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REVIEW ARTICLE

Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitor–Resistant Disease

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ABSTRACT

Purpose
EGFR-mutant lung cancer was first described as a new clinical entity in 2004. Here, we present an update on new controversies and conclusions regarding the disease.

Methods
This article reviews the clinical implications of *EGFR* mutations in lung cancer with a focus on epidermal growth factor receptor tyrosine kinase inhibitor resistance.

Results
The discovery of *EGFR* mutations has altered the ways in which we consider and treat non–small-cell lung cancer (NSCLC). Patients whose metastatic tumors harbor *EGFR* mutations are expected to live longer than 2 years, more than double the previous survival rates for lung cancer.

Conclusion
The information presented in this review can guide practitioners and help them inform their patients about *EGFR* mutations and their impact on the treatment of NSCLC. Efforts should now concentrate on making *EGFR*-mutant lung cancer a chronic rather than fatal disease.

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INTRODUCTION

EGFR-mutant lung cancer was first described as a potential distinct clinical entity in 2004.[1-3] Eight years later, multiple studies have undisputedly validated the disease as a unique subset of lung cancer, with its own clinical features, natural history, and clinical course. *EGFR*-mutant lung cancer also serves as a paradigm for an oncogene-addicted solid tumor that can be effectively treated with specific targeted therapy (ie, first-generation epidermal growth factor receptor [EGFR] tyrosine kinase inhibitors [TKIs], gefitinib [Iressa; AstraZeneca, London, United Kingdom] and erlotinib [Tarceva; Genentech, South San Francisco, CA]).[4-9] Multiple reviews have been published on the rationale for targeting EGFR in cancer and the subsequent discovery of *EGFR* mutations in lung cancer.[10-12] Here, we review new controversies and conclusions regarding the clinical implications of *EGFR* mutations in lung cancer, with a focus on EGFR TKI resistance.

DRUG-SENSITIVE *EGFR* MUTATIONS IN LUNG CANCER

EGFR is a receptor tyrosine kinase that belongs to the EGFR family, consisting of four members: EGFR, ERBB2, ERBB3, and ERBB4. Under normal circumstances, binding of ligands (eg, epidermal growth factor, transforming growth factor-alpha) activates the intracellular tyrosine kinase activity of EGFR via homo- or heterodimerization with EGFR family members.[13] In lung cancer, *EGFR* mutations occur in exons encoding the ATP-binding pocket of the kinase domain (exons 18 to 21; Fig 1). In a cohort of nearly 1,200 patients with *EGFR* mutations linked to clinical outcomes, more than 145 different types of nucleotide changes have been reported within the EGFR kinase domain.[14]

The most clinically relevant and extensively studied drug-sensitive mutations are deletions in exon 19 that eliminate a common amino acid motif (LREA) and point mutations in exon 21 that lead to a substitution of arginine for leucine at position 858 (L858R). Together, these two classes of mutations account for approximately 85% of *EGFR* mutations in the disease. They are constitutively active and oncogenic[15,16] as a result of a disruption of autoinhibitory interactions.[17] Biochemical studies indicate that these mutants preferentially bind to drugs like gefitinib and erlotinib over ATP.[17,18] Other potential drug-sensitive mutations occur at much lower frequency: G719X (3%), L861X (2%),[14] and exon 19 insertions (1%).[19] The former two were associated

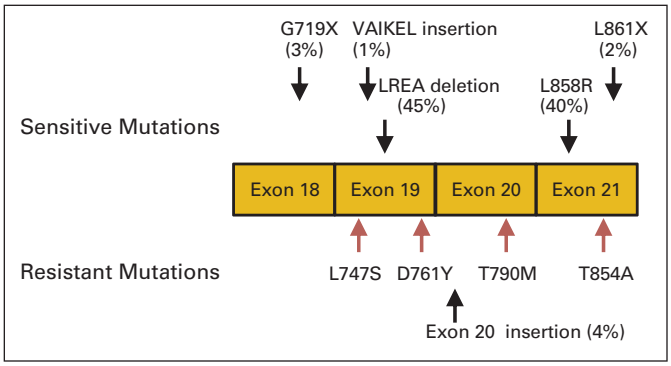

Fig 1. Distribution of *EGFR* mutations in lung cancer. Schematic of the kinase domain of epidermal growth factor receptor showing exons 18 to 21. Activating drug-sensitive mutations are shown on the top, and tyrosine kinase inhibitor (TKI)–resistant mutations are depicted on the bottom (red: acquired resistant mutations). The most common activating mutations in *EGFR* are a point mutation in exon 21, which substitutes an arginine for a leucine (L858R), and a small deletion in exon 19 that removes four amino acids (LREA). Together, these account for approximately 85% of the TKI-sensitive mutations observed in *EGFR*-mutant tumors. Many rare mutations have also been reported.[14]

with drug sensitivity in the original reports on *EGFR* mutations,[1,2] whereas the exon 19 insertions were just recently reported as drug sensitive.[19] The rarity of clinical data associated with these less frequent mutants has made it more difficult to determine how drug sensitive they are in patients, but new data are emerging.[20,21]

CLINICAL FEATURES ASSOCIATED WITH *EGFR* MUTATIONS

EGFR mutations can be found in all histologic subtypes of non–small-cell lung cancer (NSCLC), including adenocarcinoma, large-cell carcinoma, and squamous cell carcinoma.[14] In North American/European and East Asian countries, *EGFR* mutations are found in 10% and 30% of unselected NSCLCs,[22,23] respectively. Clinical features likely to be associated with *EGFR* mutations include adenocarcinoma histology, history of never smoking cigarettes (ie, fewer than 100 cigarettes in a lifetime),[3,22] and East Asian ethnicity.[22] Female sex was originally thought to be correlated with *EGFR* mutations, but data suggest that this association was made because more women are likely to be never-smokers,[24] not necessarily because of a true sex bias. Sixty percent to 80% of tumors from East Asian never-smokers with lung adenocarcinoma harbor *EGFR* mutations,[25,26] whereas only 30% to 50% of tumors from North American/European counterparts have such mutations.[3,22] The reason for this discrepancy is unclear; as of yet, no study has determined if US citizens of East Asian descent diagnosed with lung cancer have the same prevalence of *EGFR* mutations as East Asians themselves. Such a finding would suggest a genetic rather than environmental cause of *EGFR* alterations.

