Update on *Epidermal Growth Factor Receptor* Mutations in Non–Small Cell Lung Cancer

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Abstract In 2004, several investigators reported that somatic mutations in the *epidermal growth factor receptor* gene were associated with clinical responses to erlotinib and gefitinib in patients with non–small cell lung cancer. Since then, multiple groups have examined the biological properties that such mutations confer as well as the clinical relevance of these mutations in patients with non–small cell lung cancer. Although a tremendous amount of knowledge has been gained in the past 2 years, there remain a number of important epidemiologic, biological, and clinical questions.

The discovery that somatic mutations in the *epidermal growth factor receptor* (*EGFR*) gene are found in a subset of lung adenocarcinomas and are associated with sensitivity to the EGFR tyrosine kinase inhibitors (TKI) gefitinib (1, 2) and erlotinib (3) has generated excitement among clinicians and researchers studying non–small cell lung cancer (NSCLC). This information has allowed us to gain insight into the pathogenesis and treatment of this subset of lung tumors. Coupled with the observation that *KRAS* mutations are associated with resistance to gefitinib or erlotinib (4–6), these studies validate the hope for personally tailored molecular therapy in the near future. Finally, the finding of *EGFR* mutation–dependent lung adenocarcinomas confirms and extends the concept of "oncogene addiction," previously observed only in relatively rare diseases (chronic myelogenous leukemia, gastrointestinal stromal tumors, and hypereosinophilic syndrome), to a human carcinoma. Although a great deal has been learned since the initial discovery of *EGFR* mutations in lung cancer, a number of fundamental questions need to be addressed. In this article, we review the published data on *EGFR* mutations in lung cancer as of October 1, 2006, and discuss some important questions that remain. More extensive reviews on the rationale for EGFR as a target for therapy and on the clinical development of gefitinib and erlotinib have already been published (7-9).

Epidemiology of *EGFR* Mutations

Tumors and cell lines from >3,000 lung cancer patients from different institutions, a variety of geographic locations, and with a range of histologies and smoking histories have been analyzed for mutations in exons encoding the *EGFR* kinase domain (many focusing on exons 18-21; reviewed in refs. 9, 10). Tissue from other disease sites has been examined as well. Collectively, the data show that *EGFR* kinase domain mutations are almost exclusively found in a proportion of NSCLCs, with rare mutations also found in head and neck cancers, cholangiocarcinomas, and cancers of the colon, ovary, esophagus, and pancreas (11–16).

Many types of mutations have been reported, but there thus far are only four drug-sensitive mutations, validated from either *in vitro* studies (17) and/or from actual tumor responses in human patients. These are point mutations in exons 18 (G719A/C) and 21 (L858R and L861Q) and in-frame deletions in exon 19 that eliminate four amino acids (LREA) just downstream of a critical lysine residue at position 745.[4] The most common of these four drug-sensitive mutations are exon 19 deletions and the exon 21 L858R substitution, together representing 85% to 90% of *EGFR* mutations in NSCLC (Fig. 1). Thus far, three kinase domain mutations are associated with drug resistance: an exon 19 point mutation (D761Y; see the section on Acquired Resistance to Erlotinib or Gefitinib), an exon 20 point mutation (T790M), and an exon 20 insertion (D770_N771insNPG). Within lung cancers, *EGFR* kinase domain mutations are more common in adenocarcinomas, East Asians, women, and never smokers (reviewed in ref. 10). Mutations in *EGFR* may be more

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[4] EGFR has two numbering systems. The first denotes the initiating methionine in the signal sequence as amino acid −24. The second, used here, denotes the methionine as amino acid +1.

