Measuring EGFR mutation status in lung cancer is standard for therapy selection.

Advances in EGFR as a Predictive Marker in Lung Adenocarcinoma
Farah K. Khalil, MD, and Soner Altiok, MD, PhD

Background: Worldwide, lung cancer is the most common cause of mortality. Toxins from tobacco smoke are known to increase the risk of lung cancer; however, up to 15% of lung cancer–related deaths in men and up to 50% of lung cancer–related deaths in women occur in people who do not smoke. Despite the fact that chemotherapy generally provides a survival benefit for non–small-cell lung cancer, not every patient will respond to therapy and many experience therapy-related adverse events. Thus, predictive markers are used to determine which patients are more likely to respond to a given regimen.

Methods: We reviewed the current medical literature in English relating to predictive markers that may be positive, such as the presence of an activating EGFR mutation.

Results: The advances in using EGFR as a molecular predictive marker were summarized. This biomarker influences therapeutic response in patients with lung adenocarcinoma. Clinical evidence supporting its value is also reviewed.

Conclusions: The use of EGFR as a predictive factor in lung adenocarcinoma may help target therapy to individual tumors to achieve the best likelihood for long-term survival and to avoid adverse events from medications unlikely to be effective.

Survival and Response Rates
Across all treatments and tumor stages, the 5-year overall survival (OS) rate for non–small-cell lung cancer (NSCLC) is 16%.1 Patients receiving induction chemotherapy with carboplatin, paclitaxel, and bevacizumab followed by concurrent chemotherapy with erlotinib, carboplatin, paclitaxel, and bevacizumab in addition to radiation and consolidation therapy with erlotinib and bevacizumab have objective response rates between 39% and 60% and median progression-free survival (PFS) of 10.2 months to 18.4 months.2 Response rates for patients with metastatic NSCLC provided treatment are 17% to 37%; median survival rates are 6.7 to 11.3 months; and 1- and 2-year survival rates are 31% to 46% and 9% to 21%, respectively.1,3,5,6 Time to progression averages 4 to 6 months and response rate to second-line therapy is 8%.6

EGFR and Tyrosine Kinase Inhibitors
The most promising and widely reported molecular predictive factor in NSCLC is EGFR, which resides on
the short (p) arm of chromosome 7 at position 12; epidermal growth factor (EGF) was first identified by Cohen in 1962. When EGF binds to epidermal growth factor receptor (EGFR), the receptor dimerizes, autophosphorylates, and activates several pathways, including mitogen-activated protein kinase, Janus kinase 2/signal transducer and activator of transcription (STAT) 3, STAT5, and phosphatidylinositol 3-kinase (PI3K)/protein kinase B pathways, which lead to cell proliferation, metastasis, and migration while preventing apoptosis. Because the EGF normally favors growth and proliferation, mutations of its receptor render it constitutively active independent of ligand-binding, which may lead to a malignant phenotype (Fig 1). An increased EGFR copy number or overexpression may play a role in oncogenesis. For example, based on their mechanisms of action, EGFR monoclonal antibodies (eg, cetuximab, matuzumab, panitumumab), which bind the extracellular domain of EGFR and prevent ligand binding, may be beneficial to patients with tumors overexpressing surface EGFR, whereas tyrosine kinase inhibitors (TKIs) may benefit patients whose tumor proliferation and metastases are driven by EGFR autophosphorylation. Blocking the catalytic site of the receptor, which is responsible for activating downstream molecules, may prevent growth and proliferation and possibly favor apoptosis in cancer cells.

Erlotinib and gefitinib, which are first-generation reversible TKIs, target the catalytic domain of EGFR by competing with adenosine triphosphate. Compared with placebo, erlotinib has been shown in a randomized, placebo-controlled, phase 3 trial to provide significant survival benefit in unselected individuals with advanced-stage NSCLC who received prior treatment. The researchers also found that those with certain demographic characteristics responded better to erlotinib. Other studies have demonstrated that patients who are Japanese, women, and those with adenocarcinomas have high response rates to gefitinib. Patients who have never smoked also have higher response rates, longer times to progression, and longer median OS rates when treated with TKIs than those who do smoke. Although the results of the phase 3 trial demonstrated that erlotinib provides a survival benefit compared with placebo, another phase 3 trial found that gefitinib was noninferior to docetaxel and had no benefit over docetaxel even in subgroups of women, those with adenocarcinomas, those who have never smoked, or Asians.