Most importantly, *EGFR* mutations (mostly exon 19 deletions and L858R point mutations) are associated with a clinical benefit from gefitinib and erlotinib. In early phase III trials, these drugs were tested in unselected patients with NSCLC and showed less than 10% radiographic response rates (RRs) with short ($\leq$ 3 months) progression-free survival (PFS) rates[27-29] (Table 1). After the discovery of *EGFR* mutations, several prospective single-arm first-line studies enrolling only patients with *EGFR*-mutant tumors reported unprecedented RRs (73% to 91%) and prolonged PFS (7.7 to 13.3 months).[33] Thereafter, five large prospective phase III first-line trials directly compared an EGFR TKI versus platinum doublet chemotherapy in patients with NSCLC harboring *EGFR* mutations. These trials strongly confirmed the benefit of gefitinib or erlotinib in *EGFR*-mutant lung cancer, regardless of ethnic background (Table 1).[4,6-9,30-32] By comparison,

Table 1. Select Phase III Clinical Trials in Lung Cancer Involving EGFR TKIs

Trial	Year	Line	No. of Participants	Race	*EGFR* Mutant (%)	EGFR TKI	Reference Arm	TKI *v* Reference RR (%)	CR (%)	PFS (months)	OS (months)
ISEL[27]	2005	Second to third	1,692	White, 75%; Asian, 21%*	12.1†	Gefitinib	Placebo	8.0 *v* 1.3	NA	3.0 *v* 2.6	5.6 *v* 5.1
BR.21[28]	2005	Second to third	731	Asian, 12%; other, 88%	23‡	Erlotinib	Placebo	8.9 *v* < 1	0.7 *v* 0	2.2 *v* 1.8	6.7 *v* 4.7
INTEREST[29]	2008	Second	1,433	White, 75%; Asian, 21%*	14.8§	Gefitinib	Docetaxel	9.1 *v* 7.6	NA	2.2 *v* 2.2	7.6 *v* 8.0
IPASS[4,30]	2009	First	1,217	East Asian, 100%	59.7‖	Gefitinib	Platinum doublet	43.0 *v* 32.2	NA	5.7 *v* 5.8	18.8 *v* 17.4
IPASS subgroup[4,30]	2009	First	261	East Asian, 100%	100	Gefitinib	Platinum doublet	71.2 *v* 47.3	NA	9.5 *v* 6.3	21.6 *v* 21.9
WJTOG3405[6,31]	2009	First	172	East Asian, 100%	100	Gefitinib	Platinum doublet	62.1 *v* 32.2	NA	9.2 *v* 6.3	35.5 *v* 38.8
NEJ002[7]	2009	First	224	East Asian, 100%	100	Gefitinib	Platinum doublet	73.7 *v* 30.7	4.4 *v* 0	10.8 *v* 5.4	30.5 *v* 23.6
OPTIMAL[8,32]	2011	First	165	East Asian, 100%	100	Erlotinib	Platinum doublet	82 *v* 36	2 *v* 0	13.1 *v* 4.6	22.7 *v* 28.9
EURTAC[9]	2012	First	174	White, 100% (Hispanic)	100	Erlotinib	Platinum doublet	64 *v* 18	3 *v* 0	9.7 *v* 5.2	19.3 *v* 19.5

Abbreviations: CR, complete response; EGFR, epidermal growth factor receptor; EURTAC, European Tarceva Versus Chemotherapy; INTEREST, IRESSA Non-Small-Cell Lung Cancer Trial Evaluating Response and Survival Against Taxotere; IPASS, Iressa Pan-Asia Study; ISEL, IRESSA Survival Evaluation in Lung Cancer; NA, not applicable; OPTIMAL, Open Label, Phase III Study Comparing First Line Tarceva vs Cisplatin Plus Gemcitabine in Chinese Advanced/Metastatic Non-Small-Cell Lung Cancer Patients With EGFR Activating Mutations; OS, overall survival; PFS, progression-free survival; RR, response rate; TKI, tyrosine kinase inhibitor.

*Excludes people of Indian origin.
†26 positive in 215 tested samples.
‡40 positive in 177 tested samples.
§44 positive in 297 tested samples.
‖261 positive in 437 tested samples.

patients with *EGFR* wild-type tumors displayed 1% RRs and improved PFS with chemotherapy rather than a TKI.[4] To receive EGFR TKIs in many regions, such as Canada and the European Union, patients must now have a documented *EGFR* mutation. In the United States, mutation testing is available in multiple molecular diagnostics laboratories certified by the College of American Pathologists and Certified Laboratory Improvements Amendment of 1988, but the US Food and Drug Administration (FDA) has never required that only patients with *EGFR* mutations should be treated with an EGFR TKI. The rationale behind this was that the BR.21 trial, which compared survival rates in unselected patients with NSCLC treated with erlotinib versus placebo, showed a statistically significant survival benefit for patients taking the drug, even though the absolute difference was a mere 2 months (6.7 *v* 4.7 months; $P < .001$).[28] However, consistent with the notion that erlotinib is more effective against *EGFR*-mutant tumors, a recent study reported that in patients with NSCLC harboring wild-type *EGFR*, docetaxel induced a higher RR (13.9% *v* 2.2%; $P = .004$) and longer PFS (3.4 *v* 2.4 months; hazard ratio [HR], 0.69; $P = .014$) in the second-line setting than erlotinib.[34]

IMPACT OF EGFR TKIS ON *EGFR*-MUTANT LUNG CANCER

No randomized prospective studies have yet officially shown that EGFR TKIs prolong overall survival (OS) compared with chemotherapy (Table 1). One explanation for this discrepancy is that once patients in the chemotherapy arm experience disease progression, they can still display high RRs and prolonged PFS after switching to an EGFR TKI.[7] This crossover confounds subsequent survival analyses.

