Inhibitors of growth factor signalling

A E Wakeling

Cancer & Infection Research, AstraZeneca Pharmaceuticals, Alderley Park, Macclesfield, Cheshire SK10 4TG, UK
(Requests for offprints should be addressed to Alan E Wakeling; Email: alan.wakeling@astrazeneca.com)

Abstract

The therapeutic utility of trastuzumab (‘Herceptin’) in breast cancer patients with tumours that overexpress erbB2 established the principle that targeted inhibition of specific signal transduction pathways can provide a new approach to cancer treatment. The ErbB family of protein tyrosine kinases, in particular the epidermal growth factor receptor (EGFR), are commonly overexpressed in many solid human tumours and EGFR was the initial target for a drug discovery programme seeking small molecule inhibitors of the EGFR tyrosine kinase (TK) enzyme activity. The description of the anilinoquinazoline class of potent and selective TK inhibitors led to several candidate drugs from this chemical class, for example gefitinib (‘Iressa’) and erlotinib (‘Tarceva’), which are being evaluated in breast cancer patients. Rapid advances in cancer molecular genetics have identified numerous potential drug targets associated with abnormal control of cell division either downstream of the ErbBs, for example Ras and MEK, or in erbB-associated signalling networks, like Src kinase, which affect the tumour cell motility and invasiveness. Candidate drugs for several of these targets are currently being evaluated; for example, the prenylation inhibitor AZD3409, a mimetic of the CAAX box of K-Ras, inhibits protein farnesyl and geranylgeranyl transferases and a novel, selective, orally active Src kinase inhibitor AZD0530 have entered Phase I clinical trials and may have utility in breast cancer therapy.

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Epidermal growth factor signalling and breast cancer

Since the first report almost 20 years ago that the expression of the epidermal growth factor receptor (EGFR) in human breast tumours indicates a poor prognosis (Sainsbury et al. 1985), there has been an explosion of activity and interest in both receptor and non-receptor kinases as potential targets for the development of new anticancer drugs. In breast cancer patients, proof of principle for this approach was provided by the demonstration that a significant proportion of patients whose tumours overexpress erbB2 (Slamon et al. 1987) can respond favourably to treatment with trastuzumab (Herceptin), an antibody directed at the external domain of ErbB2 (Cobleigh et al. 1999). A drug-discovery programme targeting inhibition of the tyrosine kinase (TK) activity of the EGFR was initiated in 1990 at ICI Pharmaceuticals (the predecessor company of Zeneca Pharmaceuticals, in turn a predecessor company of AstraZeneca Pharmaceuticals; Wakeling 2005). EGFR rather than ErbB2 was the preferred drug target because high levels of EGFR expression are a more common manifestation of the malignant phenotype than are that of ErbB2 in many solid human tumours, including those of the lung (Gullick 1991, Salomon et al. 1995, Nicholson et al. 2001). At that time, targeting EGFR was also consistent with our wish to address new drug targets in breast cancer different from the classical endocrine pathways, and the observation that expression of EGFR is often associated with disease resistant to endocrine therapy. At the beginning of our search for inhibitors of EGFR TK activity we recognized two significant hurdles that would need to be overcome. Firstly, since EGFR is one of four receptor tyrosine kinases.
kinases composing the ErbB family with very similar kinase domains, and the ErbBs are in turn only one of many families of receptor tyrosine kinases, could inhibitors selective for EGFR be found? Secondly, since EGFR is expressed in many normal tissues as well as in solid tumours (Yano et al. 2003), would EGFR TK inhibitors (TKIs) have an acceptable toxicity profile? By analogy with our previous experience with antioestrogens we visualized that TKIs would ideally be well-tolerated cytostatic agents suitable for long-term use in cancer patients.

