Review of erlotinib in the treatment of advanced non-small cell lung cancer

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Abstract: Epidermal growth factor receptor (EGFR) is a transmembrane receptor with a cytoplasmic tyrosine kinase (TK) domain present on many solid tumors including non-small cell lung cancer (NSCLC). Once stimulated by ligand, the downstream pathway is activated leading to cell growth, survival, and carcinogenesis. There are several methods of EGFR inhibition including monoclonal antibodies directed against the external region and small molecule inhibitors of TK domain. Erlotinib and gefitinib are orally available small molecule EGFR TK inhibitors, with proven efficacy in NSCLC. The most common side effects are skin toxicity and diarrhea. Erlotinib has been shown to improve survival compared to placebo in second or third-line therapy for NSCLC. However, erlotinib in combination with chemotherapy failed to show a survival advantage in two first-line studies which could be due to the timing of chemotherapy administration. In general, patients with adenocarcinoma histology, female gender, Asian ethnicity, and never smokers have a better response when treated with erlotinib. This could be related to the presence of EGFR mutations, lack of KRAS mutations, or overexpression of EGFR as measured by fluorescent in-situ hybridization (FISH) analysis. Future studies should concentrate on further development of predictors of clinical benefit with erlotinib, overcoming resistance to erlotinib that develops in initial responders, as well as more effective sequencing of erlotinib with chemotherapy and combinations of the drug with other "targeted" therapeutic agents.
Keywords: epidermal growth factor receptor, erlotinib, non-small cell lung cancer

Epidermal growth factor receptor

Epidermal growth factor receptor (EGFR) belongs to a family of four receptors: ErbB-1 (EGFR), ErbB-2 (HER2/neu), ErbB-3 (HER3), and ErbB-4 (HER4) responsible for cell survival (Ciardiello and Tortora 2001). EGFR is a transmembrane receptor with an internal tyrosine kinase (TK) domain which is phosphorylated after the binding of the ligand to the receptor. The activation of this domain will then stimulate several internal signaling pathways which in turn affects cell proliferation, differentiation and survival (Herbst 2004). There is evidence to suggest that this process can promote cancer development and metastasis (Engebraaten et al 1993; Chan et al 1999).

There are several methods of inhibiting the EGFR pathway including monoclonal EGFR antibodies and small molecule inhibitors of TK. Cetuximab (Erbitux®; Imclone Systems Inc., Branchburg, NJ, USA) is a chimeric human/mouse monoclonal antibody directed against the extracellular domain of the EGFR and is approved for use in colorectal and head and neck cancer (Cunningham et al 2004; Saltz et al 2004; Bonner et al 2004). Cetuximab competitively blocks the binding of the EGF and other ligands to the EGFR thus preventing the activation of the downstream TK resulting in growth arrest and apoptosis (Gill et al 1984; Sato et al 1983; Baselga 2000). Another EGFR antibody is panitumomab (Vectibix®; Amgen, Thousand Oaks, CA, USA) which is fully humanized and approved for metastatic colorectal cancer. The use of EGFR targeted antibodies is under active investigation in lung cancer, but without proven efficacy at this time.

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A different method of blocking EGFR is by inhibiting the cytoplasmic TK domain. Gefitinib (Iressa®; AstraZenica Pharmaceuticals, Wilmington, DE, USA) and erlotinib (Tarceva®; Genentech, So San Francisco, USA) are both orally available small molecule EGFR TK inhibitors. Gefitinib was initially approved in the United States based on encouraging response rate and survival in phase II studies (Fukuoka et al 2003; Kris et al 2003), but was subsequently pulled from the North American market when a randomized phase III trial (ISEL) failed to show a survival benefit versus placebo (Thatcher et al 2005). Erlotinib, however, is currently approved for use as second-line or third-line therapy in patients with non-small cell lung cancer (NSCLC) based on the landmark BR.21 trial which showed a statistically significant survival advantage for the drug versus placebo (Shepherd 2005), as well as in combination with gemcitabine in locally advanced or metastatic pancreatic cancer (Moore et al 2007).

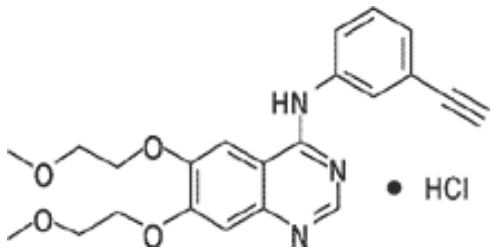

Figure 1a Structure of erlotinib.

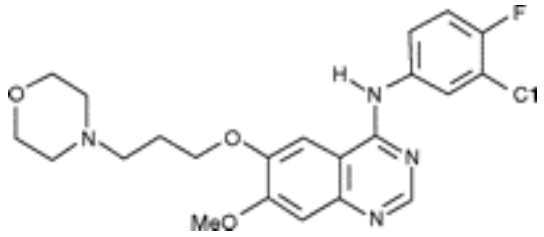

Figure 1b Structure of gefitinib.

