Published in final edited form as:
Oncogene. 2009 August ; 28(Suppl 1): S24–S31. doi:10.1038/onc.2009.198.

Activating and resistance mutations of *EGFR* in non-small-cell lung cancer: role in clinical response to EGFR tyrosine kinase inhibitors

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Abstract

The epidermal growth factor receptor tyrosine kinase inhibitors (EGFR TKIs), gefitinib and erlotinib, are reversible competitive inhibitors of the tyrosine kinase domain of EGFR that bind to its adenosine-5′ triphosphate-binding site. Somatic activating mutations of the *EGFR* gene, increased gene copy number and certain clinical and pathological features have been associated with dramatic tumor responses and favorable clinical outcomes with these agents in patients with non-small-cell lung cancer (NSCLC). The specific types of activating mutations that confer sensitivity to EGFR TKIs are present in the tyrosine kinase (TK) domain of the *EGFR* gene. Exon 19 deletion mutations and the single-point substitution mutation L858R in exon 21 are the most frequent in NSCLC and are termed ‘classical’ mutations. The NSCLC tumors insensitive to EGFR TKIs include those driven by the *KRAS* and *MET* oncogenes. Most patients who initially respond to gefitinib and erlotinib eventually become resistant and experience progressive disease. The point mutation T790M accounts for about one half of these cases of acquired resistance. Various second-generation EGFR TKIs are currently being evaluated and may have the potential to overcome T790M-mediated resistance by virtue of their irreversible inhibition of the receptor TK domain.

Keywords

epidermal growth factor receptor; mutation; non-small-cell lung cancer; tyrosine kinase inhibitor; tyrosine kinase

Introduction

The epidermal growth factor receptor (EGFR) family of receptor tyrosine kinases (TKs), referred to as the HER or ErbB family, consists of four members—EGFR (HER1/ErbB1), HER2 (ErbB2), HER3 (ErbB3) and HER4 (ErbB4)—that regulate many developmental, metabolic and physiological processes. The intracellular TK activity of EGFR is increased as a consequence of the binding of various cognate ligands, which include EGF, transforming growth factor-α, amphiregulin and others, leading to the homodimerization of two EGFRs or the heterodimerization of EGFR with other family members, most commonly HER2 (Bazley

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Conflict of interest

AF Gazdar has received consulting fees/paid advisory board fees from AstraZeneca, Boehringer and Genentech. AF Gazdar has also received lecture fees from AstraZeneca.

and Gullick, 2005). Heterodimerization with HER2, which is over-expressed in some tumors, is a more potent activator of EGFR TK than is EGFR homodimerization. The activation of receptor TK leads to the autophosphorylation of the intracellular domain of EGFR, and the phosphotyrosine residues that are formed act as a docking site for various adapter molecules, resulting in the activation of the Ras/mitogen-activated protein kinase pathway, the PI3K/Akt pathway and signal transducers and activators of transcription signaling pathways (Figure 1a) (Kumar *et al.*, 2008).

In tumor cells, the TK activity of EGFR may be dysregulated by various oncogenic mechanisms, including *EGFR* gene mutation, increased gene copy number and EGFR protein overexpression. (Ciardello and Tortora, 2008) Improper activation of EGFR TK results in increased malignant cell survival, proliferation, invasion and metastasis. EGFR overexpression is observed in tumors from more than 60% of patients with metastatic non-small-cell lung cancer (NSCLC) and is correlated with poor prognosis (Sharma *et al.*, 2007). These findings have provided a rationale for the development of novel anticancer agents that target EGFR.

Treatment with the reversible EGFR TK inhibitors (TKIs), gefitinib and erlotinib, results in dramatic antitumor activity in a subset of patients with NSCLC: clinical responses have been achieved in approximately 10% of European patients and in 30% of patients from East Asia (Fukuoka *et al.*, 2003; Kris *et al.*, 2003; Perez-Soler *et al.*, 2004; Shepherd *et al.*, 2005; Thatcher *et al.*, 2005; Sharma *et al.*, 2007). Sequencing of the *EGFR* gene revealed that a majority of tumors responding to EGFR TKIs harbored mutations in the TK domain of *EGFR* (Lynch *et al.*, 2004; Paez *et al.*, 2004; Pao *et al.*, 2004). Overall, the frequency of *EGFR* mutations is 5–20%, depending on the populations studied (Riely *et al.*, 2006). For patients whose tumors exhibit *EGFR* mutations, the response rate to gefitinib and erlotinib is approximately 75%, suggesting that these mutations, at least in part, drive malignant transformation (Jackman *et al.*, 2006; Riely *et al.*, 2006).