However, several lines of evidence clearly show that patients with *EGFR*-mutant tumors and treated with TKIs experience historically high survival rates. Multiple prospective first-line clinical trials have now demonstrated that such patients live longer than 2 years.[6,7] Such long OS was not routinely observed before the approval of EGFR TKIs (Table 1; Fig 2A). Consistent with these data, a retrospective analysis recently showed that OS in Japanese patients who started first-line chemotherapy after gefitinib was approved was much longer than the OS in patients who started chemotherapy before gefitinib approval (27.2 *v* 13.6 months; $P < .001$).[41] Furthermore, although gefitinib was withdrawn from the market in the United States in 2005, approximately 250 patients with NSCLC were still alive as of 2011 in the AstraZeneca Iressa Expanded Access Program (NCT00034879), demonstrating that long-term survival of patients with NSCLC is possible while they receive EGFR TKIs.[42]

The impact of the introduction of EGFR TKIs on the treatment of lung cancer can be further gleaned from an analysis of the US Surveillance Epidemiology and End Results (SEER) program database. Population-based resources like the SEER database do not include detailed information about patients by tumor mutation status. However, inferences can be made based on the frequency of patients with *EGFR*-mutant NSCLC among specific ethnic groups. For example, the 12-month survival rates for metastatic NSCLC from 1997 to 2008 of the three different ethnic groups represented in the SEER database (ie, white; African American; and Asian-Pacific Islander, Alaskan, and Native American) show that compared with predicted rates, there has been an increase in the survival rates of all groups. For Asians, the growth was larger than that of the other groups. This increase coincides with the widespread introduction of EGFR TKIs into the clinic; gefitinib was FDA approved for use in NSCLC in May 2003, and erlotinib was FDA approved in November 2004 (Fig 2B). As we have stated, lung tumors from Asians are more likely to harbor *EGFR* mutations and therefore to benefit the most from EGFR TKIs.

In another example, analysis of incidence and 3-year prevalence data in SEER from 2002 to 2008 shows that the incidence of metastatic NSCLC cases has decreased from 26.5 to 23.5 cases per 100,000, whereas prevalence has increased from 14.8 to 15.1 cases per 100,000 (Fig 2C). Incidence represents the number of patients diagnosed with metastatic NSCLC per year (of whom < 10% harbor *EGFR* mutations), whereas 3-year prevalence represents those patients who were diagnosed within 3 years and are still alive at the end of this time period. The 10% decline in incidence is attributable to lower smoking rates in the US population,[43] and the 2% increase in prevalence occurred concurrently with the introduction of EGFR TKIs into the population (Fig 2C). If there is no change in the average survival rate of patients during a period of observation, then the prevalence exactly mimics the behavior of the incidence curve; however, if the survival rate increases during a period of observation, then the prevalence may increase even when the incidence decreases, if the increase of survival rates is sufficiently large. Of course, other drugs (ie, pemetrexed and bevacizumab) were also approved by the FDA for NSCLC treatment during this time (2004 and 2006, respectively); thus, the changes in prevalence cannot be entirely attributed to EGFR TKIs. More dramatic observations have been made in other oncogene-driven cancers treated with TKIs, such as *BCR-ABL*–driven chronic myelogenous leukemia treated with imatinib,[44,45] but that disease is defined by the *BCR-ABL* translocation and therefore more easily analyzed using population-based databases.

EGFR MUTATIONS AND DRUG RESISTANCE

Unfortunately, approximately 30% of patients still do not experience disease responses despite harboring *EGFR*-mutant disease,[4,6-9] and less than 5% experience a complete response[7-9] (Table 1). Acquired resistance to EGFR TKIs in the metastatic setting is inevitable. Moreover, although the average PFS is 10 to 16 months, treatment duration can last as short as 1 month.[7] Thus, drug resistance remains a major problem in the clinic. Until new therapies and strategies are developed to overcome such resistance, the new prevalence rate of lung cancer (Fig 2C) will remain flat. Here, we focus on mechanisms of primary and secondary resistance to EGFR TKIs.

Primary Resistance

De novo resistant EGFR *mutations.* Tumors with mostly *EGFR* exon 20 insertions, which account for 4% of *EGFR* mutations, are associated with a lack of drug sensitivity in preclinical models and in patients.[46] Another mutation in exon 20 conferring resistance involves substitution of methionine for threonine at position 790 (T790M). This alteration is found as a heterozygous germline variant in 0.5% of never-smokers with lung adenocarcinoma[47] and may confer genetic susceptibility to *EGFR*-mutant lung cancer.[48] Efforts are being made to create an online registry of patients with germline *EGFR* T790M.[49] When the T790M mutation occurs somatically, its frequency in EGFR TKI–naive disease is somewhat controversial. Multiple studies have reported rarely detecting it pretreatment,[50,51] and mathematic modeling studies have suggested that pre-existing resistance may be present at a low frequency.[51] Others have found frequencies as high as