Erlotinib phase I trials

The initial phase I trial of erlotinib in solid tumors evaluated different doses (25, 50, 100, 150, 200 mg) and schedules (d1-3 weekly for 3 weeks every 28 days; daily for 3 weeks every 28 days; daily-uninterrupted) and found a maximum tolerated dose (MTD) of 150 mg per day (Hidalgo et al 2001). The most common toxicities were diarrhea (25%–67% depending on dose) and rash (59%). The diarrhea was mostly grade 1 and 2 and improved with anti-diarrhea agents. The cutaneous toxicities were mostly on the face and upper trunk and of a pustular acneiform type. The rash appeared 1–2 weeks post initiation of therapy and subsided by week 4 without interruption of the erlotinib. The most common skin biopsy finding was a neutriphilic infiltration of the dermal layer. Patients with skin manifestations had a higher area under the curve (AUC) concentration of erlotinib compared to those without skin changes. Higher AUC levels did not correlate with diarrhea though. The pharmacokinetics of erlotinib was not dose dependent and there was no drug accumulation with the continuous daily dosing.

The weekly regimen was explored further in patients with advanced stage NSCLC with dose escalation of 1200 mg, 1600 mg, and 2000 mg, but was discontinued due to a low response rate (5%) (Milton et al 2006).

Erlotinib as second or third-line therapy in NSCLC

Based on promising results in the phase I studies, as well as early encouraging phase II results with the related agent gefitinib (IDEAL 1 and 2) further development proceeded in NSCLC (Fukuoka et al 2003; Kris et al 2003). Fifty-seven patients with refractory or relapsed stage II or IV NSCLC were treated with erlotinib in a phase II single-agent study (Perez-Solar et al 2004). Only patients with positive EGFR-expression on immunohistochemistry (IHC) were included and an overall response rate of 12.3% was reported. Interestingly, all the patients who responded developed a rash, as did 95% of those with stable disease, compared to only 54% of those with disease progression. Patients developing a rash also had a longer median survival (no rash: 1.5 months; grade 1:8.5 months; grade 2, 3:19.6 months). Rash was the most significant predictor of survival in multivariate analysis. The intensity of EGFR staining, however, did not affect response rate or survival.

These encouraging results led to a randomized double-blind placebo controlled trial (BR.21) in patients with previously treated NSCLC (Shepherd et al 2005). Patients with one or two prior chemotherapy regimens were included. The primary endpoint was overall survival (OS) and the secondary endpoints included progress-free survival (PFS), response rate, response duration, toxicity, and quality of life (QoL) which will be discussed later in this review. Interestingly, patients with ECOG performance status of 3 were also allowed to participate in this study, a population usually excluded from chemotherapy trials. A 2:1 randomization was done with erlotinib 150 mg daily versus placebo.

The response rate in BR.21 trial with erlotinib was 8.9% with a median response duration of 7.9 months. Similar

response rates have been reported for the chemotherapy drugs approved in this setting, docetaxel and pemetrexed (Shepherd et al 2000; Fossella et al 2000; Hanna et al 2004). In the BR.21 study, the response rates were even higher in the following patients: women (14.4%), never-smokers (24.7%), those with Asian ethnicity (18.9%), and those with adenocarcinoma histology (13.9%). The number of prior regimens, age or performance status did not affect response rates. Tumors with >10% EGFR positivity by IHC and/or activating mutations within the EGFR (discussed below) also had a higher response rate. The correlative studies performed in the BR.21 study will be discussed later in this review.

As expected, the most common toxicities seen in this trial were diarrhea and rash as was seen in the previous studies. Dose reductions were carried out in 12% of patients due to rash and 5% for diarrhea. Erlotinib was discontinued only in 5% of patients due to toxicity. In contrast to most cytotoxic agents, grade 3 or 4 neutropenia was not reported with erlotinib. In the BR.21 study, pulmonary infiltrates and pneumonitis (3%) were seen equally in the erlotinib versus placebo arm. One patient died on each arm due to pneumonitis which was most likely related to the underlying lung cancer and not the drug. This is reassuring, but pneumonitis is a known toxicity with this class of agents and caution should still be exercised with this drug in patients with underlying pulmonary fibrosis.

Additional toxicity data have been presented on 4423 patients from Europe who enrolled on an expanded access open label trial of erlotinib (TRUST). Rash and diarrhea remain the predominant toxicities with a rate of serious treatment related adverse events of only 5%. Rash was seen in 70% of patients, by 84% of the time it was grade 1 or 2. Only 14% of patients required dose reductions (Gatzemeier et al 2007a).

The median PFS with erlotinib versus placebo was 2.2 months and 1.8 months respectively (adjusted hazard ratio, 0.61; 95 percent confidence interval, 0.51–0.74; $p < 0.001$). In multivariate analysis, survival was improved with statistical significance in patients with adenocarcinoma histology, never smokers or Asian ethnicity. However, sex, age, and performance status did not affect survival. Overall, the median OS was 6.7 months in the erlotinib treated patients versus 4.7 months in the placebo arm. Similar median OS have been reported with second-line docetaxel and pemetrexed (8.3 months, 7.9 months) (Hanna et al 2004). Currently, erlotinib is the only drug approved for third-line therapy in NSCLC in the United States and is one of only three drugs approved for second line therapy. The agent is also approved for use in Europe and Canada based on the encouraging results of this trial.