**Molecular Predictors of Response to Tyrosine Kinase Inhibitors and Survival Rates**

The different mutations identified in EGFR are in exons 19 (58%), 21 (36%), and 18 (6%). Approximately 90% of the mutations are either small deletions from exon 19 (codons 746–750) or substitution of arginine for leucine at position 858 in exon 21 (L858R). Another 3% are substitutions of a variety of amino acids in place of glycine at codon 719 (G719X) in exon 19, whereas tyrosine kinase inhibitors (TKIs) may benefit patients whose tumor proliferation and metastases are driven by EGFR autophosphorylation. The overall incidence of EGFR mutations in NSCLC ranges from 12.1% to 49%, depending on the patient population. Tyrosine kinase domain mutations of EGFR are rare in tumors in non-lung locations. The exon 19 deletions and L858R substitution are activating mutations that result in increased phosphorylation of EGFR independent of ligand stimulation.

![Fig 1. — EGFR involved in tumorigenesis of non–small-cell lung cancer. Akt = protein kinase B, EGF = epidermal growth factor, EGFR = epidermal growth factor receptor, MEK/MAPK = mitogen-activated protein kinase, PI3K = phosphatidylinositol 3-kinase, PTEN = phosphatase and tensin homolog, RAF = v-raf 1 murine leukemia viral oncogene homolog 1, src = proto-oncogene tyrosine-protein kinase Src.](image-url)
The usefulness of the *EGFR* mutation status as a predictive factor for response to TKIs is determined through the results of clinical trials and retrospective studies. Lynch et al\(^4\) demonstrated that *EGFR* mutations are associated with a clinical response to gefitinib and showed that in vitro *EGFR* mutants were more sensitive to gefitinib. No mutations were present in patients who did not respond to gefitinib.\(^5\) Both in vitro exon 19 deletion mutants and L858R mutants were more sensitive to gefitinib than tumors with wild-type *EGFR*.\(^6\) In addition, all 8 mutants Lynch et al\(^4\) identified were heterozygotes, indicating that these mutations act in a dominant manner, similar to other oncogenes. Paez et al\(^2\) confirmed these results, and Pao et al\(^3\) soon added that *EGFR* mutations can also be found in tumors sensitive to erlotinib. Since then, other studies have confirmed the association between *EGFR* mutation status and response to TKIs\(^30\)-\(^41\) and a review by Mitsudomi et al\(^12\) who compiled data from 1,335 patients showed that *EGFR* TKI response was observed in 70% of *EGFR*-mutant tumors compared with 10% of *EGFR* wild-type tumors. In a phase 2 trial, *EGFR*-mutant tumors responded better to gefitinib than wild-type *EGFR* tumors.\(^38\) Kalikaki et al\(^12\) demonstrated that patients with classic-activating *EGFR* mutations treated with gefitinib had a significantly longer median OS rate than those with wild-type *EGFR*; however, this relationship was not present in patients with other types of *EGFR* mutations. Significantly higher rates of objective tumor response to TKIs in *EGFR* mutants compared with wild-type *EGFR* have been seen in several studies,\(^19\),\(^29\),\(^40\),\(^43\) whereas others have found no such relationship.\(^44\)

Differential response to treatment between the different types of *EGFR* mutations have been observed by some authors. Mitsudomi et al\(^12\) reported that deletional mutations had higher response rates to gefitinib than other mutations — in particular, L858R. In addition, Hirsch et al\(^15\) found that patients who had exon 19 deletions alone had significantly higher objective response rates than patients with exon 21 mutations. By contrast, Pao et al\(^35\) found that in vitro wild-type *EGFR* and exon 19 deletion mutants similarly responded to gefitinib and erlotinib, whereas L858R mutants were approximately 10 times as sensitive. Not every patient with an activating mutation of *EGFR* responds to TKIs.\(^46\) Alternatively, some patients without *EGFR* mutations still respond to TKIs.\(^26\),\(^43\),\(^46\) Yoshioka et al\(^47\) published a phase 2 prospective study and determined that *EGFR* wild-type tumors have a modest response to erlotinib without irreversible toxicity. *EGFR* mutation status impacts treatment and has a concomitant impact on survival. Longer median survival (but not PFS) rates after first-line chemotherapy have been observed in individuals with *EGFR* mutations.\(^48\) In addition, Hotta et al\(^46\) found that OS and PFS rates following first-line chemotherapy are also significantly longer in patients with *EGFR*-mutant tumors compared with those with wild-type *EGFR* tumors. Moreover, use of gefitinib as first- or second-line therapy resulted in significantly longer PFS rates among patients with *EGFR*-mutant tumors than those treated with chemotherapy.\(^38\) In a series conducted by Kosaka et al,\(^30\) 397 individuals with *EGFR* mutations survived significantly longer than those with wild-type mutations; no survival difference was seen among those with an exon 19 deletion and L858R substitution.

