EGFR MUTATIONS IN NON-SMALL LUNG CANCER:
A Basic Science Discovery With Immediate Clinical Impact

Jonathan E. Dowell, M.D.
Assistant Professor of Internal Medicine
Division of Hematology/Oncology

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Dr. Dowell has an interest in clinical and translational research in thoracic malignancies and unknown primary cancer. He is also the Chief of Hematology/Oncology at the Dallas VA Medical Center.

Non-small cell lung cancer (NSCLC) is the leading cause of cancer death in the United States and kills greater than one million people annually worldwide. In the United States, lung cancer accounts for more deaths than breast, colon, prostate and pancreas cancers combined.(1) Approximately 50% of NSCLC patients present with metastatic disease, and historically, therapy for this group has been inadequate. A number of studies over the past 2 decades have established that chemotherapy prolongs survival and significantly improves quality of life for those patients that are fit enough to tolerate treatment. However, despite these proven benefits, the unfortunate reality is that only 30% of patients with metastatic NSCLC survive one year.(2) Clearly, novel and more effective treatment options are needed.

During this time a better understanding of the biology of NSCLC has also emerged, and with it, the discovery of rationale molecular targets for therapeutic approaches. One target that has been studied extensively is the epidermal growth factor receptor (EGFR).

The epidermal growth factor receptor

The EGFR story begins in 1962 when Stanley Cohen reported isolating a protein from mouse salivary gland that promoted incisor eruption and eyelid opening in the new-born animal.(3) This protein was ultimately named the epidermal growth factor (EGF), and in 1980, its receptor (EGFR) was identified and demonstrated to have tyrosine kinase activity.(4)

EGFR (also known as erbB1 or HER1) is now known to be part of the EGFR family of receptors that also includes HER2/neu (erbB2), HER3 (erbB3), and HER4 (erbB4) (Figure 1). EGFR is a 170-kilodalton protein that spans the cellular membrane and is composed of an extracellular ligand-binding domain, a hydrophobic

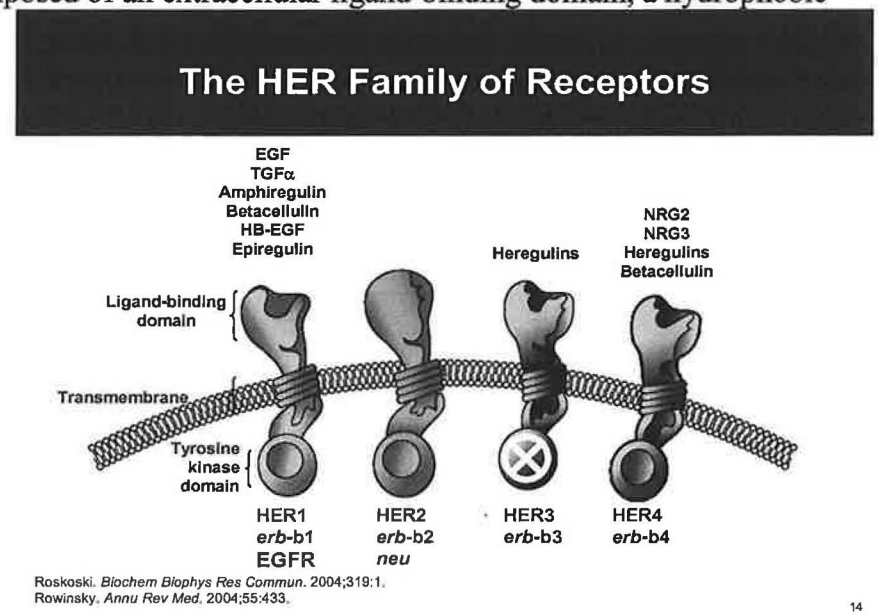


Figure 1. The HER family of receptors.

region and a cytoplasmic region that contains the tyrosine kinase domain.(5) Binding of ligand to the receptor activates a complex network of downstream signaling pathways that, under normal physiologic conditions, play a critical role in epithelial development, proliferation and organogenesis.(5) As a result, mice that lack EGFR have severe disruption of epithelial development in multiple organs including the lung, brain, kidney, gastrointestinal tract, eye, skin, and liver.(6-8)

EGFR and cancer

In normal cells, signaling through EGFR is tightly controlled. Deregulation of this system leads to malignant transformation through a number of downstream effects including promotion of proliferation and angiogenesis and inhibition of apoptosis (figure 2).(9) A number of transforming viruses induce tumor formation through upregulation of EGFR transcription, constitutive activation of EGFR or by preventing downregulation of EGFR signaling.(10-14) In addition, transfection of high levels of EGFR and its ligand leads to malignant transformation *in vitro*.(5)