Erlotinib as first-line therapy in NSCLC

For many years the standard of care for first line treatment of NSCLC has been chemotherapy doublets. Multiple trials have looked for ways to improve the 8–10 month median survival usually seen. These studies included different chemotherapy combinations of two and three drugs and the additional of "targeted" agents to chemotherapy combinations. As epitomized in the ECOG 1594 trial of 4 different platinum doublets which all had the same response rate and overall survival, a plateau in chemotherapy efficacy with doublet regimens has been reached (Schiller et al 2002). Additionally no triplet regimens have been shown to be superior in terms of survival (Delbaldo et al 2004). Trials with targeted agents have also failed to show a benefit when added to doublet chemotherapy (Herbst et al 2005a; Giaconne et al 2004). The exception to this is ECOG E4599 which compared chemotherapy alone with chemotherapy plus bevacizumab (Avastin®; Genentech, So San Francisco, CA, USA) (Sandler et al 2006a). Patients received carboplatin plus paclitaxel with or without the monoclonal anti-VEGF antibody. The response rates and the median OS were improved in the bevacizumab arm. This study resulted in the approval of bevacizumab in combination with chemotherapy for first-line therapy in patients with advanced NSCLC in the United States. However, 15 treatment related deaths were reported in the bevacizumab arm including 5 patients with pulmonary hemorrhage. A confirmatory trial in Europe (AVAiL) has been reported to show an improvement in PFS with the addition of bevacizumab to cisplatin and gemcitabine, but the overall survival data have not yet been presented (Manegold et al 2007). Toxicity was less severe than what was seen in E4599, though bleeding remains a concern. Bevacizumab plus a platinum doublet is still the only triplet regimen shown to improve survival in the first-line treatment of advanced NSCLC.

As with other targeted agents, when erlotinib was added to first-line chemotherapy, the results were disappointing. Despite encouraging preclinical studies with the combination of erlotinib and chemotherapy (Gumerlock et al 2003), two large randomized trials of erlotinib plus first-line doublet chemotherapy in advanced stage NSCLC showed no advantage with the addition of the erlotinib. The TRIBUTE trial was conducted in treatment naïve patients with advanced or metastatic NSCLC who were treated with chemotherapy plus erlotinib versus placebo (Herbst et al 2005a). Patients

received paclitaxel 200 mg/m^2 followed by carboplatin (AUC 6) every 21 days for 6 cycles plus erlotinib at 150 mg/day versus placebo. With more than a thousand patients treated there was no difference in median survival, time to progression, or objective response rates. In addition, there was no correlation between EGFR expression level and outcome. However, the response rates were higher in never smokers treated on the erlotinib arm (30% vs 11%). These patients also had an improved survival when treated with chemotherapy and erlotinib (22.5 months) versus placebo (10.1 months) which was independent of tumor histology.

Another placebo controlled randomized study (TALENT) was reported in treatment naïve unresectable stage III or IV NSCLC (Gatzemeier et al 2007b). In this study, patients received 6 cycles of cisplatin (80 mg/m^2 d1) and gemcitabine (1250 mg/m^2 d1, 8) plus erlotinib (150 mg daily) or placebo with responding patients continuing on study drug until progression. The pharmacokinetics of gemcitabine or cisplatin was not altered by erlotinib. Again, there was no difference in response rates, overall survival, time to progression, and time to symptom progression with 1172 patients enrolled. The exception to this was a survival benefit seen in a subset analysis never smokers. Based on these two randomized trials, erlotinib has no role in first-line therapy when given concurrently with chemotherapy in an unselected patient population.

Various explanations have been proposed for the negative results reported in the TALENT and TRIBUTE trials. Gumerlock argues that since erlotinib results in G1 arrest, the efficacy of chemotherapy is affected due to its reliance on mitosis (Gumerlock et al 2003). To get around this effect investigators have looked at sequencing erlotinib and chemotherapy so that cells are released from G1 arrest in time for chemotherapy to be efficacious. A phase I study evaluating the sequential administration of docetaxel and erlotinib has been completed (Davies et al 2005). There were two arms on this study with arm A receiving docetaxel (70–75 mg/m^2) every 21 days followed by erlotinib (600–800 mg) weekly on days 2, 9, 16. The MTD was docetaxel 70 mg/m^2 and erlotinib 600 mg. In arm B, patients were treated with docetaxel (70–75 mg/m^2) every 21 days and erlotinib (150–300 mg) daily on days 2–16. The MTD in this arm was docetaxel 70 mg/m^2 and erlotinib 200 mg. Responses were seen in 8 of 22 NSCLC patients (4 PR, 4 MR). Phase II studies are ongoing according to arm B. At an updated presentation in 2007 the response rate with this approach was 38% (2 CR and 12 PR of 39 patients) with a time to progression of 5.6 months and median survival not yet reached (Davies et al 2007). A similar trial design with pemetrexed is ongoing. With these encouraging results, the role of erlotinib in combination with chemotherapy for first-line therapy may be re-addressed utilizing this pulse sequencing approach.

