Current Knowledge and Future Directions of the Selective Epidermal Growth Factor Receptor Inhibitors Erlotinib (Tarceva ®) and Gefitinib (Iressa®)

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Current Knowledge and Future Directions of the Selective Epidermal Growth Factor Receptor Inhibitors Erlotinib (Tarceva®) and Gefitinib (Iressa®)

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ABSTRACT

Gefitinib (Iressa®; AstraZeneca Pharmaceuticals, Wilmington, DE, http://www.astrazeneca-us.com) and erlotinib (Tarceva®; OSI Pharmaceuticals, Inc., Melville, NY, http://www.osip.com) are so-called small molecules that selectively inhibit epidermal growth factor receptor (EGFR) tyrosine kinase activity. Both drugs received registration approval by the U.S. Food and Drug Administration (FDA) for the second- and third-line treatment of non-small cell lung cancer (NSCLC), but the failure of gefitinib to show a survival advantage over placebo has resulted in a discussion about the registration of gefitinib. Recently published results have revealed that mutations in the tyrosine kinase domain of EGFR are strongly associated with increased gefitinib and erlotinib sensitivity in patients with advanced NSCLC. Here, we present the current knowledge and the future directions of the EGFR tyrosine kinase inhibitors gefitinib and erlotinib. ***The Oncologist*** 2005;10:579–589

INTRODUCTION

During the past few years, the development of targeted anticancer therapy has become more important than the optimization of therapy with conventional anticancer drugs.

Molecular target–specific therapeutics have the potential to maximize therapeutic benefit while minimizing toxicity to normal cells. An ideal molecular target in tumors is differentially expressed or differentially functional in tumor and nontumor tissues. In addition, molecular epidemiology has identified that such a target is a dominant predictor of poor disease outcome. Some tyrosine kinases are such targets. The activation of tyrosine kinases is tightly regulated in normal cells, but in tumor cells, the signal transduction pathways are disrupted in such a way that excessive signaling results in tumor progression.

The epidermal growth factor receptor (EGFR), HER, family of tyrosine kinase receptors consists of four members: EGFR (HER1 or ERBB1), HER2/neu (ERBB2), HER3 (ERBB3), and HER4 (ERBB4). Members of the EGFR family of tyrosine kinase receptors have integral kinase activity and have extracellular ligand-binding domains, transmembrane regions, and multifunctional

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cytoplasmic tails. The tail has an ATP-binding site plus tyrosine kinase activity, and is capable of phosphorylating itself (autophosphorylation) as well as other proteins [1].

Tyrosine kinases are altered in many cancers. Excessive EGFR signaling in tumors is usually the result of EGFR overexpression and/or the excessive production and availability of receptor ligands. For example, several human cancers, including those of the upper aerodigestive tract, lung, colon, pancreas, breast, ovary, bladder, and kidney, as well as gliomas, display EGFR mRNA and/or protein overexpression [2, 3]. This overexpression is sometimes the result of *EGFR* gene amplification and is often associated with increased expression of the receptor ligands transforming growth factor alpha or amphiregulin [3]. Other possible mechanisms of aberrant EGFR signaling include heterodimerization with other ERBB receptors such as ERBB2 (HER2), transactivation by heterologous signaling networks, loss of regulatory mechanisms of receptor signaling [3], and activating mutations. The result of excessive signaling is tumor progression, including the promotion of proliferation, angiogenesis, and invasion/metastasis, and the inhibition of apoptosis [4, 5]. Expression of EGFR in tumors has been correlated with disease progression, poor survival, poor response to therapy [2], and the development of resistance to cytotoxic agents [6].

Several in vitro and in vivo studies have shown that the interruption of signaling with various EGFR inhibitors that recognize the extracellular (monoclonal antibodies) or intracellular domain of the receptor (small molecules) results in inhibition of tumor cell proliferation and/or viability [5]. These observations, together with the association of EGFR overexpression with poor patient prognosis, the ability to identify EGFR-expressing human tumors in diagnostic tissues, and the lack of a critical physiological role of EGFR in healthy persons, have all suggested this signaling network as an ideal target for novel cancer therapeutic strategies [7, 8].

The two most important anticancer drugs developed to target the EGFR tyrosine kinase signaling network are erlotinib (Tarceva®; OSI Pharmaceuticals, Inc., Melville, NY, http://www.osip.com) and gefitinib (Iressa®; AstraZeneca Pharmaceuticals, Wilmington, DE, http://www.astrazeneca-us.com). These two agents have been accepted for registration by the U.S. Food and Drug Administration (FDA), but because of recently published results, the continued registration of gefitinib is uncertain. Because of this, the development processes of gefitinib and erlotinib are discussed in great detail in this paper.

