Epidermal growth factor receptor and signal transduction: potential targets for anti-cancer therapy

Sumanta Kumar Pal[a] and Mark Pegram[a]

Agents targeting the epidermal growth factor receptor (EGFR) pathway hold particular promise for the treatment of patients with advanced disease, for whom standard chemotherapy is generally palliative. Expression of EGFR on numerous types of solid tumors, and the association of EGFR activation with tumorigenic processes including proliferation, anti-apoptosis and metastatic spread, make this pathway a particularly compelling target for rational drug design. The two classes of anti-EGFR agents in late-stage clinical testing include antibodies directed toward the extracellular EGFR domain (cetuximab, panitumumab) and small molecule tyrosine kinase inhibitors (gefitinib, erlotinib), which inactivate the receptor enzyme activity. However, important issues remain to be addressed. These include the development of appropriate predictive markers for response, such as improved tests for EGFR activity, correlation of rash with response and potential pharmacogenomic approaches; the sequencing and combination of these agents with chemotherapy and irradiation; and the possible role of these agents in the treatment of patients with earlier stage disease. *Anti-Cancer Drugs* 16:483–494 © 2005 Lippincott Williams & Wilkins.

Anti-Cancer Drugs 2005, **16**:483–494

Keywords: cetuximab, colorectal cancer, epidermal growth factor receptor, erlotinib, gefitinib, non-small cell lung cancer, squamous cell carcinoma of the head and neck

[a]UCLA Center for the Health Sciences, Los Angeles, CA 90095, USA.

Correspondence to M. Pegram, UCLA Center for the Health Sciences, 11-934 Factor Building, Mail Code 167817, Los Angeles, CA 90095, USA.
Tel: +1 310 206-9841; fax: +1 310 267-2858;
e-mail: mpegram@ucla.edu

Received 18 January 2005 **Accepted** 8 February 2005

Introduction

Conventional cytotoxic therapies are associated with significant morbidity in patients with solid tumors such as advanced colorectal cancer (CRC) and squamous cell carcinoma of the head and neck (SCCHN), and may be of limited benefit for survival [1–4]. The outcome for patients with advanced or late-stage cancers has improved only marginally over the past 30 years and in many cases chemotherapy has a palliative role at best. Therefore, the need remains for novel anti-cancer therapies that effectively and specifically target epithelial tumor cells while minimizing the toxic side-effects commonly associated with conventional cytotoxic therapies. In recent years, an improved understanding of the signal transduction pathways involved in cancer growth and spread has provided promising avenues for developing targeted anti-cancer therapy. One such target is the epidermal growth factor receptor (EGFR), a receptor tyrosine kinase with pleiotropic intracellular effects.

Malignant cells have a number of properties that distinguish them from healthy cells; these include the capacity for autocrine growth stimulation, insensitivity to growth regulatory factors such as transforming growth factor (TGF)-β, ability to evade apoptosis, unlimited replication potential (due to dysregulated telomerase activation), ability to promote angiogenesis, and capability for tissue invasion and metastasis [5]. An increasing body of evidence clearly demonstrates that, with the exception of telomerase activation, EGFR-mediated signaling pathways are involved in many, if not all, of these tumorigenic processes [6]. In addition, experimental and epidemiological evidence suggests that the activation of EGFR may promote resistance to cytotoxic or even hormonal therapies, and that patients with EGFR-expressing tumors are less responsive to conventional chemotherapy and radiation [7–9].

EGFR is expressed in all epithelial and stromal tissues, as well as in some glial and smooth muscle cells, but it is not found in hematopoietic cells [10,11]. Growth factor-induced EGFR signaling is important in many normal cellular processes, with effects ranging from apoptosis to migration and differentiation [11,12]. However, one primary biological response to EGFR stimulation is cell proliferation [10]. A significant proportion of solid tumors express the EGFR. Also, aberrant EGFR tyrosine kinase activity is observed in many tumors, and has been ascribed to dysregulated receptor expression, altered receptor structure and/or establishment of autocrine or paracrine growth-signaling pathways [13–16].