A randomized phase II trial presented at the ASCO meeting this year evaluated different strategies of pulse dose erlotinib (either 150 mg or 1500 mg) given for just 2 days prior to chemotherapy or at the higher dose for 2 days after chemotherapy (Riely et al 2007). This approach was no better than the TRIBUTE trial with response rates ranging from 18% to 35% and median overall survival of 15 months.

Erlotinib in combination with first-line chemotherapy is also under investigation in select populations such as never-smokers. The Cancer and Leukemia Group B (CALGB) trial 30406 is an ongoing randomized phase II study evaluating erlotinib alone or in combination with carboplatin/paclitaxel as first-line therapy for light and never smokers. ECOG is also considering a trial in this population which would consist of administering carboplatin and paclitaxel with or without erlotinib (and including bevacizumab for patients eligible for bevacizumab). Both of these trials also include extensive correlatives evaluating EGFR IHC, EGFR mutation status, EGFR expression by FISH, KRAS mutation status, and also proteomic analysis.

Currently, the combination of erlotinib plus first-line chemotherapy should be considered experimental and focused on determining patient selection criteria or improved sequencing. Unfortunately, like the studies of erlotinib plus chemotherapy in non-selected populations, studies of single agent erlotinib first-line have been disappointing to date. The recently completed ECOG 3503 trial of first-line erlotinib sought to gather additional data for patient selection (Kolesar et al 2007). This study enrolled 118 eligible patients to receive first-line therapy with erlotinib for advanced stage NSCLC. Patients were started at 150 mg daily and gradually escalated every 2 weeks to a maximum dose of 250 mg daily as tolerated with rash as the primary criteria. The response rate was 7% with a median survival of 7.9 months. Rash did correlate with improved survival, but not statistically.

A phase II study evaluating the role of single agent erlotinib in first-line therapy for patients with stage IIIB/IV NSCLC enrolled 53 patients and reported an ORR of 22.7% and a disease control rate of 52.8% (Giaccone et al 2006). The median duration of response for those responding was 11 months. Never smokers responded better with a 1 year OS of 54% and a median survival of 13 months. The median overall survival for all patients on the trial was 13 months. As stated previously, the median OS in E 4599 was 12.3 months

with the chemotherapy and bevacizumab combination. In the single agent erlotinib study, the following factors correlated with a better median survival: grade 2/3 skin toxicity (19.7 months vs 2.7 months in grade 0), bronchioloalveolar carcinoma (BAC) or adenocarcinoma, age <70 years, positive response to erlotinib, EGFR mutation (20 mo vs 12.6 mo in wild type), and KRAS wild type (20 mo vs 5.7 mo in mutants). Higher responses were seen in adenocarcinoma and BAC histologies and never smokers. These results were replicated by a second trial reported by Jackman.

The Jackman trial focused on elderly patients and 80 patients >70 years of age with advanced or metastatic NSCLC were treated with first-line daily erlotinib 150 mg (Jackman et al 2007) until disease progression. Toxicities were mainly grade 1 or 2 rash and diarrhea. Twelve patients (15%) were removed from study secondary to side effects including one toxic death. The disease control rate was 51% including 10% PR. The median survival of all patients was 10.9 months with a 2 year OS of 19%. Interestingly, all patients with EGFR mutation had disease control with prolonged time to progression and OS. EGFR mutation was more common in patients who had a <15 pack year history of smoking. Six patients with KRAS mutation did not respond and had a poor outcome. No patient had both KRAS and EGFR mutation. The other factor correlating with response and better survival was the development of an erlotinib-related rash.

More recently, a randomized phase II trial has been reported comparing first-line single agent erlotinib (n = 52) with carboplatin/paclitaxel (n = 51) chemotherapy in patients with advanced stage NSCLC with ECOG performance status of 2 (PS 2) (Lilenbaum et al 2006). The response rates with erlotinib versus chemotherapy were 2% and 12% respectively. The median OS was better with chemotherapy as was improvement in chest pain. However, there was no significant difference in the other QoL parameters between the two groups. Interestingly, none of 9 patients in the erlotinib arm had an EGFR mutation, whereas, 3 of 11 had the KRAS mutation. The number of patients tested is too small for any meaningful conclusions about the relationship of these mutations with the outcome of this study. Overall, this randomized phase II study suggests that chemotherapy can be given safely to patients with PS of 2 and that the response rates are better than single agent erlotinib.

The data with first-line single agent erlotinib are limited, and it should not be considered a standard approach. Ongoing trials in selected populations either felt to have a higher probability of responding, or at increased risk for toxicity are clearly warranted. There are multiple phase II studies ongoing and few reported in patients selected on clinical criteria (never-smokers) and or molecular criteria (EGFR mutations, or EGFR over-expression) (Inoue et al 2006; Paz-Ares et al 2006; Sequist et al 2007). Additionally, a phase III European trial of over 300 patients randomizes female never-smokers to either chemotherapy or erlotinib as a single agent as first-line therapy.