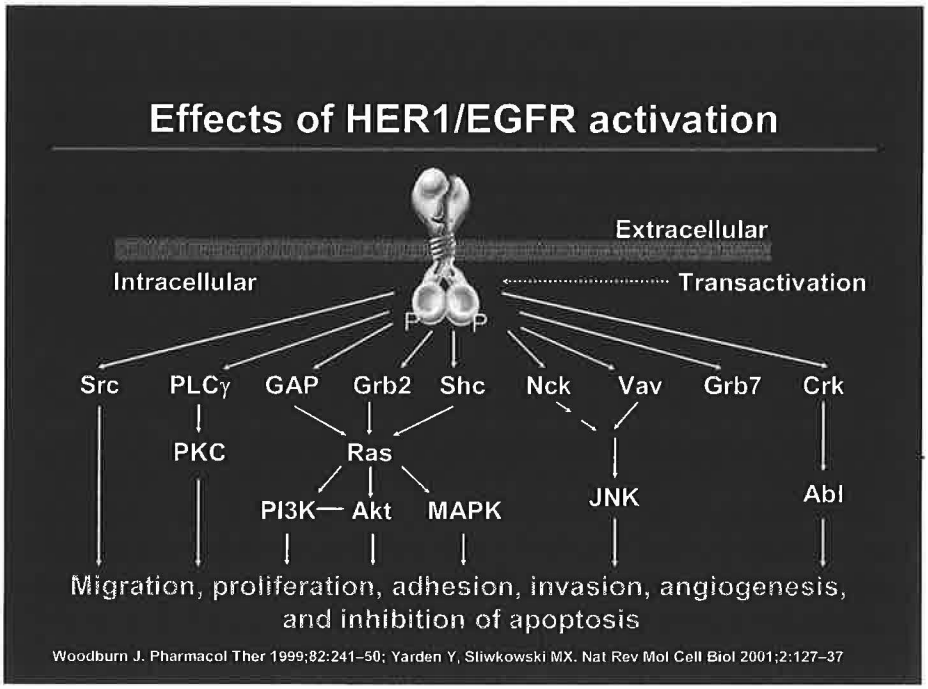


Figure 2. Effects of HER1 activation.

Also, EGFR RNA or protein overexpression occurs in a many human cancers, including head and neck, esophageal, stomach, colon, pancreas, breast, ovary, bladder, kidney, and lung. In many of these cancers, there is also evidence that EGFR overexpression is associated with a worse prognosis. (15-22)

EGFR in lung cancer

As noted above, EGFR is frequently overexpressed in lung cancer. In the normal lung, EGFR expression is limited to the basal layer of the epithelium, where proliferation occurs. In response to exposure to tobacco smoke, this epithelium becomes

initially hyperplastic, then metaplastic, and then frankly dysplastic. The amount of EGFR expression is increased in severe dysplasia when compared with the other precursor lesions, suggesting that EGFR signaling may play a role prior to the development of cancer.(9) (23-25) The amount of EGFR expression in established NSCLC is dependent on the histological subtype.(26) Overexpression is commonly seen in squamous cell carcinoma, but it is rarely observed in small cell lung cancers. Large cell and adenocarcinomas demonstrate overexpression of the protein approximately 50% of the time (Table 1).

Histology	Frequency of EGFR overexpression
Small cell	0-5%
Adenocarcinoma	40-60%
Large-cell	40-60%
Squamous cell	60-85%

Table 1. EGFR overexpression by histologic subtype (from reference 9)

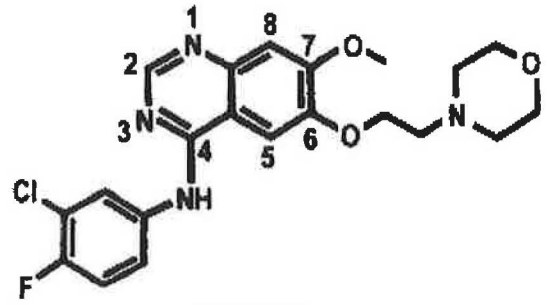
Initial reports suggested that overexpression of EGFR in NSCLC correlated with a worse prognosis. Subsequent studies, however, have not uniformly demonstrated an association, and the prognostic significance of EGFR overexpression in lung cancer is now debated (9) (27-42). A meta-analysis of 11 studies that included 2,185 patients failed to identify any association between EGFR expression and survival. Several different methods for assessing EGFR expression were utilized in these studies, and when the analysis was limited to the 8 studies that used immunohistochemistry to detect protein expression, it was shown that tumors not expressing EGFR had a significantly better survival (43). Given the lack of consistent findings in these studies, it is likely that the overall impact of EGFR expression on prognosis in NSCLC is small.

Rationale for EGFR as a therapeutic target in NSCLC

As mentioned above, the EGFR protein is frequently overexpressed in NSCLC, and EGFR signaling pathways lead to a number of downstream effects that are critically involved in a cell's acquisition of the malignant phenotype. In addition, preclinical studies have demonstrated that a number of tumor types, including NSCLC, coexpress both EGFR and its ligand (TGF-α and EGF). In these tumors, it appears that EGFR activation is occurring through an autocrine loop. Interruption of this signaling with a variety of EGFR inhibitors has been shown to decrease tumor cell viability and/or prevent proliferation both *in vitro* and *in vivo*.(5, 44) Lastly, there is no identified physiologic role for EGFR in the adult, suggesting that drugs that inhibit this receptor may not have significant side effects. For all of these reasons, EGFR is felt to be a rational target for therapeutic intervention in NSCLC.

While several different therapeutic strategies to target EGFR are being actively explored in NSCLC, the approach that has thus far shown the most promise is the use of inhibitors of the tyrosine kinase domain of EGFR. Through a random screen of small molecules, a class of compounds called the 4-aniloquinazolines was identified (Figure 3). These agents compete with ATP for binding to the tyrosine kinase domain of EGFR and

prevent the catalytic activity of the receptor. (5, 45-47) Two examples are ZD1839 (gefitinib or Iressa™) and OSI-774 (erlotinib or Tarceva™), which are orally available agents that reversibly and selectively inhibit the tyrosine kinase domain of EGFR at nanomolar concentrations. In phase I clinical trials in patients with advanced malignancies, these drugs were well-tolerated with modest rash and diarrhea being the dose-limiting toxicities. Also, in these studies anti-tumor activity was observed in a small number of patients with NSCLC.(48-52)



ZD1839
(gefitinib, Iressa)