**Molecular Alterations Conferring Resistance to Tyrosine Kinase Inhibitors**

Although many tumors with *EGFR* mutations initially respond to TKIs, disease progression will typically occur within 6 to 12 months because of the selective survival of tumor cells whose acquired mutations have conferred resistance to these agents, as well as due to pre-existing–resistant subclones, the up regulation of other parallel signaling pathways, or the transformation of tumor cells to small cell carcinoma.\(^12\),\(^18\),\(^49\),\(^51\) Even small clones of tumor cells containing a mutation conferring resistance to TKIs may have a significant clinical impact.\(^50\),\(^52\) The most common secondary *EGFR* mutation leading to TKI resistance is the substitution of methionine in place of threonine at codon 790 (T790M) within exon 20.\(^18\),\(^49\),\(^53\)-\(^55\) After identifying the presence of the T790M point mutation in a patient initially sensitive to gefitinib but who later became resistant, Kobayashi et al\(^14\) demonstrated in vitro that introducing the T790M mutation into previously gefitinib-sensitive NSCLC cells harboring either wild-type, an exon 19 deletion, or L858R-substitution *EGFR* genotypes induced gefitinib resistance. Pao et al\(^53\) also demonstrated in vitro that NSCLC cells harboring the T790M mutation with otherwise wild-type or mutated *EGFR* (either exon 19 deletion or L858R substitution) did not respond to either erlotinib or gefitinib.

Structural analysis has also shown that the T790M point mutation of *EGFR* changes tyrosine in the catalytic domain to methionine, which is bulkier and prevents erlotinib — and presumably gefitinib — from binding.\(^18\),\(^26\),\(^54\),\(^56\) The T790M mutation alters TKI binding and enhances the kinase activity of *EGFR* when coupled with the L858R mutation, thus offering cells with this mutation a survival advantage.\(^12\),\(^20\),\(^32\),\(^57\) The T790M point mutation has been identified in approximately 50% of patients who had activating *EGFR* mutations and later acquired resistance to TKI TKIs.\(^18\),\(^50\),\(^53\),\(^55\),\(^58\)-\(^59\) Engleman et al\(^60\) and Bonomi et al\(^19\) demonstrated in vitro that malignant cells with activating mutations of *EGFR* initially sensitive to gefitinib become resistant after prolonged exposure to gefitinib, and they may
also acquire the T790M mutation in EGFR. Even so, the question arises as to whether TKI treatment induces the resistance mutation or if it results in the selection of resistant clones.\(^{56}\) Depending on the sensitivity rate of the assay used, the T790M mutation has been found in 0.5% to 3.6% of patients who have never been treated with TKIs.\(^{12,50}\) Laboratory studies and clinical validation also indicate that the presence of pre-TKI EGFR T790M could act as a negative predictor of PFS in patients with EGFR-activating mutations.\(^{12,50,58,60,62}\)

The exon 20 insertion mutation of EGFR (D770insNPG) has been shown by Greulich et al.\(^{53}\) to confer erlotinib and gefitinib resistance in vitro; however, the response was obtained from the irreversible EGFR inhibitor CL-387,785. In another study, 2 women with adenocarcinoma harboring this mutation who never smoked both progressed despite gefitinib treatment.\(^{41}\) Intermediate sensitivity to erlotinib or gefitinib was observed in G719S of exon 18, and that sensitivity may be related to the specific mutation present.\(^{18,53,59}\)

Another missense mutation, D761Y in exon 19, has been reported to confer a milder degree of TKI resistance that may be clinically relevant.\(^{59}\) Greater resistance to gefitinib is produced when the T790M mutation occurs on the same DNA strand as the EGFR-activating mutation (L858R or exon 19 deletion),\(^{60}\) which is the most common scenario.\(^{65}\) This can be explained by understanding that the mutant allele alone will code for the mutant, constitutively active EGFR; likewise, binding of TKIs to these mutant, constitutively active EGFRs will be altered only if the resistance mutation has occurred on the mutant allele.