Moving erlotinib into other stages of disease

This review focuses on erlotinib in advanced stage NSCLC, but the drug is also being investigated in earlier stages of disease. An ongoing adjuvant trial, RADIANT, is open world-wide to patients with stage I-IIIA resected NSCLC who have evidence of EGFR over-expression by either IHC or FISH. Eligible patients will be randomized to either 2 years of erlotinib or observation, after completion of adjuvant chemotherapy at the discretion of the treating physician. Neo-adjuvant therapy trials with erlotinib have also been done, primarily evaluating molecular changes in the tumor that predict for response.

Further exploration of the drug in locally advanced disease is proceeding with caution given the surprising and disappointing results of SWOG 0023 with gefitinib. SWOG 0023 enrolled patients with stage IIIB NSCLC and treated them with chemotherapy and radiation, followed by consolidation chemotherapy, followed by a randomization to gefitinib or placebo (Kelly et al 2007). The surprising results showed reduced survival with the addition of the gefitinib, primarily due to excess cancer death in that arm. These results have clearly reduced enthusiasm for further study of EGFR-TKIs after completion of chemotherapy and radiation for locally advanced disease. They speak to caution in the adjuvant setting, but the RADIANT trial remains an important study.

Quality of life

One of the major advantages of erlotinib over chemotherapy is quality of life (QoL). Improvement in QoL was evaluated in the BR.21 trial (Shepherd et al 2005) by using the European organization for research and treatment of cancer (EORTC) QLQ-C30 QoL questionnaire and the QLQ-LC13 lung module (Bezjak et al 2006). The endpoint of QoL analysis was to determine the time to worsening of cough, dyspnea, and pain. Patients who were receiving erlotinib had a longer time to deterioration of symptoms and a 34%–44% improvement in lung cancer associated symptoms. The QoL

improved by 9% ($p < 0.0001$) in patients who were receiving erlotinib.

QoL was also measured in the initial phase II trial with the drug and the incidence of lung cancer symptoms including fatigue, cough, and dyspnea decreased after erlotinib was initiated in the 57 patients on the study (Perez-Soler et al 2004).

Predictors of response to erlotinib

We are moving into an era of "personalized medicine" with the hope that we may be able to predict ahead of time which agents will be best for each individual patient. Though progress is being made with traditional chemotherapeutic agents, we are much closer to this reality with the newer targeted agents, particularly erlotinib. Detection of EGFR expression and specific mutations within the gene allow for selection of patients most likely to benefit from the drug.

EGFR expression

EGFR expression has been detected in the bronchial epithelium of heavy smokers at risk of developing lung cancer (Franklin et al 2002). The degree of EGFR expression can be evaluated by immunohistochemistry (IHC) and the EGFR gene copy number by fluorescent in situ hybridization (FISH). Hirsch and colleagues evaluated 183 NSCLC tumor samples by IHC and FISH (Hirsch et al 2003). EGFR overexpression was observed in 62% of patients with NSCLC with squamous cell carcinoma the most common histology associated with high expression. Expression levels did not correlate with survival, stage, age, gender, or smoking history. Well differentiated tumors had a higher level of EGFR expression compared to poorly differentiated tumors.

The EGFR gene is on chromosome 7p12 and Hirsch found 4 major FISH patterns in the 183 tumor samples tested (Hirsch et al 2003). Low levels of EGFR expression by IHC was associated with the following 2 FISH patterns: balanced disomy (equal EGFR gene and chr 7) and balanced trisomy (similar low level gains in both EGFR gene and chr 7). High levels of EGFR expression was associated with balanced polysomy (similar high level gains in EGFR gene and chr 7) and EGFR gene amplification (unbalanced gain of EGFR gene). These FISH patterns did not correlate with patient characteristics. A low gene copy was associated with non-squamous cell histology.

In another study utilizing FISH technology, 42 small NSCLC samples from gefitinib treated patients were examined for EGFR by FISH, and for mutations in EGFR (Daniele et al 2007). DNA was extracted and sequenced by PCR. EGFR was amplified and evaluated for mutational status and gene copy number. Seven of 7 patients (100%) with EGFR mutation (6 in Exon 19 and 1 in Exon 21) responded to gefitinib compared to 5 of 35 (14%) without the mutation ($p < 0.0001$). The FISH analysis was able to detect an increase in the EGFR gene and number of chromosome 7 copies which correlated with specific EGFR mutations. In another gefitinib study, 102 NSCLC tumor samples were evaluated for number of gene copies by FISH (Cappuzzo et al 2005). EGFR gene amplification and a high polysomy was associated better response, TTP and OS (18.7 months vs 7 months). In addition, EGFR mutations, by DNA sequencing, were shown to be related to a better response and TTP but not OS.

In a recent report of patients with BAC, gene amplification in combination with EGFR mutation (exon 19, 21) was shown to a strong predictor of response to erlotinib (Miller et al 2006). Patients with both EGFR activating mutations and gene amplification had a 90% response rate and a median OS of 35 months. In comparison, patients with no mutations and no gene amplification had a 4% response rate and median OS of 15 months.

In the BR.21 study, EGFR expression in the erlotinib treated group was associated with a better response without a survival advantage (Tsao et al 2005). Thus EGFR expression by IHC alone does not seem to be useful in predicting survival after erlotinib therapy. The FISH analysis from BR.21, however, did show a striking benefit in survival for the EGFR FISH positive patients versus the FISH negative patients ($p = 0.002$).