To discover small molecules that might inhibit EGFR TK activity we used an enzyme preparation from a human tumour cell line (A431) that highly overexpresses EGFR. We tested compounds representative of the chemical diversity in the company compound inventory in a substrate tyrosine-phosphorylation assay (Ward et al. 1994). The discovery at ICI Pharmaceuticals in 1992 of the anilinoquinazoline class of inhibitors (Barker & Davies 1992, Wakeling et al. 1996) proved that potent and selective inhibitors of EGFR TK are accessible. This was subsequently confirmed by other investigators (Fry et al. 1994) and the chemistry of quinazoline-derived TKIs has since expanded to encompass many similar compounds (Bridges 2001). Discovery of this class of inhibitors was the first step in the programme that selected gefitinib (Iressa; ZD1839) as the drug candidate for clinical evaluation (Barker et al. 2001, Wakeling et al. 2002). Clinical trials in patients with non-small-cell lung cancer (NSCLC) established that a single daily oral dose of 250 mg gefitinib has antitumour activity and is sufficiently well-tolerated to permit long-term use (Fukuoka et al. 2003, Kris et al. 2003).

**ErbB inhibitors and breast cancer**

The first clinical studies of gefitinib in breast cancer patients with metastatic disease refractory to multiple previous treatments produced disappointingly low response rates (Albain et al. 2002, Baselga et al. 2003). However, in a study of ER+ patients with acquired resistance to tamoxifen treatment more than 80% responded to treatment with gefitinib (Gutteridge et al. 2004) and response was associated with decreased phosphorylation of EGFR and ERK1/2 and of the proliferative marker Ki67 (Gee et al. 2004). These clinical observations were anticipated by studies with oestrogen receptor-positive (ER+) MCF7 human breast cancer cells which showed that resistance to antioestrogens is associated with up-regulation of the EGFR signalling pathway and that combining antioestrogen and gefitinib treatment delays the development of drug resistance (McClelland et al. 2001, Knowlden et al. 2003). The importance of coexpression of ER and ErbBs in endocrine resistance is further supported by experiments with xenografts of erbB2-transfected MCF7 cells. Gefitinib treatment restored antioestrogen sensitivity and combining gefitinib with fulvestrant or oestrogen withdrawal provided long-term antitumour efficacy and greatly extended the time interval to development of drug resistance compared with single-agent treatments (Shou et al. 2004). These studies have led to clinical trials to evaluate the therapeutic efficacy of combining gefitinib and endocrine therapy; studies are underway with gefitinib plus tamoxifen or anastrazole (Arimidex) or fulvestrant (Faslodex; Kramer & Osborne 2004, Nicholson et al. 2004).

It is difficult to predict how the therapeutic use of ErbB inhibitors will develop. The great complexity of the the ErbB signalling network and the downstream transducers of these signals, in particular components of the ras/raf/mitogen-activated protein kinase (MAPK) and Akt pathways, potentially offer a large number of novel opportunities for targeted breast cancer treatments (Yarden & Sliwkowski 2001, Gullick 2005). At the level of the ErbB receptor family numerous antibody and small-molecule TKIs as well as gefitinib are being investigated in breast cancer patients (Nicholson et al. 2004, Slamon 2004), including erlotinib (Tarceva), an EGFR-selective, reversible TKI, lapatinib (GW572016), a reversible inhibitor which targets both EGFR and ErbB2 (Chu et al. 2005), and canertinib (CI-1033), an irreversible pan-ErbB inhibitor (Britten 2004). There is no obvious means to judge how these different drug-activity profiles will influence the balance between efficacy and tolerability; nor is there yet any means to match the drug and the patient. Whereas it is possible to measure the level of expression of each of the ErbB proteins in tumour cells such measurements, with the notable exception of amplification of erbB2, have failed to predict drug sensitivity (Parra et al. 2004). This should be no surprise because the mere presence of the target reveals nothing about whether the particular receptor is or is not playing a deterministic role in an individual’s disease. Much work remains to define which biomarkers change in response to TKI action, how such changes might predict which patient could benefit from treatment with a specific drug and whether protein- or gene-expression array analysis in sensitive and resistant cells or tumours could help to match patients and drugs (Campbell et al. 2004). Recent studies that related activating mutations in the kinase domain of EGFR to sensitivity to gefitinib in patients with NSCLC (Lynch et al. 2004, Paez et al.
2004) appear unlikely to translate to other tumours, including breast cancer, because no such mutations were found in other tumours (Lee et al. 2005). Thus, today we are ignorant of what determines breast cancer sensitivity to ErbB signalling inhibitors, with the notable exception of erbB2 amplification, although, with respect to gefitinib, the presence or absence of the oestrogen receptor seems to distinguish patients who might respond from those who are much less likely to receive any benefit.

