to EGFR protein expression by immunohistochemistry, *EGFR* copy number by FISH, and *EGFR* mutation status in large, placebo-controlled trials with EGFR TKIs

Author (reference)	Study description	EGFR protein, percentage positive; HRs in positive vs. negative subsets	*EGFR* copy number by FISH, percentage positive; HRs in positive vs. negative subsets	*EGFR* mutations, percentage positive; HRs in positive vs. negative subsets
Tsao (2)	BR.21, phase III trial comparing erlotinib to placebo in second or third-line treatment; trial result positive	57%; 0.68 vs. 0.93	45%; 0.44 vs. 0.85	22.6%; 0.77 vs. 0.73
Hirsch (17) and Holloway (31)	ISEL, phase III trial comparing gefitinib to placebo in second or third-line treatment; trial result negative	68%; NR	31%; 0.61 vs. 1.14	12%; NR*
Bell (21) and Bailey (16)	INTACT 1 and 2, phase III trials comparing addition of gefitinib or placebo to chemotherapy in first-line treatment; trials results negative	NS	ND	10%; 1.77 vs. 0.91
Eberhard (30) and Herbst (35)	TRIBUTE, phase III trial comparing addition of erlotinib or placebo to chemotherapy in first-line treatment; trial result negative	NR; 1.00 vs. 1.02	ND	13%; NS

NOTE: HR comparing EGFR TKI versus placebo.
Abbreviations: ND, not done; NS, not significant [exact value(s) not reported].
*Not reported due to too few events.

mutations is ~10% to 23% in Western NSCLC patient populations (2, 21, 29) compared with 22% to 45% for *EGFR* gene amplification or high polysomy (2–4, 10), and identification of relatively small subset of mutant patients with increased response rate clearly does not explain the survival difference observed in positive BR.21 trial. Not all patients with *EGFR* mutations respond to EGFR TKIs (2), *EGFR* mutations poorly predict disease control, a substantial contributing factor to survival benefit (3), and, most importantly, *EGFR* mutations carry positive prognostic information (i.e., prolonged survival regardless of treatment with EGFR TKIs, for example in patients treated with chemotherapy alone; refs. 21, 30).

The exact data breaking down EGFR FISH-positive patients into those with mutations and those with wild-type EGFR are not available from the above studies, but the molecular analysis of the ISEL study indicates that most patients with *EGFR* mutations are included in a larger FISH-positive subset (31).

The predictive value of *EGFR* mutations may be different in East Asia, where *EGFR* mutations are more prevalent, response rates seem higher, and improved survival was consistently reported in retrospective studies (22, 32–34). Unfortunately, there are no data from placebo-controlled trials in these populations to derive any meaningful conclusion on the clinical value of mutations and on combination of these biomarkers.

Current and Future Clinical Trials Exploiting Biomarkers to Select Patients for Treatment with EGFR TKIs

Consistent findings from independent institutions and from prospective placebo-controlled studies indicate improved survival from EGFR TKIs in NSCLC patients with tumors characterized by high *EGFR* copy number detected by FISH, supporting further validation of this marker in trials with enriched populations. The strategy of selecting patients for EGFR TKI therapy based on a positive FISH result is very similar to the successful strategy selecting breast cancer patients for treatment with trastuzumab, a monoclonal antibody against HER-2. In the United States, there is an ongoing randomized phase II study comparing erlotinib alone to chemotherapy alternating with erlotinib in untreated advanced NSCLC patients who are FISH positive or immunohistochemistry positive. A randomized trial of erlotinib or placebo after surgery and adjuvant chemotherapy in patients selected by FISH and immunohistochemistry is planned. The evidence to select patients based solely on EGFR immunohistochemistry is less clear, although combination of *EGFR* gene copy number by FISH and immunohistochemistry seems promising. A phase II trial of gefitinib in *EGFR* mutation-positive patients has been conducted in Japan, and other studies may be contemplated, although there is no evidence from placebo-controlled trials to support the selection of patients based on *EGFR* mutations.