Clinical studies indicate that EGFR expression may be associated with poor prognosis in bladder, cervical, ovarian and esophageal cancers, and in SCCHN; it may also be linked to reduced survival rates in gastric, breast, colorectal and endometrial cancers [17]. It is likely, however, that the clinical significance of EGFR activity

0959-4973

has been underestimated because of the lack of standardization of EGFR detection methods in these retrospective analyses [17]. The apparent association of EGFR with poor patient outcome may also reflect its role in the promotion of tumor invasion and metastasis [18,19], which together account for most cancer deaths.

The high level of redundancy in growth factor networks, coupled with the increased requirement of tumor cells for growth stimulatory mechanisms, is such that the selective inhibition of signaling pathways involved in cancer, such as those initiated by EGFR activation, should be possible without causing significant disruption to healthy cells [20,21].

Human epidermal receptor (HER) family of receptor tyrosine kinases

EGFR is one of a family of four structurally related transmembrane growth factor receptors: EGFR (also known as HER1/ErbB-1), HER2/*neu* (ErbB-2), HER3 (ErbB-3) and HER4 (ErbB-4). Each of these receptors is composed of an extracellular ligand-binding domain and a cytoplasmic signal transduction domain, separated by a hydrophobic membrane-spanning region. Each receptor interacts with a set of preferred growth factor ligands, which appear to bind to the extracellular domain with one-to-one stoichiometry [10]. Ligands specific for EGFR include EGF, amphiregulin and TGF-α. The ligands β-cellulin, epiregulin and heparin-binding epidermal growth factor (HB-EGF) bind to both EGFR and HER4, whereas neuregulin binds to HER3 and HER4, as well as their respective heterodimers [11,22]. To date, no ligand has been identified for HER2.

Binding of cognate ligand to receptor induces subsequent homodimerization of EGFR or heterodimerization of EGFR with other HER family members, affording a 2:2 ligand:receptor complex that activates the receptor intracellular kinase activity through a proximity effect [23]. This leads to the phosphorylation of critical tyrosine residues on the cytoplasmic domain, which form docking sites for intracellular effector and adaptor proteins that are then released in active form to stimulate complex signal transduction networks [12]. This mechanism is regarded as the primary, although not necessarily the only, mechanism for the transmission and amplification of HER family growth factor signals [10].

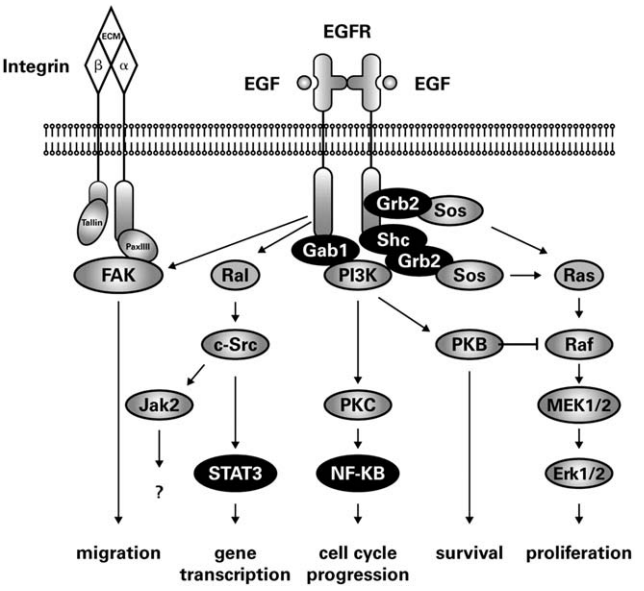
The receptor–ligand complex is then removed from the cell surface by endocytosis through clathrin-coated pits. Subsequent sorting in the early endosome determines whether the receptor–ligand complex is degraded by way of a lysosome pathway or recycled to the cell surface. The time to endocytosis and the eventual fate of the complex are influenced by the composition of the receptor dimer and the nature of the bound ligand. EGFR homodimer complexes are internalized rapidly and tend to remain stable within the early endosome, thus allowing ubiquitination of the receptor and its degradation by the lysosome. In contrast, EGFR–HER2 heterodimers remain at the cell surface longer and are less stable in the early endosome, preventing ubiquitination and thus sending the receptors through the default recycling pathway [12]. In addition, pH-sensitive ligands such as TGF-α promote recycling by dissociating readily from the receptor in the mildly acidic endosome, whereas EGF remains bound to the receptor and directs receptor degradation [11]. The net result of internalization is down-regulation of EGFR, leading to the attenuation of growth factor signaling.

