Mechanisms of Acquired Resistance to Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors in Non–Small Cell Lung Cancer

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Abstract Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors gefitinib and erlotinib are effective therapies for non–small cell lung cancer patients whose tumors harbor somatic mutations in *EGFR*. All patients, however, ultimately develop resistance to these agents. Thus, there is a great need to understand how patients become resistant to develop effective therapies for these cancers. Studies over the last few years have identified two different EGFR tyrosine kinase inhibitor resistance mechanisms, a secondary mutation in *EGFR, EGFR* 790M, and amplification of the *MET* oncogene. These findings have led to clinical trials using newly designed targeted therapies that can overcome these resistance mechanisms and have shown promise in laboratory studies. Ongoing research efforts will likely continue to identify additional resistance mechanisms, and these findings will hopefully translate into effective therapies for non–small cell lung cancer patients.

Background

Epidermal growth factor receptor (EGFR)–specific tyrosine kinase inhibitors (TKI) gefitinib and erlotinib have been developed as therapeutic agents for non–small cell lung cancer (NSCLC) treatment (1, 2, 3). Both agents were found to have evidence of antitumor activity in phase II clinical trials but only erlotinib was associated with a survival benefit in a phase III clinical trial (4, 5). Although the benefits of erlotinib were statistically significant, they were clinically modest (median survival of 6.7 months versus 4.7 months for erlotinib and placebo, respectively; $P < 0.0001$) and have prompted studies to identify those patients most likely to benefit from erlotinib therapy. In clinical studies, gefitinib and erlotinib are most effective in never-smokers with NSCLC (4, 5).

Subsequent molecular studies identified somatic mutations in *EGFR* as a major determinant underlying the dramatic clinical responses following treatment with gefitinib (6, 7) and erlotinib (8). Somatic mutations in *EGFR* are found in 10% to 15% of Caucasian and in 30% to 40% of Asian NSCLC patients. *EGFR* mutations are present more frequently in never-smokers, females, those with adenocarcinoma, and in patients of East Asian ethnicity (9). These are the same groups of patients previously clinically identified as most likely to benefit from gefitinib or erlotinib (1, 2, 4). *EGFR* mutations associated with increased response to gefitinib and erlotinib are found in the first four exons (exons 18-21) of the tyrosine kinase domain of EGFR. The mutations reported thus far have been predominantly of two types: 45% are deletions involving at least 12 nucleotides in exon 19, eliminating a conserved LREA motif, and 40% are single point mutations in exon 21 (L858R). The exon 19 deletions and L858R are the most common *EGFR* mutations and are also the ones that have been most extensively evaluated to date and closely linked to the sensitivity of gefitinib and erlotinib (reviewed in ref. 10).

Six prospective clinical trials treating chemotherapy naïve patients with *EGFR* mutations with gefitinib or erlotinib have been reported to date (11–16). Cumulatively, these studies have prospectively identified and treated more than 200 patients with *EGFR* mutations. Together, they show radiographic response rates ranging from 55% to 82% and median times to progression of 9.4 to 13.3 months in the patients treated with gefitinib and erlotinib. These outcomes are 3- to 4-fold greater than historically observed with platin-based chemotherapy (20% to 30% and 3-4 months, respectively) for advanced NSCLC (17). Despite the dramatic efficacy of erlotinib and gefitinib in NSCLC patients with *EGFR* mutations, however, all patients will ultimately develop resistance (i.e., acquired resistance) to these agents. It is critical to understand the mechanisms of acquired resistance because it may lead to the development of effective therapies for patients who clinically develop acquired resistance to gefitinib or erlotinib.

Clinical studies have also shown that a small population of patients with amplified, wild-type *EGFR* lung cancers also benefit from gefitinib or erlotinib (18, 19). In addition, a significant portion of NSCLC patients develops stable disease after treatment with EGFR inhibitors (20). The mechanism(s)

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of underlying sensitivity to gefitinib or erlotinib, however, are not well characterized in this patient population. Acquired resistance mechanisms have been studied most extensively in *EGFR* mutant cancers and will be the subject of this review. It remains to be determined if these resistance mechanisms are shared with wild-type *EGFR* cancers.