As a result of these findings, a large amount of data on *EGFR* mutations occurring in patients with NSCLC have recently become available. This article reviews the types of activating and resistance *EGFR* mutations and the pivotal role they have in the sensitivity and resistance of NSCLC tumors to gefitinib and erlotinib. Advances in understanding *EGFR* mutations have led to strategies for novel EGFR TKIs that hold promise in the improvement of clinical outcomes for patients with advanced NSCLC.

Activating mutations of the *EGFR* gene

EGFR mutations are the most prevalent and well characterized in NSCLC, owing their relationship to clinical responses to EGFR TKIs. Because of the high frequency of EGFR mutations in NSCLC, these somatic mutations are thought to represent very early genetic events leading to the development of lung cancer (Politi *et al.*, 2006; Gazdar and Minna, 2008). Furthermore, the susceptibility to EGFR TKIs validates the fundamental dependence of NSCLC tumors on EGFR mutations for maintaining the malignant phenotype. All of the somatic activating *EGFR* mutations involve the adenosine triphosphate (ATP)-binding pocket in the receptor TK domain, which is the binding site for the TKIs erlotinib and gefitinib. Kinase domain mutations in *EGFR* are referred to as ‘activating mutations’ because they lead to a ligand-independent activation of TK activity. In some tumors, partially activated mutant EGFRs can be rendered fully ligand independent and, therefore, constitutively active by a second mutation.

The activating mutations of the *EGFR* gene are found in the first four exons (18 through 21) of the TK domain (Figure 1b) (Shigematsu and Gazdar, 2006; Kumar *et al.*, 2008). These mutations fall into three major classes, with the majority of EGFR TKI-sensitizing mutations falling into class I and II. Class I mutations are in-frame deletions in exon 19; these deletions

almost always include amino-acid residues leucine-747 to glutamic acid-749 (ΔLRE), and account for about 44% of all EGFR TK mutations. Class II mutations are single-nucleotide substitutions that cause an amino-acid alteration. The predominant single-point mutation is in exon 21, which substitutes an arginine for a leucine at codon 858 (L858R). L858R has the highest prevalence of any single-point activating mutation in EGFR TK and accounts for about 41% of all EGFR TK activating mutations. Other class II activating mutations result in a glycine-719 (G719) change to serine, alanine or cysteine (4% of all EGFR TK activating mutations), and other missense mutations account for another 6% of EGFR mutations. Class III mutations are in-frame duplications and/or insertions in exon 20. These account for the remaining 5% of EGFR TK activating mutations. A variety of other activating mutations have been detected with low frequency, including V765A and T783A (<1%) in exon 20 (Sharma *et al.*, 2007). Many of the sensitizing mutations have been detected in tumors from drug responders.

Overall, deletions in exon 19 and the point mutation of L858R constitute about 90% of all EGFR activating mutations, and are termed ‘classical’ activating mutations. Although the signaling events that are affected as a result of *EGFR* mutations are not fully understood, it is well established that the ‘on–off’ equilibrium of EGFR TK states is altered (Kumar *et al.*, 2008). Specifically, an equilibrium shift occurs between active and inactive states of the TK that favors the activated state, resulting in a net increase in kinase activity. As a consequence, tumor cells, in which *EGFR* activating mutations are present, display an oncogene addiction to EGFR, with consequent selective growth and survival advantages (Gazdar and Minna, 2005; Sharma *et al.*, 2007). Crystallographic analysis suggests that this equilibrium shift is the result of structural alterations induced by activating mutations (Kumar *et al.*, 2008). It has been postulated that these mutations cause a constitutive activation of the kinase by destabilizing the autoinhibited conformation that is normally found in the absence of ligand binding (Zhang *et al.*, 2006; Yun *et al.*, 2008).

A kinetic analysis of the intracellular domains of EGFR L858R and EGFR Del (746–750) has shown that both mutants are active but show a higher K_M for ATP and a lower K_i for erlotinib, relative to wild-type receptor (Carey *et al.*, 2006). Thus, mutant kinases demonstrate a reduced affinity for ATP, which provides a molecular explanation for the increased sensitivity to erlotinib and gefitinib (Carey *et al.*, 2006). It is notable that when expressed in a cell line that does not express EGFR or other ErbB receptors, both mutations activate downstream EGFR signaling pathways and promote cell-cycle progression.

