Overview of tyrosine kinase inhibitors in clinical breast cancer

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Abstract

Studies of cell models and profiling of clinical breast cancer material to reveal the mechanisms of resistance to anti-oestrogen therapy, and to tamoxifen in particular, have reported that this phenomenon can be associated with increased expression and signalling through erbB Type 1 growth factor receptors, notably the epidermal growth factor receptor (EGFR) and HER2. Further molecular studies have revealed an intricate interlinking between such growth factor receptor pathways and oestrogen receptor (ER) signalling. Inhibition of receptor tyrosine kinase activity involved in the EGFR signalling cascade forms the basis for the use of EGFR specific tyrosine kinase inhibitors exemplified by gefitinib (ZD1839, Iressa) and erlotinib (OSI-774, Tarceva). Such agents have proved promising in pre-clinical studies and are currently in clinical trials in breast cancer, where gefitinib has been studied more extensively to date. Here, we present an overview of the current development of gefitinib in clinical breast cancer. This includes results from our clinical breast cancer trial 1839IL/0057 that demonstrate the efficacy of gefitinib within ER-positive, tamoxifen-resistant patients with locally advanced/metastatic disease, where parallel decreases in EGFR signal transduction and the Ki67 (MIB1) proliferation marker can be detected as predicted from model system studies. We also consider trials examining combination treatment with gefitinib and anti-hormonal strategies that will begin to address the clinically important question of whether gefitinib can delay/prevent onset of anti-hormone resistance.

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Introduction

The erbB (HER/neu 1–4) receptors are a family of Type 1 transmembrane receptors frequently expressed at high levels in human tumours. erbB receptors are activated by various growth factor ligands triggering a network of intracellular signalling pathways leading to uncontrolled growth of cancer cells. Signalling through this receptor family invariably involves heterodimerization between the family members.

HER2/neu is a transmembrane tyrosine kinase receptor which forms part of this erbB family signalling network. Aberrant signalling by this network is reportedly present in a cohort of breast carcinomas (Arteaga et al. 2002). Slamon and colleagues reported in 1987 that HER2 gene amplification independently predicted overall survival (OS) and disease-free survival (DFS) in a multivariate analysis in node-positive patients. Subsequent studies have confirmed this finding in node-positive breast cancer (Ross & Fletcher 1998), although there is as yet no consensus on its prognostic value in node-negative disease (Paik et al. 1990, Clark & McGuire 1991, Dati et al. 1991). The epidermal growth factor receptor (EGFR/HER1), a further erbB receptor, is expressed in a large number of breast cancers, with a positivity rate of 14–91% reported. Overexpression of EGFR has again been linked to a more aggressive breast tumour phenotype, involving...
increased potential for invasiveness and metastasis. This has been linked to poorer patient prognosis, as extensively reviewed by Klijn and colleagues (1992). More recently, Tsutsui and colleagues (2002) reported in a large series of patients that EGFR expression was a significant prognostic factor, although this remains controversial; Ferrero et al. (2001) and Rampaul et al. (2004), for example, both report that EGFR is not prognostic.