Gefitinib and Erlotinib

Gefitinib (molecular weight, 446.9 Da) and erlotinib (molecular weight, 429.9 Da) are orally active EGFR tyrosine kinase inhibitors that compete for the ATP-binding site in the cytoplasmic tail [9]. The result is inhibition of cellular proliferation, angiogenesis, tumor invasion, and metastasis. Several carcinomas of the upper aerodigestive tract, including non-small cell lung cancer (NSCLC), display EGFR overexpression [10]. Gefitinib is currently available in Japan, the U.S., and Australia, and was conditionally approved by the FDA in May 2003 based on response rates in uncontrolled phase II studies. Erlotinib received full approval from the FDA in November 2004 based on a survival advantage. Gefitinib is available as brown film-coated tablets that contain 250 mg of gefitinib. The chemical structure of gefitinib is shown in Figure 1. Erlotinib tablets are available in three dose strengths: 25 mg, 100 mg, and 150 mg. The chemical structure of erlotinib is shown in Figure 2. Erlotinib shares with gefitinib a common chemical backbone structure.

Development Procedure

Phase I Trials

Gefitinib and erlotinib followed the same development procedure. Both drugs are indicated for the second- and third-line treatment of patients with advanced NSCLC after failure of at least one prior platinum treatment. Phase I studies of gefitinib and erlotinib were performed in patients with a variety of solid tumors, including patients with the diagnosis of advanced NSCLC [11, 12]. Both drugs were well tolerated. Gefitinib exhibited encouraging antitumor

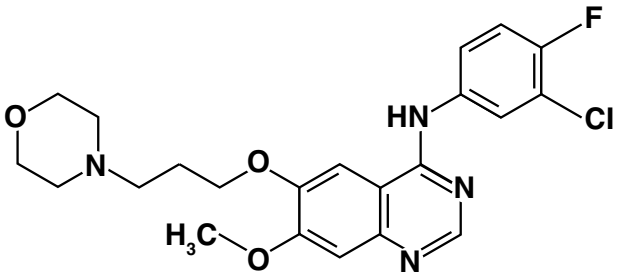

Figure 1. The chemical structure of gefitinib.

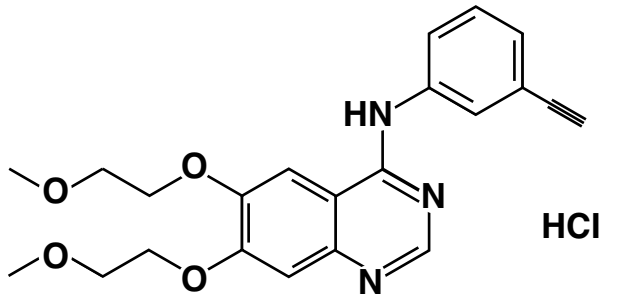

Figure 2. The chemical structure of erlotinib.

activity across the dose range of 150–700 mg/day. Despite interpatient variability in exposure, biologically relevant plasma concentrations (exposure levels well above the 90% inhibitory concentration [IC_{90}] for carcinoma cells) were maintained at the doses of 150 mg/day and above. Fixed doses of 250 and 500 mg/day were, therefore, selected for subsequent phase II and phase III trials; 250 mg/day is higher than the lower dose level at which objective tumor regression was seen, whereas 500 mg/day is the highest dose that was well tolerated when taken chronically in phase I trials. The erlotinib phase I trials identified a dose of 150 mg/day for further clinical development. The most frequently observed drug-related adverse events were rash and diarrhea (grade 1 and 2). The incidence and severity of the adverse events generally increased as the dose increased.

Trials in First-Line NSCLC Treatment

Four randomized chemotherapy combination trials with gefitinib (Iressa® non–small cell lung cancer trial assessing combination treatment [INTACT]-1 and INTACT-2) [13, 14] and erlotinib (TALENT and TRIBUTE) [15, 16] were performed in chemotherapy-naïve patients with advanced NSCLC. In those studies, chemotherapy plus gefitinib or erlotinib was compared with chemotherapy alone. In the INTACT-1 ($n = 1,093$) and TALENT ($n = 1,172$) trials, the chemotherapy regimen consisted of cisplatin (Platinol®; Bristol-Myers Squibb, Princeton, NJ, http://www.bms.com) and gemcitabine (Gemzar®; Eli Lilly and Company, Indianapolis, http://www.lilly.com). In the INTACT-2 ($n = 1,037$) and TRIBUTE ($n = 1,059$) trials, the chemotherapy regimen was carboplatin (Paraplatin®; Bristol-Myers Squibb) and paclitaxel (Taxol®; Bristol-Myers Squibb). Unfortunately, none of those studies definitively showed any benefit to adding an EGFR inhibitor to standard combination chemotherapy in patients with NSCLC.

The rationale for those studies was based on preclinical in vitro and in vivo data. In vitro studies revealed greater cytotoxicity of cisplatin, more DNA-adduct formation, and less DNA repair of platinum-DNA adducts when combined with gefitinib [17]. Preclinical studies combining erlotinib with cisplatin, doxorubicin (Adriamycin®; Bedford Laboratories, Bedford, OH, http://www.bedfordlabs.com), gemcitabine, or paclitaxel showed an additive effect on antitumor activity with no increase in toxicity [18]. Thus, the combination trials have not shown proof of concept of the synergistic preclinical studies.