More recently, it has been suggested that NSCLC tumors from Western populations negative for EGFR by FISH and IHC do not respond to gefitinib (Hirsch et al 2007). In this study, patients with EGFR positive tumors by both FISH and IHC had a median survival of 21 months compared to 6 months with FISH and IHC negative tumors. Similar results were found in the ONCOBELL trial treating NSCLC patients who had EGFR positive tumors with gefitinib (Cappuzzo et al 2007). FISH is a more accurate diagnostic modality for EGFR-TKI patient selection, than IHC. The recent TRUST, open access European erlotinib study, also confirmed the increased benefit of FISH versus IHC. Patients with EGFR IHC positive tumors had a HR for survival of 0.75, $p = 0.1$, but those with FISH positive tumors had a HR for survival of 0.53, $p = 0.02$ (Schneider et al 2007). There was high concordance with EGFR positivity by FISH and IHC on this study though (93%) (Laack et al 2007). The ability of EGFR over-expression by FISH analysis to predict response

to EGFR-TKIs has been clearly demonstrated but the debate about the relative strength of FISH analysis versus EGFR mutational analysis is ongoing.

EGFR mutation

The phenomenal responses seen in a small number of patients led investigators to sequence EGFR and several activating mutations have now been identified. This was initially seen in 2004 when two groups simultaneously published small series of patients with excellent responses to gefitinib with specific mutations in EGFR. Paez and colleagues examined tumors from 119 Japanese and Caucasian NSCLC patients treated with gefitinib (Paez et al 2004). Somatic mutations were found in 5 of 5 responders and 0 of 4 non-responders ($p = 0.0027$). Most mutations were found in Japanese females with adenocarcinoma. In a report by Lynch, 8 of 9 gefitinib responders had mutations of the TK domain compared to 0 of 7 non-responders (Lynch 2004). All amino acid deletions were seen in exon 19 while substitutions were in exons 18 and 21. Tumors with these somatic mutations have a better prognosis (Bunn et al 2002) and are normally associated with never smokers, Asian race, female gender, and adenocarcinoma histology. In one study, 7 of 15 non-smokers had mutations compared to 4 of 81 smokers ($p = 0.0001$) (Pao et al 2004). Moreover, EGFR mutations have been reported to be more common in light smokers (Jackman et al 2006; Sequist et al 2007). In one study, 68 of 278 (24%) patients had EGFR somatic mutations (Sequist et al 2007). The presence of the mutation correlated with smoking history with a 5% decrease in chance of a mutation with each pack-year smoking history. The EGFR mutated tumors responded better to EGFR-TKI but not to chemotherapy.

In the BR.21 mutational analysis study, 40 of 177 (23%) samples were positive for mutations in exons 18–21 (Tsao et al 2005). EGFR mutations were found at varying levels in the following subgroups: males (22%), females (24%), Asians (50%), non-Asians (21%), never smokers (31%), and adenocarcinoma (28%). In this trial surprisingly, the presence of mutations did not correlate with response or survival even in patients with classic exon 19 or 21 mutations. This was attributed to low number of patients positive for mutations in this study. There was a trend towards better response rate in those with mutations, but not of statistical significance. In another study, all patients with EGFR mutations had disease control with prolonged time to progression and OS (Jackman et al 2007). These results were also seen in a study by Cappuzzo, except that the survival advantage was not statistically significant (Cappuzzo et al 2005). There is a lot to be learned about these mutations especially since the BR.21 investigators found 24 novel mutations not previously described. The majority of activating mutations are of 3 dominant types, deletions in exon 19, insertions in exon 20 or a single point mutation L858R. Another report confirms that E746_A750 exon 19 deletion and L858R missense mutation to be the most common EGFR mutations (Janne et al 2005). It is important to note that the tyrosine kinase domain is encoded by exons 18–24 and the EGFR mutations have only been found in this domain (Paez et al 2004; Pao et al 2004).

More recent data have focused on patients with known mutations in EGFR who initially respond and subsequently become resistant to erlotinib. Secondary mutations have been identified in some of these patients, predominantly in the T790M location (Vikis et al 2007). Over-expression of MET has also been identified in other patients who initially responded to EGFR-TKI and subsequently became resistant (Engleman et al 2007). Several novel TKIs are in development to overcome resistance in this setting.

Other genes

KRAS is downstream to EGFR and its mutation has been shown to affect the efficacy of erlotinib. In the TRIBUTE trial, DNA was extracted from tumor samples and the EGFR exons 18–21 and KRAS exon 2 were amplified (Eberhart et al 2005). Only 12.7% of tumors had EGFR mutations which translated into a better response rate with the chemotherapy and erlotinib combination. In this study, EGFR mutation was found to be a favorable prognostic factor in patients with NSCLC. The overall response rate with chemotherapy and erlotinib was 53% compared to 21% with chemotherapy alone. However, patients with the KRAS mutation did poorly with the addition of erlotinib in this trial. In a BAC study, KRAS mutation was associated with resistance to erlotinib (Miller et al 2006). KRAS mutations and EGFR mutations are generally mutually exclusive (Tam et al 2006; Bae et al 2007; Mounawar et al 2007). The recent TRUST open access erlotinib study in Europe also showed the KRAS was associated with decreased survival, though without statistical significance (Schneider et al 2007).