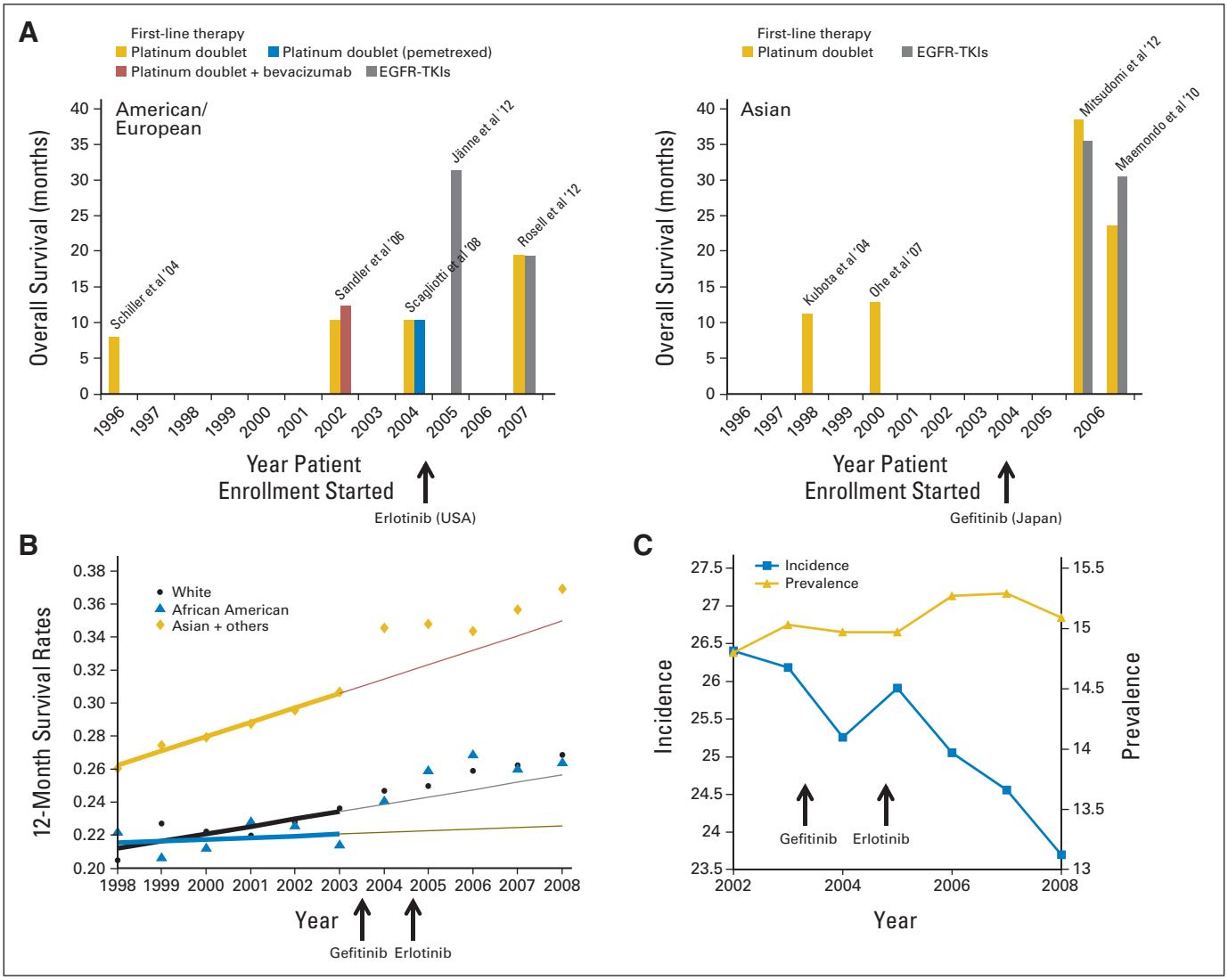

Fig 2. Impact of epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) on survival in patients with lung cancers harboring *EGFR* mutations. (A) The graph depicts the overall survival (OS) rates achieved in various prospective trials conducted in the United States/Europe (left panel) and East Asia (right panel) before and after the introduction of EGFR TKIs. After 2006, OS was consistently longer than 18 months in patients with *EGFR*-mutant tumors.[6,7,9,35-40] Five trials[35-37,39,40] were conducted in unselected patients with non–small-cell lung cancer (NSCLC), and the other trials were performed in patients with *EGFR*-mutant tumors.[9,6,7] One trial[38] was for never- or light former smokers; the OS data were from the subgroup of patients with *EGFR*-mutated lung cancers treated with erlotinib. (B) Age-adjusted 12-month survival rates in patients with metastatic NSCLC in the SEER database. The rate increases especially in the Asian population from 2003 to 2004 onward. The thick lines are the trends for the years 1998 to 2003, and the thin lines are the predicted improvement in 12-month survival, given the 1998 to 2003 trends. (C) Age-adjusted incidence and prevalence (per 100,000 people in the United States) of NSCLC in the SEER database. The incidence decreases starting from 2002, whereas the prevalence displays a small increase. (A to C) Arrows indicate years in which drugs were approved.

35%.[52-54] By contrast, the data are much more consistent in showing that more than half of patients with acquired resistance to gefitinib or erlotinib develop the T790M mutation. Patients whose tumors harbor somatic T790M mutations before treatment experience a shorter PFS.[52,54]

Suboptimal drug exposure. Suboptimal drug exposure may result in a lack of antitumor effect. In an interesting case report, disease in a patient with *EGFR*-mutant lung cancer (exon 19 deletion) progressed after only 2 months of erlotinib at the standard dose (150 mg orally once per day). The patient was found to have a low plasma concentration of drug, so the dose was increased. At 300 mg orally once per day, a significant response was achieved. Further investigation implicated a drug-drug interaction with fenofibrate. Erlotinib is extensively metabolized by the monooxygenase, cytochrome P450 3A4 (CYP3A4), which can be induced by fenofibrate. Subsequent withdrawal of fenofibrate led to suprahigh levels of erlotinib along with concomitant adverse effects, necessitating a reduction of erlotinib back to 150 mg orally once per day.[55] How often such suboptimal dosing occurs is unknown. In an analogous manner, smoking has also been shown to affect erlotinib dosing through the upregulation of CYP1A1.[56] Polymorphisms in the genes involved in erlotinib metabolism could further influence drug concentrations in individual patients, as seen with sunitinib in patients with renal cell carcinoma.[57]