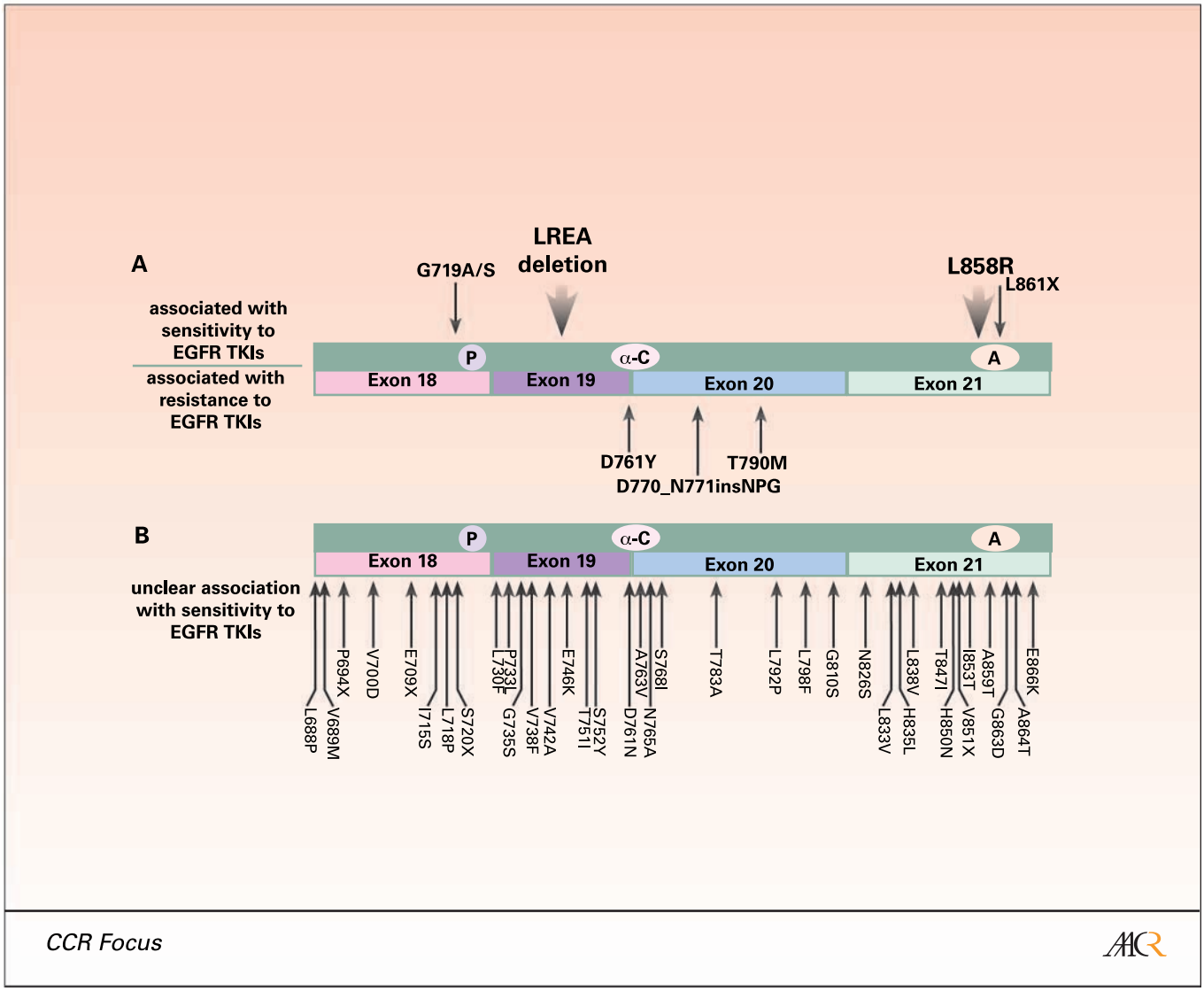

Fig. 1. Schematic diagram of reported mutations in *EGFR* in NSCLC tumors. *A,* mutations associated with sensitivity to gefitinib or erlotinib (*top*). The relative frequencies of mutations are indicated by size of arrows. Mutations associated with resistance to gefitinib or erlotinib (*bottom*). *B,* mutations isolated from NSCLC tumors with unclear association with response to erlotinib or gefitinib. X, multiple substitutions have been reported at an amino acid. P, P-loop. A, activation domain. α-C, α-C helix domain (1–4, 17, 41, 44, 45, 47, 48, 55–57, 71, 76, 85).

common in women because the majority of never smokers are women (18). These characteristics had been previously noted as clinical predictors of response to gefitinib and erlotinib (19–21). Mutations outside the exons encoding the kinase domain are rare in lung cancers (1–3), but the EGFRvIII mutations commonly found in gliomas (22) have been found in some NSCLCs, especially in those with squamous cell histology (23).

Although virtually all work in this area has confirmed that *EGFR* mutations associated with response to gefitinib or erlotinib are somatic mutations not identified in either adjacent normal lung or peripheral blood leukocytes, a family with germ line mutations in *EGFR* has been identified (24). In that family of European descent, six family members in three generations had lung cancer, three with the bronchioloalveolar carcinoma subtype of adenocarcinoma. The *EGFR* T790M mutation, which has been associated with acquired resistance (see below), was isolated from tumor and peripheral blood specimens in the two patients for which samples were available. One patient with measurable disease was treated with gefitinib and did not respond.

Biological Consequences of *EGFR* Mutations

The precise mechanisms by which *EGFR* mutations induce lung cancer and why mutant-bearing tumors are more sensitive to treatment remain to be fully elucidated. Using NSCLC cell lines with mutations or a variety of transfected cells (mouse fibroblasts, human bronchial epithelial cells, mouse mammary epithelial cells, and mouse pre-B cells), multiple groups have

shown that the EGFR exon 19 deletion and L858R mutants confer ligand-independent activation and prolonged receptor kinase activity after ligand stimulation (1, 2, 25). Kinetic analysis of the purified intracellular domains of the L858R mutant and a deletion mutant reveals that both mutants are active but exhibit a higher K_m for ATP and a lower K_i for erlotinib relative to the wild-type receptor (26). Separate *in vitro* kinase activity assays show that the catalytic efficiency (k_{cat}/K_M) of the L858R mutant form of the kinase domain is ~20-fold higher than that for the wild-type kinase domain, suggesting that whereas the wild-type kinase domain is autoinhibited, the L858R mutant is constitutively active, probably because the L → R amino acid substitution destabilizes the inactive EGFR conformation (27). The structural basis for the enhanced sensitivity of the deletion mutants is not apparent from previously published reports of crystal structure data of EGFR TKIs with the kinase domain of EGFR (28, 29). Further insights may be gained by the characterization of a cocrystal structure of erlotinib (or gefitinib) with the L858R and deletion mutants.