EGFR Mutant Tumors Are Addicted to EGFR Signaling

To understand how *EGFR* mutant NSCLCs can develop resistance to EGFR TKIs, it is first critical to understand the normal signaling mechanisms in these tumors. *EGFR* mutant tumors are dependent or "addicted" to EGFR signaling for their growth and survival (21–23). Studies to date suggest that, in these cancers, several (if not all) of the critical downstream signaling pathways, including the phosphoinositide 3-kinase (PI3K)/Akt, signal transducers and activators of transcription, and extracellular signal-regulated kinase 1 and 2 pathways, are solely controlled by EGFR. Thus, when the tumors are exposed to EGFR inhibitors, these intracellular pathways are turned off and the cancer cells undergo apoptosis (21, 22, 24, 25). In contrast, EGFR does not singularly regulate these pathways in most other lung cancers, and these cancers are impervious to EGFR inhibitors.

The detailed molecular events that lead to activation of these downstream signaling events are just beginning to be understood. This understanding facilitates the discovery of potential resistance mechanisms because cancers adopt novel ways of activating these pathways to circumvent the effects of EGFR inhibition (see below). EGFR is one of a family of four erbB family members. Two other family members, HER2 and erbB3, are highly implicated in promoting EGFR activation of downstream signaling. ErbB3 is a unique member of this family in that it is believed to be "kinase dead" (Fig. 1A). On heterodimerization with other erbB family members, however, ErbB3 is phosphorylated on tyrosines and serves as a scaffold to activate downstream signaling. In lung cancers that are sensitive to EGFR inhibitors, PI3K/Akt is activated by binding to phosphorylated erbB3 (26). In contrast, cancers that are not sensitive to EGFR inhibitors primarily use non-erbB3 mechanisms for activating PI3K (26). There are now several studies reporting a correlation between gefitinib sensitivity and erbB3 expression in NSCLC cell lines (25–27). In fact, erbB3 expression analysis identified patients that most benefited from EGFR inhibitors (28).

HER2 (erbB2) also seems to play a prominent role in EGFR mutant cancers. *HER2* amplification, as determined by fluorescence *in situ* hybridization analysis, was identified as a positive predictor of response to EGFR TKIs (29). In a small study, the most powerful predictor of response was the presence of both *HER2* amplification and an *EGFR* mutation (29). HER2 expression may increase EGFR recycling to the membrane and prevent its degradation. Furthermore, HER2 may increase signaling to erbB3 in an EGFR-dependent manner via lateral signaling (30).

It is also clear that EGFR activity regulates extracellular signal-regulated kinase 1 and 2 signaling pathways as well in TKI-sensitive cancers. The detailed molecular mechanisms leading from EGFR kinase to extracellular signal-regulated kinase 1 and 2 activation, however, remain to be elucidated. Similarly, signal transducer and activator of transcription 3 seems to be active in EGFR mutant cancers. Although a study found that EGFR kinase activity was not necessary for signal transducer and activator of transcription 3 tyrosine phosphorylation, it reported that EGFR kinase activity was necessary for its serine phosphorylation, a process often required for its full activation (31). In *EGFR* mutant cancers, it is clear that inhibition of EGFR turns off these downstream signaling events and, to become resistant, these cancers seem to find ways to maintain their activity.

Secondary *EGFR* Mutations

Two main mechanisms of acquired resistance have been identified. The first is a secondary *EGFR* mutation, T790M, that renders gefitinib and erlotinib ineffective inhibitors of EGFR kinase activity (Fig. 1B; refs. 32, 33). *EGFR* T790M has been detected both from tumors of *EGFR* mutant NSCLC patients who have developed clinical resistance to gefitinib or erlotinib and from *in vitro* gefitinib-resistant *EGFR* mutant cell lines (32–37). To date, the *EGFR* T790M mutation is found in ~50% of tumors (24 of 48) from patients that have developed acquired resistance to gefitinib or erlotinib (34–36). In one patient, another secondary *EGFR* mutation, D761Y, has also been reported (35).

The *EGFR* T790M mutation occurs in an analogous position to known resistance mutations to imatinib in other kinases (T315I in *ABL*, T674I in *PDGFRA*, and T670I in *KIT*; refs. 38–40). The conserved threonine residue among these different kinases, located near the kinase active site, is often referred to as the gatekeeper mutation. The exact mechanism through which T790M causes gefitinib/erlotinib resistance is not completely understood. In ABL, the T315I mutation causes a steric hindrance and causes imatinib binding (38, 41). Whether this same mechanism also occurs in *EGFR* T790M remains to be determined.