Failure of apoptosis induction. Induction of the proapoptotic BH-3–only molecule, BIM, is essential for apoptosis triggered by

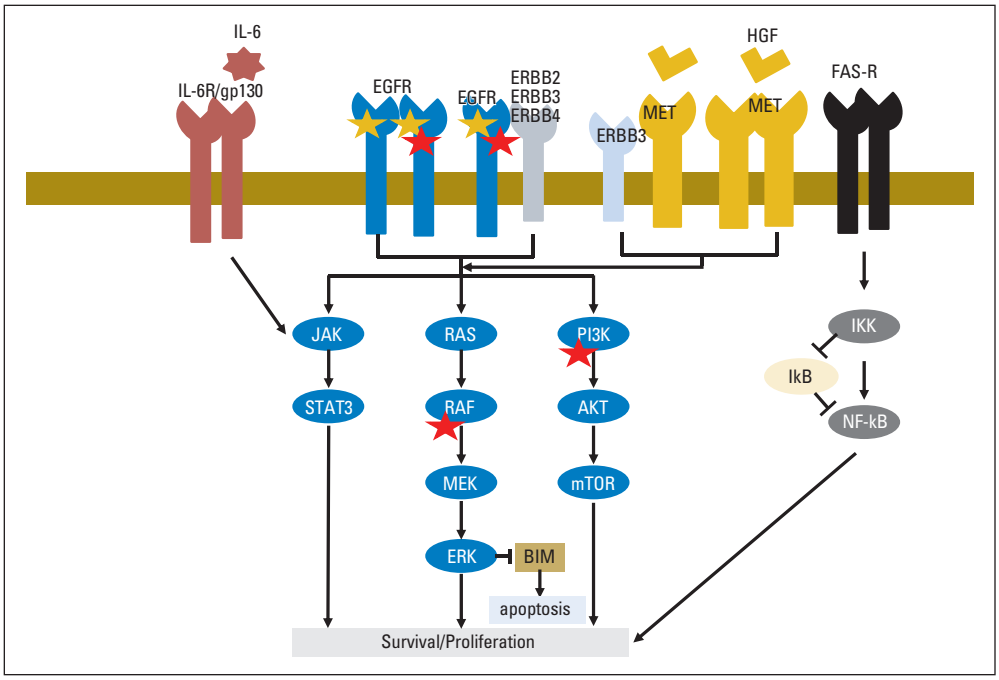

Fig 3. Schematic representation of the epidermal growth factor receptor (EGFR) signaling pathway and molecules that may affect drug resistance. Gold star indicates mutations in *EGFR*. Red star indicates genes found to be mutated in tumor samples from patients with acquired resistance to EGFR tyrosine kinase inhibitors. HGF, hepatocyte growth factor; mTOR, mammalian target of rapamycin.

EGFR kinase inhibitors in mutant *EGFR*–dependent lung adenocarcinomas both in vitro and in vivo.[58] Low expression levels of BIM in primary tumors have been associated with shorter PFS in patients treated with EGFR TKIs.[59] Functional variants of BIM that impair its function could also explain variability of response.[60]

Other potential mechanisms. Other cell intrinsic factors may affect TKI sensitivity. Approximately 50% of NSCLCs, especially adenocarcinomas, harbor recurrent somatic alterations in genes that encode components of major signaling pathways, including *ALK*, *ROS1*, *RET*, *HER2*, *KRAS*, *NRAS*, *PIK3CA*, *AKT1*, *BRAF*, and *MEK1*.[61-63] Among these, only *PIK3CA* mutations thus far have been shown to commonly co-occur with *EGFR* mutations.[64] Introduction of PIK3CA into *EGFR*-mutant cells confers resistance to EGFR TKIs,[65] and *PIK3CA* mutations have been shown to be acquired after patients develop resistance (Fig 3). The full spectrum of genome-wide genetic alterations associated with untreated *EGFR*-mutant lung cancer remains to be established. In another example, activation of the FAS/NFκB signaling pathway may modulate EGFR dependence in lung cancer cells (Fig 3).[66] In this study, high expression of NFκB correlated with a significantly shorter PFS in patients treated with EGFR TKIs.

Exogenous factors may also affect EGFR TKI resistance in *EGFR*-mutant tumors. For example, in one study, hepatocyte growth factor (HGF), the ligand of the MET receptor tyrosine kinase, was found to be overexpressed in 29% of primary resistant lung tumors with drug-sensitive *EGFR* mutations (13 of 45).[67] This result suggests that activation of the MET signaling pathway through HGF stimulation might be associated with primary resistance as well as acquired resistance (Fig 3). In another example, inflammation has been implicated as a resistance mechanism via activation of the interleukin-6/JAK2/STAT3 pathway (Fig 3).[68] In xenograft models, administration of an anti–interleukin-6 antibody restores drug sensitivity.

Secondary Resistance

As we have stated, all patients with metastatic *EGFR*-mutant lung cancer will eventually develop disease progression. For more than 60% of patients, a plausible mechanism of resistance has been identified (Fig 4). The key to these studies has been analysis of new tumor tissue after patients develop resistance,[69,70] a practice which should be considered standard to help guide therapy. Here, we review known mechanisms observed in human lung tumors as well as potential mechanisms found in preclinical models.