While the prognostic significance of EGFR and HER2 expression in breast cancer remains some matter of debate, there is strong pre-clinical evidence to link an importance for EGFR expression and its signalling to de novo and acquired anti-hormone resistance in oestrogen receptor-positive (ER+), as well as ER-negative (ER−), breast tumour growth. erbB receptor signal transduction has invariably been associated with resistance to anti-oestrogens such as tamoxifen (Knowlden et al. 2003), as well as to long-term oestrogen deprivation in model systems (Martin et al. 2003). For example, our in vitro studies of resistant sublines of MCF-7 breast cancer cells developed by prolonged exposure to the anti-oestrogen tamoxifen (TAM-R) or Faslodex (ICI 182, 780, fulvestrant, AstraZeneca, Macclesfield, UK; FASR) to mimic the development of acquired resistance in the clinic have revealed increases in expression of EGFR and HER2 mRNA and protein (McClelland et al. 2001, Nicholson et al. 2002, 2004, Knowlden et al. 2003). Immunoprecipitation studies revealed increased heterodimerization between these receptors in the TAM-R cell line. Western blotting also revealed increased phosphorylation (and hence activity) of these receptors compared with the parental anti-hormone responsive MCF-7 cells. Increased basal levels of ERK1/2 MAP kinase phosphorylation (pMAPK) were also observed in TAM-R cells. TAM-R cells were capable of generating a range of EGFR-specific ligands and increased expression of transforming growth factor-alpha (TGFα) or amphiregulin were observed in these cells. Immunocytochemical analysis confirmed these various signalling data (Knowlden et al. 2003). Interestingly, patients expressing increased HER2 or EGFR, together with ligands for the EGFR such as TGFα, or increased downstream signalling through pMAPK in their breast tumours as measured immunohistochemically, exhibit an increased likelihood of being resistant to anti-hormonal agents, notably tamoxifen (Nicholson et al. 1993, 1994a,b, Giai et al. 1994, Archer et al. 1995, Elledge et al. 1998, Gee et al. 2001).

Given the emerging importance of erbB receptor signalling in anti-hormone-resistant breast cancer, it is encouraging that several therapeutic strategies are being developed to target such receptors. First, there are extracellular approaches i.e. monoclonal antibodies to the extracellular domain of particular erbB receptors. For example, monoclonal antibodies have been developed such as trastuzumab (rhuMAbHER2, Herceptin, Genentech, San Francisco, US), cetuximab (Erbitux, Merck KGaA, Darmstadt, Germany) and pertuzumab (rhuMAb 2C4, Omnitarg, Roche, Basel, Switzerland) that act to inhibit HER2, EGFR and HER2 dimerization respectively. Secondly, there are intracellular approaches i.e. small molecule tyrosine kinase inhibitors (TKIs), notably those which selectively inhibit the EGFR tyrosine kinase (Ciardiello 2000, Mendelsohn & Baselga 2000, Solignac 2000). Of note in this regard are gefitinib (ZD1839, Iressa) and erlotinib (OSI-774, Tarceva). There are also now further TKIs that block activity of both EGFR and HER2, such as lapatinib (GW572016; Rusnak et al. 2001), while CI-1033, which is in the pre-clinical stage of development, is an agent purported to inhibit all four erbB receptors. Therapeutic approaches such as anti-sense, anti-growth factor targeted therapies, and toxin conjugated monoclonal antibodies are also of some interest. To date, the Nottingham-Tenovus group have been involved primarily in the pre-clinical and clinical development of the EGFR TKI, gefitinib, in anti-oestrogen-resistant breast cancer, and this agent will therefore comprise the principal focus of this overview.

**EGFR TKI, gefitinib, in pre-clinical breast cancer studies**

Gefitinib is a low molecular weight inhibitor with highly selective and reversible tyrosine kinase inhibition properties directed at the EGFR, being a competitive inhibitor of adenosine triphosphate binding to this receptor. It is a potent inhibitor of proliferation not only in cells overexpressing EGFR but also in those that additionally overexpress HER2, possibly mediated by gefitinib reduction of EGFR/HER2 heterodimer phosphorylation (Anderson et al. 2001). Pre-clinically, our studies showed that gefitinib treatment (1 μM) was associated with a loss of phosphorylated EGFR in the acquired tamoxifen-resistant TAM-R cell line, shown both by Western blotting and immunocytochemistry, with an additional partial inhibition of phosphorylation of the principle EGFR heterodimerization partner in these cells, HER2. Downstream, pMAPK was markedly depleted as a consequence of this agent in TAM-R cells. Importantly, gefitinib subsequently induced a concentration-dependent inhibition of TAM-R cell growth, where 1 μM gefitinib reduced proliferation significantly by...
approximately 60% (Nicholson et al. 2002, 2004, Knowlden et al. 2003). While resistance to gefitinib ultimately emerged in culture (Jones et al. 2004), the duration of response was long-lasting. Treatment with trastuzumab to block HER2 heterodimerization with EGFR was also able to reduce pMAPK activity in TAM-R cells and inhibit growth (Knowlden et al. 2003). Obvious inhibitory effects of gefitinib were similarly noted in acquired tamoxifen-resistant T47D breast cancer cells (approximately 70% growth inhibition), and also in cells that had acquired resistance to Faslodex (approximately 50% growth inhibition, McClelland et al. 2001, Nicholson et al. 2004). Gefitinib has also been reported to be inhibitory on growth of de novo tamoxifen-resistant HER2-overexpressing MCF-7/HER2-18 breast cancer cells (Shou et al. 2004). However, in marked contrast to its effects in tamoxifen-resistant cells, gefitinib exerted only a small inhibition of the anti-hormone responsive MCF-7 cells that exhibit minimal levels of EGFR signalling. In such cells there was only approximately 15% reduction in growth at 1 μM gefitinib (Nicholson et al. 2002, 2004, Knowlden et al. 2003).