Nevertheless, mutations in the *EGFR* kinase domain are sufficient for oncogenic transformation. *In vitro* work has shown that selected mutations in *EGFR* (exon 18 G719S, exon 19 deletion, exon 21 L858R, and exon 20 insertion) can transform both fibroblasts and lung epithelial cells (17, 26). Additionally, tetracycline-regulatable mouse model systems indicate that expression of either *EGFR* exon 19 deletions or L858R alleles in mouse lung epithelia leads to formation of tumors analogous to human lung cancers (30, 31).

Because EGFR activation requires homodimerization or heterodimerization for downstream signaling, investigators have begun to look at the binding of EGFR mutants. Initial

Table 1. Selected retrospective studies analyzing *EGFR* mutations and response to treatment with erlotinib or gefitinib

		Reference	No. patients	RR (%)	Median PFS/TTP (mo)	Median OS (mo)
Gefitinib	IDEAL	(44)				
	Mutation		14	46	4	
	Wild type		65	10	2	
	Mitsudomi et al.	(50)				
	Mutation		33	83		
	Wild type		26	10		
	Takano et al.	(52)				
	Mutation		39	82	13	20
	Wild type		27	11	2	7
	Shih et al.	(56)				
	Mutation		29	69	9	14
	Wild type		33	9	2	5
	Chou et al.	(45)				
	Mutation		33	71	8	15
	Wild type		17	31	2	5
	Han et al.	(47)				
	Mutation		17	65	22	31
	Wild type		73	14	2	7
	Taron et al.	(57)				
	Mutation		17	94		NR
	Wild type		51	12		10
	Cappuzzo et al.	(40)				
	Mutation		15	53	10	21
	Wild type		74	5	3	8
	Tokumo et al.	(53)				
	Mutation		9	89		25
	Wild type		12	17		14
	Cortes-Funes et al.	(46)				
	Mutation		10	60	12	13
	Wild type		73	8	4	5
Gefitinib with chemotherapy	INTACT	(44)				
	Mutation		23	72	NR	15
	Wild type		197	55	6	9
Erlotinib	BR.21	(41)				
	Mutation		24	30		
	Wild type		177	8		
Erlotinib with chemotherapy	TRIBUTE	(4)				
	Mutation		15	53	13	NR
	Wild type		99	18		

Abbreviations: RR, response rate; PFS, progression-free survival; TTP, time to progression; OS, overall survival; NR, not reached.

Table 2. Prospective trials of erlotinib or gefitinib in patients with *EGFR* mutations

	No. patients	RR (%)
Gefitinib		
Inoue et al. (62)	16	75
Morikawa et al. (63)	20	65
Sunaga et al. (65)	12	75
Sutani et al. (66)	26	81
Erlotinib		
Paz-Ares et al. (64)	21	90

work suggests that ErbB3 preferentially associates with EGFR mutants (32). Others have shown that increased expression of ErbB2 can increase the sensitivity to treatment with gefitinib of some cell lines (33). Consistent with these data, lung adenocarcinoma cells that depend on EGFR for survival were found to constitutively activate the receptor through overexpression of EGFR dimeric partners and their ligands (6). These data are reviewed elsewhere in this issue of *Clinical Cancer Research*.

Regulation of downstream events after EGFR activation is an area of intense investigation. Early biochemical analyses of NSCLC cell lines and transfectants indicate that, in cell lines bearing mutations in *EGFR*, signal transducers and activators of transcription (STAT) 3 and 5 and AKT are preferentially activated, whereas extracellular signal-regulated kinase and SHC phosphorylation remain largely unchanged, suggesting selective activation of prosurvival pathways without alteration of proliferation pathways (25, 34–36). These mutant cell lines are more sensitive to inhibition of STAT 3 or AKT (35, 36). A recent report showed that the SRC-ABL kinase inhibitor dasatinib selectively induces apoptosis in *EGFR*-mutant lung cancer cells, implicating SRC or ABL as critical downstream molecules (37). However, whether the effect of dasatinib was due to inhibition of SRC or ABL or of the mutant EGFRs themselves was unclear, as there did seem to be inhibition of autophosphorylation of EGFR itself in the treated cells and in surrogate kinase assays; moreover, at the concentrations used to induce apoptosis, dasatinib has been shown to inhibit other kinases as well, including mutant EGFRs (38). That kinase inhibition leads to apoptosis in cells with mutant *EGFR* supports the notion that these cells are "addicted" to signaling via the mutant proteins.