Second-site EGFR *mutations.* Second-site *EGFR* mutations are the most frequent mechanism of acquired resistance to EGFR TKIs in lung cancer, found in more than 50% of patients. Among the reported mutations—L747S, D761Y, T790M, and T854A—more than 90% are composed of the T790M gatekeeper mutation[11,69-72] (Figs 1 and 4). The T790M substitution alters proper binding of the drug to the ATP pocket of EGFR and/or restores the affinity for ATP versus drug back to the level of wild-type *EGFR*.[18]

Suboptimal drug exposure in the brain. Up to 33% of patients with *EGFR*-mutant lung cancer treated with EGFR TKIs will experience disease progression in the CNS.[73,74] Thus far, the second-site *EGFR* T790M mutation has been found in only four (13%) of 30 examined, a frequency far lower than that seen in peripheral organs.[74-79] Concurrently, multiple studies have shown that the concentration of drug achievable in the brain is approximately 1% to 5% of the level found in the plasma.[75,77,80] Presumably, the selection pressure for second-site mutations is thus different in the brain versus the periphery. In those patients who experience disease progression only in the brain but not in the rest of the body, brain metastasis treatment should be administered, but erlotinib at standard daily doses can be resumed after completion of the radiation course. In the setting of leptomeningeal disease, which has been historically difficult to treat with conventional chemotherapy, high-dose EGFR TKI has

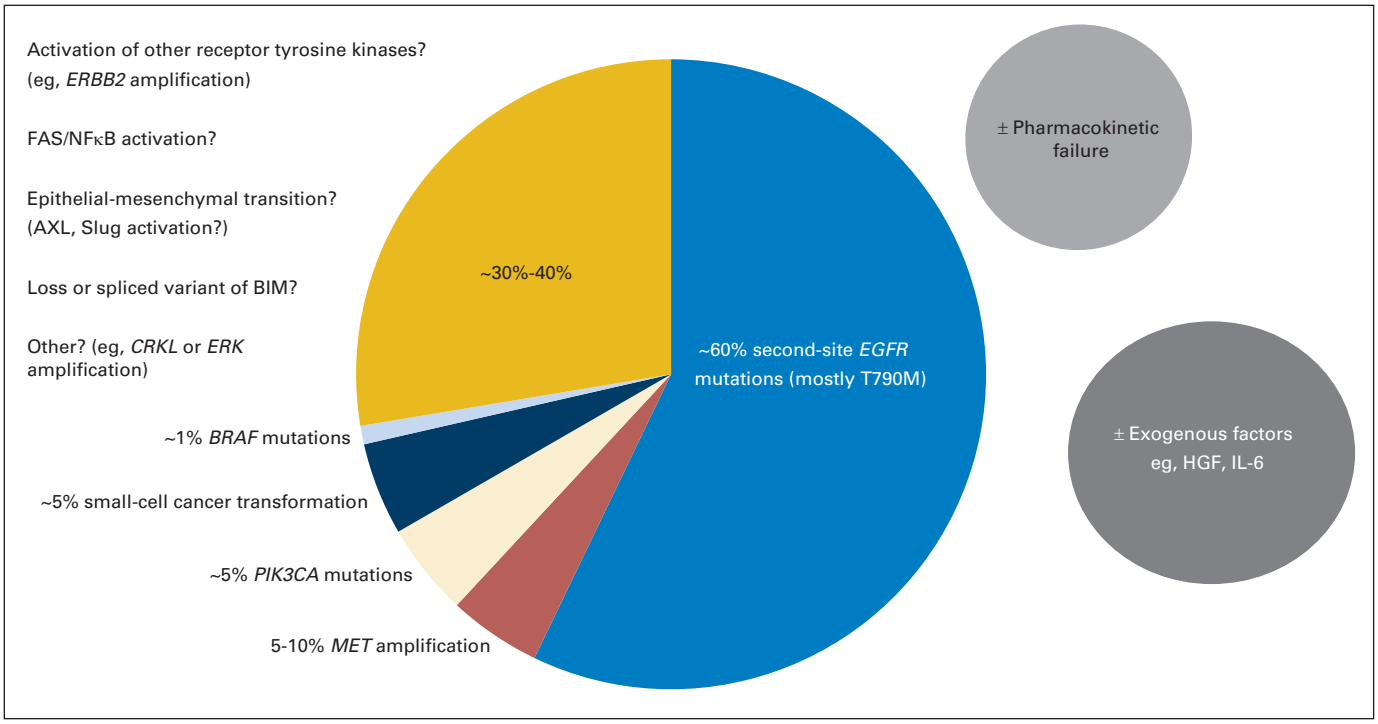

Fig 4. Mechanisms of acquired resistance to epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors. Multiple mechanisms have been elucidated in human samples and preclinical models. Some factors may overlap. HGF, hepatocyte growth factor; IL-6, interleukin-6.

been shown to be clinically tolerable and potentially of some benefit with no dose-limiting toxicity.[75,77,78,81] Such a dosing regimen leads to higher levels of drug in the cerebrospinal fluid (CSF; gefitinib 1,250 mg once per day: serum, 3,730 nmol/L and CSF, 39.4 nmol/L; erlotinib 1,500 mg orally once per week: plasma, 11,300 nmol/L and CSF, 130 nmol/L).[75,77] Whether plasma drug concentrations decrease over time when EGFR TKIs are taken at standard doses remains unclear. In chronic myelogenous leukemia, studies have shown that imatinib levels can decrease the longer patients receive the drug.[82]

Activation of EGFR signaling pathways via other aberrant molecules. Another 5% to 10% of cases of acquired resistance will display amplification of the gene encoding MET.[69,70,83,84] Overexpression of MET activates the PI3K/AKT pathway via interaction with ERBB3, rendering cells less solely dependent on mutant *EGFR* for survival.[83] In a tumor cell population, HGF may help expand pre-existing minor populations of cells harboring *MET* amplification (Figs 3 and 4).[85,86]