OSI-774
(erlotinib, Tarceva)

Figure 3. Chemical structure of the quinazoline EGFR TKI's erlotinib and gefitinib (reproduced from reference 46)

Two large trials (Iressa Dose Evaluation in Advanced Lung Cancer; IDEAL 1 and 2) examined the efficacy of single agent gefitinib in 431 previously treated patients with advanced NSCLC.(53, 54) Both trials were randomized phase II studies that compared 2 different doses of gefitinib (250 mg and 500 mg). The trials were essentially identical in design, except that the IDEAL 2 trial stipulated that patients must have failed 2 prior therapies for advanced NSCLC while IDEAL 1 required only one prior treatment. In IDEAL 1 and 2, gefitinib produced an objective tumor response in 18% and 10% of patients respectively, and symptom improvement occurred in approximately 40% of patients. No significant difference in anti-tumor activity was seen in either trial between the 250 mg and 500 mg doses. While the studies were not placebo controlled, significant

symptom improvement was seen in 77-96% of patients with radiographic tumor responses, arguing against a substantial placebo effect. The toxicity of gefitinib was modest, with mild rash and diarrhea being the most frequent events. These studies proved the hypothesis that inhibition of the EGFR tyrosine kinase results in tumor regressions and symptom improvement in some patients with pretreated advanced NSCLC. Based on this data, gefitinib (at a dose of 250 mg daily) was approved by the United States Food and Drug Administration (FDA) for use as third-line therapy in metastatic NSCLC. This approval was contingent on the developer of gefitinib (AstraZeneca) performing a randomized, placebo controlled trial to demonstrate a survival advantage for the drug in this population.

A second orally active EGFR TKI, erlotinib (Tarceva™), has also been studied in NSCLC. A single-arm phase II trial demonstrated that erlotinib had similar antitumor activity to gefitinib in pretreated patients with advanced NSCLC.(55) The National Cancer Institute of Canada has now completed an international randomized, double-blind phase III comparison of erlotinib versus placebo in 731 NSCLC patients that had failed 1 or 2 prior treatments for metastatic disease (the NCIC BR.21 trial).(56) The patients that received erlotinib had significantly higher objective tumor response rates (8.9% vs 0.9%), median survival (6.7 months vs 4.7 months), and 1-year survival (31.2% vs 21.5%) than those that received placebo (figure 4). These results lead to the FDA approval of erlotinib in November 2004 as second-line therapy for metastatic NSCLC.

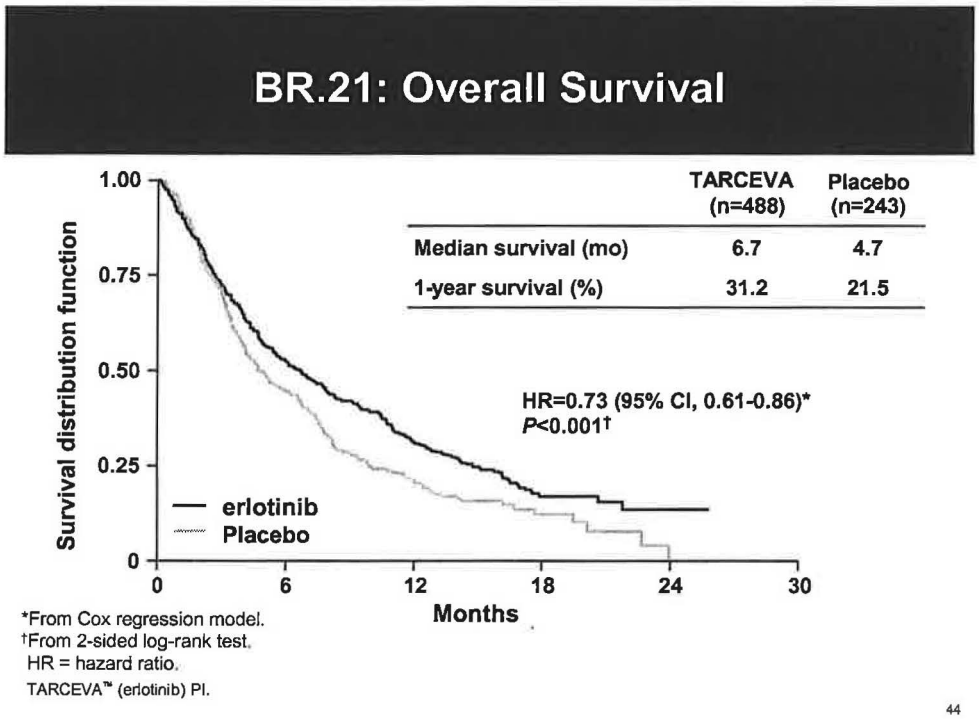


Figure 4. Overall survival curves for erlotinib and placebo from the NCIC BR.21 trial (from reference 56).

Unfortunately, not all of the trials performed with these agents have been positive. In December 2004, AstraZeneca made available through a press release the results of the FDA mandated randomized, double-blind phase III comparison of gefitinib versus placebo as second or third-line therapy for advanced NSCLC (the Iressa Survival Evaluation in Lung Cancer or ISEL trial). This trial failed to demonstrate a significant difference in the primary endpoint of median survival between the patients that received gefitinib and those that received placebo, with reported median survivals of 5.6 months and 5.1 months, respectively ($p = .11$)(www.astrazeneca.com). In addition, in 4 randomized trials of either gefitinib or erlotinib in combination with standard chemotherapy as first-line therapy for advanced NSCLC, the addition of an EGFR TKI to standard chemotherapy did not produce any improvement in survival over chemotherapy alone.(57-60)

Who benefits from treatment with an EGFR TKI?