Low PTEN expression is an important reason for tumors becoming resistant to TKIs.\(^{54,66}\) PTEN normally functions to down-regulate the PI3K pathway (downstream of EGFR; it mediates growth, proliferation, and survival). Loss of PTEN leads to persistent activation of the PI3K pathway independent of EGFR signaling, making this proliferation signal unresponsive to the EGFR blockade.\(^{64,65}\) Among gefitinib-resistant cells lacking PTEN, Bianco et al.\(^{65}\) demonstrated that the reconstitution of PTEN reversed EGFR-independent PI3K pathway activity and restored gefitinib sensitivity. Zhuang et al.\(^{64}\) demonstrated that irradiating TKI-resistant tumors with low PTEN expression may help reverse the resistance by increasing PTEN expression levels. Although low PTEN expression may induce resistance to TKIs, a relationship may not exist between PTEN expression and rate of survival.\(^{10}\)

Some authors have reported acquired resistance to EGFR-resistant TKIs in tumors with MET amplification.\(^{12,60,66}\) It has also been suggested that MET activation (which may be induced by hepatocyte growth factor binding, overexpression, or structural alterations) may be used in place of MET amplification to determine sensitivity to TKIs.\(^{56}\) The results of an in vitro study by Rho et al.\(^{66}\) suggested that sensitivity to TKIs was not associated with MET activation in the absence of MET amplification.

### Methods to Overcome Acquired Resistance to Tyrosine Kinase Inhibitors

Although T790M makes cells resistant to gefitinib and erlotinib (which are reversible TKIs), the effect of irreversible TKIs (which covalently bind the EGFR kinase domain) is not changed by T790M. Irreversible TKIs include afatinib, dacomitinib, neratinib, pelitinib, canertinib, and CL-387,785.\(^{54,56,58,60,67}\) Irreversible EGFR inhibitors such as afatinib inhibit EGFR and downstream molecules more potently than gefitinib or erlotinib in cells harboring the T790M mutation, although not to the degree observed in cells without the T790M mutation.\(^{54,60,66}\) Regales et al.\(^{69}\) demonstrated in mice that tumors harboring the T790M TKI–resistance mutation had more shrinkage (and a complete response in most tumors) when treated with combination afatinib/cetuximab (an EGFR-specific antibody) when compared with other antitumor agents. This combination resulted in lower total and phosphorylated EGFR.\(^{49}\) Neither agent alone induced a complete response in any of the tumors in the study.\(^{69}\) Another study showed that CUDC-305, a heat-shock protein 90 inhibitor, had inhibited tumor growth in mouse xenograft models of NSCLC with EGFR T790M mutations.\(^{68}\)

Blocking signaling molecules downstream of EGFR has the potential to overcome the resistance induced by various mutations that alter the binding of drugs to EGFR.\(^{69,70}\) Src is one such downstream signaling molecule vital to maintaining the malignant phenotype of EGFR-mutant cells.\(^{69}\) In vitro, Src inhibitors have been shown to prevent oncogenesis caused by EGFR mutations, suggesting that Src inhibitors may be of clinical benefit in TKI-resistant EGFR-mutated NSCLC.\(^{69}\) Faber et al.\(^{70}\) demonstrated in vitro that blocking 2 major pathways downstream from EGFR resulted in tumor shrinkage, even in cell lines with various TKI-resistant mutations, including T790M. Afatinib and dacomitinib have shown sustained control of disease progression in patients with NSCLC who have primary or acquired TKI resistance.\(^{51}\) The LUX-Lung 1 phase 2/2B trial investigated afatinib compared with placebo in patients with NSCLC who were progressing following treatment with erlotinib or gefitinib and combination platinum-based chemotherapy. No significant improvement in OS rate was seen when compared with placebo.\(^{51}\) Individuals naive to EGFR-TKI treatment with stage 3B and stage 4 NSCLC
who harbored activating EGFR mutations were studied during the LUX-Lung 2 trial.51 These study volunteers were given once-daily dosing of afatinib (40 or 50 mg), and similar overall response rates were seen; however, more adverse events were seen among those given the higher dose.51 The phase 3 trial, LUX-Lung 3, randomized 345 individuals with EGFR mutation-positive adenocarcinoma to either once-daily 40 mg afatinib or combination cisplatin/pemetrexed.71 In that trial, 72% of study volunteers were of Asian descent.71 The afatinib group showed a prolonged PFS rate (11.1 vs 6.9 months) compared with the cisplatin/pemetrexed group.71 Patients with common EGFR mutations (eg, L858R, exon 19 deletion) had a maximum PFS rate of 13.6 months when treated with afatinib, whereas those assigned to chemotherapy had a maximum PFS rate of 6.9 months.51,72