Open Discussion

Dr. Thomas Lynch: We have seen different data from different groups, and some of the differences may be related to methodologic issues. When we talk about immunohistochemistry, is everyone using the same techniques for determining what are considered to be IHC-positive cells?

Dr. Bunn: The answer is that there are completely different methodologic approaches. First, there are different antibodies, but I don't think the antibody matters. I think the definition does matter. These cells can be scored in two ways once they are stained. One way is to score the intensity as 0, 1+, 2+, 3+. Another way is to score the fraction of cells that are positive, 0 to 100. A third approach, which is what we have done, is to multiply those two together, intensity times the percent positive. There, you get a numeric score of 0 to 400. Using a cutoff of 200, our pathologists found 61% of the patients were positive. I would submit that, at the moment, nobody knows what is the best test and the best cutoff.

Dr. Lynch: In the study that Daphne Bell is publishing (J Clin Oncol, 2005), she did not find a relationship between amplification and IHC positivity. That was defined as the fraction of cells that was positive, correct?

Dr. Daniel Haber: Pathologists typically use the percentage of cells positive, but if there were a reliable way of quantifying the amount of expression per cell, that would make more sense in correlating with gene amplification. However, quantifying the degree of staining or extent of staining by IHC is very difficult because you need standards for each assay.

Dr. Lynch: Dr. Bunn, did you find a relationship between the degree of amplification and the intensity of immunohistochemical staining in your samples?

Dr. Bunn: There is a significant correlation between amplification by FISH and protein by IHC, but in a multivariable analysis, they are independent. In our experience, 61% of patients are EGFR protein positive, but only a third are FISH positive. FISH is different from quantitative PCR. There are positive patients in one and not the other. It still remains to be determined which is a better test. For whatever reason, in our hands, FISH is a better test with correlating with the clinical outcome than quantitative PCR.

Dr. Haber: When you define a FISH-positive tumor, what fraction of cells are FISH positive for specific EGFR amplification versus those that may be aneuploid? How do you deal with tumor heterogeneity?

Dr. Bunn: The good news is that different groups have gotten the same result in six different cohorts now. For high polysomy, you have to have four copies in more than 40% of the cells. So, even in high polysomy, there may be plenty of diploid cells.

Dr. Matthew Meyerson: I'm curious about the reproducibility of the distinction between high polysomy and low polysomy. If you take different sections and have it measured by different observers, what is the precision of that distinction?

Dr. Bunn: We haven't done a formal study of that, and it needs to be done. When Dr. Cappuzzo was at our institution, all of the specimens were read by Dr. Varella-Garcia and Dr. Cappuzzo. When they disagreed, they got together and discussed it. The rate of agreement was very high, it was over 90%. But we have not done that scientifically so that I can give you an accurate number.

Dr. Rogerio Lilenbaum: Assuming that IHC and FISH will have the importance that we hope in terms of selecting patients and therapies, how does the situation compare to breast cancer and how they establish HER2 status? They have been dealing with this for over a decade. From a clinician's perspective, is the

EGFR methodology much different than the methodology that is used for HER-2/*neu*?

Dr. Bunn: No, they are identical in technology, differing only in the specific process. I think the results are also similar for their ability to select patients. I think the biology is very similar, and clinically, I believe that is what we are all going to be doing 2 or 3 years from now. Every patient going on a trial in our institution has these tests done, but we don't test routinely at the moment. Until we have prospective trials, I'm not sure that we should be doing this testing clinically.

Dr. Meyerson: Are any large-scale trials being planned to validate different diagnostic approaches and to see is any of them can determine who would be the best patients to receive EGFR inhibitors?

Dr. Lynch: Yes, actually, those trials are either about to open or in the works. One of the difficulties is that the data are all from retrospective studies involving a fraction of the patients on trial. As Dr. Haber pointed out this morning, we have to consider what might be the bias in terms of which subset of patients you have tissue for. So it really is critical to do this prospectively.