Cancers that become resistant to kinase inhibitors through a secondary mutation are still likely to be dependent on the activated kinase for their growth and survival. Thus, alternative strategies of inhibiting EGFR T790M may be therapeutically efficacious. This has prompted the preclinical and clinical development of second-generation kinase inhibitors (41). For EGFR, second-generation irreversible EGFR inhibitors have shown activity in gefitinib-resistant preclinical models of NSCLC containing *EGFR* T790M (42, 43). Irreversible inhibitors, including HKI-272 and PF00299804, are able to inhibit EGFR phosphorylation and lead to growth inhibition of NSCLC or Ba/F3 cell lines containing *EGFR* T790M (42, 43). These agents are also ATP mimetics similar to gefitinib and erlotinib but, unlike gefitinib or erlotinib, they covalently bind Cys-797 of EGFR. How the irreversible nature of these agents allows them to inhibit EGFR phosphorylation is unclear. It is possible that by covalently binding to EGFR, the local concentration of these agents increases substantially (compared with gefitinib or erlotinib, which is a reversible inhibitor), thus providing a means of inhibiting EGFR phosphorylation despite the presence of a T790M mutation.

Alternative Mechanisms for Activating Downstream Signaling

As mentioned previously, if an *EGFR* mutant cancer can maintain activity of downstream signaling pathways in the

presence of gefitinib or erlotinib, this may lead to resistance. Indeed, several preclinical studies have shown that continued activation of downstream signaling, especially the PI3K pathway, is sufficient to confer resistance to EGFR TKIs. Most, if not all, laboratory models of acquired resistance show continued activation of the PI3K pathway despite TKI treatment (36, 37, 44, 45). Additionally, activation of PI3K/Akt signaling by an ectopically expressed p110α-activating mutant (*PIK3CA*) confers an EGFR mutant cancer resistant to TKIs (44). Similarly, in *HER2*-amplified breast cancers, the presence of an activating