Although common EGFR mutations have been well studied in preclinical models (*in vitro* and *in vivo*) and their effects on response to TKIs have been observed in patients, relatively little is known about rarer mutations. We now realize that not all mutations are activating, and that some activating mutations may be associated with resistance to TKIs (Kancha *et al.*, 2009). In particular, insertion mutations in exon 20 are associated with a lack of response to TKIs.

Effect of the activating mutations on clinical response

Despite the modest response rate and overall survival benefit observed with EGFR TKIs in patients with advanced NSCLC, significant clinical benefits were achieved in a subset of 10–30% of patients. In 2004, two independent studies were published that probed the molecular basis for the dramatic responses to gefitinib observed in a series of patients with advanced NSCLC (Lynch *et al.*, 2004; Paez *et al.*, 2004). Somatic activating mutations in the *EGFR* TK domain (exons 18, 19 and 21) were found in tumor specimens from 13 of 14 patients who experienced objective responses to gefitinib. These mutations were absent in tumors from patients with progressive disease. Another study reported activating *EGFR* mutations in tumors from patients who responded to gefitinib or erlotinib (Pao *et al.*, 2004). *EGFR* mutations were

subsequently examined in several studies of unselected NSCLC tumor specimens. Activating EGFR TK mutations are significantly more common in East Asians, women, never smokers and patients with adenocarcinoma histology (Table 1) (Jänne and Johnson, 2006). Thus, the frequency of the *EGFR* mutation mirrors the clinically defined subgroups of patients who were most likely to achieve radiographic responses to EGFR TKIs (Miller and Kris, 2004). A germ line transmission of *EGFR* mutations has also been described within families that show a high incidence of lung cancer (Ikeda *et al.*, 2008).

The presence of *EGFR* activating mutations impacts not only on response rate but also progression-free survival and overall survival in patients with NSCLC treated with EGFR TKIs (Table 2) (Bonomi *et al.*, 2007). In four single-arm studies of EGFR TKIs in patients with metastatic NSCLC, a significantly longer overall survival was observed in patients with EGFR mutations (Cortez-Funes *et al.*, 2005;Han *et al.*, 2005;Mitsudomi *et al.*, 2005;Takano *et al.*, 2005). In a study of NSCLC patients treated with gefitinib 250 mg/day, response and time to progression were statistically significantly correlated with *EGFR* mutations and there was a trend toward longer overall survival in patients harboring these mutations (Cappuzzo *et al.*, 2005). When data from all of these studies are combined, the response rate for patients with EGFR mutations (n=110) is 60% (Bonomi *et al.*, 2007). However, EGFR mutations were not found to be significantly associated with longer survival times in a trial comparing erlotinib with placebo, in which hazard ratios (HRs) for death were similar for patients with classical activating mutations, novel mutations and wild-type *EGFR* (HR, 0.65, 0.67 and 0.73, respectively) (Shepherd and Tsao, 2006). These investigators proposed that *EGFR* activating mutations may be a prognostic factor for NSCLC rather than being a predictive factor of EGFR TKI efficacy. This possibility is supported by a subset analysis from a phase III trial of erlotinib plus chemotherapy versus chemotherapy alone, which revealed significantly longer survival times in patients with *EGFR* mutations compared with those who had wild-type *EGFR* when treated with chemotherapy alone (Eberhard *et al.*, 2005).

In contrast, EGFR mutations did show a predictive value in the INTEREST study, which compared docetaxel with gefitinib in patients with NSCLC that had progressed or recurred after chemotherapy. Patients with mutations had a significantly longer progression-free survival (PFS) with gefitinib than with docetaxel (7.0 vs 4.1 months; HR, 0.16; P=0.001), whereas PFS among patients with wild-type EGFR trended in favor of docetaxel (1.7 vs 2.6 months; HR 1.24; P=0.135) (Douillard *et al.*, 2008). A similar association was found in recently reported results from the I-PASS trial, which compared first-line gefitinib with carboplatin/paclitaxel in Asian patients with advanced NSCLC and with no history of substantial smoking. In this study, patients harboring EGFR mutations had a significantly longer PFS with gefitinib (HR, 0.48; P<0.001), whereas those with wild-type EGFR had a better PFS with chemotherapy (HR, 2.85; P<0.001) (Mok *et al.*, 2008).