Trials in Second-Line NSCLC Treatment

Five single-agent, phase II studies with gefitinib or erlotinib in patients with NSCLC have been reported. Gefitinib was studied in two multicenter trials: the Iressa® Dose Evaluation in Advanced Lung Cancer (IDEAL)-1 (Japan, Europe, and Australia) and IDEAL-2 (North America) trials [19, 20]. Patients enrolled in the IDEAL-1 trial were required to have failed only one prior platinum-containing regimen [19], whereas patients enrolled in the IDEAL-2 trial were required to have failed a platinum-containing regimen and docetaxel (Taxotere®; Aventis Pharmaceuticals Inc., Bridgewater, NJ, http://www.aventispharma-us.com) [20]. Patients were randomized to receive 250–500 mg/day gefitinib. In the IDEAL trials, the response rates ranged from 9%–19%. The median survival ranged from 5.9–8 months. Grade 3 and 4 toxicities were relatively uncommon. The recommended dose was 250 mg/day, because treatment at 500 mg/day led to seemingly greater toxicity (higher rates of diarrhea, rash, acne, dry skin, nausea, and vomiting) without additional benefit. Based upon the response rate data from the IDEAL trials, the FDA registered gefitinib at a dose of 250 mg/day for the treatment of advanced NSCLC.

A randomized, phase II study was also performed with erlotinib in patients with previously treated advanced NSCLC ($n = 57$). Patients received either 150 mg/day of erlotinib or best supportive care. In that study, erlotinib produced an objective response rate of 12.3%. The median survival time was 8.4 months, and the 1-year survival rate was 40%. No grade 4 toxicity was observed and grade 3 toxicity was minimal [21, 22].

Two studies (one with gefitinib at a dose of 500 mg/day and one with erlotinib at a dose of 150 mg/day) were performed in the front-line or second-line therapy of patients with bronchoalveolar carcinoma [23, 24]. The response rates were 12%–19% for gefitinib and 26% for erlotinib.

Single-Agent Trials

Two large trials were initiated in second- and third-line NSCLC patients to investigate the survival benefit of gefitinib or erlotinib monotherapy compared with placebo. The Iressa® Survival Evaluation in Lung Cancer (ISEL) trial investigated gefitinib compared with placebo. That study included 1,692 patients who had progressed or could no longer tolerate chemotherapy. The results showed a statistically significant greater tumor shrinkage in the gefitinib arm, but the overall survival durations were similar in the two arms: 5.6 months in treated patients versus 5.1 months in patients receiving placebo [25]. Because of these results, the continued registration of gefitinib is under discussion.

The BR.21 trial investigated erlotinib compared with best supportive care with placebo. That trial included 731 patients who were randomized 2:1 to receive either erlotinib at a dose of 150 mg/day or placebo (488 erlotinib, 243 placebo) [26]. Study end points included overall survival,

response rate, and progression-free survival (PFS). The response rate was 9% after erlotinib treatment and <1% after receiving placebo. The results showed a significantly longer PFS time following treatment with erlotinib (median, 9.9 weeks versus 7.9 weeks for placebo). Furthermore, erlotinib produced a significantly longer overall survival time ($p = .001$) by 2 months compared with placebo (6.7 months versus 4.7 months). Based on that study, erlotinib received approval by the FDA. This was the first randomized trial to confirm that an EGFR tyrosine kinase inhibitor can prolong survival after first- or second-line chemotherapy. These results also formed the basis for the registration in Europe by the European Agency for the Evaluation of Medicinal Products (EMEA).

Pharmacodynamics

The pharmacodynamics of gefitinib were studied in two phase I trials in which the effects of gefitinib on EGFR tyrosine kinase activity in cancer patients were evaluated [27]. The pharmacodynamics of erlotinib were studied in the phase II trial in patients with advanced NSCLC [21, 22]. Skin expresses EGFR tyrosine kinase, and the results of the phase I and phase II trials showed that it can serve as a surrogate tissue for detecting EGFR tyrosine blockade. Cutaneous rash can be used as a surrogate marker of clinical benefit for both erlotinib and gefitinib [21, 22, 28]. Besides skin, it was shown that high levels of EGFR expression could also be detected in human hair follicles [29]. The use of hair is minimally invasive, in comparison with skin biopsies, and offers the possibility of being used as a surrogate tissue to quantitate the pharmacodynamics of EGFR inhibitors. The results of the phase I and phase II trials also showed that, besides EGFR, downstream molecules of the EGFR signaling pathway also can serve as markers. For example, after treatment with gefitinib, expression of mitogen-activated protein kinase (MAPK) was also reduced [30]. Recently, the erlotinib marker identification program was designed to identify and investigate predictive or surrogate markers other than rash. A large number of clinical samples will be analyzed from patients enrolled in the TALENT, TRIBUTE, and BR.21 trials. The identification of predictive or surrogate markers of response would permit selection of patients most likely to respond to such treatment. Markers could consist of tumor characteristics, such as those of the receptor or downstream signaling molecules and determinants of resistance [31]. Recently, it was shown that an increased EGFR gene copy number, based on fluorescence in situ hybridization analysis, could be used as a predictive marker for sensitivity to gefitinib and erlotinib [32]. Another study revealed that mutations in the downstream GTPase K-Ras were associated with a lack of sensitivity to gefitinib and erlotinib [33]. Tumors with K-*ras* exon 2 mutations were associated with response rates of 0% to both gefitinib and erlotinib. If confirmed in other studies, mutations in K-*ras* could be used as predictive markers of response to gefitinib and erlotinib.