While the identification of molecularly targeted drugs with activity in NSCLC represents a major step forward in the treatment of this disease, it is clear that not every patient benefits from treatment with erlotinib or gefitinib. What is tantalizing are the occasional reports of dramatic and durable tumor responses (what some have termed Lazarus responses because the patient appeared to be brought back from the dead)(45), and these observations prompted investigations to determine which patients were most likely to benefit from treatment with an EGFR TKI.

One factor that does not consistently predict for the efficacy of these agents is the level of tumor expression of EGFR as detected by immunohistochemistry. In retrospective analyses of the IDEAL trials, no significant association between level of EGFR expression and clinical efficacy of the TKI was identified.(55, 61) In the NCIC BR.21 trial, erlotinib conferred a survival advantage on those patients whose EGFR protein expression by immunohistochemistry was either positive or unmeasured. A survival advantage in the EGFR negative subgroup could not be excluded given the relatively small number of patients and the resultant wide confidence intervals.(62) In addition, a retrospective evaluation of a relatively small group of patients treated with gefitinib (and/or erlotinib) at the University of Colorado and in Italy found that those patients with both high levels of tumor expression of EGFR and evidence of EGFR gene amplification by fluorescence *in situ* hybridization were more likely to respond to treatment than those patients with only high protein expression or gene amplification or those patients with neither.(63) Ideally, this needs to be validated prospectively in larger group of patients. At present, demonstration of EGFR protein expression or gene amplification is not a prerequisite for consideration of treatment with erlotinib or gefitinib.

Certain clinical and pathological characteristics are independent predictors of response to an EGFR TKI. In IDEAL 1, which included a large number of patients from Japan, the objective tumor response rate observed with gefitinib was significantly higher for Japanese patients than non-Japanese patients (28% v 10%), and in the planned multivariate analysis, female gender, adenocarcinoma histology, a good functional status, and a history of having received prior immuno or hormonal therapy for lung cancer all predicted for response to the EGFR TKI.(54) Similarly, in IDEAL 2, significantly higher tumor response rates were observed in adenocarcinoma histology (13% v 4%) and

women (19% v 3%).(53) Female gender, adenocarcinoma histology, and being a never smoker also predict for response to erlotinib.(62) In addition, a retrospective review of 139 NSCLC patients treated with gefitinib at Memorial Sloan-Kettering demonstrated that significantly greater tumor response rates were seen in those patients with adenocarcinoma versus other NSCLC histologies (19% versus 0%), adenocarcinoma with any bronchioloalveolar features versus other adenocarcinomas (38% versus 14%), and no smoking history versus those with a history of smoking (36% versus 8%). A multivariate analysis of these features identified only adenocarcinoma with any bronchioloalveolar features and no smoking history as independent predictors of response.(64) The available evidence therefore suggests that certain demographic features and tumor histology may be correlated with response to treatment with erlotinib or gefitinib.

Identification of Somatic Mutations in the Tyrosine Kinase Domain of EGFR

While the identification of clinical and pathological features that predict for the efficacy of EGFR TKI's is helpful, they are not perfect discriminators of those patients that will benefit from treatment with these compounds. Subsequent investigations centered on finding specific molecular markers that would identify the population of NSCLC patients that are sensitive to EGFR tyrosine kinase inhibition. Two groups at Harvard simultaneously investigated the hypothesis that the presence of somatic mutations in the tyrosine kinase domain of EGFR might predict for response to an EGFR TKI.

Lynch and colleagues sequenced the entire coding region for *EGFR* in tumor specimens from nine NSCLC patients with a significant objective tumor response to gefitinib and from seven patients without a response.(65) Eight of the nine responders had somatic heterozygous missense mutations in the tyrosine kinase domain of *EGFR* while none were identified in the non-responders. Of note, all nine of the patients with a response to gefitinib had either adenocarcinoma or bronchioloalveolar carcinoma histology, none were current smokers (six had never smoked) and six of the nine were women (Figure 5).

Lynch and colleagues also sequenced the *EGFR* coding region in 25 NSCLC tumor samples (including 15 bronchioloalveolar samples) from patients who had not received gefitinib, and similar mutations were found in the tumors of two patients with bronchioloalveolar carcinoma.

Table 1. Characteristics of Nine Patients with Non–Small-Cell Lung Cancer and a Response to Gefitinib.

Patient No.	Sex	Age at Beginning of Gefitinib Therapy	Pathological Type*	No. of Prior Regimens	Smoking-Status†	Duration of Therapy	Overall Survival‡	*EGFR* Mutation§	Response¶
		yr				*mo*			
1	F	70	BAC	3	Never	15.6	18.8	Yes	Major; improved lung lesions
2	M	66	BAC	0	Never	>14.0	>14.0	Yes	Major; improved bilateral lung lesions
3	M	64	Adeno	2	Never	9.6	12.9	Yes	Partial; improved lung lesions and soft-tissue mass
4	F	81	Adeno	1	Former	>13.3	>21.4	Yes	Minor; improved pleural disease
5	F	45	Adeno	2	Never	>14.7	>14.7	Yes	Partial; improved liver lesions
6	M	32	BAC	3	Never	>7.8	>7.8	Yes	Major; improved lung lesions
7	F	62	Adeno	1	Former	>4.3	>4.3	Yes	Partial; improved liver and lung lesions
8	F	58	Adeno	1	Former	11.7	17.9	Yes	Partial; improved liver lesions
9	F	42	BAC	2	Never	>33.5	>33.5	No	Partial; improved lung nodules

* Adenocarcinoma (Adeno) with any element of bronchoalveolar carcinoma (BAC) is listed as BAC.
† Smoking status was defined as former if the patient had not smoked any cigarettes within 12 months before entry and never if the patient had smoked less than 100 cigarettes in his or her lifetime.
‡ Overall survival was measured from the beginning of gefitinib treatment to death.
§ *EGFR* denotes the epidermal growth factor receptor gene.
¶ A partial response was evaluated with the use of response evaluation criteria in solid tumors; major and minor responses were evaluated by two physicians in patients in whom the response could not be measured with the use of these criteria.