In total, the model system data evaluating EGFR signal transduction and gefitinib response in various anti-hormone resistant cell lines, coupled with available immunohistochemical profiling of the EGFR pathway in clinical tamoxifen resistance, indicate the considerable potential for the EGFR-selective TKI, gefitinib, to efficiently treat anti-hormone resistant breast cancer (Nicholson et al. 2004). EGFR TKIs are thus being studied intensively through various clinical trials in this disease.

**EGFR TKI, gefitinib, in clinical breast cancer trials**

**1839IL/0156**

An open label study, 1839IL/0156, of the EGFR TKI, gefitinib, in actively progressive, measurable, metastatic breast carcinoma in 63 patients with no limit on prior chemotherapy or anti-hormonal therapy has revealed clinical benefit (CB=complete response (CR)+partial response (PR)+stable disease (SD)≥6 months) in 3 patients (4.8%), and a further 6 patients (9.5%) with stable disease up to 6 months (Albain et al. 2002). A phase II multi-centre study of gefitinib monotherapy at 500 mg/day did not appear to be efficacious in taxane and anthracycline metastatic pre-treated breast cancer patients (57 patients, with 98.3% non-responders) (von Minckwitz et al. 2005).

The efficacy of gefitinib in these breast cancer studies was thus relatively low, although these studies did focus on heavily pre-treated groups. This observation also appears to extend to another EGFR TKI, namely erlotinib. A phase II trial of erlotinib (as monotherapy of 150 mg/day) in locally advanced or metastatic breast cancer (n=69) was conducted with 2 arms, both of which contained heavily pretreated patients. Cohort 1 (n=47) examining disease progression on or after treatment with an anthracycline, a taxane, and capecitabine revealed 1 PR (23 weeks) and 6 SD (all >12 weeks). Cohort 2 (n=22) examining disease progression on or after at least one chemotherapy regimen revealed 1 PR (16+ weeks) and 2 SD (both >8 weeks) (Winer et al. 2002). Again, the efficacy in these study groups was at best modest and the most common side effects were grade 1 and 2 skin rashes (78%) and diarrhoea (59%). Another phase II trial based on pre-clinical data of additional efficacy of combination of capecitabine with erlotinib had 24 patients in the trial. It found that erlotinib (100 mg/day), capecitabine and docetaxel were generally well tolerated and that there was no pharmacokinetic interaction between erlotinib, capecitabine and docetaxel or the metabolites of these drugs (Jones et al. 2003).

**Iressa trial 57**

To date, the use of EGFR TKIs in metastatic disease refractory to multiple previous treatments has proved relatively disappointing, despite demonstrating acceptable tolerability. However, a recent study by our group has revealed that there is a cohort of breast cancer patients that appear to obtain more substantial benefit from EGFR TKI treatment.