Role of *EGFR* Amplification

In NSCLC cell lines, *EGFR* mutations are commonly associated with amplification. In H3255, which has an *EGFR* L858R mutation and is one of the most drug-sensitive cell lines identified to date, *EGFR* is amplified ~11-fold (39). These data highlight the notion that drug sensitivity could be associated with both mutation and amplification. Several groups have investigated the predictive value of amplification in patients treated with gefitinib or erlotinib on clinical trials (40, 41). In these studies, patients with amplification or polysomy of *EGFR* were more likely to respond to erlotinib or gefitinib compared with patients with normal *EGFR* copy number. Patients with amplification or high polysomy also had longer median time to progression and overall survival. In most studies, amplification of *EGFR* has been associated with somatic mutation in *EGFR* (reviewed in ref. 42). Whether amplified wild-type *EGFR* contributes to lung cancer oncogenesis and susceptibility to erlotinib and gefitinib remains to be established. A431 cells, which contain amplified wild-type *EGFR*, are sensitive to gefitinib and erlotinib but are derived from a vulvar, squamous tumor. In the absence of ligand, wild-type *EGFR* is not transforming in mouse fibroblasts or bronchial epithelial cells (17, 43). Experiments in transgenic mice may shed light on this issue.

Clinical Aspects of *EGFR* Mutations

EGFR *mutations are associated with response to erlotinib and gefitinib.* The association of sensitivity to gefitinib and erlotinib with *EGFR* mutation is very consistent. Initial data are based largely on retrospective data collected from patients treated on trials designed for gefitinib or erlotinib before *EGFR* mutations were known to exist (Table 1). Collectively, these studies show an ~75% response rate for patients whose tumors have mutations compared with a response rate of <10% for those with wild-type *EGFR* (1–5, 40, 41, 44–60).

Because mutational analysis was not originally planned, molecular studies of tumors from patients on previously completed prospective clinical trials of erlotinib or gefitinib have had relatively low rates of tumor acquisition. For example, in multicenter randomized trials of patients with NSCLC treated with chemotherapy along with erlotinib (TRIBUTE) or gefitinib (INTACT), only 21% and 28% of patients had their tumors analyzed (4, 44). In the molecular analysis of BR.21, a large, randomized trial of single-agent erlotinib versus placebo, usable sequence data on just 28% (202 of 731) of patients enrolled was collected (41, 61). Nevertheless, these studies all showed a statistically significant association between mutation and response.

The retrospective data have now been confirmed in five studies conducted specifically to determine prospectively the response rates in Caucasian and East Asian patients with drug-sensitizing *EGFR* mutations to gefitinib or erlotinib (Table 2; refs. 62–66). Collectively, these showed that 74 of 95 (78%) patients whose tumors had either exon 19 deletions or L858R mutations had radiographic responses to either TKI. Although overall survival data were not yet mature enough to report, these studies confirm that *EGFR* mutation status is a bona fide predictor of radiographic response to EGFR TKIs. As a comparison, the standard for molecularly targeted therapy thus far has been the monoclonal antibody trastuzumab (Herceptin), which "targets" breast cancer patients whose tumors overexpress the drug target HER2. In a single-arm study used in part as the basis for Food and Drug Administration approval of trastuzumab, only ~33% of tested patients were eligible to receive drug (i.e., had breast tumors that overexpressed HER2), and the drug induced only a 14% response rate in this enriched patient cohort (67).

Although multiple retrospective studies have shown that patients with *EGFR* mutations treated with gefitinib live longer

than those without *EGFR* mutations (45, 47, 50, 52, 56, 57), retrospective molecular subgroup analyses of the prospective BR.21 trial failed to associate *EGFR* mutations with improved overall survival (Table 1).

One major confounding factor in all mutation analyses is the sensitivity of the mutation detection assay used. By convention, direct Sanger sequencing has been used in most studies, but this technique has a relatively low sensitivity for detection of mutations in available clinical specimens, and, as has been recently shown, results can also be obscured by allelic dilution if one copy of the gene is amplified (68). Several groups using more sensitive techniques have identified multiple patients with *EGFR* mutation–positive tumors not detected by direct sequencing (69–71). Some of the more sensitive assays (69, 72–74) require previous knowledge of the mutation being analyzed, whereas others (70, 71) are able to capitalize on mismatch between wild-type and mutant DNA to identify novel mutations in very small amounts of material, making them particularly appropriate for exploratory studies.

