Published in final edited form as:
Pharmacogenet Genomics. 2013 November ; 23(11): 636–642. doi:10.1097/FPC.0b013e3283655091.

PharmGKB Summary: Very Important Pharmacogene Information for Epidermal Growth Factor Receptor (*EGFR*)

Ugur Hodoglugil[1], **Michelle W. Carrillo**[1], **Joan M. Hebert**[1], **Niki Karachaliou**[2], **Rafael C. Rosell**[3], **Russ B. Altman**[1,4], and **Teri E. Klein**[1]

[1]Department of Genetics, Stanford University Medical Center, Stanford, CA, USA

[2]Breakthrough Cancer Research Unit, Pangaea Biotech, Dexeus University Institute, Barcelona, Spain

[3]Medical Oncology Service, Catalan Institute of Oncology, Hospital Germans Trias i Pujol, Spain

[4]Department of Bioengineering, Stanford University Medical Center, Stanford, CA, USA

Keywords

Epidermal growth factor receptor (*EGFR*); tyrosine kinase inhibitor; erlotinib; gefitinib; pharmacogenomics

Introduction

Epidermal growth factor receptor (*EGFR*) encodes a transmembrane glycoprotein. This protein is a member of the protein kinase superfamily, which consists of EGFR (ErbB1/HER1), HER2 (ErbB2), HER3 (ErbB3) and HER4 (ErbB4). All family members contain an extracellular ligand-binding domain, a single membrane-spanning region, a juxtamembrane nuclear localization signal, and a cytoplasmic tyrosine kinase domain. They are collectively called HER receptors and are ubiquitously expressed in various cell types, primarily in those of epithelial, mesenchymal and neuronal origin. Under homeostatic conditions, receptor activation is tightly regulated by the availability of ligands, which together form the epidermal growth factor (EGF) family [1]. From those ligands, EGF, transforming growth factor alpha and amphiregulin bind specifically to EGFR [2]. Binding of the EGFR or other family members to a ligand induces receptor dimerization and tyrosine autophosphorylation and leads to cell proliferation.

The EGFR involvement in carcinogenesis has been well established and mutations in *EGFR* can be utilized as predictive markers in the treatment of cancer. In this review, we will focus on the effects of genetic variants (somatic and germline) in the treatment of non-small cell lung cancer (NSCLC) with tyrosine kinase inhibitors (TKI) and will provide a pharmacogenomics overview of *EGFR* in humans. This Very Important Pharmacogene (VIP) summary is available with interactive links to gene variants and drugs on the PharmGKB website http://pharmgkb.org/gene/PA7360 [3].

EGFR Gene, Molecular Structure and Function

EGFR maps on chromosome 7p11.2; it covers 188.3 kb, from 55,086,725 to 55,275,031, on the positive strand. *EGFR* is composed of 28 exons and encodes a protein of 1210 amino

Correspondence to Teri E. Klein, PhD, Department of Genetics, Stanford University, 1501 S. California Avenue, Palo Alto, CA 94304, USA, Tel: +1 650 725 0659; fax: +1 650 725 3863; feedback@pharmgkb.org.

acids (ENST00000275493, Ensembl v69) [4]. Multiple alternatively spliced transcript variants that encode different protein isoforms have been found [5].

EGRF activation by binding of growth factor leads to the autophosphorylation of the intracellular tyrosine kinase domain and results in the formation of receptor homodimers or heterodimers with other HER family members. The phosphorylated tyrosine residues act as a docking site for various adapter molecules, and this results in the activation of downstream signaling pathways including Ras/Raf/MEK/ERK and PI3K/Akt [6, 7], driving different biological processes including cell cycle progression and differentiation, increased cell invasiveness, apoptosis and angiogenesis [8, 9]. Thus, overexpression of EGFR is believed to have a critical role in tumor progression [8–10].

The principal cause of cancer-related mortality is lung cancer, and non-small cell lung cancer (NSCLC) constitutes almost 80% of all lung cases. NSCLC arises from lung epithelial cells, and comprises diverse histological subtypes including adenocarcinoma, bronchioloalveolar, squamous, anaplastic and large-cell carcinomas. About half of the NSCLC patients manifest advanced disease at the time of diagnosis, thus making treatment difficult [11]. Various oncogenic mechanisms, including *EGFR* gene mutations, increased *EGFR* copy number and EGFR protein overexpression may impair the regulation of tyrosine kinase activity of EGFR in tumor cells [12, 13] and may result in increased malignant cell survival, proliferation, invasion and metastasis [14]. The present procedure is that patients with specific types and stages of cancer are treated according to standardized, predetermined protocols [15]. However, understanding the molecular genesis of NSCLC, along with advances in the field of pharmacogenomics, can lead to targeted therapies.

EGFR as cancer drug target

EGFR has been linked to the growth of many human epithelial malignancies, including NSCLC, metastatic colorectal cancer (CRC), head and neck squamous-cell carcinoma (HNSCC), and pancreatic cancer [10, 16, 17]. Intensive laboratory and clinical research have facilitated development of EGFR inhibitors. There are two main types of EGFR inhibitors: tyrosine kinase inhibitors and monoclonal antibodies against EGFR (http://pharmgkb.org/pathway/PA162356267).