Our previous immunohistochemical study of primary human tumours had reported that increased EGFR expression was significantly associated with lack of anti-hormone sensitivity de novo in both ER+ and ER– breast cancers (Nicholson et al. 1994a). Examination of the in vivo breast tumour tissue showed obvious overexpression of several erbB signalling components in the ER+ de novo resistant tumours i.e. an increase in EGFR, HER2, TGFα and pMAPK (and also the Ki67 proliferation marker), approaching levels seen in the ER– tumours (Nicholson et al. 1993, 1994a,b, Gee et al. 2001). Furthermore, ER+ breast cancer patients who developed acquired tamoxifen resistance showed more modest increases in expression of EGFR, TGFα, HER2 and activation of ERK1/2 MAPK at the time of relapse (Gee et al. 2002, Nicholson et al. 2004). These observations, coupled with pre-clinical studies showing the importance of EGFR signalling and anti-tumour effects of gefitinib in various anti-oestrogen-resistant models, formed the
basis for the design of the gefitinib breast cancer trial 57 (1839IL/0057). This comprised two stratified arms of locally advanced/metastatic disease patients with either ER+ tamoxifen-resistant disease or ER− tumours. The target recruitment was 54 patients, with 27 patients in each arm. The early results of this study were presented when 33 patients had been recruited: 13 in the ER+ arm and 20 in the ER− arm. The common side effects with gefitinib treatment were again skin rash and diarrhoea. Six patients discontinued gefitinib 500 mg/day within 3 months owing to these side effects. A minority of patients was also reported to have a degree of alopecia. Interestingly, of the 27 evaluable patients, clinical benefit was seen in 6 out of 9 patients in the ER+ arm (66%, comprising PR = 1 and SD = 5). There was a lower efficacy in the ER− arm, although 2 out of 18 patients in this group gained clinical benefit with gefitinib (11%, comprising PR = 1 and SD = 1) (Robertson et al. 2003). Median time to progression was also longer in the ER+ tamoxifen-resistant group compared with the ER− group (Gutteridge et al. 2004). Since then, further recruitment continued up to 53 patients by January 2005: 26 patients in the ER+ arm and all 27 patients in the ER− arm. The initial difference in therapeutic efficacy seen between the ER+ tamoxifen-resistant group and ER− patients has been maintained.

As part of trial 57, patients with palpable disease were asked for consent to carry out sequential biopsies which were taken before commencement of treatment (T0), at 8 weeks (T1) and 6 months (T3) on treatment, and on progression of disease. Patients who obtained a CB with gefitinib expressed lower pre-treatment levels of EGFR than patients with progressive disease (PD) (Robertson et al. 2003, Gee et al. 2004, Gutteridge et al. 2004). Some degree of EGFR expression was, however, detectable in all CB patients. It thus appears that it is largely patients with ER+ acquired tamoxifen-resistant disease whose tumours express relatively modest EGFR that obtain clinical benefit from this EGFR TKI, rather than a predominance in the ER−, EGFR overexpressing cohort. These data directly parallel the successes of gefitinib in ER+ acquired anti-oestrogen-resistant cell models, including our TAM-R cells. The somewhat paradoxical lack of direct association between high EGFR expression and the gefitinib response has previously been noted within in vitro and xenograft models (Wakeling et al. 2002). Efficacy of gefitinib was also independent of ER expression in the IDEAL non-small cell lung carcinoma (NSCLC) trials. It was reported that the percentage of membrane staining for EGFR was approximately 20% higher in 69 non-responsive subjects compared with 18 responders in IDEAL 1 (although the opposite was seen in IDEAL 2, where staining was approximately 25% lower in 62 non-responders compared with 8 responders). There was thus no consistent association of relevance of EGFR membrane staining and response to classify NSCLC patients for gefitinib treatment (Bailey et al. 2003).