***Natural history of patients with mutant* EGFR *versus wild-type* EGFR.** Emerging clinical data suggest that NSCLC tumors with *EGFR* mutations exhibit a unique biology in comparison with *EGFR* wild-type NSCLC. Although some retrospective series have noted prolonged survival of patients with *EGFR* mutation tumors compared with wild-type tumors for patients treated with erlotinib or gefitinib, this prolonged survival may even occur in the absence of treatment with TKI (patients treated with primary surgery or standard cytotoxic chemotherapy). In the molecular analysis of patients with NSCLC enrolled on the TRIBUTE and INTACT trials (large phase 3 trials in which patients with NSCLC were randomized to receive either chemotherapy or chemotherapy in combination with an EGFR TKI) among patients who received chemotherapy alone, patients with *EGFR* mutations (TRIBUTE, $n = 14$; INTACT, $n = 9$) had prolonged progression-free and overall survival compared with patients with *EGFR* wild-type tumors (TRIBUTE, $n = 99$; INTACT, $n = 83$). The differences in overall survival were >10 months (4, 44). Some have found similar results for patients who had primary treatment with surgery and were never treated with erlotinib or gefitinib (75), whereas others have found no difference in overall survival in patients never treated with a TKI (76).

Natural history and clinical course of patients with exon 19 deletions versus L858R point mutations. Different mutations in *EGFR* may confer different tumor activation profiles that lead to variations in both natural history and clinical course after treatment with erlotinib or gefitinib. In NSCLC patients treated with surgery alone, patients with *EGFR* point mutations ($n = 31$) have a prolonged overall survival when compared with patients with exon 19 deletions ($n = 31$; ref. 76). In contrast, retrospective data from our group (77) and others (78) suggest that after treatment with gefitinib or erlotinib, patients with *EGFR* exon 19 deletions have a longer overall survival when compared with patients with *EGFR* L858R (34 versus 8 months; log-rank, $P = 0.01$). The molecular basis for this observation remains to be elucidated, although recent kinetic analyses of EGFR-mutant proteins suggest that the off-rate for erlotinib may be slower for the deletion mutant, compared with the L858R mutant, thus prolonging the duration of erlotinib binding to the deletion mutant (26). Interestingly, patients with gastrointestinal stromal tumors treated with imatinib similarly have differential survival rates when treated with imatinib; patients with mutations in *KIT* exon 11 have a significantly longer overall survival when compared with patients with mutations in *KIT* exon 9 (79). Clearly, prospective evaluation of the different responses to treatment is necessary and will require the collaboration of multiple institutions to accrue a statistically sufficient number of patients with *EGFR* mutations. All studies investigating the response to treatment of patients with *EGFR* mutations should express survival, time to progression, and response stratified by the presence or absence of mutation and by type of *EGFR* mutation.

Acquired resistance to erlotinib or gefitinib. Despite an initial response to *EGFR* TKIs, patients with *EGFR* mutations rarely achieve a complete radiographic or pathologic response. The presence of tumor and continued treatment with gefitinib or erlotinib provides a selective pressure for the development of tumor cells with acquired resistance to gefitinib or erlotinib. The mechanisms of this acquired resistance are beginning to be elucidated. Tumors from a small number of patients who showed initial sensitivity to gefitinib or erlotinib and subsequently developed acquired resistance have been analyzed, either by biopsies done as a part of clinical trials or by obtaining autopsy tissue. These studies have shown that additional mutations in *EGFR* are found in specimens with acquired resistance (80–82). The major lesion identified to date is an EGFR T790M mutation that has been reported in about half of patient tumors after disease progression (80–86). *In vitro*, EGFR T790M is resistant to inhibition by gefitinib and erlotinib (82). This T790M substitution in EGFR is predicted to block binding of erlotinib or gefitinib to the kinase ATP-binding pocket and is analogous to amino acid changes seen in acquired resistance to imatinib in GIST and chronic myelogenous leukemia. Acquired resistance may also be influenced by anatomic site, as at least in two patients with widely metastatic disease, T790M mutations have been found in visceral sites but not in the central nervous system (85, 87); this observation suggests that the selective pressure for resistance mutations could be different in the central nervous system, where levels of drug seem to be lower than in the periphery (87). Consistent with this, a different mutation (D761Y in exon 19) has been found in a patient with acquired resistance to gefitinib that developed in the brain (85). Others have suggested that alterations in receptor turnover may also play a role in acquired resistance, although this is has not been shown *in vivo* (81).