Figure 5. Patient characteristics for 9 patients with response to gefitinib (reproduced from reference 65).

To assess the functional properties of the mutated receptor, they expressed 2 of the identified EGFR mutant constructs in cultured cells. *In vitro*, the *EGFR* mutants displayed a 2-3 fold increase in tyrosine kinase activity in response to EGF as well as a significant prolongation in the duration of activation when compared with the wild-type receptor. In addition, these mutant receptors were more sensitive to inhibition by gefitinib. The wild-type EGFR was completely inhibited at a gefitinib concentration of 2 micromolar, while the mutant EGFR required a concentration of only 0.2 micromolar to achieve the same level of inhibition.

Paez et al simultaneously reported finding similar *EGFR* mutations in a group of 119 unselected primary NSCLC tumors that included 58 samples from Japan.(66) Notably, again the presence of a mutation was strongly associated with the clinical and

pathological characteristics previously identified as predictors for response to gefitinib. Mutations were more common in adenocarcinomas (21%) than other NSCLC histologies (2%), more prevalent in women (20%) than men (9%), and more frequent in the Japanese patients (26%) than in those from the United States (2%). The highest incidence of mutations was seen in the Japanese women with adenocarcinomas (57%). They also identified *EGFR* mutations in 5/5 tumors from NSCLC patients that had responded to gefitinib, while no mutations were found in the tumors from four non-responders. All of these patients were from the United States and were Caucasian. In addition, Paez et al. determined the mutation status and response to gefitinib in four NSCLC cell lines. They found that one of the four (H3255) did contain a mutation in *EGFR*. They also demonstrated that this cell line was fifty times more sensitive to treatment with gefitinib than those without a mutation, again suggesting that the presence of an *EGFR* mutant confers extraordinary drug sensitivity.

Several groups have now reported similar findings. Pao and colleagues from Memorial Sloan-Kettering Cancer Center sequenced exons 18-24 of *EGFR* from 10 patients with an objective tumor response or marked clinical improvement when treated with gefitinib.(67) Seven tumors (70%) had mutations in *EGFR*. Of those seven patients, 5 were never smokers, 6 had adenocarcinoma histology with BAC features, and none were of East Asian origin. No mutations were detected in 8 tumors from patients that were refractory to treatment with gefitinib. This difference was statistically significant (p=.004). They were also the first group to look for *EGFR* mutations in patients that had received erlotinib. In an analysis of 7 tumors from patients who demonstrated a partial response to erlotinib, 5 of 7 tumors (71%) harbored mutations, while no mutations were detected in 10 tumors from patients who did not respond to erlotinib treatment (p=.003). Four of the 5 patients with mutations were never smokers. In addition, *EGFR* sequencing was performed on 15 adenocarcinomas from patients known to be never smokers and 7 of 15 (47%) were identified to have mutations. Conversely, in 81 randomly selected tumors form the same tumor bank, mutations were detected in only 4 (5%). This suggests that tumors likely to have mutations can be identified using specific clinical criteria such as histology and smoking status.

Huang and colleagues from Taiwan analyzed 101 unselected NSCLC tumors for the presence of *EGFR* mutations and reported an overall mutation rate of 38.6%.(68) All of the mutations except one occurred in patients with adenocarcinoma, and the one exception was a tumor of mixed histology that was classified as adenosquamous. When the analysis was limited to those patients with adenocarcinoma, 55% of tumors were found to have a mutation, and, unlike in the previous series, in the group of adenocarcinomas with an *EGFR* mutation, no strong association between female gender or smoking status and the presence of a mutation was detected. The significance of this finding is questionable, however, given that the vast majority (57 of 69 or 83%) of patients with adenocarcinoma in this series were non-smokers. In a separate mutation analysis of 16 NSCLC patients that had received gefitinib, 7 of the 9 patients with gefitinib responsive tumors had mutations, while only 1 of 7 tumors refractory to gefitinib contained a mutated *EGFR*.

Two groups from Japan have evaluated banked NSCLC samples for the presence of mutations in *EGFR*. In a series of 277 patients, Kosaka et al. reported detecting mutations in 111 (40%).(69) Mutations were observed significantly more frequently in

females (59%) than males (26%), never smokers (66%) than those that smoked (22%), and in patients with adenocarcinomas (49%) than those with other NSCLC histologies (2%). In a multivariate analysis of these 3 variables, being a never smoker and having adenocarcinoma were significantly associated with the presence of a mutation, while female gender was not. Again, the only mutation seen in a non-adenocarcinoma was in an adenosquamous tumor. Tokumo and colleagues sequenced the tyrosine kinase domain of *EGFR* in 120 NSCLC samples.(70) Mutations occurred in 38 cases (32%), and in logistic regression models, being a never smoker and having adenocarcinoma histology were the only independent predictors for the presence of a mutation. Twenty-one of their cases had been treated with gefitinib. Eight of the 10 cases with objective response to gefitinib had *EGFR* mutations, as did one of the 11 cases without a response. The difference in mutation rate between gefitinib responders and non-responders was statistically significant ($p = .002$).