Our immunohistochemical analysis of 15 of the breast cancers in trial 57 performed to date has studied erbB signalling elements and the Ki67 (MIB1) proliferation marker in paired pre-treatment and 8 weeks (T1) after gefitinib (500 mg/day) samples. Patients studied were categorized as having CB (n = 9; all ER+) or PD (n = 6; all ER−) at 6 months. These studies revealed maintenance of a significant association between CB and ER (and progesterone receptor, PgR) positivity in the pre-treatment and post-treatment samples. Change in the Ki67 proliferation marker paralleled subsequent outcome, with a significant decline between the pre-treatment and post-treatment levels in patients with CB compared with PD (Gee et al. 2004). Phosphorylated (activated) EGFR (pEGFR) was generally low at pre-treatment and unrelated to outcome with gefitinib in trial 57. However, 5/9 patients with CB showed falls in pEGFR during treatment, with parallel decreases in pMAPK and proliferation. In pharmacodynamic immunohistochemical studies of 16 paired pre-treatment and post-treatment normal skin biopsies in patients participating in gefitinib phase I clinical trials receiving escalating daily doses of gefitinib for solid tumours (Albanell et al. 2001, 2002, Baselga et al. 2002), there was similarly a statistically significant decrease in pEGFR, pMAPK and Ki67 post gefitinib treatment.

CB is thus achievable with gefitinib in ER+/PgR+ tamoxifen-resistant breast cancer patients expressing relatively low EGFR, with proliferation changes at T1 paralleling the subsequent response. Patients with CB can exhibit reduced pEGFR and pERK1/2 MAPK during treatment, in accordance with the inhibitory effects of gefitinib on EGFR signal transduction and proliferation observed in pre-clinical studies in TAM-R cells. However, decreases in pEGFR were not universal in patients with CB in trial 57, suggesting that a non-classical gefitinib response mechanism does exist in some patients.

In breast cancer patients exhibiting PD with gefitinib, some increase in EGFR expression was noted following treatment, while the pEGFR level was unchanged and could even increase. Thus, significantly elevated EGFR expression and pEGFR were recorded
post-treatment in patients with PD versus CB (Gee et al. 2004). While increases in EGFR expression could perhaps underlie the absence of pEGFR decline in de novo gefitinib resistance, EGFR phosphorylation mediated in an EGFR kinase-independent manner by other receptors (perhaps additional erbB receptors or the insulin-like growth factor receptor (IGF-1R)) may be contributory. (Gee et al. 2004, Gutteridge et al. 2004). Interestingly, tumours from PD patients in this study also commonly exhibited increased activity of the signalling molecule AKT, an element that has been linked pre-clinically both to IGF-1R signalling and to gefitinib resistance (Jones et al. 2004).

Iressa trial 225

Acquired tamoxifen resistance in MCF-7 cells and in clinical breast cancer has been reported to be associated with some increases in EGFR signalling through ERK1/2 MAPK, where gefitinib blocks such signalling activity in tamoxifen-resistant models and, as stated above, in some ER+ tamoxifen-resistant patients. Further pre-clinical mechanistic studies have revealed an intricate interlinking between such signalling and the oestrogen receptor signalling pathway at the level of ER phosphorylation and ER co-activator recruitment (Nicholson et al. 2004). In contrast, however, EGFR/MAPK signalling is minimal in the anti-hormone responsive MCF-7 cells and treatment with gefitinib alone has no anti-proliferative effect. This ‘switch’ to the use of EGFR/MAPK signalling that appears integral to the evolution of anti-oestrogen resistance can be blocked by the combination of tamoxifen and gefitinib during the anti-hormone responsive phase in vitro (Gee et al. 2003). Such a strategy improves the growth inhibitory effect of the anti-oestrogen and, moreover, delays/prevents development of acquired resistance in ER+ breast cancer models (Gee et al. 2003, Shou et al. 2004).