EGFR signal transduction cascade and tumorigenesis

The complexity of the signal transduction cascades initiated by EGFR activation is such that individual stimuli generally cannot be traced through intracellular pathways to a specific cellular response [12]. However, some of the basic features of EGFR signal transduction have been elucidated (Fig. 1) and in recent years the importance of signaling between receptors (receptor 'cross-talk') also has become apparent.

One of the better-understood EGFR signal transduction pathways is the serine/threonine mitogen-activated protein (MAP) kinase cascade. Phosphorylated EGFR is known to recruit the adaptor proteins Shc and Grb2, which act through the exchange factor Sos to activate Ras. The subsequent activation of Raf and MAP kinase/extracellular signal-regulated kinase (ERK), or MEK,

Fig. 1



Signaling pathways induced by EGFR activation.

leads to the phosphorylation of the MAP kinase ERK1/2, which induces the expression of transcription factors such as Elk-1 and c-*fos* [22]. The activation of the MAP kinase pathway is associated with cell division and its aberrant activity may be involved in the uncontrolled cell proliferation that occurs in tumors.

EGFR also promotes cell division and survival through activation of the lipid kinase phosphotidylinositol-3 kinase (PI3K). The adaptor protein Gab1 is thought to activate PI3K, setting up a positive feedback loop that enhances Gab1 recruitment to the receptor. Activated PI3K phosphorylates protein kinase B (PKB) and leads to anti-apoptotic signaling through the transcription factor nuclear factor (NF)-κB. PI3K also activates NF-κB through the induction of protein kinase C (PKC), a pathway known to be involved in cell cycle progression in some cancers [22].

A third pathway initiated by EGFR activation is mediated by c-Src, a cytoplasmic tyrosine kinase involved in many cellular processes, including mitogenic signaling [22]. There is a range of known c-Src substrates, including EGFR itself. However, the c-Src-mediated induction of the signal transducer and activator of transcription (STAT) family of transcription factors appears to be of particular importance in the proliferation and survival of cancer cells. Indeed, activation of STAT3 is required for the TGF-α-induced autocrine growth of transformed epithelial cells [24], while down-modulation of STAT3 in SCCHN cells has been shown to inhibit tumor cell growth and stimulate apoptosis [25]. Of note, EGFR-induced activation of the STAT3 pathway has also been implicated in the resistance of tumor cells to cytotoxic therapy [9].

Exposure to EGF increases the invasiveness of many normal and malignant cell lines, and several EGFR-mediated signaling pathways thought to promote cancer cell motility have been identified. EGFR-induced activation of the phospholipase C (PLC) γ pathway has been linked to increased cell motility, possibly by initiating a 'communication' signaling pathway between EGFR and cell adhesion receptors [19]. The activation of PLCγ is required, but not sufficient, for EGF-induced motility, which also appears to involve MEK activation [19]. The interaction of EGFR with the integrin signaling pathway, mediated by focal adhesion kinase (FAK), is also of interest in tumor invasion and metastasis. Phosphorylation of the EGFR tyrosine kinase has been shown to inactivate FAK, leading to the disruption of cell–cell and cell–matrix interactions, and promoting cell motility and invasiveness [26].

Finally, it has become apparent that EGFR activation stimulates the production of pro-angiogenic factors through multiple signaling pathways. For example, in addition to its proliferative effects, STAT3 regulates the production of the angiogenic factor vascular endothelial growth factor (VEGF) [27]. Similarly, activation of the MEK pathway results in the activator protein (AP)-1-dependent production of both interleukin (IL)-8 and VEGF, while activation of PI3K results in IL-8 production through an NF-κB-dependent pathway [28].