Pharmacokinetics

The pharmacokinetics of gefitinib have been evaluated in several phase I trials [34, 35]. A summary of the gefitinib and erlotinib pharmacokinetics is shown in Table 1. In patients with advanced malignancies treated with gefitinib, the maximum concentration (C_{max}) was achieved within 3–7 hours of oral dosing and the elimination half-life ($t_{1/2}$) ranged from 24–58 hours. The cytochrome P450 enzyme, CYP3A4, is mainly responsible for the metabolism of gefitinib. Excretion was predominantly by feces (86%). The major metabolite is O-desmethyl gefitinib. This metabolite is 14-fold less potent than gefitinib in inhibiting the EGFR tyrosine kinase. The area under the concentration–time curve (AUC) showed interpatient variability and increased linearly with once-daily dosing ranging from 10–100 mg. The mean oral bioavailability was ± 60%. Bioavailability was not significantly altered by food. Gefitinib has a high affinity (90%) for binding to plasma proteins, mostly to albumin and α–1-acid glycoprotein.

The pharmacokinetics of erlotinib have been evaluated in several phase I trials [36, 37]. In patients with advanced malignancies, C_{max} was achieved within 2–4 hours of oral dosing, and the elimination $t_{1/2}$ ranged from 10–20 hours. The cytochrome P450 enzyme, CYP3A4, is mainly responsible for its metabolism. Excretion was predominantly by feces (68%). No significant relationships between clearance and patient age, body weight, or gender were observed. Smokers had a 24% higher rate of erlotinib clearance. It is not advised to escalate doses of erlotinib in smokers, but patients should stop smoking when they are treated with erlotinib. The major metabolite is O-desmethyl erlotinib. The AUC showed moderate interpatient variability and was roughly proportional to the erlotinib dose in the range of 25–200 mg/day [38]. The mean oral bioavailability following a 150-mg oral dose was ± 59%. Food increased the bioavailability substantially, to almost 100%. Erlotinib has a high affinity (95%) for binding to plasma proteins, mostly to albumin and α–1-acid glycoprotein.

For both gefitinib and erlotinib, clinically important drug–drug interactions have been described. Precaution should be taken if patients use CYP3A4 inducers or inhibitors. Substances that are inducers of CYP3A4 activity increase the metabolism of gefitinib and erlotinib and decrease its plasma concentration. For example, rifampin (Rifadin®; Aventis Pharmaceuticals Inc.), a CYP3A4

Table 1. Summary of gefitinib and erlotinib pharmacokinetics

	Gefitinib	Erlotinib
Administration	Oral	Oral
Bioavailability	60%	59%
Effect of food	Of no importance	Increases bioavailability to almost 100%
Binding to plasma proteins	90%, mostly to albumin and α-1-acid glycoprotein	95%, mostly to albumin and α-1-acid glycoprotein
Metabolizing enzyme	CYP3A4	CYP3A4
Major metabolite	O-desmethyl gefitinib	O-desmethyl erlotinib
Excretion	Feces (86%) and urine (<4%)	Feces (68%) and urine (13%)
Plasma pharmacokinetics	C_{max} 3–7 hours after dosing	C_{max} 2–4 hours after dosing
	$t_{1/2}$ 24–58 hours	$t_{1/2}$ 10–20 hours
	Mean AUC rose linearly with dose from 10–100 mg	Mean AUC was roughly proportional to the dose in the range of 25–200 mg/day
Effect of hepatic dysfunction	Patients with moderately and severely elevated biochemical liver abnormalities had gefitinib pharmacokinetics similar to those of individuals without liver abnormalities	In vitro and in vivo evidence suggests that erlotinib is cleared primarily by the liver; therefore, erlotinib exposure may be increased in patients with hepatic dysfunction
Effect of renal dysfunction	No clinical studies conducted, but a decrease in total body clearance is not expected in patients with renal insufficiency	No clinical studies conducted, but a decrease in total body clearance is not expected in patients with renal insufficiency

Abbreviations: AUC, area under the concentration–time curve; C_{max}, maximum concentration; CYP3A4, cytochrome P450 3A4 enzyme; $t_{1/2}$, half-life.

inducer, reduced the mean AUC of gefitinib by 85% and of erlotinib by 67% [37, 39]. Substances that are potent inhibitors of CYP3A4 activity decrease gefitinib and erlotinib metabolism and increase its plasma concentration. When itraconazole (Sporanox®; Janssen Pharmaceutica Products, L.P., Titusville, NJ, http://www.janssen.com), a CYP3A4 inhibitor, was concomitantly administered with gefitinib, the mean AUC of gefitinib was increased by 88% [39]. When ketoconazole (Nizoral®; Janssen Pharmaceutica Products, L.P.), a CYP3A4 inhibitor, was concomitantly administered with erlotinib, the mean AUC of erlotinib was increased by 67% [37]. Furthermore, it was shown that drugs that cause significant sustained elevation in gastric pH, like ranitidine (Zantac®; GlaxoSmithKline, Philadelphia, http://www.gsk.com) or cimetidine (Tagamet®; GlaxoSmithKline), may reduce plasma concentrations of gefitinib and therefore potentially may reduce its efficacy. The mechanism for this reduction is the poor solubility of gefitinib above a pH of 7 [39]. A summary of the drug–drug interactions is shown in Table 2.