Cumulatively, these data not only supported the potential clinical utility of gefitinib in anti-oestrogen-resistant breast cancer but also suggested the possibility of delaying or preventing resistance by the early use of a combination of gefitinib with anti-oestrogens such as tamoxifen (Wakeling et al. 2001). This led to the designing of a trial known as Iressa 225 (1839IL/0225). This is a randomized phase II trial intended to recruit 274 women with ER+ metastatic breast cancer derived from 2 strata of patients: strata 1 comprises patients who have newly diagnosed disease or who have completed adjuvant tamoxifen at least one year prior to recruitment, while strata 2 comprises patients with recurrent disease during or after adjuvant aromatase inhibitor (AI) or failing first-line treatment with AI. Patients are then equally randomized to two arms: tamoxifen (20 mg/day) plus gefitinib (250 mg/day) or tamoxifen plus placebo. The primary endpoint of the study is comparative time to progression in the two arms and the secondary endpoints are the recurrence rate (RR) and clinical benefit rate. This study should begin to answer the clinically important question of whether gefitinib can delay/prevent acquired resistance to tamoxifen. Further immunohistochemical exploration would examine downstream effectors of the erbB family, and also key co-activators such as AIB-1 that may enable interplay between erbB and ER signalling. No results are thus far available for this study. A very similar trial in the United States plans to recruit 174 patients, with the only difference being that it will assess acquired resistance to anastrozole instead of tamoxifen.

Iressa trials 0219 and 0223

There are also two studies assessing changes induced by gefitinib in tumour samples obtained pre-surgically that should prove mechanistically informative. The pre-surgical biological trial 1839IL/0219 intends to recruit about 50 patients (stages I, II, IIIa) and will compare immunohistochemical biomarker changes in pre-gefitinib tissue samples (tumour and skin biopsy) with those taken after 14 days of gefitinib (250 mg/day) (McKillop et al. 2004). A second trial combines the principles of the Iressa 225 study into a neoadjuvant protocol using anastrozole rather than tamoxifen (1839IL/0223). This is a randomized blinded parallel group, placebo-controlled, multi-centre trial. It intends to recruit 175 evaluable patients with stage II–IIIb treatment-naïve ER+ tumours. In a neoadjuvant study of 3–4 months there is usually insufficient time for tumours to respond and then establish acquired resistance. The hypothesis therefore being tested in such a trial is whether gefitinib further sensitizes ER+ tumours to anastrozole (both given as a neoadjuvant) and can reverse de novo resistance. The patients are randomized into three arms: arm A (n = 29) comprising patients who would receive anastrozole (1 mg four times a day) + gefitinib (250 mg four times a day) for 16 weeks prior to surgery, arm B (n = 73) comprising patients receiving anastrozole + placebo for 2 weeks and then placebo replaced by gefitinib for the remaining 14 weeks, and arm C (n = 73) comprising patients receiving anastrozole + placebo for the full 16 weeks. The study would assess biomarker changes (including Ki67, pMAPK, pEGFR) and measure mRNA expression in tumour biopsy, plasma and urine samples.
Our in vivo study of the effect of Faslodex (fulvestrant), a pure anti-oestrogen with no partial agonist activity on the ER, given for 7 days pre-operatively in clinical breast cancer did not show any short-term alteration of EGFR or TGFα protein levels even though ER protein was significantly suppressed (McClelland et al. 1996). However, our in vitro studies that established an acquired fulvestrant-resistant breast cancer cell line (FASR) by maintenance of the anti-hormone responsive parental MCF-7 cells in steroid-depleted, fulvestrant-supplemented medium showed that EGFR protein and mRNA, growth responses to TGFα, and pMAPK were all increased by 3-months exposure to this anti-oestrogen. Unlike the parental MCF-7 cells, FASR cells were sensitive to growth inhibition by gefitinib or by an inhibitor of the activation of MEK1 (MAPKK) and hence pMAPK, PD098059. Furthermore, the parental MCF-7 cells were markedly inhibited by combination fulvestrant + gefitinib treatment, with prominent cell death and reduced rates of cellular proliferation (McClelland et al. 2001), a strategy consequently abrogating emergence of resistance. Thus, gefitinib may be a viable option after resistance to fulvestrant, and may again delay development of resistance to this agent. The Nottingham-Tenovus group have built on these promising pre-clinical findings and initiated a phase II trial, 1839IL/0004, of gefitinib (250 mg daily) second-line therapy in metastatic breast cancer patients who have developed acquired resistance to the pure anti-oestrogen fulvestrant that they received as first-line anti-hormonal therapy. The exploratory objectives in this study are correlation between EGFR expression and response, and evaluation of changes in tissue and serum biomarkers, the main clinical endpoints being RR and CB.