A recent prospective study in first-line gefitinib-treated patients with NSCLC reported that *EGFR* activating mutations were the most important independent predictors for time to treatment failure compared with other mutations, among which exon 19 deletion and L858R mutations were the best predictors for longer time to treatment failure in a multivariate analysis (Yang *et al.*, 2008). However, additional prospective studies are needed to clarify the prognostic and predictive implications of *EGFR* activating mutations.

Interestingly, despite the impact of the *EGFR* mutation on outcomes in advanced NSCLC, the mutational status may not have a dramatic effect on the outcome of patients with early-stage NSCLC. In a study in 277 Japanese patients with early-stage lung cancer who had undergone surgical resection, a Kaplan–Meier analysis that excluded patients treated with gefitinib, as well as patients undergoing surgery for recurrent or second primary cancers, indicated that

EGFR mutations did not affect the survival of these patients ($P = 0.9933$). However, it is noteworthy that the median follow-up period was short (788 days) (Kosaka et al., 2004).

Not all activating mutations necessarily lead to a full or constitutive EGFR TK activity. Therefore, the type of EGFR mutations in NSCLC tumors seems to influence the sensitivity of the tumor to gefitinib and erlotinib. For example, NSCLC cells expressing the L858R mutant are significantly more sensitive to gefitinib than are those that express the G719S mutant (Jiang *et al.*, 2005). Response rates to EGFR TKIs are higher in patients with NSCLC, whose tumors have exon 19 mutations (70–100%), than in patients with exon 21 mutations (20–67%) (Mitsudomi *et al.*, 2005; Hirsch *et al.*, 2006; Jackman *et al.*, 2006; Riely *et al.*, 2006). These differential response rates translated into longer survival, whereby patients with an exon 19 (ΔLRE) deletion mutation had a median overall survival ranging from 26 to 34 months and patients with exon 21 (L858R) had a median overall survival ranging from 8 to 17 months (Hirsch *et al.*, 2006; Jackman *et al.*, 2006; Paz-Ares *et al.*, 2006).

EGFR mutations and resistance to EGFR TKIs

Although EGFR kinase mutations are associated with an enhanced sensitivity to gefitinib and erlotinib, not all tumors that have activating mutations are associated with an enhanced response. Tumors that fail to respond to EGFR TKIs despite the presence of an activating mutation might have an additional genetic lesion that relieves the tumor of its dependence on the EGFR signaling pathway. One mechanism that has been linked to insensitivity of NSCLC to EGFR TKIs is the occurrence of insertion point mutations in exon 20 of the *EGFR* gene. These include the exon 20 insertion mutants D770_N771 (ins NPG), D770_(ins SVQ) and D770_(ins G) N771T (Greulich *et al.*, 2005; Sharma *et al.*, 2007). In an *in vitro* model system, insertion mutations in exon 20 render transformed cells less responsive to EGFR TKIs compared with the sensitizing mutations of exons 19 and 21 (Greulich *et al.*, 2005). However, exon 20 mutations are relatively rare, suggesting that other mechanisms probably contribute to EGFR TKI primary resistance in metastatic NSCLC. For many of the rare point mutations, the effect on responsiveness to EGFR TKIs remains unknown.

Acquired resistance occurs in virtually all NSCLC tumors that initially respond to EGFR TKI therapy. It is now recognized that the efficacy of gefitinib and erlotinib is of limited duration owing, in large part, to the emergence of drug resistance conferred by a second point mutation in the TK domain. The threonine-790 to methionine (T790M) point mutation is found in approximately 50% of all patients at the time of acquired resistance to EGFR TKI therapy (Kobayashi *et al.*, 2005; Balak *et al.*, 2006; *Kosaka et al.*, 2006). This so-called gatekeeper mutation is believed to be acquired through selective pressure during treatment, as it is rarely detected in tumors from untreated patients (Pao *et al.*, 2005a). Interestingly, using a highly sensitive allele-specific assay, Maheswaran *et al.* (2008) recently detected low levels of T790M in pretreatment NSCLC tumor samples from 10 of 26 patients. Although significant responses were achieved with EGFR TKIs in these patients, the presence of T790M before treatment was associated with a significantly shorter progression-free survival compared with that in TKI-naive patients o without detectable T790M (7.7 vs 16.5 months; $P < 0.001$). These results suggest that T790M may be a useful pretreatment biomarker for identifying patients who are unlikely to achieve durable responses with reversible EGFR TKIs (that is, erlotinib and gefitinib).