Toxicity

Safety evaluation was primarily based on the results of trials in which patients received gefitinib or erlotinib monotherapy. A summary of the most common nonhematological toxicities by worst common toxicity criteria (CTC) grade reported at the recommended 250-mg daily dose of gefitinib is shown in Table 3. The most common nonhematological toxicities were diarrhea, rash, acne, dry skin, nausea, and vomiting [39]. Adverse events reported to a lesser extent were pruritus, anorexia, asthenia, weight loss, peripheral edema, amblyopia, dyspnea, conjunctivitis, vesiculobullous rash, and mouth ulceration [30, 39, 40]. The phase I studies showed a relationship between the AUC of gefitinib and the development of cutaneous rash and diarrhea. Patients who developed toxicity had higher AUC levels than patients who did not experience significant toxicity [39].

A summary of the most common nonhematological toxicities by worst CTC grade reported at the recommended 150-mg daily dose of erlotinib is shown in Table 3. The most common nonhematological toxicities were rash, diarrhea, anorexia, fatigue, dyspnea, nausea, and vomiting [37]. Adverse events reported to a lesser extent were cough, stomatitis, pruritus, dry skin, and conjunctivitis [37]. Liver function test abnormalities (elevated aspartate aminotransferase, alanine aminotransferase, and bilirubin) have been observed. These elevations were mainly transient. Again, a relationship was found between the C_{max} and AUC of erlotinib and the development of cutaneous rash. Patients who developed rashes had higher C_{max} and AUC values.

Cases of interstitial lung disease (ILD) have been observed in patients receiving gefitinib and erlotinib. The overall incidences in gefitinib- and erlotinib-treated patients from all studies were approximately 1% and 0.6%, respectively [37, 39]. In the event of acute onset or worsening of pulmonary symptoms (dyspnea, cough, fever), gefitinib

Table 2. Drug–drug interactions

Agent[a]	Effect	Mechanism
CYP3A4 inducers (e.g., phenytoin, carbamazepine, rifampin, barbiturates, St. John's Wort)	Decreases gefitinib/erlotinib plasma concentration and reduces efficacy	Enhances gefitinib/erlotinib CYP3A4 metabolism
CYP3A4 inhibitors (e.g., ketoconazole, erythromycin, clarithromycin, protease inhibitors, grapefruit juice)	Increases gefitinib/erlotinib plasma concentration and increases toxicity	Decreases gefitinib/erlotinib CYP3A4 metabolism
Proton pump inhibitors (e.g., omeprazole)	Reduces gefitinib absorption (not documented for erlotinib)	Sustained elevation of gastric pH
Histamine H_2-receptor antagonists (e.g., ranitidine, cimetidine, famotidine)	Reduces gefitinib absorption (not documented for erlotinib)	Sustained elevation of gastric pH

[a]Trade names and manufacturers' information are as follows: phenytoin (Dilantin®; Pfizer Pharmaceuticals, New York, http://www.pfizer.com), carbamazepine (Tegretol®; Novartis Pharmaceuticals Corporation, East Hanover, NJ, http://www.pharma.us.novartis.com), rifampicin (Rifadin®; Aventis Pharmaceuticals Inc., Bridgewater, NJ, http://www.aventispharma-us.com), ketoconazole (Nizoral®; Janssen Pharmaceutica Products), omeprazole (Prilosec®; AstraZeneca Pharmaceuticals, Wilmington, DE, http://www.astrazeneca-us.com), ranitidine (Zantac®; GlaxoSmithKline, Philadelphia, http://www.gsk.com), cimetidine (Tagamet®; GlaxoSmithKline), and famotidine (Pepcid®; Merck & Co., Inc., Whitehouse Station, NJ, http://www.merck.com).

Table 3. Drug-related nonhematological toxicities by worst common toxicity criteria (CTC) grade

Adverse event	CTC grade 1-2	CTC grade 3-4
Gefitinib, 250 mg/day, $n = 102$		
Diarrhea	47%	1%
Rash	43%	–
Acne	25%	–
Dry skin	13%	–
Nausea	12%	1%
Vomiting	11%	1%
Erlotinib, 150 mg/day, $n = 485$		
Rash	67%	8%
Diarrhea	48%	6%
Anorexia	43%	9%
Fatigue	34%	18%
Dyspnea	13%	28%
Nausea	30%	3%
Vomiting	21%	2%

Values are expressed as the percentage of patients experiencing toxicity.

and erlotinib therapy should be interrupted [37, 39]. ILD is primarily treated with corticosteroids, like dexamethasone (Decadron®; Merck & Co., Inc., Whitehouse Station, NJ, http://www.merck.com) [41]. Concomitant treatment with gefitinib or erlotinib and dexamethasone is not advised because dexamethasone is a potent CYP3A4 inducer [42]. Physicians may also decide to supplement patients with oxygen to prevent the complications of hypoxia [41].

No significant myelosuppression was observed in patients treated with gefitinib or erlotinib as monotherapy [35, 37].

EGFR Mutations and Sensitivity

In in vitro studies, it was shown that gefitinib and erlotinib target the EGFR tyrosine kinase and compete with ATP for binding to the cytoplasmic tail [43]. Recently, it was shown that mutations in the *EGFR* gene are significantly associated with response to gefitinib and erlotinib.