Phosphorylated MAP kinase is also involved in the EGFR signaling pathway had is under investigation as well as a predictor of response to EGFR-TKIs.

Proteomic analysis

A recent publication assesses the use of mass spectrometry proteomic analysis of response of NSCLC patients to

erlotinib and gefitinib (Taguchi et al 2007). The study used serum from NSCLC patients prior to therapy with erlotinib or gefitinib and found patterns predictive of good or poor outcome. These results were validated in different cohorts with testing at two separate institutions with good concordance. This technology will be the basis for an ECOG trial currently in development.

Cutaneous toxicity

Cutaneous toxicity is common in patients treated with erlotinib. Dose reductions and discontinuation of therapy are needed in some patients. There are several theories on the etiology of these skin reactions. EGFR is expressed on the keratinocytes, sebaceous gland cells, and the outer sheath of hair follicles (Lee et al 2004). EGFR inhibition can result in follicle occlusion and acneiform eruption with inflammation (Journagan 2006). Individuals normally present with a papulopustular rash affecting the face and the upper trunk occurring in the first weeks of therapy (Luu et al 2007). Topical antibiotics are routinely used, with escalation to oral antibiotics as necessary even though there has never been an association with any infectious organism. Topical steroids are also utilized, though with controversy. The rash normally clears with these measures even with continuation of erlotinib therapy. In addition, the rash has been reported to worsen with photoexposure and patients should be advised to use sunscreen (Luu et al 2007). There are currently no formal guidelines available for the management of cutaneous toxicity related to erlotinib use. A recent presentation at ASCO (Jatoi et al 2007) explored the use of tetracycline to alleviate the rash and found that though the rate of development of rash was not reduced by tetracycline, the severity was diminished with this agent.

Erlotinib metabolism and drug adjustments

Cytochrome P450-3A (CYP3A) is one of the major subfamilies of the CYP450 family of genes present in the liver (Guengerich 1995). CYP3A is composed of the following isoforms: CYP3A4, CYP3A5, CYP3A7, and CYP3A43. These genes are responsible for the metabolism of up to half of drugs used in humans. Of note, genetic variations have been described which may cause altered drug metabolism (Eichelbaum and Burk 2001).

CYP3A4 is the major isoform responsible for erlotinib metabolism (Li et al 2007). Caution should be exercised when combining erlotinib to CYP3A4 inhibitors or inducers as this may increase or decrease the erlotinib AUC (Table 1).

Table 1 CYP3A4 inhibitors and inducers (concise list)

Inhibitors	Inducers
Ketoconazole	Rifabutin
Atanazavir	Rifapentin
Clarithromycin	Phenytoin
Indinavir	Carbamazepine
Itraconazole	Phenobarbitol
Nefazodone	St. John's wort
Nelfinavir	Rifampicin
Ritonavir	
Saquinavir	
Telithromycin	
Troleandomycin	
Voriconazole	
Grape juice	

A dose reduction in erlotinib should be considered if a patient is on a CYP3A4 inhibitor as an increase in toxicity could be experienced due to an increase in the level of erlotinib.

Smoking also dramatically increases activity of CYP1A2, another enzyme involved in erlotinib metabolism and this is hypothesized to be one reason for lack of efficacy of the drug in smokers, due to increased clearance (Li et al 2007). Ongoing trials are exploring dose escalation of erlotinib in smokers to see if this can be overcome.

Novel drug combinations

There are many novel/targeted agents currently in clinical trials in combination with erlotinib (Table 2). Completed phase I/II studies are discussed below.

Bevacizumab was combined with erlotinib in a phase I/II study in patients with relapsed non-squamous NCSLC (Herbst et al 2005b; Sandler et al 2006b). Blocking the EGFR inhibits the synthesis of angiogenic factors including vascular endothelial growth factor (VEGF) which in turn prevents endothelial response to VEGF with the addition of bevacizumab. Furthermore, there is some evidence to suggest that bevacizumab inhibits the EGFR autocrine function (Petit et al 1997; Hirata et al 2002). There was no DLT reported in the phase I portion and 34 patients were treated at the phase II doses with erlotinib 150 mg daily and bevacizumab 15 mg/kg every 21 days. The most common toxicities were diarrhea, rash, hematuria, and proteinuria with no treatment related deaths. There was a 20% PR and a 65% SD with a median OS of 12.6 months and a PFS of 6.2 months. Nine tumors were tested for EGFR mutations in exons 19–21 and 23 and only 2 had the mutation (1 PR and 1 SD). Confirmation of these exciting preliminary results is being sought in two international phase III trials, ATLAS, and Beta. The ATLAS trial (N = 1150) is a randomized double-blind, placebo-controlled