Tyrosine Kinase Inhibitors (TKIs)

TKIs are synthetic molecules that block ligand-induced receptor autophosphorylation by binding to the ATP-binding pocket of the intracellular tyrosine kinase domain and disrupting tyrosine kinase activity, thus eliminating intracellular downstream signaling [6, 7]. Gefitinib and erlotinib are specific for EGFR (HER1), whereas afatinib, lapatinib and neratinib inhibit both EGFR and HER2; pelitinib and dacomitinib inhibit EGFR, HER2 and HER4; and vandetanib inhibits EGFR, vascular endothelial growth factor receptor (VEGFR) and the RET-tyrosine kinases [16].

The FDA approved gefitinib through an accelerated process in May 2003 as monotherapy for the treatment of advanced NSCLC patients after failure of both platinum-based and docetaxel chemotherapies. As a condition of accelerated approval, the FDA required demonstration of a survival benefit in a subsequent clinical trial. Three large, prospective studies showed no improvement in overall survival [18–20]; therefore, the original FDA approval for gefitinib was modified. Currently gefitinib is indicated as monotherapy for the continued treatment of advanced NSCLC patients who are benefiting from or who have benefited from gefitinib after failure of both platinum-based and docetaxel chemotherapies [15, 16, 21].

In Europe, gefitinib is not approved for the treatment of patients with locally advanced or metastatic NSCLC unless they also harbor EGFR mutations. In November 2004, erlotinib monotherapy was approved by the FDA for the treatment of advanced NSCLC patients after failure of prior chemotherapy regimen. The FDA also approved erlotinib in combination with gemcitabine for advanced pancreatic cancer patients who have not received previous chemotherapy [15, 16, 21, 22].

Previously, treatment outcomes of erlotinib or gefitinib were studied in unselected patients, which led to conflicting results depending on the type of patient population enrolled in each study. However, the discovery that response to erlotinib or gefitinib is associated with the presence of activating somatic *EGFR* mutations in NSCLC has led to the design of clinical trials in which patients were selected on the basis of *EGFR* mutational status [15, 23]. This pharmacogenetic approach and its results are discussed below.

Other TKIs (lapatinib, neratinib, pelitinib and vandetanib) either have been approved or are in clinical trial phases for cancers other than NSCLC [16]. Several clinical trials are continuing for afatinib, and preliminary result of one of these trials [24] will be discussed in the context of treatment of advanced NSCLC harboring activating somatic *EGFR* mutations.

Monoclonal antibodies

Cetuximab and panitumumab are monoclonal antibodies that specifically target the extracellular domain of EGFR. Cetuximab functions by blocking endogenous ligand binding to the extracellular domain of EGFR and enhancing receptor internalization and degradation [25, 26]. Cetuximab and panitumumab were approved for the treatment of patients, other than NSCLC, with EGFR-expressing metastatic CRC refractory to chemotherapy [27–29]. Cetuximab was also approved for the treatment of advanced HNSCC in combination with radiation therapy [30, 31]. Since cetuximab and panitumumab block the extracellular domain of EGFR, not the TK domain, activating mutations might not affect treatment outcome.

Genetic variation of *EGFR*: Somatic mutations & germline SNPs

Somatic Mutations

The COSMIC database provides information about somatic mutations and related details of human cancers (release v62, date of access: 12/03/2012, http://www.sanger.ac.uk/cosmic) [32].

Out of 68,986 unique samples deposited in the COSMIC database for *EGFR* (partial or full sequence and genotype data) including all cancers examined, 13,201 (19.1%) samples have somatic mutations and about 1.3% of all samples have more than one mutation. There are 842 unique location entries for somatic *EGFR* mutations. Among the mutation bearing patients, six of the mutations have a frequency ≥ 1% and five have 0.1%–1% frequency. The remaining somatic mutations are spread out along EGFR, and are mostly missense substitutions, but there are also insertions and deletions. The six common somatic mutations (≥1%) constitute ~93% of all mutations and are in the tyrosine kinase (TK) domain (between amino acids 712 and 968, exon 18–24) of EGFR (Table 1). The most common set of mutations is in Exon 19 (codon 729–761); it is not a simple mutation, but rather, a collection of different deletions and a few missense substitutions concentrated in codons 744–753 of exon 19. The most frequent mutation in this group is the E746_A750del mutation. Exon 19 mutations comprise 48.3% of all mutations.

The second most common mutation is L858R (rs121434568, T-to-G change at the middle base of the codon) which comprises 36.2% of all mutations. Other missense mutations also

have been observed in this codon as monoallelic or biallelic mutation combinations (L858K, L858M, L858Q, L858R and L858L) in small numbers of subjects. The third most common mutation, T790M (rs121434569) comprises 3.8% of mutations. Exon 20 mutations, a group of different insertions concentrated in codons 763–774, comprise 2.3% of mutations. Mutations at codon 719, including rs28929495 (G719S/G719C), mutations G719A or G719D, and others comprise 1.6% of mutations. L861Q (rs121913444) comprises ~1% of mutations. L861R and L861V mutations are also observed. Five relatively rare mutations (0.1%–0.3% individual frequency, totaling together ~1% of mutations) are A289V, G598V, E709K, S768I and L833V (Table 1).

Mutations in the TK domain of *EGFR* (exon 18–21) also appear in non-lung cancers, including 38 different cancer tissues. *EGFR* mutations in the TK domain are observed in 7.4% of lung cancer samples and 1–2% of salivary gland, eye, peritoneum, upper aerodigestive tract, adrenal gland, and thyroid cancer tissues. Of 39 cancer tissues in COSMIC, 22 of them have *EGFR* mutations ranging from rare (0.1% in pancreas, hematopoietic and stomach tissues) to moderately frequent (7.4% in lung).