Understanding in part the basis for acquired resistance has led to the identification of agents which may overcome acquired resistance. *In vitro* data have suggested that irreversible EGFR TKIs (including HKI-272, EKB-569, and CI-1033; some of which also inhibit the HER2 kinase) may have activity in patients with acquired resistance (38, 80, 81). Initial phase 1 trials, which have accrued multiple patients with NSCLC, have failed to note significant activity for most of these agents

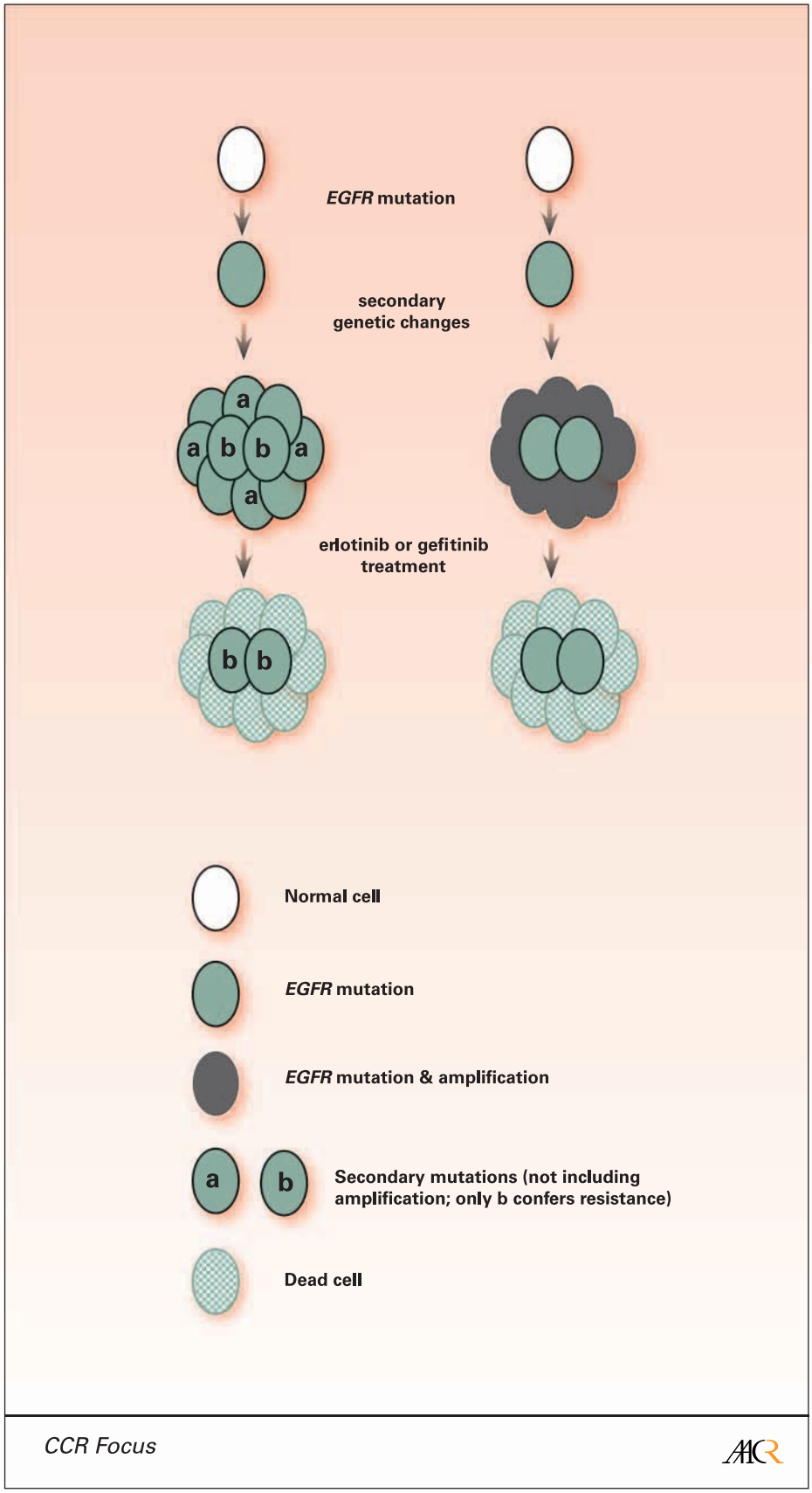

Fig. 2. A model for EGFR-dependent tumor maintenance that accounts for the lack of complete response to treatment with gefitinib or erlotinib (see text for details).

in unselected NSCLC populations. A multicenter, phase 2 clinical trial of HKI-272 has recently begun. An aim of this trial is to determine the efficacy of the drug in patients who have progressed after initial treatment with erlotinib or gefitinib. Additional strategies may also be successful. For example, *in vitro* data suggest that treatment with the hsp90 inhibitor geldanamycin or its derivatives may be able to overcome acquired resistance to erlotinib or gefitinib (88).

Areas for Further Study

Epidemiology

Why are* EGFR *mutations found disproportionately in women, Asians, and never smokers? Although the majority of patients with NSCLC have a significant history of cigarette smoking, the majority of patients with NSCLC with *EGFR* mutations are never smokers. The lack of the most commonly implicated carcinogen in this latter set of lung cancer patients raises the possibility that other genetic and environmental factors contribute to the development of *EGFR* mutations. A variety of factors may lead to an underlying genetic instability, including genetic variants of DNA mismatch repair or exposure to other carcinogens, including radiation or second-hand smoke. Comprehensive epidemiologic studies should help elucidate risk factors that explain the unique distribution of *EGFR* mutations in NSCLC.