Dr. Panos Fidias: So on the one hand we have to do trials, but at the same time we have to treat people. For me, the difficulty has been to say, what do these studies add to day-to-day decision-making? The clear thing that has emerged is the importance of smoking status. Smoking status is much more important than female sex or adenocarcinoma or BAC histology in predicting response to an EGFR TKI. In my mind, the main importance in decision-making for knowing EGFR mutation or amplification would be for those patients who do not have clinical characteristics. So, for those patients who were double positive, do you know their smoking status?

Dr. Bunn: The question in my mind isn't who to treat, it is who not to treat. If the patient has the mutation or is a never smoker, I have no doubt that patient should be treated with an EGFR inhibitor. But those are 10% of the patients. There are many more patients than that who are going to benefit from these drugs, and there are many more patients than that who are going to have better survival with these pills than they are going to get with triple-agent chemotherapy. So the clinical question is, which patients should not get a pill first. I would argue that it is the double-negative patients for protein by IHC and copy number by FISH.

Dr. Fidias: Even if they are nonsmokers?

Dr. Bunn: Even if they are nonsmokers. There is a statistical correlation between never smoking and FISH positivity. There is a statistical correlation between never smoking and amplification. But in a multivariable analysis, because there are so many more FISH-positive patients, FISH comes up as an independent prognostic variable even when smoking status is in the equation.

References

1. Baselga J, Arteaga CL. Critical update and emerging trends in epidermal growth factor receptor targeting in cancer. J Clin Oncol 2005;23:2445–59.
2. Tsao MS, Sakurada A, Cutz JC, et al. Erlotinib in lung cancer—molecular and clinical predictors of outcome. N Engl J Med 2005;353:133–44.
3. 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;97:643–55.
4. Hirsch FR, Varella-Garcia M, McCoy J, et al. Increased epidermal growth factor receptor gene copy number detected by fluorescence *in situ* hybridization associates with increased sensitivity to gefitinib in patients with bronchioloalveolar carcinoma subtypes: a Southwest Oncology Group Study. J Clin Oncol 2005;23:6838–45.
5. Shepherd FA, Rodrigues PJ, Ciuleanu T, et al. Erlotinib in previously treated non-small-cell lung cancer. N Engl J Med 2005;353:123–32.
6. Fukuoka M, Yano S, Giaccone G, et al. Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer (the IDEAL 1 trial). J Clin Oncol 2003;21:2237–46.
7. Kris MG, Natale RB, Herbst RS, et al. Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: a randomized trial. JAMA 2003;290:2149–58.
8. Solan MJ, Werner-Wasik M. Prognostic factors in non-small cell lung cancer. Semin Surg Oncol 2003;21:64–73.
9. Thatcher N, Chang A, Parikh P, et al. Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: results from a randomised, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer). Lancet 2005;366:1527–37.
10. Hirsch FR, Varella-Garcia M, Bunn PA, Jr., et al. Epidermal growth factor receptor in non-small-cell lung carcinomas: correlation between gene copy number and protein expression and impact on prognosis. J Clin Oncol 2003;21:3798–807.
11. Hirsch FR, Scagliotti GV, Langer CJ, et al. Epidermal growth factor family of receptors in preneoplasia and lung cancer: perspectives for targeted therapies. Lung Cancer 2003;41:S29–42.
12. Meert AP, Martin B, Delmotte P, et al. The role of EGF-R expression on patient survival in lung cancer: a systematic review with meta-analysis. Eur Respir J 2002;20:975–81.
13. Niemiec J, Kolodziejski L, Dyczek S, Gasinska A. Prognostic significance of epidermal growth factor receptor in surgically treated squamous cell lung cancer patients. Folia Histochem Cytobiol 2004;42:111–8.
14. Selvaggi G, Novello S, Torri V, et al. Epidermal growth factor receptor overexpression correlates with a poor prognosis in completely resected non-small-cell lung cancer. Ann Oncol 2004;15:28–32.
15. Bailey LR, Kris MG, Wolf M, et al. Tumor EGFR membrane staining is not clinically relevant for predicting response in patients receiving gefitinib ("Iressa," ZD1939) monotherapy for pretreated advanced non-small-cell lung cancer: IDEAL1 and 2 [abstract LB-170]. Proc Am Assoc Cancer Res 2003;44:1362.
16. Bailey LR, Janas M, Schmidt K, et al. Evaluation of epidermal growth factor receptor as a predictive marker in patients with non-small-cell lung cancer (NSCLC) receiving first-line gefitinib combined with platinum based chemotherapy [abstract 7013]. J Clin Oncol 2004;22(14s):620s.
17. Hirsch FR, Varella-Garcia M, Bunn PA, et al. Molecular analysis of EGFR gene copy number, EGFR expression, and Akt activation status in advanced non-small-cell lung cancer (aNSCLC) treated with gefitinib or placebo (ISEL trial) [abstract A268]. Clin Cancer Res 2005;11:9031s.
18. Cappuzzo F, Varella-Garcia M, Shigematsu H, et al. Increased HER2 gene copy number is associated with response to gefitinib therapy in epidermal growth factor receptor-positive non-small-cell lung cancer patients. J Clin Oncol 2005;23:5007–18.
19. Engelman JA, Janne PA, Mermel C, et al. ErbB-3 mediates phosphoinositide 3-kinase activity in gefitinib-sensitive non-small cell lung cancer cell lines. Proc Natl Acad Sci U S A 2005;102:3788–93.
20. Cappuzzo F, Toschi L, Domenichini I, et al. HER3 genomic gain and sensitivity to gefitinib in advanced non-small-cell lung cancer patients. Br J Cancer 2005;93:1334–40.
21. Bell DW, Lynch TJ, Haserlat SM, et al. Epidermal growth factor receptor mutations and gene amplification in non-small-cell lung cancer: molecular analysis of the IDEAL/INTACT gefitinib trials. J Clin Oncol 2005;23:8081–92.
22. 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;23:6829–37.
23. Dziadziuszko R, Witta SE, Cappuzzo F, et al. Epidermal growth factor receptor messenger RNA expression, gene dosage, and gefitinib sensitivity in non-small cell lung cancer. Clin Cancer Res 2006;12:3078–84.
24. Hirsch FR, McCoy J, Cappuzzo F, et al. FISH and immunohistochemistry can be used to select NSCLC patients, who will not benefit from gefitinib treatment. Lung Cancer 2005;49:S38.
25. Amann J, Kalyankrishna S, Massion PP, et al. Aberrant epidermal growth factor receptor signaling and enhanced sensitivity to EGFR inhibitors in lung cancer. Cancer Res 2005;65:226–35.
26. 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;350:2129–39.
27. 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 2005;11:5878–85.
28. Riely GJ, Pao W, Pham DK, 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;12:839–44.