In a recent study, whole exome and genome sequences of 183 lung adenocarcinoma tumor/normal DNA pairs revealed *EGFR* mutations in 17.5% of patients, with a few patients having more than one mutation. The L858R (rs121434568) and exon 19 deletions constituted half of the *EGFR* mutations [33]. In contrast, whole exome sequencing of 31 NSCLC revealed a L858R mutation in only one patient (3.2%) [34]. Several somatic mutations were also observed in genes other than *EGFR* [33, 34].

L858R (rs121434568) and exon 19 deletions: *EGFR* mutations that lead to increased response to epidermal growth factors are called activating mutations; these mutations produce a significant and persistent activation of intracellular signaling pathways, resulting in increased cell proliferation. On the other hand, lower concentrations of TKIs are required to inhibit TK phosphorylation, because the mutant receptor has reduced ATP affinity that accounts for increased sensitivity to drugs as compared with wild type EGFR [8, 35, 36]. *EGFR* kinase domain mutations that are clustered around the ATP-binding pocket of the enzyme (exon 19 mutations, L858R, G719X (G719C, G719S and G719A) and L861Q) increase the kinase activity of EGFR and are activating mutations [35, 36]. There are many rare mutations in this region whose functions have not been determined. The L858R and exon 19 mutations constitute between 85% and 91% of all mutations; therefore many studies use these two mutations in their analysis [32, 37, 38].

Clinical responses to both erlotinib and gefitinib differ among NSCLC patients; approximately 10% of patients had clinical responses when treated with TKIs [18–20, 39, 40]. Sequencing of the *EGFR* in tumor samples from these responders showed somatic gain-of-function (e.g. activating) mutations, and these findings led to new clinical trials or retrospective analysis in which patients were chosen depending on the activating *EGFR* mutational status.

In retrospective or prospective studies; patients with an activating somatic *EGFR* mutation had significantly increased response rate (RR)[41–45] and longer progression-free survival (PFS) [42–49] time when treated with erlotinib or gefitinib compared to patients who have no somatic mutation. Although none of the prospective studies reported a statistical overall survival (OS) advantage, a few relatively small studies suggested that OS was increased in mutation-harboring East Asian NSCLC patients when treated with gefitinib [45, 48, 49]. *EGFR* mutations were present in most cases of NSCLC patients who responded well to TKIs, yet approximately 10–20% of patients who show a partial response to gefitinib do not have identifiable *EGFR* mutations, indicating that *EGFR* mutations are not the sole

determinants of TKI response [21]. Most studies present their results according to activating somatic *EGFR* mutation status, regardless of the mutation type, but all or a majority of these mutations are either rs121434568 (L858R) or exon 19 deletion(s).

In prospective phase III randomized trials comparing TKIs and chemotherapy as first-line therapy in patients with advanced NSCLC who harboured activating *EGFR* mutations, erlotinib [50, 51] gefitinib [52, 53] and afatinib [24] treatment arms had significantly increased RR and longer PFS time, whereas OS did not show any clinical benefits when compared to standard chemotherapy [50, 52, 53]. Similarly, in a phase III trial where previously untreated East Asian NSCLC patients who were nonsmokers/former light smokers were treated with gefinitib or carboplatin/paclitaxel (IPASS trial), activating mutation-harboring patients treated with gefitinib had significantly longer PFS time compared to the carboplatin/paclitaxel group; on the contrary, the *EGFR* mutation negative group had significantly shorter PFS time when treated with gefinitib [54, 55]. OS did not differ between two treatments arm (gefitinb vs. carboplatin/paclitaxel)[55].

Many studies analyzed L858R and exon 19 mutations together to increase power. A few studies compared the clinical outcomes for common L858R and exon 19 mutations and failed to show any differential benefits [55–58] between the two types of mutations, except for two studies which suggested that the exon 19 deletions group had longer PFS [59, 60] and OS [60] compared to L858R in TKI-treated NSCLC patients.

Demographic differences of the incidence of *EGFR* mutations in NSCLC patients were observed. Activating mutations in *EGFR* are more frequent in women (38% vs. 10% in men), nonsmokers (47% vs. 7% in smokers), adenocarcinomas (30% vs. 2% in non-adenocarcinoma) and Asian populations (26–36% vs. 7–12% in Whites) [32, 36, 38, 61]. *EGFR* mutations in all NSCLC patients (whether smokers or not) will be important, as inhibition of this receptor has considerable clinical benefits [61]. This observation was particularly clear in Asian patients with *EGFR* mutations treated with gefitinib in the IPASS trial [54].

T790M (rs121434569): Acquired resistance to TKIs: The majority of patients with an activating *EGFR* mutation received clinical benefits when treated with erlotinib/gefitinib, but the magnitude and the duration of the clinical response vary significantly and patients experience disease progression within 9–12 months of treatment [15, 23]. Mutation type (L858R vs. exon 19 del) seems to have little effect on the clinical outcome [55–58].

The most frequent mechanism of acquired resistance to TKIs is the T790M (rs121434569) mutation [62–65]. This mutation may reduce the binding capability of TKIs to the TK domain of EGFR by an allosteric mechanism [63] and increase the affinity to ATP, so that a much higher concentration of TKIs is required to inhibit EGFR [66]. The T790M mutation was originally thought to be acquired by tumors cells during treatment with TKIs; however, when more sensitive methods were used for mutation detection, the presence of the T790M mutation was shown in a small fraction of tumors cells before treatment with TKIs and usually co-exists in these cells with other activating mutations [67, 68]. The tumor cell clones carrying both the activating and the T790M mutations will eventually develop resistance to the TKIs and will be responsible for the progression or recurrence of the disease; this hypothesis was confirmed by findings that patients harboring T790M mutation before the start of the treatment had a significantly shorter PFS [69–71] and decreased response rate (RR) [68] compared with those not having T790M mutation.