Other mutant signaling proteins may also confer resistance. Up to 5% of patients with acquired resistance examined in one study developed new *PIK3CA* mutations.[70] In another study of nearly 200 tumor samples from patients with acquired resistance, we recently found that approximately 1% of patients harbor *BRAF* mutations.[87] No common mutations in *KRAS*, *NRAS*, or *MEK1* signaling genes were detected.[87] We have also recently shown that ERBB2 amplification may be associated with acquired resistance, especially in patients without detected T790M mutations.[88] Other studies have suggested that *CRKL* or *ERK* amplification may be a mediator of disease progression.[89,89a]

Histologic transformation. In a handful of cases, rebiopsy of growing tumors has obtained cells that no longer display adenocarcinoma histology. Although they still harbor a drug-sensitive *EGFR* mutation, the cells display features of small-cell lung cancer[70,90] or epithelial-to-mesenchymal transition (EMT).[70,91] How cells transform to a different histology is not well understood. The RB and p53 pathways may have a role in the transformation to small-cell cancer,[92] and the TGFβ pathway may play a role in EMT.[68] In preclinical models, cells with EMT features seem to be no longer dependent on EGFR signaling for survival, but drug sensitivity may be restored by treatment with histone deacetylase inhibitors[93] or inhibition of the kinase AXL or the zinc finger protein Slug.[91,94]

Strategies to Overcome Resistance

To overcome EGFR TKI resistance, several new drugs or drug combinations are being developed. To date, however, no agents have been approved for either the first-line setting or patients with acquired resistance.

Second- and third-generation EGFR TKIs. Second-generation EGFR TKIs include canertinib, neratinib, afatinib, and dacomitinib (Table 2). These irreversible ATP-competitive agents make covalent bonds with a cysteine residue at position 797 in EGFR. They are more potent than gefitinib and erlotinib and also affect other EGFR family members (eg, ERBB2, ERBB4). However, they still inhibit *EGFR* drug-sensitive mutations at lower concentrations of drug as compared with the common T790M mutant and therefore eventually select for cancer cells with *EGFR* T790M in preclinical models.[51,98] In humans, the concentration of drug needed to overcome T790M-mediated resistance may be not achievable in the absence of significant toxicity.

Among these four drugs, afatinib has progressed the farthest in development. In a phase III trial for TKI-naive patients with *EGFR*-mutant tumors, afatinib was superior to platinum doublet chemotherapy in terms of RR (56.1% *v* 22.6%; $P < .001$) and PFS (11.1 *v* 6.9

Table 2. Small-Molecule EGFR TKIs Clinically Available or in Development

Drug Name	Generic Name	Trade Name	Manufacturer	Target	Recommended Dose	MTD	Status
Reversible							
ZD1839	Gefitinib	Iressa	AstraZeneca, Wilmington, DE	EGFR	250 mg once per day	750 mg once per day	Approved (Asia/EU)
OSI-776	Erlotinib	Tarceva	Genentech, South San Francisco, CA	EGFR	150 mg once per day	150 mg once per day	Approved
BPI-2009H	Icotinib	Conmana	BetaPharma, Branford, CT	EGFR	150 mg once every 8 hours	Not reached	Approved (China)
TAK-165	Mubritinib	NA	Takeda, Osaka, Japan	EGFR/ERBB2	NA	NA	Phase I*
XL647	NA	NA	Kadmon, New York, NY	EGFR/ERBB2†	300 mg once per day	300 mg once per day	Phase II*
ZD6474	Vandetanib	Zactima	AstraZeneca	EGFR/VEGFR2/RET	300 mg once per day	300 mg once per day	Phase III*‡
GW572016	Lapatinib	Tykerb	GlaxoSmithKline, Philadelphia, PA	EGFR/ERBB2	1,250-1,500 mg once per day	Not reached	Preclinical*§
Irreversible							
EKB-569	Pelitinib	NA	Wyeth/Pfizer, New York, NY	EGFR	50 mg once per day	75 mg once per day	Phase I*
CI-1033	Canertinib	NA	Pfizer, New York, NY	EGFR/ERBB2/ERBB4	150 mg once per day	150 mg once per day	Phase II*
HKI-272	Neratinib	NA	Puma Biotechnology, Los Angeles, CA	EGFR/ERBB2	320 mg once per day‖	320 mg once per day	Phase II*
BIBW2992	Afatinib	Tomtovok	Boehringer Ingelheim, Ingelheim, Germany	EGFR/ERBB2/ERBB4	50 mg once per day	50 mg once per day	Phase III
PF-00299804	Dacomitinib	NA	Pfizer	EGFR/ERBB2/ERBB4	45 mg once per day	45 mg once per day	Phase III
Third generation							
CO-1686	NA	NA	Clovis/Avila, Boulder, CO	EGFR T790M	NA	NA	Phase I/II
WZ4002	NA	NA	NA	EGFR T790M	NA	NA	Preclinical
Other							
AP26113	NA	NA	Ariad Pharmaceuticals, Cambridge, MA	ALK/EGFR¶	NA	NA	Phase I/II

Abbreviations: EGFR, epidermal growth factor receptor; EU, European Union; FDA, US Food and Drug Administration; MTD, maximum tolerated dose; NA, not applicable; NSCLC, non–small-cell lung cancer; TKI, tyrosine kinase inhibitor; VEGFR2, vascular endothelial growth factor receptor 2.

*Currently, no additional trials are being planned for lung cancer.

†XL647 inhibits EGFR, ERBB2, VEGFR2, FLT-4, and EPHB4.

‡Vandetanib failed to show a survival benefit *v* placebo in unselected patients after prior therapy with EGFR TKI,[95] showed efficacy equivalent to that of erlotinib in unselected patients treated with one prior anticancer therapy for advanced NSCLC,[96] and showed an additive effect with docetaxel in unselected patients treated with one prior anticancer therapy for advanced NSCLC.[97] The FDA has approved vandetanib as an RET inhibitor for medullary thyroid cancers under the trade name Caprelsa (AstraZeneca, London, United Kingdom).