Tumor biology

How do mutations affect intrinsic* EGFR *activity? What are the relative contributions of* EGFR *mutation and amplification of either mutated or wild-type EGFR to lung cancer oncogenesis? How do the dimerization profiles and activation patterns of wild-type and mutant* EGFR *differ? What are the similarities and differences in tumor biology of the multiple ERBB network mutations (i.e.,* EGFR, HER2, PIK3CA, BRAF, *and* KRAS*)? The importance of the ERBB signaling pathway in lung cancer oncogenesis is supported by the identification of mutations of multiple sites in this network in lung adenocarcinomas. Mutations in *EGFR, HER2, PIK3CA, KRAS, BRAF, LKB/STK11*, and *SHP2* have all been identified in lung adenocarcinomas and seem to be predominantly mutually exclusive (76, 89–95), except for *PI3KCA* (96). The frequency of mutations in this pathway and the relative absence of overlapping mutations (for at least *EGFR, KRAS, HER2*, and *BRAF*) suggest that single mutations at any point in the ERBB signaling network are sufficient for transformation. Determining how EGFR and other molecules in the ERBB signaling network of additional therapies.

Clinical outcomes

Is there a role for routine* EGFR *mutational analysis in lung cancer? A number of prospective trials are now ongoing or planned that address the role of *EGFR* mutation testing in advanced/metastatic NSCLC and the initial treatment of patients with *EGFR* mutations. For example, there are ongoing trials in Japan, Europe, and the United States, in which tumors from untreated patients are tested for mutations. Patients with wild-type *EGFR* receive standard chemotherapy, whereas patients with mutations in *EGFR* are treated with gefitinib or erlotinib. Other trials being conducted in Asia randomize patients with *EGFR* mutations to receive either chemotherapy or gefitinib. These studies should help determine the importance of mutation testing in selecting therapy for subsets of patients with lung cancer, providing prospective data on response rates, time to progression, and survival with and without mutations treated with either gefitinib or erlotinib. Other analyses should also be done to determine the most sensitive and cost-effective methods for determining mutation status from either archival paraffin-embedded and/or fresh-frozen tissues in real-time clinical settings. Should these studies show convincing evidence that *EGFR* mutations are associated with response to, and survival on, EGFR TKIs, we envision that, as HER2 testing is standard for breast cancer patients, testing for *EGFR* and possibly other mutations (such as *KRAS*) will become standard for many lung cancer patients.

This notwithstanding, we do note a major caveat that currently does not limit the use of EGFR TKIs in NSCLC. The relatively minimal side-effect profile of erlotinib and its Food and Drug Administration approval for use in unselected, previously treated patients with NSCLC makes it likely that erlotinib will be continue to be prescribed to patients with metastatic NSCLC without the use of mutational analysis, especially those patients with poor performance status. However, as a wider range of targeted therapies become available in the future, oncologists may use mutational analysis to help them choose among possible treatments and to guide the most rational order with which these therapies should be given for individual patients. The widespread use of mutational analysis is currently hindered by the routine use of very small fragments of tissue to establish the diagnosis of NSCLC. These small amounts of diagnostic material are usually inadequate for any molecular analysis. In addition, the time to determine mutation status can be quite lengthy, sometimes taking longer than 2 weeks. As mutational analysis becomes more useful in the treatment of patients with advanced NSCLC, oncologists will need to rely upon diagnostic procedures that obtain larger amounts of tissue (such as core needle biopsies) and laboratory methods that yield results more quickly.

In the treatment of metastatic NSCLC, should erlotinib or gefitinib be used alone or in combination with cytotoxic chemotherapy? Four large, randomized, prospective trials (TRIBUTE, TALENT, INTACT-1, and INTACT-2) failed to show a benefit for the use of combinations of chemotherapy and EGFR TKIs in unselected groups of patients with NSCLC. However, subset analysis of one of these trials showed that, among never smokers, patients treated with the combination of erlotinib, carboplatin, and paclitaxel had an overall survival of >20 months, suggesting that this subgroup, enriched for tumors with *EGFR* mutations, had significant benefit from the combination of all three drugs. To determine the relative contribution of each component of this treatment, the Cancer and Leukemia Group B is conducting a randomized phase 2 study of erlotinib versus erlotinib, carboplatin, and paclitaxel in patients with lung adenocarcinoma with <10 pack-years history of smoking. Tissue adequate for *EGFR* mutational analysis is necessary for entry. In addition, an Asian cooperative group is

investigating a similar population of patients and randomizing them to either gefitinib or chemotherapy alone. The results of these trials, complete with molecular analysis of *EGFR*, will help to determine the ideal initial treatment for both never smokers and patients with *EGFR* mutations.