The effective afatinib dose has toxicity limitations that has necessitated continual studies, thus leading to third-generation, irreversible TKIs, such as rociletinib, AZD9291, and HM61713, which have shown profound and sustained regression in T790M-mutant–selective resistance in NSCLC.73,74 Preliminary reports have shown response rates of 58%, 64%, and 29% for rociletinib, AZD9291, and HM61713, respectively.75,76 AZD9291 does not have the same severe adverse events as those seen with first-generation TKIs because it does not specifically target wild-type EGFR, it is associated with reduced rates of skin rash and diarrhea, and it has an overall response rate of 64% in patients positive for T790M.74 A recent trial showed that no dose-limiting toxicities were present at doses between 20 and 240 mg/day.75

Correlation of Histological Subtype of Adenocarcinoma With EGFR-Mutation Status
NSCLC and small cell carcinoma are 2 major categories of lung cancer based on histology and response to chemotherapy. The most common cell type of NSCLC is adenocarcinoma. The International Association for the Study of Lung Cancer, the American Thoracic Society, and the European Respiratory Society International proposed a new classification of lung adenocarcinoma in 2011.77 This international, multidisciplinary classification divided adenocarcinoma into histological subtypes based on survival data that indicated 5-year survival rates of 100% among patients with adenocarcinoma in situ (previously known as bronchioloalveolar carcinoma) and minimally invasive adenocarcinoma.77 Invasive adenocarcinoma subtypes include lepidic, acinar, papillary, micropapillary, and solid predominant patterns. Mucinous, colloid, fetal, and enteric histology have been described as variants of adenocarcinoma.77 Results from clinicopathological studies have identified a correlation between histological subtypes and the clinical outcome in which adenocarcinoma in situ and minimally invasive adenocarcinoma had excellent outcomes; lepidic, acinar, and papillary predominant adenocarcinoma had intermediate outcomes, whereas solid and micropapillary predominant and invasive mucinous adenocarcinoma had poor outcomes.78 Fig 2 illustrates invasive adenocarcinoma of the lung with a micropapillary growth pattern.

Reclassification studies of adenocarcinoma based on the recommendations from the International Association for the Study of Lung Cancer, the American Thoracic Society, and the European Respiratory Society International indicate that the lepidic pattern is associated with 44% of EGFR-mutant lung cancers as compared with the acinar pattern, which is associated with 69% of EGFR–wild-type lung cancers.77,79

Conclusions
Of all the molecular alterations that may have predictive value in non–small-cell lung cancer, testing for EGFR mutations is usually the first step in determining the course of adjuvant therapy. Activating EGFR mutations and amplification predict the response of non–small-cell lung cancer to tyrosine kinase inhibitors. The use of these predictive factors help target therapy to individual tumors to achieve the best likelihood for long survival; they are also used to help avoid unnecessary adverse events related to therapies unlikely to have any effect. Studies showing post–tyrosine kinase inhibitor resistance due to mutations not detected prior to treatment impress the utility of reassessing tumors following treatment. Additional, large cohort studies are required to accurately determine the association between adenocarcinoma subtypes and EGFR-mutation status. Mutational heterogeneity within a single tumor and morphological associations will most likely mandate the continual assessment of each type of cancer as it progresses from primary to metastatic and pretreatment to post-treatment status.
in patients with non-small-cell lung cancer treated with chemotherapy alone.