Marchetti and colleagues in Italy evaluated 860 consecutive primary NSCLC specimens for the presence of *EGFR* mutations.(71) No mutations were detected in 454 squamous cell carcinomas or 31 large cell carcinomas. A total of 39 mutations (10%) were found in the 375 adenocarcinomas. In addition, mutations were present in 26% of the 86 bronchioloalveolar carcinomas (BAC) and in only 6% of the 289 conventional adenocarcinomas ($p=.000002$). In a multivariate analysis, only BAC histology, being a never-smoker, and female gender were independently associated with the presence of an *EGFR* mutation with odds ratio of 4.5, 3.6 and 2.9 respectively.

John Minna and Adi Gazdar from UT-Southwestern have also recently published the results of their analysis of 617 NSCLC specimens.(72) The tumors included samples from Japan ($n = 263$), Taiwan ($n = 93$), the United States ($n = 160$) and Australia ($n = 101$). Five-hundred and nineteen of the specimens were collected sequentially, and the remainder was selected from patients with well-documented smoking histories from the United States and Australia. In the 519 unselected tumors, mutations were detected in 120 (23%). The frequency of mutations was significantly greater for patients of East Asian descent versus those of other origins (30% versus 8%, $p < .001$), females versus males (42% versus 14%, $p < .001$), never smokers versus those with a smoking history (51% versus 10%, $p < .001$), and adenocarcinomas versus other histologies (40% versus 3%, $p < .001$). When they limited their analysis to those patients with the highest frequencies of mutations (never smokers with adenocarcinoma), a significantly higher mutation rate was still observed for those patients of East Asian ethnicity as compared with a predominantly Caucasian population from the United States and Australia (64% versus 36%, $p = .003$), and this difference persisted after adjustment for gender.

The available data clearly indicates that mutations in the tyrosine kinase domain of *EGFR* can be reliably detected in a subgroup of patients with NSCLC. A review of the published information reveals the following (Table 2). The mutation rate in Eastern Asia greatly exceeds that for patients of Western ethnicity. In unselected cases obtained from Japan and Taiwan, 306 of 912 (34%) samples harbor *EGFR* mutations. Conversely, only 61 of 1165 (5%) samples from the United States, Italy, and Australia were found to contain mutated *EGFR*. It is also clear that adenocarcinoma histology is significantly associated with the presence of a mutation. In the series from East Asia, 297 of 625 (48%) adenocarcinomas had mutations, while in the Western patients with adenocarcinoma, *EGFR* was mutated in 58/499 (12%). In those patients with non-

adenocarcinoma histology (regardless of country of origin), only 10 of 840 cases (1.1%) were found to contain mutations in *EGFR*. Tumors from NSCLC patients who experience an objective tumor response or marked clinical improvement on an EGFR TKI (gefitinib or erlotinib) are also significantly more likely to have *EGFR* mutations than those from non-responders. Forty of 50 (80%) EGFR TKI responsive tumors have been shown to have mutations as compared with only 2 of 47 (4%) non-responsive tumors. The published series also agree that smoking status (i.e. being a never smoker) is an independent predictor for the presence of a mutation. They disagree, however, on the importance of gender. Several of the above reports showed that female sex does independently predict for the presence of a mutation, while a number failed to find a significant association.

Table 2. EGFR mutation frequencies in specific clinical subsets of NSCLC based on a compilation of the published data.

	EGFR mutation frequency
Western patients	61/1165 (5%)
Eastern patients	306/912 (34%)
EGFR TKI responders	40/50 (80%)
EGFR TKI non-responders	2/47 (4%)
Smokers	68/1263 (5%)
Never-smokers	140/332 (42%)
Adenocarcinoma (Western patients)	58/499 (12%)
Adenocarcinoma (Eastern patients)	297/625 (48%)
Non-adenocarcinoma histology	10/840 (1%)

Structural and functional effects of *EGFR* mutations

All of the reported *EGFR* mutations to date are typically heterozygous, somatically acquired and occur within the first 4 exons (exons 18-21) of the seven that encode for the tyrosine kinase domain of EGFR. Three types of mutations account for approximately 90% of those described. They include deletions surrounding 3 or 4 codons of exon 19, a single missense point mutation in exon 21 (L858R), and duplications and/or insertions in exon 20. The remainder are rare point mutations predominantly in exon 18, though they have been described in exons 20 and 21 as well. All of these mutations appear to target residues around the ATP binding cleft of EGFR, which is also the binding site for the EGFR TKI's. It has been hypothesized that these mutations result in repositioning of amino acid residues and a stabilization of their interaction with both ATP and the EGFR TKI's (Figure 6).(45, 65, 70)

Several groups have examined the functional consequences of the *EGFR* mutations. As mentioned above, Lynch and colleagues found that cultured cells which expressed EGFR with the L858R mutation or one of the common exon 19 deletions had increased kinase activity and 10-fold greater sensitivity to inhibition by gefitinib.(65) Several other groups have also demonstrated that mutations in the tyrosine kinase domain of *EGFR* result in increased kinase activity *in vitro* as well as exquisite sensitivity to treatment with an EGFR TKI. In addition, in *in vitro* models, NSCLC cell lines that contained these mutations displayed an increase in EGFR copy number, suggesting that

gene amplification is another effect of *EGFR* mutation. Three groups have also shown that *EGFR* mutations lead to activation of anti-apoptosis pathways, and that inhibition of these pathways by the EGFR TKI's appears to contribute to these drugs' efficacy.(73-75)