Preclinical studies support clinical findings implicating T790M as an underlying mechanism of resistance. It has been demonstrated *in vitro* that this mutation can substantially suppress the inhibitory effects of erlotinib and gefitinib, whereas TK activity is maintained (Kobayashi *et al.*, 2005; Pao *et al.*, 2005a). Similarly, the introduction of T790M into gefitinib-sensitive tumor cells that show activating *EGFR* mutations or an increased *EGFR* copy number also

confers resistance to gefitinib treatment (Greulich *et al.*, 2005). It is not clear how T790M imparts resistance to reversible EGFR TKIs. The T790M mutation results in an alteration of the topology of the ATP-binding pocket (Kumar *et al.*, 2008). It has been suggested that this change in topology precludes the binding of reversible EGFR TKIs through steric hindrance, thereby resulting in resistance (Kobayashi *et al.*, 2005; Kwak *et al.*, 2005; Pao *et al.*, 2005b). However, another mechanism was proposed in a recent study showing that T790M increases the affinity of the kinase domain for ATP (Yun *et al.*, 2008). The authors suggested that this increased affinity results in reduced potency of any ATP-competitive agent.

Other resistance point mutations, such as aspartic acid-761 to tyrosine (D761Y), have been reported, some of which may weaken the interaction of EGFR TKI with its target (Balak *et al.*, 2006). Clinically, the challenge remains how to best detect tumors with the T790M and other resistance point mutations on limited quantities of post-treatment tumor samples. A molecular analysis of circulating cells may provide an alternative approach for monitoring tumor mutations (Maheswaran *et al.*, 2008). In a recent report, it was shown that the actual number of activating EGFR mutant molecules could be detected in the plasma of patients with NSCLC using a procedure called micro-fluidics digital polymerase chain reaction, which is capable of detecting single input template molecules (Yung *et al.*, 2009). In addition, it was found that the concentration of mutant sequences from sequential measurements correlated with response to therapy (that is, decreased concentration correlated with clinical response, whereas persistence of the mutant sequence correlated with progression). These results indicate that an examination of the plasma may be a suitable surrogate test when tumor tissue is not available for determining therapy selection.

Strategies for optimizing response to EGFR TKIs

Insights gained from the treatment of patients with metastatic NSCLC with gefitinib and erlotinib are dramatically changing drug development and treatment strategies, as well as clinical outcomes. Because acquisition of the secondary resistance point mutation T790M reduces the efficacy of ATP-competitive inhibitors, one strategy for preventing or overcoming EGFR TKI resistance would be to identify novel agents that bind and inhibit EGFR by a distinct, non-ATP competitive mechanism. A second strategy may be to irreversibly inhibit the binding of ATP to the TK domain with an irreversible rather than a reversible inhibitor. As a class, the irreversible EGFR inhibitors, including BIBW 2992, HKI-272 and PF00299804, are able to inhibit EGFR phosphorylation and inhibit growth in gefitinib-resistant NSCLC or Ba/F3 cell lines that contain the *EGFR* T790M mutation (Kwak *et al*., 2005; Wong, 2007; Li *et al.*, 2008; Engelman *et al.*, 2008). For example, the irreversible EGFR/HER2 inhibitor, BIBW 2992, suppresses wild-type and activated *EGFR* and HER2 mutants, including *EGFR* and HER2 inhibitor-resistant isoforms (Li *et al.*, 2008). BIBW 2992 has a higher affinity for binding to EGFR with the T790M resistance mutation than do first-generation EGFR TKIs (Table 3) (Li *et al.*, 2008), and induces dramatic tumor regression in an *L858R/T790M EGFR*-driven lung cancer model. Late-stage clinical trials are evaluating BIBW 2992 and HKI-272 for the treatment of patients with NSCLC who relapse after a successful previous treatment with gefitinib or erlotinib.

Another approach for overcoming resistance to reversible EGFR TKIs involves targeting parallel- or convergent signaling pathways. The mammalian target of the rapamycin (mTOR) signaling pathway integrates nutrient and mitogen signals to regulate cell proliferation, survival and angiogenic pathways, and has been implicated in resistance to EGFR inhibitors. In both sensitive and resistant tumor cell lines, the mTOR inhibitor, everolimus, reduces the expression of EGFR signaling effectors and cooperates with gefitinib to overcome resistance (Bianco *et al.*, 2008). In patients with resistance to first-generation EGFR TKIs generated by *MET* amplification, it is unlikely that an irreversible EGFR inhibitor alone would be effective, but

the combination of an irreversible EGFR inhibitor and an mTOR inhibitor may be an effective strategy for overcoming resistance (Li *et al.*, 2008). These hypotheses suggest novel therapeutic strategies that are yet to be validated in clinical studies.