Rosell et al. [70] assessed the T790M mutation in pretreatment diagnostic specimens from 129 EGFR TKI treated advanced NSCLC patients with EGFR mutations, and found that the

EGFR T790M mutation was present in 45 of 129 patients (35%). PFS was 12 months in patients with and 18 months in patients without the T790M mutation (P = 0.05). Additionally, it was found that low BRCA-1 levels neutralized the negative effect of the T790M mutation and were associated with longer PFS with erlotinib treatment, whereas high levels of BRCA-1 may lead to *de novo* resistance through increased DNA damage repair capacity, suggesting that pretreatment assessment of both T790M mutation status and BRCA1 expression could be useful to predict outcome [70]. In the EURTAC trial the T790M mutation was detected in 38% of the pre-treatment specimens analyzed [51]. Fujita et al. [72] evaluated the incidence of T790M in pretreatment tumor specimens using the highly sensitive colony hybridization technique, and this mutation was detected in 30/38 (79% of) resected tumor tissues of patients with EGFR mutations. The median time to treatment failure (TTF) was 9 months for the patients with pretreatment T790M and 7 months for the patients without the T790M mutation (p = 0.44), which suggested that patients with the T790M allele may have a relatively favorable prognosis [72].

The T790M (rs121434569) mutation, along with other secondary mutations in EGFR, was observed as a germline mutation in four siblings of European descent family in which multiple members developed NSCLC [73]. The T790M mutation was not observed in a cohort of ~400 subjects [73] and dbSNP (build 137) does not show its existence in the general population.

Other resistance mechanisms to EGFR-targeted therapy

The T790M mutation is detectable in about 50% of patients with NSCLC patients who develop resistance to TKI treatment [64, 65, 67], and may not explain all resistance cases. One of the mechanisms of resistance to TKIs involves the mesenchymal epithelial transition factor gene (*MET)* amplification that occurs in 5–20% of patients [74]. *MET* gene amplification leads to EGFR-independent activation of the PI3K/AKT pathway through MET/ErbB-3 heterodimers and may be responsible for the resistance to TKIs [74]. MET amplification in NSCLC was identified in a very small proportion of tumor cells even before exposure to TKIs, and this population of cells expands following TKI treatment [75]. The other mechanism involves the *KRAS* oncogene, which is mutated in approximately 15–30% of NSCLC [76]. Mutations in *KRAS* and those in *EGFR* seem to be mutually exclusive, and *KRAS* mutation-harboring patients do not respond to TKI therapy [77]. KRAS is a downstream mediator of EGFR-induced cell signaling, and mutations confer constitutive activation of the signaling pathway(s), independent of EGFR activation [78].

BIM (known as BCL2-like 11) is also involved in resistance to TKI treatment and is a proapoptotic protein that is overexpressed in different malignancies [79, 80]. Various chemotherapeutic agents use BIM as a mediating executioner of cell death. Hence, BIM suppression supports metastasis and chemoresistance. BIM upregulation is required for apoptosis induction by EGFR tyrosine kinase inhibitors (EGFR-TKIs) in EGFR-mutant NSCLC. Low BIM mRNA levels can lead to gefitinib resistance in NSCLC with EGFR mutations and may be a marker of primary resistance. The extracellular regulated kinase (ERK) pathway also negatively regulates BIM expression in NSCLC with EGFR mutations [81–83]. Components that cause induction of BIM may have a role to overcome resistance to EGFR TKI in NSCLC with EGFR mutations. Recent studies have showed that HDAC inhibition can epigenetically restore BIM function *in vitro* and death sensitivity of EGFR-TKI, in cases of EGFR mutant NSCLC where resistance to EGFR-TKI is associated with a common BIM polymorphism [84]. Thus, NSCLC has a significant level of plasticity, being able to activate several different mechanisms leading to resistance to EGFR-TKIs.

EGFR gene copy number and protein expression in NSCLC

Mutations, gene copy number, and protein expression are three EGFR-related biomarkers that have been extensively studied in clinical trials in order to obtain better predictive and prognostic values for treatment modalities. Although *EGFR* gene amplification frequently correlates with EGFR protein overexpression and tumor progression [85], EGFR gene amplification and protein overexpression studies yielded controversial results in terms of prognostic significance and clinical benefits [15, 23, 47]. In this regard, biomarkers associated with EGFR-TKI activity was evaluated in the IPASS trial (>1200 NSCLC patients) [55]. *EGFR* mutations were the strongest predictive biomarker for PFS and objective RR to first-line gefitinib versus carboplatin/paclitaxel treatment, and post hoc analysis suggested that the predictive value of *EGFR* gene copy number was driven by coexisting *EGFR* mutations [55].

Germline SNPs

EGFR contains over 800 SNPs found in ≥ 1% of samples (dbSNP build 137) and a few of them may have some biological importance.

Intron 1 (CA)n repeat (rs11568315): This is a simple sequence repeat polymorphism; dinucleotides range from 9 to 23, with the majority clustered around 15 to 21 CA repeats (dbSNP build 137). Association of the intron 1 CA repeat polymorphism to better clinical response in NSCLC patients treated with gefitinib was analyzed in four different studies, all having less than 100 study subjects [86–89]. Alleles with 16 or fewer CA repeats were grouped together and considered to be short alleles, and those with 17 or more CA repeats were considered to be long. NSCLC patients carrying one or two short alleles are more likely to have better clinical response (increased RR, increased PFS and increased OS) when treated with gefitinib as compared to patients who have two long alleles [86–89]. Well-powered studies are needed to replicate the beneficial clinical effect of rs11568315 in NSCLC patients.