**Future prospects**

Among an extensive AstraZeneca portfolio of other signal transduction inhibitors, targeting cancer cell proliferation by inhibition of Ras farnesylation or MEK (MAPK/extracellular-signal-regulated kinase (ERK) kinase), or cancer cell invasion by inhibition of Src kinase, might be of particular interest in breast cancer. Signalling through the ras/raf/MAPK pathway is often activated in breast cancers so it is not surprising that inhibitors of Ras have demonstrated efficacy (Head & Johnston 2004), and inhibition of MEK, a critical integrator of signals from multiple growth factors in addition to the ErbB family, could be beneficial. The prenylation inhibitor AZD3409 is a mimic of the CAAX box of K-Ras but differs from currently available Ras inhibitors in having activity against geranylgeranyl transferase as well as farnesyl transferase (Stephens et al. 2003). In the absence of any consensus on the clinically relevant target for Ras inhibitors, broader prenylation inhibition might confer an advantage but this remains to be tested in the clinic. Inhibition of MEK with AZD6244 (ARRY-142886; Wallace et al. 2004) should prevent activation and nuclear translocation of ERK1/2, the proximal target of MEK. AZD6244 is a potent selective inhibitor of MEK1/2 that does not compete with ATP and comes from a different chemistry than the TKIs. AZD6244 is a benzoimidazole derivative in contradistinction from the anilinoquinazoline TKIs, which are competitive with ATP. Clinical studies of both AZD3409 and AZD6244 have begun with Phase I trials. Phase I trials of a Src kinase inhibitor AZD0530 have also commenced. Src family kinases function as gatekeepers for many signal transduction pathways and overexpression or hyperactivity of Src kinases is common in human epithelial tumours including breast cancers (Bromann et al. 2004, Ishizawar & Parsons 2004). AZD0530 is a novel selective, orally active, inhibitor of Src-family kinases that has low antiproliferative activity but inhibits tumour cell adhesion, migration and invasion (Green et al. 2004). The direct anti-metastatic activity of Src kinase inhibition has been demonstrated with AZM475271 in a human pancreatic cancer model grown orthotopically in nude mice (Yezhelyev et al. 2004).

**Conclusions**

Inhibition of ErbB signalling seems likely to play an increasingly important role in the treatment of breast cancer. The outcome of Phase II clinical trials currently in progress with several first-generation TKIs representing different profiles of selectivity for ErbB kinase inhibition should begin to answer important questions about how inhibitor profile affects the balance between efficacy and toxicity. Pre-clinical studies on the mechanisms of resistance to endocrine treatment have provided a cogent case that combining TKIs with hormonal therapy, particularly with anti-oestrogens, might delay or even prevent the development of resistance to antioestrogen treatment. Other signal transducers that work either downstream of the ErbBs, like Ras and MEK, or in concert with ErbBs, like Src, have now been targeted with novel small-molecule inhibitors that have recently entered clinical trials. One or more of these compounds may prove useful in breast cancer patients because hyperproliferative or invasive phenotypes are common but, drawing on the lessons learned from the absence of any simple correlation between ErbB expression and sensitivity to TKIs, it is probable that effective use of these new agents will depend on the discovery of biomarkers that relate drug action to response in individual patients.

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