§The FDA has approved lapatinib as an ERBB2 inhibitor for breast cancers.

‖Dose was reduced to 240 mg orally once per day during trial because of toxicity.

¶AP26113 is reported to reversibly inhibit ALK and EGFR mutant proteins, including EGFR T790M.

months; $P < .001$).[99] In a separate randomized phase IIb/III trial of afatinib versus placebo for patients who "had disease progression after at least 12 weeks of treatment with erlotinib or gefitinib"[100(p529)] (not necessarily with *EGFR*-mutant tumors), RR and PFS were 7% versus < 1% and 3.3 versus 1.1 months, respectively.[100] OS was 10.8 months in the afatinib arm and 12.0 months in the placebo arm.[100] In a phase II trial, dacomitinib also has shown promising results in patients with untreated *EGFR*-mutant tumors (RR, 74%; preliminary median PFS, 17 months).[101]

Third-generation EGFR inhibitors include WZ4002[102] and CO-1686.[103] Whereas first- or second-generation EGFR TKIs have a quinazoline core, WZ4002 has an anilinopyrimidine core, which fits better into the ATP pocket of *EGFR* T790M. The structure of CO-1686 has not yet been released publicly. Notably, these reagents were designed to specifically inhibit the *EGFR* T790M mutant. To date, no clinical trials for WZ4002 have been initiated, but a phase I/II clinical trial for CO-1686 started in January 2012 (NCT01526928). Another new EGFR inhibitor is AP26113. This drug was originally characterized as an ALK inhibitor but has also been shown in preclinical models to inhibit *EGFR* mutants including *EGFR* T790M.[104] A phase I/II clinical trial for AP26113 began in September 2011 (NCT01449461).

Drug combinations. Several studies have examined the addition of an anti-EGFR antibody to an EGFR TKI to overcome resistance. The combination of erlotinib with cetuximab showed no effect in patients who acquired resistance to EGFR TKIs (RR, 0%).[12] However, the combination of afatinib and cetuximab has led to highly promising results (RR, 36% in eight of 22 patients).[105] Results from this trial importantly demonstrate in patients that tumors remain dependent on EGFR signaling for survival even after developing resistance. Interestingly, 50% of the responders to this combination did not harbor secondary *EGFR* T790M mutations, suggesting that there exist EGFR signaling pathway–dependent but *EGFR* T790M–independent mechanisms of resistance.

Other trials have assessed the combination of EGFR TKIs with other classes of inhibitors (eg, mammalian target of rapamycin inhibitors, SRC inhibitors, HSP90 inhibitors, and so on), but results have been disappointing.[12] If there is no universal Achilles' heel in tumors, specific combinations may need to be directed against the genetic makeup of individual tumors (eg, adding a MET inhibitor for tumors displaying no *EGFR* T790M but *MET* amplification, or adding a PI3K inhibitor for tumors displaying a secondary *PI3KCA* mutation, and so on[65,83]).

Treatment beyond progression. Finally, recent preclinical and clinical studies have revealed interesting properties of EGFR TKI–resistant tumors. In standard practice, cytotoxic cancer drugs are usually discontinued when acquired resistance develops. However, many reports have demonstrated that patients who acquire resistance can re-respond to EGFR TKIs after a drug holiday.[106] Multiple studies have now shown that patients who acquire *EGFR* T790M may have a favorable clinical outcome as compared with those who do not.[79,107,108] Consistent with these findings, preclinical studies have demonstrated that cells that acquire *EGFR* T790M can actually grow slower than parental cells, and after several passages in the absence of TKIs, they become resensitized.[51] These findings indicate that resistant tumors are composed of mixed populations of sensitive and resistant cancer cells and suggest a benefit of continued EGFR TKI administration even after the acquisition of resistance. Several clinical studies support this hypothesis,[109-112] and prospective trials to test whether an EGFR TKI beyond progression is better than stopping the drug at the time of resistance are currently in progress (A Study of IRESSA Treatment Beyond Progression in Addition to Chemotherapy Versus Chemotherapy Alone [IMPRESS]) as well as in development.

Novel combinations. Evolutionary mathematic cancer modeling in conjunction with in vitro experimental data was recently used to predict alternative dosing strategies that delay the outgrowth of pre-existing resistance using currently available EGFR TKIs.[51] The most promising strategy administers low-dose continuous EGFR TKI (eg, erlotinib) in combination with high-dose pulsed doses (eg, afatinib) with the goal of preventing both the replication of EGFR TKI–sensitive cells as well as optimally delaying the emergence of resistant clones. Such modeling also suggests that pulsed high doses alone will not be sufficient to suppress resistance. The clinical utility of this strategy should be validated. This integrated mathematic modeling and experimental approach represents a new roadmap for the rational design of clinical trials that can also be applied to other cancer types treated with targeted therapy.

Other novel combinations could involve TKIs plus immunologic therapies. BMS-936558 (MDX-1106), an antibody against programmed death-1, a T-cell inhibitory receptor, has shown promising activity in lung cancer with minimal toxicity.[113] Addition of agents like BMS-936558 to erlotinib in TKI-naive patients or potentially with more potent TKIs after the development of resistance should be explored.

DISCUSSION

In conclusion, *EGFR* mutations define a distinct clinical entity of lung cancer. EGFR TKIs such as gefitinib and erlotinib have increased the OS of patients with *EGFR*-mutant lung cancer. However, despite rapid progress over the past 8 years, a new plateau for survival of patients with *EGFR*-mutant lung cancer already seems to have been reached. Greater efforts need to be made not only to overcome acquired resistance but also to induce greater responses and longer PFS in the first-line setting. The goal should now be to become as creative as possible with new strategies and therapies to make *EGFR*-mutant lung cancer a chronic rather than fatal disease.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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