The -216G>T (rs712829): GT and TT genotypes are associated with increased PFS time when treated with gefitinib in NSCLC patients [86] and with decreased severity of diarrhea when treated with erlotinib in neoplasm patients [90] as compared to patients with GG genotypes. Both studies involved few patients, and well-powered studies are needed to replicate suggested associations [86, 90].

GWAS studies on tumor risk and EGFR: Two well-powered genome wide association studies (GWAS) showed that SNPs in *EGFR* were significantly associated with risk of glioma, the most common primary brain tumor [91, 92]; implications of these finding on treatment of glioma or other cancers are yet to be seen.

Conclusion

Extensive molecular, cancer genome sequencing and recent GWAS studies demonstrate that *EGFR* is an important gene for many biological processes and particularly tumorigenesis. NSCLC patients harboring activating somatic *EGFR* mutations have better clinical outcomes when treated with TKIs compared to the patients who do not have mutations. Clinical and treatment associations with germline *EGFR* SNPs are not strong; more studies are necessary to clarify the role of germline SNPs in treatment of NSCLC.

Acknowledgments

PharmGKB is supported by the National Institutes of Health/National Institute of General Medical Sciences (R24 GM61374).

References

1. Yarden Y, Sliwkowski MX. Untangling the ErbB signaling network. Nat Rev Mol Cell Biol. 2001; 2:127–137. [PubMed: 11252954]
2. Hynes NE, Lane HA. ERBB receptors and cancer: the complexity of targeted inhibitors. Nat Rev Cancer. 2005; 5:341–354. [PubMed: 15864276]
3. Whirl-Carrillo M, et al. Pharmacogenomics knowledge for personalized medicine. Clin Pharmacol Ther. 2012; 92(4):414–7. [PubMed: 22992668]
4. Hubbard T, et al. The Ensembl genome database project. Nucleic Acids Res. 2002; 30(1):38–41. [PubMed: 11752248]
5. Thierry-Mieg D, Thierry-Mieg J. AceView: a comprehensive cDNA-supported gene and transcripts annotation. Genome Biol. 2006; 7(Suppl 1):S12, 1–14. [PubMed: 16925834]
6. Kumar A, et al. Structure and clinical relevance of the epidermal growth factor receptor in human cancer. J Clin Oncol. 2008; 26(10):1742–51. [PubMed: 18375904]
7. Jorissen RN. Epidermal growth factor receptor: mechanisms of activation and signaling. Exp Cell Res. 2003; 284:31–53. [PubMed: 12648464]
8. Krause DS, Van Etten RA. Tyrosine kinases as targets for cancer therapy. N Engl J Med. 2005; 353:172–187. [PubMed: 16014887]
9. Raymond E, Faivre S, Armand JP. Epidermal growth factor receptor tyrosine kinase as a target for anticancer therapy. Drugs. 2000; 60(Suppl 1):15–23. discussion 41–2. [PubMed: 11129168]
10. Normanno N. Epidermal growth factor receptor (EGFR) signaling in cancer. Gene. 2006; 366:2–16. [PubMed: 16377102]
11. Jemal A, et al. Cancer statistics, 2008. CA Cancer J Clin. 2008; 58(2):71–96. [PubMed: 18287387]
12. Ciardiello F, Tortora G. EGFR antagonists in cancer treatment. N Engl J Med. 2008; 358(11): 1160–74. [PubMed: 18337605]
13. De Luca A, Normanno N. Predictive biomarkers to tyrosine kinase inhibitors for the epidermal growth factor receptor in non-small-cell lung cancer. Curr Drug Targets. 2010; 11(7):851–64. [PubMed: 20388064]
14. Gazdar AF, Minna JD. Deregulated EGFR signaling during lung cancer progression: mutations, amplicons, and autocrine loops. Cancer Prev Res (Phila). 2008; 1(3):156–60. [PubMed: 19138950]
15. Mayo C, et al. Pharmacogenetics of EGFR in lung cancer: perspectives and clinical applications. Pharmacogenomics. 2012; 13(7):789–802. [PubMed: 22594511]
16. Wheeler DL, Dunn EF, Harari PM. Understanding resistance to EGFR inhibitors-impact on future treatment strategies. Nat Rev Clin Oncol. 2010; 7(9):493–507. [PubMed: 20551942]
17. Funke S, Brenner H, Chang-Claude J. Pharmacogenetics in colorectal cancer: a systematic review. Pharmacogenomics. 2008; 9(8):1079–99. [PubMed: 18681783]
18. Giaccone G. Gefitinib in combination with gemcitabine and cisplatin in advanced non-small-cell lung cancer: a phase III trial[mdash]INTACT 1. J Clin Oncol. 2004; 22:777–784. [PubMed: 14990632]
19. Herbst RS. Gefitinib in combination with paclitaxel and carboplatin in advanced non-small-cell lung cancer: a phase III trial[mdash]INTACT 2. J Clin Oncol. 2004; 22:785–794. [PubMed: 14990633]
20. Thatcher N. Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small cell lung cancer: Results from a randomized, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer). Lancet. 2005; 366:1527–1537. [PubMed: 16257339]
21. Sharma SV, et al. Epidermal growth factor receptor mutations in lung cancer. Nat Rev. 2007; 7:169–181.