As of November 2004, 192 EGFR tyrosine kinase domain mutations have been reported [43–48]. One hundred sixty-five (85.9%) of these 192 mutations occur in two "hot spots": exons 19 and 21. Ninety-two (55.8%) of those 165 mutations are deletions that eliminate four highly conserved amino acids (LREA) encoded by exon 19. The other 73 (44.2%) of the 165 mutations are point mutations in exon 21 that result in a specific amino acid substitution at position 858 (L858R). The remaining 27 (14.1%) of the 192 mutations include (a) a deletion downstream of the LREA amino acids encoded by exon 19, (b) mutations scattered throughout exons 18 through 21, and (c) duplications and insertions in exon 20 [49]. It is important to note, however, that among all the various mutations found in the EGFR tyrosine kinase domain, only the following have actually been associated with response to gefitinib or erlotinib: G719C (exon 18), some of the common exon 19 deletions (LREA), L861Q (exon 21), and L858R (exon 21) [49]. In addition to these mutations, another mutation was found that was associated with erlotinib sensitivity. Analysis revealed an amino acid substitution at position 776 (R776C) in exon 20. This mutation was found in an erlotinib-sensitive tumor that also harbored an L858R mutation [46]. The significance of the R776C mutation alone was not reported. All the previously described mutations result in conformational changes that lead to increased sensitivity for tyrosine kinase inhibitors. Several studies showed that higher rates of these mutations were found in

females, patients with adenocarcinomas, the Japanese population, and never-smokers [50, 51]. A summary is shown in Table 4. These results indicate that screening of patients for EGFR tyrosine kinase domain mutations before treatment with gefitinib or erlotinib can partly predict the clinical benefit of the treatment.

Mechanisms of Resistance

Tumor cells can develop several mechanisms that may result in resistance to gefitinib and erlotinib. Besides mutations in the *EGFR* gene that make the target sensitive to gefitinib and erlotinib treatment, it has been shown that other mutations in the *EGFR* gene are associated with resistance to gefitinib and erlotinib [52]. Patients who relapse after an initial response frequently have mutations in the *EGFR* gene. The mutation can take place in the ATP-binding pocket, with the result that the target becomes insensitive to gefitinib or erlotinib. Recently, it was shown that patients who relapsed after initially responding had secondary mutations in exon 20, in addition to primary drug-sensitive mutations in EGFR [53, 54]. The secondary mutation was a substitution of methionine for threonine at position 790 (T790M) in the kinase domain. Biochemical analyses of transfected cells and growth inhibition studies with lung cancer cell lines demonstrate that the T790M mutation confers resistance to EGFR mutants usually sensitive to either gefitinib or erlotinib. The T790M mutation leads to steric hindrance of erlotinib and gefitinib binding in the ATP-binding pocket [53].

Mechanisms like drug efflux and protein binding could also prevent gefitinib and erlotinib from binding to its target.

Studies have revealed that additional oncogenic changes downstream of the EGFR (e.g., changes in the phosphatidylinositol 3′ kinase–Akt pathway or K-ras) could also result in resistance to gefitinib and erlotinib [33, 55, 56]. When Akt is phosphorylated, it inactivates proapoptotic and cell cycle regulatory molecules, thus enhancing tumor cell survival and proliferation. When EGFR is inhibited by gefitinib or erlotinib, Akt can still be active due to Akt gene amplification and overexpression, as well as loss of the PTEN phosphatase that can turn off Akt activity [57]. In this case, combined blockade of the EGFR tyrosine kinase and Akt should be considered as a therapeutic approach.

Table 4. Epidemiology of response (gefitinib and erlotinib)

Nonsmokers	27%	Smokers	5%
Females	15%	Males	5%
Adenocarcinoma	33%	Not adenocarcinoma	8%
Japanese	27%	White	11%

Recently, it was shown that mutations in the downstream K-ras were also associated with primary resistance to gefitinib and erlotinib [33, 56]. That study suggested that treatment decisions regarding the use of gefitinib or erlotinib might be improved by determining the mutational status of both EGFR and K-ras.