29. Shigematsu H, Lin L, Takahashi T, et al. Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. J Natl Cancer Inst 2005;97:339–46.

30. 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;23:5900–9.

31. Holloway B, Thatcher N, Chang A, et al. Epidermal growth factor receptor (EGFR), K-ras, and B-Raf mutation status and clinical outcome to gefitinib (Iressa) in a phase III placebo-controlled study (ISEL) in advanced non-small cell lung cancer (aNSCLC) [abstract 269]. Clin Cancer Res 2005;24:9031–2s.

32. 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;23:2493–501.

33. 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 2005;23:2513–20.

34. Zhang XT, Li LY, Mu XL, et al. The EGFR mutation and its correlation with response of gefitinib in previously treated Chinese patients with advanced non-small-cell lung cancer. Ann Oncol 2005;16:1334–42.

35. Herbst RS, Prager D, Hermann R, et al. TRIBUTE: a phase III trial of erlotinib hydrochloride (OSI-774) combined with carboplatin and paclitaxel chemotherapy in advanced non-small cell lung cancer. J Clin Oncol 2005;23:5892–9.

Clinical Cancer Research

Selecting Lung Cancer Patients for Treatment with Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors by Immunohistochemistry and Fluorescence *In situ* Hybridization—Why, When, and How?

Rafal Dziadziuszko, Fred R. Hirsch, Marileila Varella-Garcia, et al.

Clin Cancer Res 2006;12:4409s-4415s.