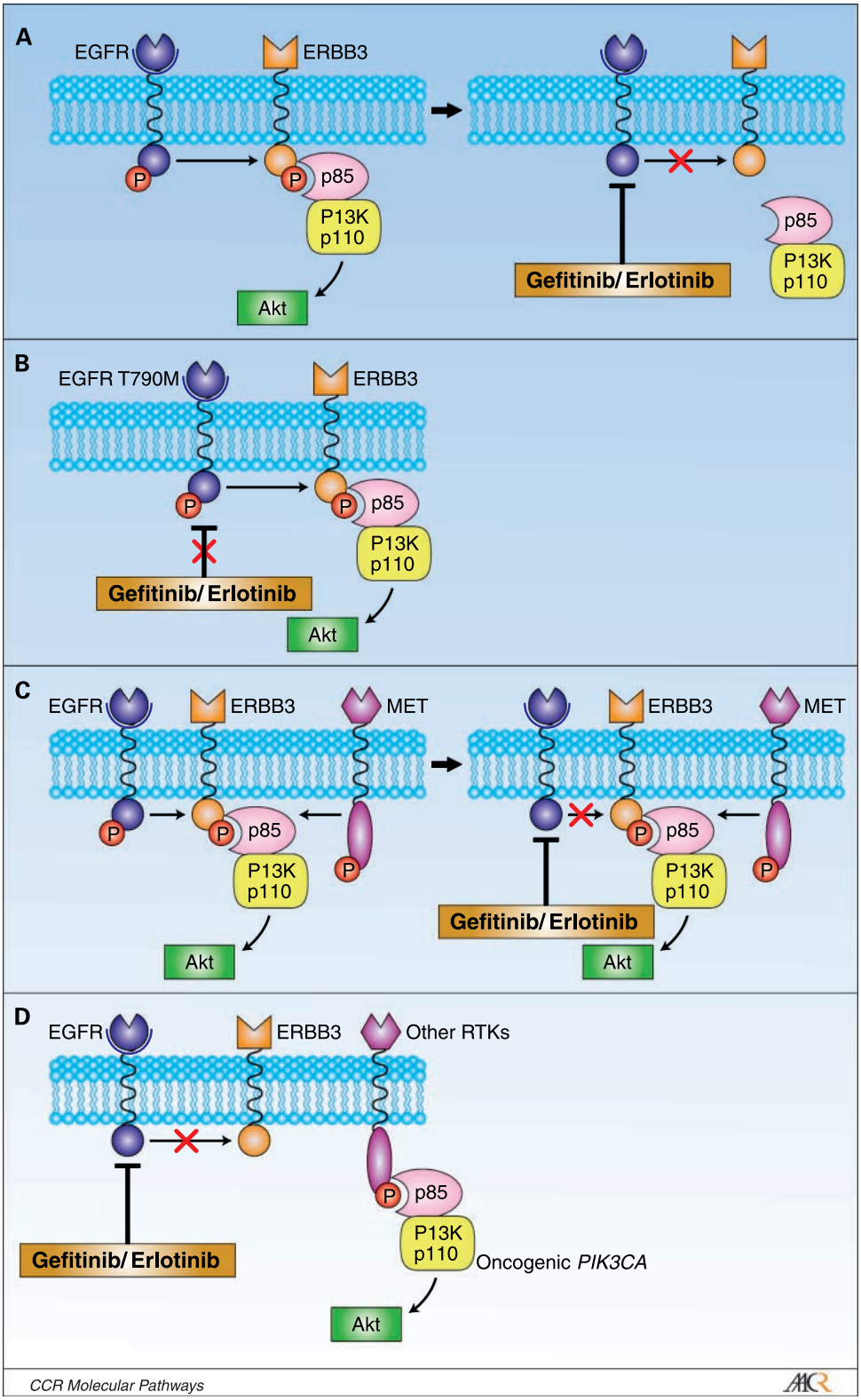

Fig. 1. EGFR signaling in gefitinib/erlotinib – sensitive and gefitinib/erlotinib – resistant *EGFR* mutant NSCLCs. *A,* EGFR phosphorylates erbB3 to activate PI3K/Akt signaling in gefitinib/erlotinib – sensitive NSCLCs. In such cancers, following gefitinib/erlotinib treatment, EGFR, erbB3, and Akt phosphorylations are turned off. *B,* gefitinib/erlotinib are unable to inhibit EGFR phosphorylation in the presence of *EGFR* T790M. EGFR signaling persists in the presence of gefitinib/erlotinib, leading to persistent erbB3 and Akt phosphorylation. *C,* MET can also activate PI3K/Akt signaling through erbB3. In NSCLCs with *MET* amplification, gefitinib/erlotinib can still inhibit EGFR phosphorylation but not erbB3 phosphorylation. This leads to persistent activation of PI3K/Akt signaling via erbB3 in an EGFR-independent manner. *D,* other potential mechanisms of gefitinib/erlotinib resistance. These potential mechanisms include alternative ways of maintaining PI3K/Akt signaling, such as by an oncogenic *PIK3CA* or by other receptor tyrosine kinases that could activate PI3K/Akt signaling in an erbB3-independent fashion. In such cancers, gefitinib/erlotinib would be expected to inhibit EGFR and erbB3 phosphorylation but not Akt phosphorylation. Adapted by permission from Macmillan Publishers Ltd. (Arteaga CL. HER3 and mutant EGFR meet MET. Nat Med 2007;13:675–7), copyright 2007.

p110α mutation or PTEN loss predicts a lack of response to trastuzumab (46). Although loss of PTEN or acquisition of a *PIK3CA* mutation has not been identified as a mechanism of resistance in lung cancer specimens, these analogous findings and preclinical studies suggest that if a cancer can find a way to effectively activate PI3K independent of EGFR activity, it will become resistant to EGFR TKIs.