Future Directions

The development of gefitinib and erlotinib has led to more options in the treatment of patients with advanced NSCLC. Gefitinib was the first selective EGFR inhibitor and received accelerated approval by the FDA based not on survival advantage but on preliminary data from the phase II (IDEAL) trials [19, 20]. The ISEL trial, which randomly assigned either gefitinib or placebo to patients with advanced NSCLC, showed that there was no survival advantage for gefitinib monotherapy [25]. This is in contrast with the erlotinib trial (BR.21) that showed improvement in overall survival by 2 months over placebo [26]. The different results of the gefitinib and erlotinib trials surprised many researchers, and the question is why gefitinib failed to show any survival advantage. A possible explanation could be that gefitinib and erlotinib do not have the same mechanisms of action. Recently published results [46, 49] have shown that some mutations in the EGFR tyrosine kinase domain are associated with response to gefitinib and erlotinib. Although these mutations overlap between gefitinib and erlotinib, it is not known if both drugs are equally active for every mutation. It could be that gefitinib has a lower affinity for certain EGFR mutations than erlotinib. Another explanation could be that the gefitinib trials were performed with a dose less than the maximum-tolerated dose (MTD) [25]. The phase I trials of gefitinib and erlotinib have resulted in the selection of continuous, fixed daily oral administration of 250 mg gefitinib and 150 mg erlotinib as the recommended doses. In the case of gefitinib, a dose of 250 mg is less toxic and as effective as a dose of 500 mg, as shown in the IDEAL trials [19, 20]. Therefore, 250 mg is close to an optimal biological dose. In the case of erlotinib, the dose of 150 mg meets the classic definition of the MTD [38]. To achieve maximum EGFR tyrosine kinase inhibition and inhibition of downstream signaling pathways, tyrosine kinase inhibitors should be administered at the highest dose possible. At the 150-mg dose, erlotinib results in an AUC of 38.42 μg*h/ml [38]. Similar exposure (AUC 36.08 μg*h/ml) is achieved with gefitinib at 700 mg/day, the approximate MTD [58]. The combination of the higher AUC/mg erlotinib dose and the greater affinity of erlotinib for EGFR (IC_{50}, 2 nM compared with 5 nM for gefitinib) [49] gives a significant advantage to erlotinib over gefitinib. For orally administered agents, many

factors influence the amount of active drug that eventually reaches the receptor. In particular, interpatient differences can have a marked effect on the absorption and metabolism of gefitinib and erlotinib. The actual dose that reaches the EGFR receptor is different for each patient. This may not be important if these agents exert their therapeutic effect more as antihormonal than cytotoxic agents, but this must be investigated further [59].

Both gefitinib and erlotinib showed no benefit in the combination trials (gefitinib, INTACT-1 and -2; erlotinib, TALENT and TRIBUTE) [13, 14, 15, 16]. The rationale for these studies was based on preclinical in vivo evidence of synergism between cytotoxic agents and gefitinib or erlotinib in human xenografts with high levels of EGFR expression. A reason for the failure of the combination trials could be the use of a combination schedule significantly different from that in the preclinical studies, which could be antagonistic. In the preclinical studies, animals did not receive gefitinib or erlotinib for 48 hours (weekend) before the weekly administration of the cytotoxic agents, thus probably releasing the tumor cells from G_1–S arrest and sensitizing them to the effects of the cytotoxic agents. In the clinical studies, gefitinib and erlotinib were given continuously without interruption [59].

With all the current information known about gefitinib and erlotinib, the question is: What are the future directions? The development of both gefitinib and erlotinib has resulted in the unexpected insight that EGFR mutations are found in a substantial number of patients with NSCLC, particularly in never-smokers with adenocarcinomas [50]. These discoveries promise to alter the approach toward NSCLC treatment in many ways.

It is essential to incorporate EGFR mutational profiling into future clinical trials, particularly to determine if a patient will derive clinical benefit from treatment with gefitinib or erlotinib, or other EGFR inhibitors [59]. A recent study has shown that HKI-272 is highly effective in tumors with the T790M mutation that confers resistance to gefitinib and erlotinib [60]. Approved tests for detecting EGFR mutations have been established at many academic medical centers and may soon become widely available, for example, sensitive polymerase chain reaction assays that are able to detect the common mutations in exons 19 and 21. Whether other genes, like K-*ras* and *akt*, should concurrently be profiled is currently an active area of research [33]. The ISEL trial, which resulted in no survival benefit for gefitinib versus placebo, could have been biased because of the lack of mutational screening before treatment. It could be that too few patients with an EGFR mutation were enrolled, compared with the erlotinib trial (BR.21), thus diluting an overall beneficial effect.

Furthermore, it is important to initiate clinical trials with EGFR tyrosine kinase inhibitors in different regions of the world. Studies may show different results because of geographic differences in the incidences of mutations in EGFR and, potentially, other genes. In particular, response rates to certain drugs may be lower or higher in certain areas (East Asia versus North America/Europe) [59].

Currently, gefitinib and erlotinib are used for the treatment of advanced NSCLC after failure of at least one prior platinum treatment. Given the effectiveness of erlotinib against both squamous carcinomas and adenocarcinomas, the administration of erlotinib should be considered as the first option for second-line treatment, rather than conventional chemotherapy like gemcitabine, pemetrexed (Alimta®; Eli Lilly and Company), or docetaxel. Besides NSCLC, studies have shown that tumors of the upper aerodigestive tract, lung, colon, pancreas, breast, ovary, bladder, and kidney, as well as gliomas, also display EGFR overexpression. Mutational screening of these tumors could provide more information for the further development of gefitinib and erlotinib as monotherapy or in combination. Encouraging results have already been shown when gefitinib was combined with FOLFOX4 for the treatment of colorectal cancer [61] or with celecoxib (Celebrex®; Pfizer Pharmaceuticals, New York, http://www.pfizer.com) for the treatment of squamous cell carcinoma of the head and neck (SCCHN) [62]. Erlotinib has also shown positive results when combined with capecitabine (Xeloda®; Hoffmann-La Roche Inc., Nutley, NJ, http://www.rocheusa.com) and oxaliplatin (Eloxatin®; Sanofi-Synthelabo Inc., New York, http://www.sanofi-synthelabo.us) for the treatment of patients with colorectal cancer [63] and with bevacizumab (Avastin®; Genentech, Inc., South San Francisco, CA, http://www.gene.com) for the treatment of SCCHN [64]. Recently, encouraging results were found with gefitinib and erlotinib in combination with radiotherapy [65, 66]. Furthermore, investigation is ongoing to find the optimal paradigms for various subgroups of patients with NSCLC. EGFR inhibitors could be effective as first-line treatment in patients with early-stage disease and sensitizing EGFR mutations, and also as neoadjuvant or adjuvant treatment, but this needs further investigation [49].