Other TKI trials

There are two further phase II studies of gefitinib + fulvestrant in patients with advanced disease. One is being carried out in Spain, where 60 metastatic breast cancer patients are to be recruited to first-line therapy. Along with clinical end-points, this study will examine EGFR and HER2 signalling components. The second is an Eastern Co-operative Oncology Group (ECOG) trial comparing gefitinib (250 mg/day) + fulvestrant (250 mg i.m. monthly) with gefitinib (250 mg/day) + anastrozole (1 mg/day). The number of patients to be recruited to the latter study is 204, to be divided equally between the two arms. Again, the endpoints will be biomarkers and the correlation of the two arms in terms of efficacy. As yet no data are reported for either trial.

Conclusions

Initial phase II studies have suggested that the EGFR TKIs, gefitinib and erlotinib, do not have a high efficacy in a heavily pre-treated population of patients with metastatic breast cancer, particularly post chemotherapy. Surprisingly, there is also evidence of only minimal efficacy in ER−, EGFR overexpressing breast cancer. Importantly, however, in patients with tamoxifen-resistant ER+ tumours, gefitinib does appear to have a significant therapeutic effect. While such studies indicate that EGFR overexpression is not related to response to gefitinib in breast cancer, the available data suggest that the EGFR is required to be present at detectable levels before patients’ tumours can respond to this agent. Indeed, previous clinical profiling has detected modest levels of various EGFR signalling elements in tamoxifen-resistant material, suggesting EGFR pathway functionality in such tumours. However, the presence of ER was the most important biological marker in study 57 indicating an increased chance of responding to gefitinib—this significant relationship with ER positivity reflects the gefitinib-sensitive population of acquired tamoxifen-resistant tumours in this study. Equally important findings of this study were the decreases in the Ki67 proliferation marker and in some of the downstream markers of EGFR signal transduction during the gefitinib response. In total, these findings closely parallel the pre-clinical data demonstrating the effectiveness of gefitinib in blocking the EGFR signalling pathway and the growth of ER+ tamoxifen-resistant breast cancer cell models. Such data should be a valuable guide in determining which patient populations to target with EGFR TKIs in future studies. However, future biomarker studies examining the impact of erbB signalling, additional growth factor receptors and the ER pathway on the gefitinib response are likely to be of key importance in further discriminating EGFR TKI-sensitive/resistant patients. Microarray studies may also be important in this regard to reveal EGFR TKI-associated gene changes (Yang et al. 2004). Alongside these various profiling studies, of potential interest may be the study of EGFR mutational status. It has been observed that in patients with NSCLC, specific mutations in the EGFR gene, mostly those in exons 19, 21 and also 18, have a direct correlation with the response to gefitinib (Lynch et al. 2004, Paez et al. 2004). EGFR mutations were also found in a lung adenocarcinoma cell line that was hypersensitive to gefitinib inhibition (Paez et al. 2004). In gefitinib-responsive NSCLC primary tumours, somatic mutations in the tyrosine kinase
domain of the EGFR gene comprise small, in-frame deletions or amino acid substitutions clustered around the ATP binding pocket of the tyrosine kinase domain (Lynch et al. 2004). In vitro, such EGFR mutants demonstrated enhanced tyrosine kinase activity in response to EGF and increased sensitivity to inhibition by gefitinib (Lynch et al. 2004). The EGFR mutations have similarly been linked to erlotinib sensitivity in lung cancer (Pao et al. 2004). In contrast, however, there have been reports of patients with further EGFR mutation developing resistance to gefitinib (Kobayashi et al. 2005). A second point mutation was found in an EGFR-mutant, gefitinib-responsive advanced NSCLC patient who relapsed after two years of complete remission with gefitinib. This mutation resulted in a threonine to methionine change at position 790 in the EGFR (Kobayashi et al. 2005). Clearly, there is an equivalent requirement to examine the mutational status of EGFR in breast cancers, and such studies are ongoing.