Conclusion

Various clinical characteristics and molecular factors have been associated with sensitivity and resistance to EGFR TKIs. The discovery and characterization of *EGFR* activating mutations and their relationship to sensitivity to gefitinib and erlotinib have provided a basis for transforming NSCLC from a disease treated with conventional combination chemotherapy to one in which subsets of patients with specific EGFR mutations can be effectively treated with targeted therapy. It is reasonable to suggest that personalized therapy for NSCLC patients should include a genetic assessment of the *EGFR* mutational status for individual patients. Current research is directed at optimizing the accuracy and sensitivity of *EGFR* mutational testing so that it might be introduced into routine clinical practice. The appropriate role of an *EGFR* mutation analysis in the treatment of patients with NSCLC continues to evolve, awaiting prospective clinical studies with an adequate documentation of the *EGFR* mutational status.

Several novel targeted therapies are currently in clinical development for patients with advanced NSCLC. The irreversible EGFR TKIs are one class of agents that may have the potential to prevent and overcome resistance that emerges during treatment with gefitinib and erlotinib. Results of ongoing phase III studies on this class of compounds in erlotinib-resistant NSCLC populations are eagerly awaited.

Acknowledgments

I thank Johnathan C Maher, PhD of BlueSpark Healthcare Communications for medical and editorial assistance with this article. Financial support for medical and editorial assistance was provided by Boehringer Ingelheim Pharmaceuticals Inc. Supported by grant P50CA70907 from the Specialized Programme for Research Excellence in Lung Cancer from the National Cancer Institute, Bethesda, MD, USA.

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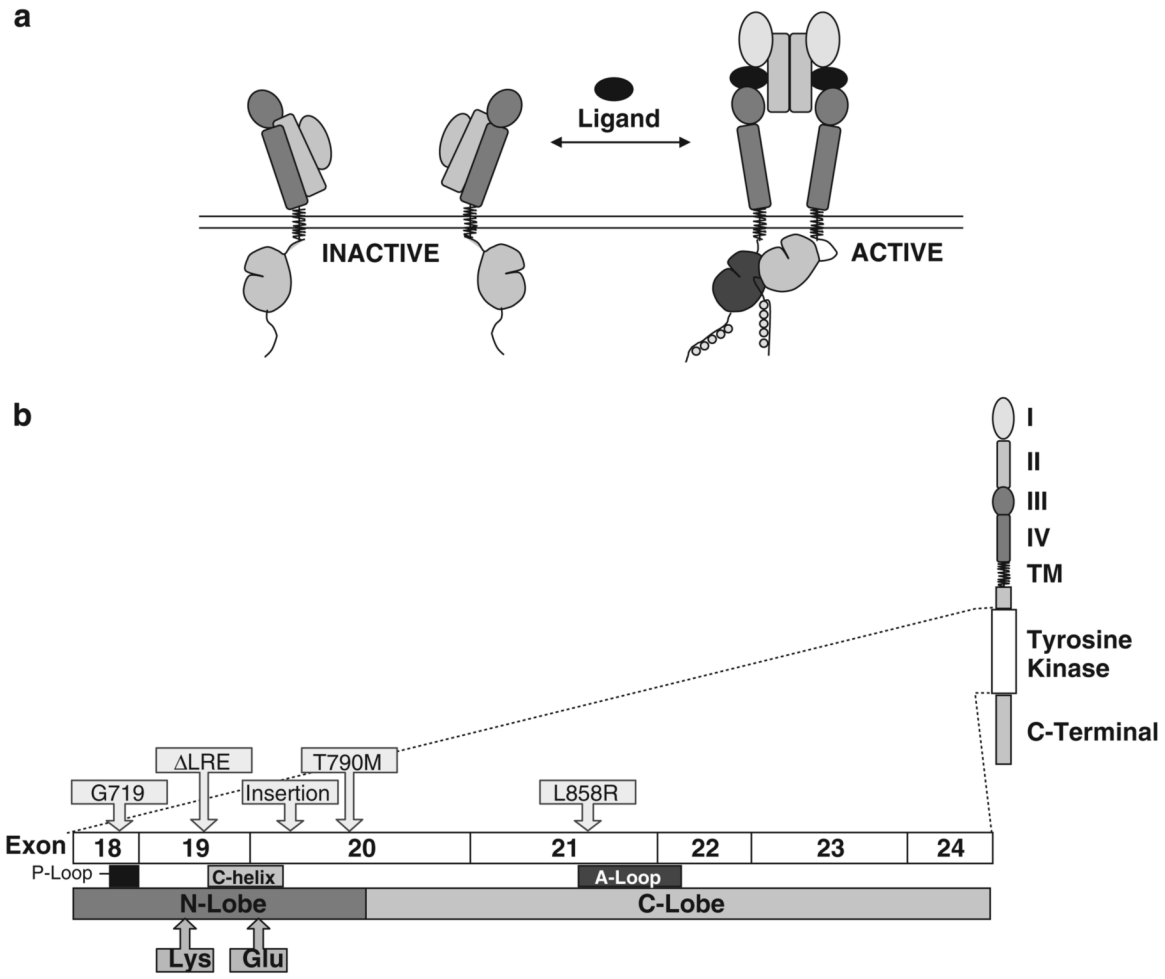