22. Yang ZY, et al. Gemcitabine plus erlotinib for advanced pancreatic cancer: a systematic review with meta-analysis. PLoS One. 2013; 8(3):e57528. [PubMed: 23472089]
23. Rossi A, et al. Should epidermal growth factor receptor tyrosine kinase inhibitors be considered ideal drugs for the treatment of selected advanced non-small cell lung cancer patients? Cancer Treat Rev. 2012
24. Yang JC, et al. LUX-Lung 3: A randomized, open-label, phase III study of afatinib versus pemetrexed and cisplatin as first-line treatment for patients with advanced adenocarcinoma of the lung harboring EGFR-activating mutations. J Clin Oncol. 2012; 30(15S):480s.
25. Kawamoto T. Growth stimulation of A431 cells by epidermal growth factor: identification of high-affinity receptors for epidermal growth factor by an anti-receptor monoclonal antibody. Proc Natl Acad Sci USA. 1983; 80:1337–1341. [PubMed: 6298788]
26. Yang XD, et al. Development of ABX-EGF, a fully human anti-EGF receptor monoclonal antibody, for cancer therapy. Crit Rev Oncol Hematol. 2001; 38:17–23. [PubMed: 11255078]
27. Giusti RM. U.S. Food and Drug Administration approval: panitumumab for epidermal growth factor receptor-expressing metastatic colorectal carcinoma with progression following fluoropyrimidine-, oxaliplatin-, and irinotecan-containing chemotherapy regimens. Clin Cancer Res. 2008; 14:1296–1302. [PubMed: 18316547]
28. Van Cutsem E. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. N Engl J Med. 2009; 360:1408–1417. [PubMed: 19339720]
29. Jonker DJ. Cetuximab for the treatment of colorectal cancer. N Engl J Med. 2007; 357:2040–2048. [PubMed: 18003960]
30. Vermorken JB. Platinum-based chemotherapy plus cetuximab in head and neck cancer. N Engl J Med. 2008; 359:1116–1127. [PubMed: 18784101]
31. Bonner JA. Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. N Engl J Med. 2006; 354:567–578. [PubMed: 16467544]
32. Forbes SA, et al. COSMIC: mining complete cancer genomes in the Catalogue of Somatic Mutations in Cancer. Nucleic Acids Res. 2011; 39(Database issue):D945–50. [PubMed: 20952405]
33. Imielinski M, et al. Mapping the hallmarks of lung adenocarcinoma with massively parallel sequencing. Cell. 2012; 150(6):1107–20. [PubMed: 22980975]
34. Liu P, et al. Identification of somatic mutations in non-small cell lung carcinomas using whole-exome sequencing. Carcinogenesis. 2012; 33(7):1270–6. [PubMed: 22510280]
35. Lynch TJ. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med. 2004; 350:2129–2139. [PubMed: 15118073]
36. Kancha RK, et al. Functional analysis of epidermal growth factor receptor (EGFR) mutations and potential implications for EGFR targeted therapy. Clin Cancer Res. 2009; 15:460–467. [PubMed: 19147750]
37. Murray S, et al. Somatic mutations of the tyrosine kinase domain of epidermal growth factor receptor and tyrosine kinase inhibitor response to TKIs in non-small cell lung cancer: an analytical database. J Thorac Oncol. 2008; 3(8):832–9. [PubMed: 18670300]
38. Yatabe Y, Mitsudomi T. Mutations of the epidermal growth factor receptor gene and related genes as determinants of epidermal growth factor receptor tyrosine kinase inhibitors sensitivity in lung cancer. Cancer Sci. 2007; 98:1817–1824. [PubMed: 17888036]
39. Shepherd FA. Erlotinib in previously treated non-small cell lung cancer. N Engl J Med. 2005; 353:123–132. [PubMed: 16014882]
40. Kim ES. Gefitinib versus docetaxel in previously treated non-small-cell lung cancer (INTEREST): a randomised phase III trial. Lancet. 2008; 372:1809–1818. [PubMed: 19027483]
41. Chen YM, et al. Plasma epidermal growth factor receptor mutation analysis and possible clinical applications in pulmonary adenocarcinoma patients treated with erlotinib. Oncol Lett. 2012; 3(3): 713–717. [PubMed: 22740981]
42. Han JY, et al. First-SIGNAL: first-line single-agent iressa versus gemcitabine and cisplatin trial in never-smokers with adenocarcinoma of the lung. J Clin Oncol. 2012; 30(10):1122–8. [PubMed: 22370314]