Table 2 Selected targeted drug combinations with erlotinib in clinical trials

a. Anti-angiogenesis
   ADH-1
   AVE0005
   Bevacizumab
   Volociximab
   Vandetanib
b. EGFR inhibitors
   Cetuximab
   Vandetanib
c. Multiple receptor TKI
   Sorafenib
   Sunitinib
   Vandetanib (ZD 6474)
   Dasatinib
d. Triple combinations
   Bevacizumab and cetuximab
   Bevacizumab and docetaxel
   Bevacizumab and pemetrexed
   Bevacizumab and temsirolimus
   Carboplatin and docetaxel
   Cisplatin and gemcitabine
e. Miscellaneous
   Bexarotene (BATTLE trial)
   Temsirolimus (CCI-779)
   RAD001
   Perifosine
   Promune (PF-3512676)
   hydroxychloroquine
   Bortezomib
   Celecoxib
   Digoxin
   Docetaxel
   Enzastaurin
   Fulvestrant
   Vorinostat (SAHA)
   Hydrochloroquine
   Pemetrexed
   Perifosine
   Promune
   Vorinostat
   Satraplatin

phase IIIb trial that compares bevacizumab with or without erlotinib after completion of first-line chemotherapy with bevacizumab for advanced NSCLC (non-squamous). The Beta trial (N = 650) randomizes patients requiring second-line therapy to erlotinib with or without bevacizumab.

These promising results with dual EGFR/VEGFR inhibition have also been seen with single drugs that target both receptors. The one furthest in development is ZD6474 (vandetanib). This compound has been directly compared to gefitinib in phase II testing with favorable results (Natale et al 2006). This has led to an ongoing trial of the compound versus erlotinib.

Erlotinib is also being studied in combination with radiation, both in the thorax and for central nervous system metastasis.

Future direction

Erlotinib has a clear role in second or third-line treatment of NSCLC. The decision of when to administer (either second- or third-line) can be challenging. Certain patients, such as never-smokers and those with known EGFR mutations, will obviously be offered the drug second-line (if they were not given it first-line as part of a trial). For those patients without favorable clinical or molecular predictors of response though, it is difficult to know if erlotinib is as efficacious as either docetaxel or pemetrexed. The ongoing TITAN trial will hopefully answer this question. This phase III trial randomizes patients to receive either erlotinib or docetaxel or pemetrexed. Several of the ongoing randomized studies with erlotinib are listed in Table 3.

The other major question in second line therapy is when to start treatment. Older trials had shown no advantage to continuing beyond 4–6 cycles of standard doublet chemotherapy, but more recent data, bring back the question of whether we should be offering second line therapy sooner (Fidias et al 2007). The ongoing SATURN study randomizes patients to either placebo or erlotinib after completion of first-line chemotherapy and will hopefully help further in resolving this controversy. The ATLAS trial also randomizes patients to receive either erlotinib or placebo after completion of 4 cycles of a platinum based regimen, but with the addition of bevacizumab to both arms.

Table 3 Ongoing phase III trials with erlotinib

- RADIANT – Erlotinib or placebo following complete resection and adjuvant chemotherapy for resected stage I-IIIA NSCLC for patients whose tumors over-express EGFR either by IHC or FISH
- SATURN – Erlotinib or placebo following 4 cycles of carboplatin/paclitaxel for advanced stage NSCLC
- ATLAS – Erlotinib or placebo plus bevacizumab following 4 cycles of doublet chemotherapy plus bevacizumab for advanced stage NSCLC
- Beta – Erlotinib plus placebo versus erlotinib plus bevacizumab for second-line therapy of advanced stage NSCLC
- TITAN – Erlotinib versus docetaxel or pemetrexed for second-line therapy of advanced stage NSCLC
- Erlotinib versus vandetanib (ZD6474) for advanced stage NSCLC
- Erlotinib versus chemotherapy in women never smokers as first-line therapy for advanced NSCLC
- Erlotinib or placebo after completion of concurrent carboplatin/paclitaxel/radiation for inoperable stage III NSCLC

Further investigation of erlotinib in first-line therapy, either alone or in combination with chemotherapy is also ongoing. Ideally we will be able to select, based on markers such as EGFR expression by FISH, and mutational analysis, who should receive erlotinib and at what point in their therapy. We are certainly closer to this step in personalized cancer therapy than we are in predicting the same issues with conventional chemotherapy.

The best therapy for those who have initially responded to erlotinib and have subsequently lost their response either through development of the T790M mutation or another resistance mechanism, remains another area of active research. Hopefully at least one of the newer TKIs in development will help in this situation.

Conclusions

EGFR-TKIs belong to a new class of targeted agents that have shown to be beneficial in patients with advanced NSCLC. Erlotinib is currently approved in many countries as a single agent for use in the second or third line setting for this disease. Though response rates in the general population are no better than those seen with standard chemotherapy, a certain group of patients have striking and durable responses. Some clinical characteristics help predict response, including never-smokers, women and those of East Asian ethnicity. These characteristics correlate with activating mutations in the EGFR gene. Higher gene copy number as predicted by FISH analysis is also correlated with better survival with treatment with this agent. A survival benefit has been demonstrated in an unselected patient population as well though, in the landmark BR.21 trial. Ongoing trials seek to improve therapy with this agent further with combination regimens and better understanding of predictors of benefit with therapy. Though we are not yet in an era of personalized medicine, we are close with erlotinib which has become a crucial part of the lung cancer arsenal.

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