Recently, amplification of *MET*, a receptor tyrosine kinase, was identified as another acquired resistance mechanism (36). This was originally identified in the HCC827 cells (*EGFR* exon 19 mutation and amplified) that had been made resistant to gefitinib *in vitro*. Interestingly, MET causes resistance because it activates erbB3-dependent activation of PI3K (Fig. 1C). Furthermore, it was determined that MET signals through erbB3 in most MET-amplified cancers (36). This redundant activation of erbB3 permits the cells to transmit the same downstream signaling in the presence of EGFR inhibitors. Thus, concomitant inhibition of both EGFR and MET is required to kill the resistant cells. In the initial study, 22% (4 of 18) of NSCLCs with acquired resistance to gefitinib/erlotinib had *MET* amplification in the resistant specimens. It is interesting to speculate that *MET* amplification is a prevalent resistance mechanism because it activates PI3K signaling in the same way as EGFR, via erbB3.

EGFR T790M and *MET* amplification account for ~60% to 70% of all known causes of acquired resistance to gefitinib or erlotinib. Thus, other mechanisms of acquired resistance are likely to be discovered. Based on the preclinical models to date, such mechanisms are likely to lead to maintenance of PI3K/Akt signaling in the presence of gefitinib/erlotinib. This could occur through erbB3 (such as for *EGFR* T790M or *MET* amplification) or by an erbB3-independent mechanism (Fig. 1D). It will be important to continue to study preclinical models and tumors from NSCLC patients that have developed gefitinib/erlotinib resistance to uncover novel resistance mechanisms.

Clinical Translation Advances

Several clinical trials are under way, aimed at inhibiting known resistance mechanisms in NSCLC patients that have clinically developed acquired resistance to gefitinib or erlotinib. Ongoing trials are evaluating irreversible EGFR inhibitors, the combination of EGFR and MET kinase inhibitors, or Hsp90 inhibitors as strategies to overcome acquired resistance in NSCLC patients (36, 42, 43, 47, 48). There are several challenges in translating the preclinical studies into effective clinical therapies, however. The first challenge is accurately identifying which patients have which mechanism of resistance. The vast majority of NSCLC patients who develop acquired resistance to gefitinib or erlotinib do not undergo repeated tumor biopsies at the time when their cancer develops resistance. This is critical in that the therapeutic strategy aimed at overcoming resistance may not be effective in all resistant patients. For example, irreversible EGFR inhibitors are not effective in preclinical models of gefitinib resistance that are mediated by *MET* amplification (43). A second challenge is that, unlike in preclinical models that focus on single mechanisms of resistance, multiple mechanisms of resistance can occur concurrently in the same patient. Both *MET* amplification and *EGFR* T790M have been detected in the same resistant tumor specimen (36, 49). In addition, they have been found to occur independently in different metastatic sites in the same patient (36). Thus, a therapeutic strategy aimed solely at inhibiting *EGFR* T790M or *MET* amplification may not be very effective or may lead only to regression of a subset of metastases that contains the particular mechanism of resistance. A more comprehensive and potentially effective strategy may be a combination of an irreversible EGFR and a MET kinase inhibitor. At present, there are no ongoing clinical trials combining these classes of agents. Alternatively, strategies such as Hsp90 inhibitors may also be effective as both EGFR and MET are known Hsp90 client proteins (47, 50). A third challenge relates to the biological definition and detection of resistance mechanisms. As most *EGFR* mutant NSCLCs also contain a concurrent copy gain at the *EGFR* locus, the *EGFR* T790M mutation can sometimes be present as a minor allele and yet be sufficient to cause drug resistance (18, 44). In such cases, *EGFR* T790M may go undetected by conventional sequencing techniques and more sensitive mutation detection methods are necessary for accurate identification of *EGFR* T790M (44). Similarly, there are challenges with the detection of *MET* amplification. The definition of what constitutes clinically significant *MET* amplification (i.e., that causes gefitinib/erlotinib resistance) is also not currently well defined. This too will be important in deciding on which patients should be treated with MET kinase inhibitors for their gefitinib/erlotinib–resistant NSCLC.

Gefitinib and erlotinib are effective therapies for patients with *EGFR* mutant NSCLC (11–16). It will continue to be important to identify and study the mechanisms of resistance that develop to these agents as a means of rationally designing the next-generation clinical studies.

Disclosure of Potential Conflicts of Interest

P.A. Jänne has received commercial research support from Pfizer, has consulted with AstraZeneca, Roche and Boehringer Ingelheim, and received royalties from Genzyme and is part of a pending patent application on EGFR mutations. J. A. Engleman has consulted with Roche.

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