Does erlotinib or gefitinib have a role in the adjuvant treatment of NSCLC tumors with* EGFR *mutations? Over the last few years, considerable evidence has supported the use of adjuvant chemotherapy in the treatment of resected, early-stage NSCLC (reviewed in ref. 97). A logical question then is whether patients with *EGFR* mutations would benefit from adjuvant therapy with erlotinib or gefitinib. A trial to evaluate adjuvant gefitinib in an unselected population of patients with NSCLC was closed following the report that gefitinib conferred no survival benefit in patients with advanced NSCLC (98), but studies specifically targeting patients with *EGFR* mutations are still ongoing (99).

If the oncogene addiction hypothesis pertains to mutant* EGFR*–dependent lung cancers, why does treatment with erlotinib or gefitinib not lead to complete radiographic and pathologic response? Multiple lines of evidence suggest that *EGFR* mutations are an initiating event in lung cell transformation and that the tumors remain addicted to EGFR signaling. By inhibiting tyrosine kinase activity, erlotinib and gefitinib block tumor cell proliferation and induce apoptosis. Clinically, however, we only rarely see complete remissions, and patients eventually have disease recurrence. Two distinct, but not necessarily mutually exclusive, scenarios could account for this phenomenon (Fig. 2). In the first situation, *EGFR* mutation alone is not sufficient to confer "oncogene addiction" and drug sensitivity. The tumor cells also need additional genetic lesions, such as *EGFR* amplification, and only cells with both mutations and amplification proliferate more quickly and become dependent on mutant *EGFR* signaling. If a tumor contains a heterogeneous population of cells, some with mutations only and some with mutations and amplification, then treatment with gefitinib or erlotinib kills only the latter rapidly dividing, EGFR-dependent population, leaving residual cells that may undergo initial growth arrest but could eventually cause disease after acquiring additional genetic lesions. In a second scenario, dividing tumor cells with *EGFR* mutations acquire other unidentified genetic lesions not involving *EGFR* itself. Treatment with TKI kills the *EGFR*-mutated cells without additional lesions but leaves the cells with secondary mutations that reduce sensitivity to drug. Both of these scenarios are experimentally testable.

What causes acquired resistance to erlotinib or gefitinib in the absence of T790M mutations? How can patients with acquired resistance to erlotinib or gefitinib be treated? Do treatments that suppress the development of acquired resistance exist? Although we have identified *EGFR* T790M in a proportion of patients with acquired resistance to erlotinib or gefitinib, the mechanisms of acquired resistance for about half of patients remains unknown. Continued examination of biopsy and autopsy specimens from patients who have acquired resistance will help to define the frequency of the T790M and discover additional mechanisms of acquired resistance. Trials to assess the efficacy of newer kinase inhibitors in patients with acquired resistance to erlotinib or gefitinib are ongoing and may suggest novel treatment strategies to prevent or delay the development of acquired resistance. Potentially, as has been shown analogously in the treatment of HIV infection, combination drug treatment may be useful in delaying the development of acquired resistance to erlotinib or gefitinib or treating it once it has emerged.

Is there a role for anti-EGFR antibodies in the treatment of NSCLC? What are the determinants of tumor types that respond to treatment with antibodies or TKIs? Small-molecule kinase inhibitors are not the only agents that target EGFR in the clinic. In parallel with gefitinib and erlotinib, anti-EGFR antibodies have been developed. The most well studied to date is cetuximab (IMC-C225, Erbitux), a human/murine chimeric anti-EGFR antibody that inhibits proliferation of EGFR-overexpressing cells *in vitro* and *in vivo* (100). In contrast to small-molecule TKIs, like gefitinib or erlotinib, that compete with ATP in the ATP-binding site of the EGFR kinase domain, crystal structure analyses have indicated that cetuximab binds exclusively to an extracellular domain (domain III), partially occluding the ligand-binding region on this domain and sterically preventing the receptor from adopting the extended conformation required for receptor dimerization (101).

Thus far, cetuximab seems to be active in only a limited number of cancers. The drug was approved by the Food and Drug Administration in February 2004 for use in treating advanced-stage, EGFR-expressing colorectal cancer (102), and it also seems to confer additional benefit when added to radiation for head and neck cancers (103). Somewhat surprisingly, trials of cetuximab as a single agent in NSCLC have shown relatively low response rates (104, 105). Conversely, gefitinib and erlotinib seem to have very little activity in colorectal cancers, where *EGFR* kinase domain mutations are very rare (106, 107). Consistent with this, early human studies suggest that lung cancer patients whose tumors harbor EGFR mutations do not respond to cetuximab, whereas tumors with the wild-type sequence do (108). Moreover, cetuximab does not significantly affect EGFR phosphorylation in *EGFR*-mutant *NSCLC* cell lines (104). Collectively, these observations indicate that anti-EGFR antibodies and EGFR TKIs target tumors in different patient populations. The challenge of both preclinical and clinical work will be tailoring anti-EGFR agents to the right populations.

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