43. Miller VA, et al. Molecular characteristics of bronchioloalveolar carcinoma and adenocarcinoma, bronchioloalveolar carcinoma subtype, predict response to erlotinib. J Clin Oncol. 2008; 26(9): 1472–8. [PubMed: 18349398]
44. Satouchi M, et al. Predictive factors associated with prolonged survival in patients with advanced non-small-cell lung cancer (NSCLC) treated with gefitinib. Br J Cancer. 2007; 96(8):1191–6. [PubMed: 17387341]
45. Han SW, et al. Clinical predictors versus epidermal growth factor receptor mutation in gefitinib-treated non-small-cell lung cancer patients. Lung Cancer. 2006; 54(2):201–7. [PubMed: 16956694]
46. Cadranel J, et al. Impact of systematic EGFR and KRAS mutation evaluation on progression-free survival and overall survival in patients with advanced non-small-cell lung cancer treated by erlotinib in a French prospective cohort (ERMETIC project--part 2). J Thorac Oncol. 2012; 7(10): 1490–502. [PubMed: 22982650]
47. Brugger W, et al. Prospective molecular marker analyses of EGFR and KRAS from a randomized, placebo-controlled study of erlotinib maintenance therapy in advanced non-small-cell lung cancer. J Clin Oncol. 2011; 29(31):4113–20. [PubMed: 21969500]
48. Han SW, et al. Intron 1 CA dinucleotide repeat polymorphism and mutations of epidermal growth factor receptor and gefitinib responsiveness in non-small-cell lung cancer. Pharmacogenet Genomics. 2007; 17(5):313–9. [PubMed: 17429313]
49. Ichihara S, et al. The impact of epidermal growth factor receptor gene status on gefitinib-treated Japanese patients with non-small-cell lung cancer. Int J Cancer. 2007; 120(6):1239–47. [PubMed: 17192902]
50. Zhou CC. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. Lancet Oncol. 2011; 12:735–742. [PubMed: 21783417]
51. Rosell R, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol. 2012; 13(3):239–46. [PubMed: 22285168]
52. Mitsudomi T. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. Lancet Oncol. 2010; 11:121–128. [PubMed: 20022809]
53. Maemondo M. Gefitinib or chemotherapy for non small-cell lung cancer with mutated EGFR. N Engl J Med. 2010; 362:2380–2388. [PubMed: 20573926]
54. Mok TS. Gefitinib or carboplatin-paclitaxel in pulmonary adenococarcinoma. N Engl J Med. 2009; 361:947–957. [PubMed: 19692680]
55. Fukuoka M, et al. Biomarker analyses and final overall survival results from a phase III, randomized, open-label, first-line study of gefitinib versus carboplatin/paclitaxel in clinically selected patients with advanced non-small-cell lung cancer in Asia (IPASS). J Clin Oncol. 2011; 29(21):2866–74. [PubMed: 21670455]
56. Sutani A. Gefitinib for non-small-cell lung cancer patients with epidermal growth factor receptor gene mutations screened by peptide nucleic acid-locked nucleic acid PCR clamp. Br J Cancer. 2006; 95:1483–1469. [PubMed: 17106442]
57. Costa DB, et al. Pooled analysis of the prospective trials of gefitinib monotherapy for EGFR-mutant non-small cell lung cancers. Lung Cancer. 2007; 58:95–103. [PubMed: 17610986]
58. Inoue A. Prospective phase II study of gefitinib for chemotherapy-naive patients with advanced non-small-cell lung cancer with epidermal growth factor receptor gene mutations. J Clin Oncol. 2006; 24:3340–3346. [PubMed: 16785471]
59. Won YW, et al. Comparison of clinical outcome of patients with non-small-cell lung cancer harbouring epidermal growth factor receptor exon 19 or exon 21 mutations. J Clin Pathol. 2011; 64(11):947–52. [PubMed: 21725039]
60. Rosell R, et al. Screening for epidermal growth factor receptor mutations in lung cancer. N Engl J Med. 2009; 361(10):958–67. [PubMed: 19692684]
61. Mitsudomi T, Kosaka T, Yatabe Y. Biological and clinical implications of EGFR mutations in lung cancer. Int J Clin Oncol. 2006; 11(3):190–8. [PubMed: 16850125]