The pre-clinical studies and available clinical trial data indicate that the use of EGFR TKIs as mono-therapies, of which gefitinib is the most investigated to date in breast cancer, is likely to be most successful when applied to specific sub-groups of patients (i.e. ER+ tamoxifen-resistant disease). However, pre-clinical studies also suggest that a particularly rewarding avenue could be to use EGFR TKIs in combination with anti-hormonal agents, where anti-tumour effects are significantly improved and emergence of resistance is delayed or even prevented. Trial 225 builds upon these promising pre-clinical data, and is looking to establish if gefitinib can prevent or delay the onset of tamoxifen resistance in the clinic. Other studies are looking to answer similar questions with respect to the aromatase inhibitor, anastrozole, although the preclinical supportive data in this field is much more limited. There is also some pre-clinical data showing the potential for gefitinib to treat, and in combination, prevent resistance to fulvestrant, and studies assessing the clinical efficacy of gefitinib to subvert resistance to fulvestrant are also emerging.

Finally, it should be remembered that EGFR is only one member of the erbB family, and in addition to homodimerization it can be activated following heterodimerization with other erbB receptors (Deb et al. 2001). Additional erbB receptors might therefore be able to activate the EGFR in a manner independent of EGFR kinase activity and to generate signalling that is insensitive to inhibitors of the EGFR tyrosine kinase domain. Furthermore, there is evidence of erbB interplay with alternative growth factor pathways, including IGF-1R signalling that contributes to breast cancer growth and is able to promote EGFR activation (Nicholson et al. 2004). Emerging experimental data suggest these complexities may be able to promote resistance to EGFR TKIs, and, as such, strategies targeting multiple erbB interactions are worthy of therapeutic investigation and may improve anti-tumour effects of the EGFR TKIs. The recently developed agent, pertuzumab (rhuMAb 2C4), a recombinant humanized monoclonal antibody, is of some interest in this regard. It binds to the extracellular domain II of the HER2 receptor and blocks its ability to dimerize with other erbB receptors. This disrupts ligand-dependent HER2 signalling. Pertuzumab thus represents a new class of targeted therapeutics known as HER2 ‘dimerization inhibitors’ that block both homo- and heterodimerization of HER2 (Agus et al. 2005). In pre-clinical studies, pertuzumab proved inhibitory to breast, prostate and NSCLC tumour models, comprising those overexpressing HER2 as well as non-overexpressing cells. A pre-clinical study evaluated combining pertuzumab with the EGFR TKI erlotinib in the HER2/EGFR overexpressing human breast cancer cell line, MDA175. Sub-optimal doses of the combination treatment revealed superior 66% growth inhibition of the cell line compared with 6% and 34% for pertuzumab or erlotinib alone (Totpal et al. 2002). Another pre-clinical study examined the effect of combining pertuzumab with the anti-HER2 antibody trastuzumab in the HER2-overexpressing BT474 breast cancer cell line. This study revealed synergistic inhibition of cell growth, in part because of increased apoptosis. Combination drug treatment reduced levels of total and phosphorylated HER-2 protein and blocked subsequent signalling through Akt (Nahta et al. 2004). In phase I clinical trials, pertuzumab has shown activity in a number of human cancers, and a phase II programme is in progress (Badache & Hynes 2004, Bianco 2004). With further regard to inhibitors of multiple erbB receptors, clinical activity of lapatinib that blocks activity of both EGFR and HER2 has also been reported in a phase I trial (Spector et al. 2003). In total, these recent results and developments suggest that combining one HER-targeting agent with another or with an EGFR-specific TKI may be a more effective and futuristic therapeutic strategy in breast cancer.

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