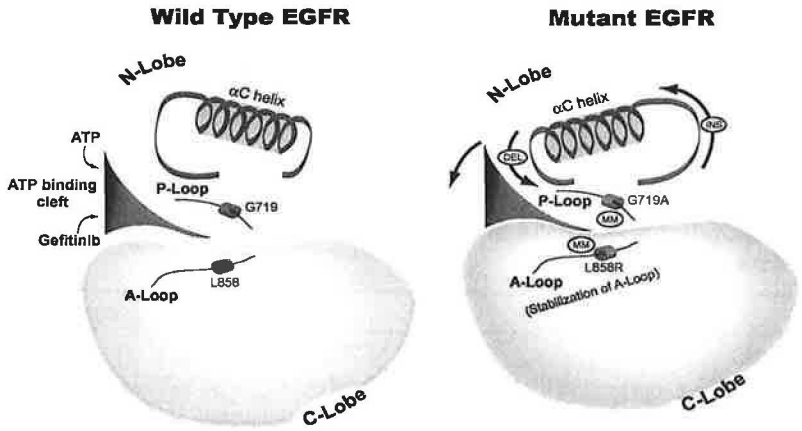


Figure 6. Effects of tyrosine kinase mutations on the ATP binding. The mutation appears to stabilize the interaction with both ATP and its competitive inhibitors (gefitinib and erlotinib)(reproduced from reference 45)

Not all of the preclinical data on the functional effects of *EGFR* mutations confirms these findings, however. Pao and colleagues, using autophosphorylation of tyrosine residues as a surrogate for kinase activity, found no evidence for increased EGFR activation in cultured cells with the L858R mutation or a deletion in exon 19. In addition, they found that although the L858R mutant displayed 10-fold greater sensitivity to inhibition with an EGFR TKI, the *EGFR* mutant with an exon 19 deletion had the same sensitivity as wild-type EGFR.(67)

Despite the discrepancies in the preclinical data noted above, the consensus opinion is that at least the most commonly observed mutations in *EGFR* are activating mutations. They may then be an example of what Bernard Weinstein termed "oncogene addiction."(76) Weinstein suggested that certain cancers may rely on the persistent activation of specific oncogenes. This dependence or "addiction" may also make these cancers especially vulnerable to therapies that target the products of these genes. In other words, while mutations in *EGFR* may accelerate the development of NSCLC, they also result in enhanced susceptibility to the EGFR TKI's that target this pathway.(77)

Future directions and unanswered (or partially answered) questions

What do EGFR mutations tell us about the pathogenesis of NSCLC in never smokers and the East Asian population?

Previously described genetic alterations in NSCLC are uniformly more prevalent in smokers than non-smokers. Examples include the well-described mutations in the *KRAS* genes, which like mutations in *EGFR* are especially common in adenocarcinomas.(78, 79) Shigematsu et al. found that while mutations occurred in either *KRAS* or *EGFR* in 47% of adenocarcinomas, no tumors had mutations in both genes. In addition, they observed that *KRAS* mutations were significantly more common in smokers than non-smokers and in patients from Western countries than in patients from East Asia.(72) Similarly, Kosaka and colleagues reported that *KRAS* mutations occurred in only 13% of adenocarcinomas from Japanese patients, and that they were more frequently observed in smokers than non-smokers. Again, no tumors were seen to harbor both *KRAS* and *EGFR* mutations.(69) In addition, an analysis of 60 lung adenocarcinomas by Pao et al. revealed that zero of the 21 tumors with *KRAS* mutations also had *EGFR* mutations, and none were sensitive to treatment with gefitinib or erlotinib.(80) This provides at least preliminary evidence that the presence of a *KRAS* mutation leads to primary resistance to an EGFR TKI.

The finding that *KRAS* and *EGFR* mutations are mutually exclusive also suggests that there may be two distinct molecular pathways involved in the pathogenesis of lung adenocarcinomas.(45) Given the strong association of *EGFR* mutations and never-smoking status, it may also be reasonable to hypothesize that some yet unidentified carcinogen may be responsible for these mutations and may be the major pathogenetic factor responsible for NSCLC in non-smokers. For example, in Taiwan, both human papilloma virus (types 16 and 18) and cooking oil fumes have been shown to be possibly associated with NSCLC in non-smoking women.(81, 82) In addition, the finding that *EGFR* mutations occur preferentially in East Asian patients suggests that certain populations may display greater genetic susceptibility to these hypothetical carcinogens.(72, 77)

What is the mechanism of acquired resistance to an EGFR TKI?

Although NSCLC patients whose tumors contain mutations in *EGFR* exhibit dramatic and durable responses to treatment with EGFR TKI's, unfortunately all patients eventually relapse and succumb to their disease. Until recently, the mechanism of "secondary" resistance to these drugs was unclear. In February, two groups simultaneously reported the discovery of a novel mutation in *EGFR* in 4 patients that had become resistant to treatment with gefitinib or erlotinib.(83, 84) This mutation is in exon 20 of the kinase domain and leads to a substitution of methionine for threonine at position 790 (T790M). Structural models suggest that this mutation introduces a bulkier amino acid (methionine) that leads to steric hindrance that prevents EGFR TKI binding. These models also predict that the mutation will not interfere with ATP binding and therefore will not alter kinase activity in response to ligand. In the 4 cases described, all of the tumors contained one of the described activating mutations in *EGFR* prior to initiation of treatment with gefitinib or erlotinib. After the development of tumor progression, rebiopsy of the tumor was performed and both the original and the new *EGFR* mutations (T790M) were detected. In *in vitro* models, cells transfected with both the L858R

activating mutation and the T790M mutation maintained their kinase activity but were insensitive to inhibition with erlotinib or gefitinib.