62. Pao W, et al. Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. PLoS Med. 2005; 2(3):e73. [PubMed: 15737014]
63. Kobayashi S, et al. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. N Engl J Med. 2005; 352(8):786–92. [PubMed: 15728811]
64. Sequist LV, et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. Sci Transl Med. 2011; 3(75):75ra26.
65. Bean J, et al. MET amplification occurs with or without T790M mutations in EGFR mutant lung tumors with acquired resistance to gefitinib or erlotinib. Proc Natl Acad Sci U S A. 2007; 104(52): 20932–7. [PubMed: 18093943]
66. Yun CH, et al. The T790M mutation in EGFR kinase causes drug resistance by increasing the affinity for ATP. Proc Natl Acad Sci U S A. 2008; 105(6):2070–5. [PubMed: 18227510]
67. Arcila ME, et al. Rebiopsy of lung cancer patients with acquired resistance to EGFR inhibitors and enhanced detection of the T790M mutation using a locked nucleic acid-based assay. Clin Cancer Res. 2011; 17(5):1169–80. [PubMed: 21248300]
68. Inukai M, et al. Presence of epidermal growth factor receptor gene T790M mutation as a minor clone in non-small cell lung cancer. Cancer Res. 2006; 66(16):7854–8. [PubMed: 16912157]
69. Su KY, et al. Pretreatment epidermal growth factor receptor (EGFR) T790M mutation predicts shorter EGFR tyrosine kinase inhibitor response duration in patients with non-small-cell lung cancer. J Clin Oncol. 2012; 30(4):433–40. [PubMed: 22215752]
70. Rosell R, et al. Pretreatment EGFR T790M mutation and BRCA1 mRNA expression in erlotinib-treated advanced non-small-cell lung cancer patients with EGFR mutations. Clin Cancer Res. 2011; 17(5):1160–8. [PubMed: 21233402]
71. Maheswaran S, et al. Detection of mutations in EGFR in circulating lung-cancer cells. N Engl J Med. 2008; 359(4):366–77. [PubMed: 18596266]
72. Fujita Y, et al. Highly sensitive detection of EGFR T790M mutation using colony hybridization predicts favorable prognosis of patients with lung cancer harboring activating EGFR mutation. J Thorac Oncol. 7(11):1640–4. [PubMed: 22899358]
73. Bell DW, et al. Inherited susceptibility to lung cancer may be associated with the T790M drug resistance mutation in EGFR. Nat Genet. 2005; 37(12):1315–6. [PubMed: 16258541]
74. Engelman JA, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science. 2007; 316(5827):1039–43. [PubMed: 17463250]
75. Turke AB, et al. Preexistence and clonal selection of MET amplification in EGFR mutant NSCLC. Cancer Cell. 2010; 17(1):77–88. [PubMed: 20129249]
76. Eberhard DA, et al. Mutations in the epidermal growth factor receptor and in KRAS are predictive and prognostic indicators in patients with non-small-cell lung cancer treated with chemotherapy alone and in combination with erlotinib. J Clin Oncol. 2005; 23(25):5900–9. [PubMed: 16043828]
77. Linardou H, et al. Assessment of somatic k-RAS mutations as a mechanism associated with resistance to EGFR-targeted agents: a systematic review and meta-analysis of studies in advanced non-small-cell lung cancer and metastatic colorectal cancer. Lancet Oncol. 2008; 9(10):962–72. [PubMed: 18804418]
78. Normanno N, et al. Implications for KRAS status and EGFR-targeted therapies in metastatic CRC. Nat Rev Clin Oncol. 2009; 6(9):519–27. [PubMed: 19636327]
79. Akiyama T, Dass CR, Choong PF. Bim-targeted cancer therapy: a link between drug action and underlying molecular changes. Mol Cancer Ther. 2009; 8(12):3173–80. [PubMed: 19934277]
80. Wang Z, et al. Constitutive production of NF-kappaB2 p52 is not tumorigenic but predisposes mice to inflammatory autoimmune disease by repressing Bim expression. J Biol Chem. 2008; 283(16): 10698–706. [PubMed: 18281283]
81. Cragg MS, et al. Gefitinib-induced killing of NSCLC cell lines expressing mutant EGFR requires BIM and can be enhanced by BH3 mimetics. PLoS Med. 2007; 4(10):1681–89. discussion 1690. [PubMed: 17973573]
82. Gong Y, et al. Induction of BIM Is Essential for Apoptosis Triggered by EGFR Kinase Inhibitors in Mutant EGFR-Dependent Lung Adenocarcinomas. PLoS Med. 2007; 4(10):e294. [PubMed: 17927446]

83. Costa DB, et al. BIM mediates EGFR tyrosine kinase inhibitor-induced apoptosis in lung cancers with oncogenic EGFR mutations. PLoS Med. 2007; 4(10):1669–79. discussion 1680. [PubMed: 17973572]
84. Nakagawa T, et al. EGFR-TKI resistance due to BIM polymorphism can be circumvented by in combination with HDAC inhibition. Cancer Res.
85. Yatabe Y, Takahashi T, Mitsudomi T. Epidermal growth factor receptor gene amplification is acquired in association with tumor progression of EGFR-mutated lung cancer. Cancer Res. 2008; 68(7):2106–11. [PubMed: 18381415]
86. Liu G, et al. Epidermal growth factor receptor polymorphisms and clinical outcomes in non-small-cell lung cancer patients treated with gefitinib. Pharmacogenomics J. 2008; 8(2):129–38. [PubMed: 17375033]
87. Ma F, et al. Polymorphisms of EGFR predict clinical outcome in advanced non-small-cell lung cancer patients treated with Gefitinib. Lung Cancer. 2009; 66(1):114–9. [PubMed: 19201048]
88. Tiseo M, et al. Predictors of gefitinib outcomes in advanced non-small cell lung cancer (NSCLC): study of a comprehensive panel of molecular markers. Lung Cancer. 2010; 67(3):355–60. [PubMed: 19473722]
89. Nie Q, et al. The epidermal growth factor receptor intron1 (CA) n microsatellite polymorphism is a potential predictor of treatment outcome in patients with advanced lung cancer treated with Gefitinib. Eur J Pharmacol. 2007; 570(1–3):175–81. [PubMed: 17597605]
90. Rudin CM, et al. Pharmacogenomic and pharmacokinetic determinants of erlotinib toxicity. J Clin Oncol. 2008; 26(7):1119–27. [PubMed: 18309947]
91. Sanson M, et al. Chromosome 7p11.2 (EGFR) variation influences glioma risk. Hum Mol Genet. 2011; 20(14):2897–904. [PubMed: 21531791]
92. Rajaraman P, et al. Genome-wide association study of glioma and meta-analysis. Hum Genet. 2012; 131(12):1877–88. [PubMed: 22886559]

Table 1

Incidence of the Common (≥1%) and Rare (0.1%–1%) Somatic Mutations of *EGFR*.

Mutation	Incidence
Exon 19 mutations	
Collection of different deletions and a few missense substitutions; the most frequent one is E746_A750del	48.3%
L858R: rs121434568	
Other missense mutations were also observed in this codon at extremely low frequency	36.2%
T790M: rs121434569	3.8%
Exon 20 mutations	
A group of different insertions concentrated in codons 763–774	2.3%
Codon 719 mutation	
Mutation at the first base of codon G719S or G719C (rs28929495), 0.82%	
Mutation at the second base of codon G719A or G719D, 0.77%	1.6%
L861Q: rs121913444	
L861R and L861V mutations were also observed on this codon in extremely low frequency	~1.0%
A289V, G598V, E709K, S768I and L833V: combined incidence	~1.0%

Incidences are derived from COSMIC database. 68,986 unique samples have been deposited for *EGFR*, of which 69% are from lung cancer tissues.