Figure 1.

Schematic of EGFR TK activation and *EGFR* kinase domain mutations. (**a**) Upon binding of the extracellular ligand, the EGFR receptor dimerizes, leading to the activation of cytoplasmic TK activity. (**b**) This exon boundary map shows the location of regions within the EGFR TK domain wherein mutations activate the kinase activity by a ligand-independent mechanism. Deletions in exon 19 and the point mutation of L858R are common activating mutations and these 'classical' mutations are associated with sensitivity to gefitinib and erlotinib in patients with NSCLC. T790M is a secondary point mutation found in tumors that were previously responsive to these agents, but have developed acquired resistance. Adapted from Kumar *et al.*, 2008.

Table 1

Frequency of *EGFR* mutations in different NSCLC patient subgroups

	Total, %	Non-east Asian, %	East Asian, %
All subgroups	19	10	30
Smokers	11	4	17
Nonsmokers	54	35	60
Adenocarcinoma	42	16	49
Non-adenocarcinoma	3	1	4
Male	16	1	22
Female	46	20	58

Adapted from Jänne and Johnson, 2006.

Table 2

Wild-type *EGFR* vs *EGFR* mutations related to response rate, progression-free survival, and overall survival in patients treated with EGFR TKIs

Investigator	*Patients,* n	*Mutation, %*	*Response rates*		*PFS*		*OS*	
			WT/mutation, %	P	*WT/mutation, mo*	P	*WT/mutation, mo*	P
Cappuzzo (2005)	89	19	5/53	<0.001	2.6/9.9	0.02	8.4/20.4	0.9
Cortez-Funes (2005)	83	12	9/60	0.001	3.6/12.3	0.002	4.9/13	0.002
Han (2005)	90	19	14/65	<0.001	1.8/21.7	<0.001	6.6/30.5	<0.0001
Mitsudomi (2005)	59	56	10/84	<0.0001	NA	—	—	0.0496
Takano (2005)	66	59	11/82	0.005	1.7/12.6	<0.0001	6.9/20.4	0.0001
Tsao (2005)	100	37	7/16	0.37	NA	—	—	0.45

Abbreviations: NA, not available; OS, overall survival; PFS, progression-free survival. Adapted with permission from Bonomi *et al.*, 2007.

Table 3

In vitro inhibitory activities of BIBW 2992, lapatinib, canertinib and gefitinib on the receptor TK activities of wild-type and mutated EGFR

	IC_{50} (nm)			
	BIBW 2992	**Lapatinib**	**Canertinib**	**Gefitinib**
$EGFR^{WT}$	0.5	3	0.3	3.0
$EGFR^{L858R}$	0.4	8	0.4	0.8
$EGFR^{L858R/T790M}$	10.0	>4000	26.0	1013.0

Abbreviations: $EGFR^{WT}$, wild-type epidermal growth factor receptor; $EGFR^{L858R}$, epidermal growth factor receptor harboring the L858R resistance mutation; $EGFR^{L858R/T790M}$, epidermal growth factor receptor harboring the L858R-activating mutation and the T790 resistance mutation. Adapted from Li *et al.*, 2008.