Several additional features of the T790M mutation are worth mentioning. Acquired *KRAS* mutations were not observed in any of the 6 patients that developed resistance to an EGFR TKI which suggest that such mutations are not a cause of "secondary resistance."(84) In addition, in 3 of the 6 NSCLC patients with *EGFR* activating mutations who had developed resistance to erlotinib or gefitinib, no new *EGFR* mutation was detected on rebiopsy. This indicates that the T790M mutation is not the only cause of acquired resistance, and thus, the identification of additional mechanisms of resistance is critical. In addition, it is clear that in NSCLC tumors from patients that have not received erlotinib or gefitinib, the T790M mutation is exceedingly rare. Thus far, it has been described in only 1 of the nearly 1300 tumors in which sequencing of exons 18-21 of *EGFR* has been performed, and in that one case, it was not reported whether the biopsy in question had been obtained after treatment with an EGFR TKI.(84) It is also noteworthy that the T790M mutation is analogous to a mutation described in the ABL tyrosine kinase domain that leads to resistance to the tyrosine kinase inhibitor imatinib in patients with chronic myelogenous leukemia. In addition, the T790M mutation had been introduced into EGFR two years previously and was shown to confer resistance to the EGFR TKI's. These findings have major implications for future drug development because they suggest that common mechanisms of resistance may exist for tyrosine kinase inhibitors that can be predicted from the beginning. Knowledge of these mechanisms should allow for quicker development of agents that overcome this resistance.(83-86)

Are EGFR activating mutations present in other tumors?

A number of additional tumor types have now been screened for the presence of somatic mutations in the tyrosine kinase domain of *EGFR*. Shigematsu et al. evaluated 243 epithelial carcinomas (including prostate, bladder, breast, colorectal and gallbladder primaries) and found no mutations in *EGFR*. In addition, no mutations were detected in DNA extracted from neuroendocrine lung tumors (including 6 small cell lung cancers, 25 bronchial carcinoids, and 5 large cell neuroendocrine tumors). A group from Korea performed mutational analysis on DNA from 537 tissue samples (98 colon cancers, 185 gastric adenocarcinomas, 93 breast cancers, 73 hepatocellular carcinomas, and 88 acute adult leukemias).(87) Only one *EGFR* mutation was found in a ductal carcinoma of the breast, and this mutation was a silent mutation that does not affect kinase function. Huang and colleagues also found only wild type *EGFR* in 30 hepatocellular carcinomas.(68) Thus, it currently appears that these activating mutations of *EGFR* are specific to NSCLC.

Do only patients with activating mutations in EGFR benefit from treatment with an EGFR TKI?

Approximately 50% of NSCLC patients derive "clinical benefit" (objective tumor response, stable disease, or symptom improvement) from treatment with an EGFR TKI. The prevalence of activating EGFR mutations (5% in Western populations and 35% in East Asians) does not appear to account for all of this advantage. Thus far, there is no published data on the frequency of *EGFR* mutations in patients with stable disease as the

best response to treatment with an EGFR TKI, but these analyses are underway.(88, 89) It is certainly conceivable that other genetic aberrations may be identified in this group of patients. In addition, approximately 20% of the EGFR TKI responsive tumors analyzed to date did not harbor *EGFR* mutations, and conversely, 4% of the EGFR TKI non-responsive tumors contain mutant *EGFR*. Therefore, at the present time, it is reasonable to consider all NSCLC patients that have progressed after standard chemotherapy as candidates for a trial of erlotinib or gefitinib, and the presence of an activating mutation in *EGFR* (or the clinical features that predict for the presence of a mutation) should not be a prerequisite for this therapy.

What are potential future clinical applications for the EGFR TKI's in NSCLC?

The therapeutic impact of the EGFR TKI's is being assessed in virtually every stage of NSCLC. There is theoretical evidence to suggest that these agents may be most effective in the setting of "low bulk" disease, i.e. the adjuvant setting after curative intent surgery. Several trials are currently underway in unselected NSCLC patients evaluating the efficacy of EGFR TKI's in preventing recurrence after surgery, and similar trials including only patients with *EGFR* mutations are in development. These drugs also enhance tumor cell sensitivity to radiation *in vitro*, and, therefore, several groups are incorporating erlotinib or gefitinib into chemotherapy and radiation regimens for locally advanced NSCLC. Although the original trials in combination with standard chemotherapy for metastatic disease failed to show a benefit with the addition of an EGFR TKI, post hoc subgroup analyses of these trials suggest that the group of patients with mutations in *EGFR* did derive benefit from the combination treatment (Giaccone, personal communication). There is thus some rationale for replicating these trials in only those NSCLC patients with mutant *EGFR*. Given the profound tumor responses seen in patients with mutations in *EGFR*, others have advocated assessing the efficacy of single agent erlotinib or gefitinib as first-line therapy for metastatic disease in this group of patients (thereby potentially avoiding standard chemotherapy altogether). Finally, investigations are underway to determine if these mutations occur in preneoplastic lung lesions (hyperplasia, dysplasia). If so, it may be rational to evaluate the EGFR TKI's as chemopreventive agents.

Conclusions

The identification of mutations in the tyrosine kinase domain of *EGFR* has galvanized the lung cancer research community because of the promise of immediate clinical applications. In order to fulfill this promise, answers to the questions raised above will need to be answered. The answers will not only define the optimal use of the EGFR TKI's in NSCLC treatment and prevention, but should also provide insight and accelerate research on additional "druggable targets" in NSCLC and other malignancies.

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