In conclusion, EGFR tyrosine kinase inhibitors are promising anticancer drugs for the future, but further research is warranted to select the patient group that will benefit optimally from treatment. The ISEL trial has taught investigators that the development process for targeted drugs should be strengthened. It is important to fully understand the target pharmacodynamics before the drug is developed clinically. This will help to select the most successful development strategies in the interest of patients with cancer.

Addendum

During the publication process of our manuscript, "Current Knowledge and Future Directions of the Selective Epidermal Growth Factor Receptor Inhibitors Erlotinib (Tarceva®) and Gefitinib (Iressa®)," new information became available about these two drugs. This field is developing rapidly, and therefore this commentary contains the recent available information about these drugs and drug applications.

In the August issue of ***The Oncologist***, two papers were published about the FDA drug approval of erlotinib [67] and the current situation of the application of erlotinib and gefitinib in non-small cell lung cancer (NSCLC) [68]. The first paper outlines in detail the subset analyses of the BR.21 trial. This trial compared erlotinib treatment (150 mg/day) with placebo in a 2:1 randomized scheme. A total of 488 patients were treated in the erlotinib arm and 243 patients in the placebo arm. Epidermal growth factor receptor (EGFR) status was determined for 238 of the 731 study patients (33%) of whom tissue samples were available prior to the study. There were 127 EGFR-positive patients (78 treated with erlotinib, 49 receiving placebo). A positive EGFR expression status was defined as having at least 10% of cells staining for EGFR using the DAKO EGFR pharmDx™ kit (DakoCytomation, Glostrup, Denmark, http://www.dakocytomation.com).

An additional objective of this trial was to correlate EGFR levels with treatment outcome. The subset analyses revealed that erlotinib resulted in a survival benefit in patients who were EGFR-positive and who never smoked. These analyses were based on relatively small numbers of patients, the confidence intervals were overlapping between EGFR-positive and EGFR-negative groups and smokers and never-smokers. These results should therefore be interpreted with caution. However, a survival benefit was only statistically significant compared with placebo in the EGFR-positive subgroup.

The second paper published in ***The Oncologist*** outlines the current situation for gefitinib and erlotinib [68]. The negative results of the gefitinib ISEL trial and the current information about erlotinib resulted in a New Labeling and Distribution Program for gefitinib by the FDA on June 17, 2005. This program limits the administration of gefitinib to patients in the following circumstances: patients currently receiving and benefiting from the drug, patients who have previously received and benefited from gefitinib, and previously enrolled patients or new patients in non-Investigational New Drug clinical trials approved by an Investigational Review Board prior to June 17, 2005 (http://www.fda.gov/cder/drug/advisory/iressa.htm). Erlotinib remains the only EGFR inhibitor approved by the FDA for the treatment of patients with locally advanced or metastatic NSCLC after failure of at least one prior chemotherapy regimen. On June 23, 2005, the Committee for Medicinal Products for Human Use of the European Medicines Evaluation Agency (http://www.emea.eu.int/pdfs/human/opinion/13384605en.pdf) adopted a positive opinion, recommending to grant a marketing authorization for 25-, 100-, and 150-mg erlotinib tablets intended for treatment of patients with locally advanced or metastatic NSCLC after failure of at least one prior chemotherapy regimen [69]. The label contains a statement that factors associated with prolonged survival should be taken into account when prescribing erlotinib. The screening of patients for EGFR status is strongly advised, and the benefit of treatment with erlotinib appears to be limited to high (>10%) expression of EGFR. Detailed conditions for the use of erlotinib in Europe will be described in the Summary of Product Characteristics, which will be published in the European Public Assessment Report that will become available after marketing authorization has been granted by the European Commission, which is expected in September or October of 2005. Prospective studies should, however, be performed to further explore the relationship between EGFR expression and treatment outcome of erlotinib in NSCLC. In addition, the possible implications of activating mutations in EGFR and treatment benefit of erlotinib should be determined prospectively in larger populations. A recent study published in August in Clinical Cancer Research reveals a response benefit of gefitinib in patients harboring EGFR mutations, never-smokers, Asians, younger patients and patients receiving a greater number of prior chemotherapy regimens [70].

The conclusion of this study was that the presence of EGFR mutations is a major determinant of gefitinib response, and targeting EGFR should be considered in preference to chemotherapy as first-line treatment in lung adenocarcinomas that have demonstrable EGFR mutations. These results indicate that, besides EGFR expression, it is essential to incorporate EGFR mutational profiling in future studies with erlotinib, and results of those studies may have important consequences for the selection of patients who will benefit most of this therapy.

Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflicts of interest.

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