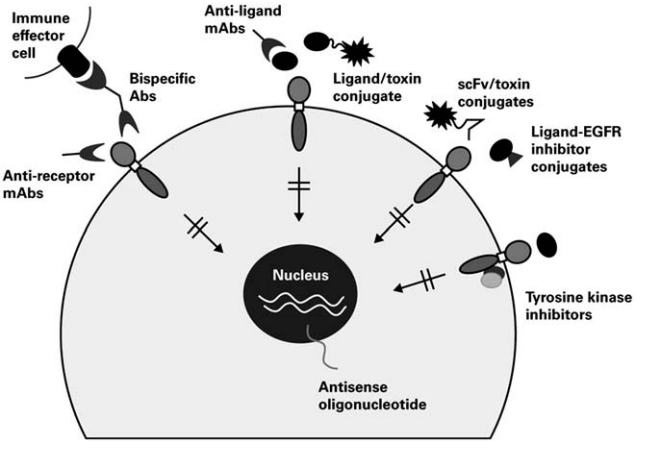
Targeting EGFR pathways in cancer therapy

The growing realization that multiple tumorigenic processes are regulated by EGFR-signaling pathways has resulted in an abundance of research over the last two decades into the mechanisms and effects of EGFR inhibition. Various strategies have been employed to elicit EGFR pathway blockade, including monoclonal antibodies (mAbs) directed toward EGFR or one of its ligands, bispecific antibodies, immunotoxin conjugates, ligand–EGFR inhibitor conjugates, ligand–toxin conjugates, antisense oligonucleotides and tyrosine kinase inhibitors (Fig. 2). Many of these agents are currently undergoing clinical testing (Table 1). Two of the more extensively tested strategies are the anti-receptor mAbs, such as cetuximab (IMC-C225; Erbitux), and inhibitors of the receptor tyrosine kinase, such as gefitinib (ZD1839; Iressa) and erlotinib (OSI-774; Tarceva). Numerous studies conducted in cell culture and in mouse xenograft models of human cancer have demonstrated that EGFR blockade inhibits many mechanisms of tumor progression.

Inhibition of proliferation

In preclinical studies, cetuximab, gefitinib and erlotinib inhibited tumor cell proliferation by inducing G_1 cell cycle arrest in a variety of carcinoma cell lines, an effect

Fig. 2



Strategies to inhibit EGFR signaling pathways. scFv, single-chain Fv (variable region-containing fragment) antibodies.

Table 1 EGFR inhibitors in clinical trials

EGFR inhibitor	Sponsor	Description	Trial status
mAbs			
Cetuximab [IMC-C225 (Erbitux)]	ImClone Systems/Bristol-Myers Squibb	human:murine chimeric anti-EGFR mAb	II/III (FDA approved for metastatic CRC)
ABX-EGF	Abgenix	human anti-EGFR mAb	II/III
EMD 72000	Merck	humanized anti-EGFR mAb	I/II
hR3 (TheraCIM)	YM BioSciences	humanized anti-EGFR mAb	II
ICR62	Institute for Cancer Research	rat anti-EGFR mAb	I/II
Bispecific antibodies			
MDX-447	Medarex	humanized anti-EGFR/anti-CD64 antibody	II
Tyrosine kinase inhibitors			
Gefitinib [ZD1839 (Iressa)] (FDA approved for NSCLC)	AstraZeneca	quinazoline reversible EGFR tyrosine kinase inhibitor	II/III
Erlotinib [OSI-774 (Tarceva)] (FDA approved for NSCLC)	OSI Pharmaceuticals	quinazoline reversible EGFR tyrosine kinase inhibitor	II/III
EKB-569	Wyeth-Ayerst	3-cyanoquinoline irreversible EGFR tyrosine kinase inhibitor	II
PKI-166	Novartis	pyrrolo-pyrimidine irreversible EGFR tyrosine kinase inhibitor	I
GW-572016 (Lapatinib)	GlaxoSmithKline	quinazoline reversible inhibitor of EGFR and HER2 tyrosine kinases	II/III
CI-1033	Pfizer	quinazoline irreversible inhibitor of multiple HER family tyrosine kinases	II
EGFR ligand–toxin conjugate			
TP-38	Duke University/National Cancer Institute	TGF-α fused to a mutated form of Pseudomonas exotoxin	I/II

that was attributed to EGFR blockade-induced up-regulation/accumulation of the cyclin-dependent kinase inhibitors $p27^{Kip1}$ and/or $p21^{CIP1/WAF1}$ [29–33].

Cetuximab- and gefitinib-induced growth suppression of human tumor cell lines has also been shown to correlate with inhibition of ERK1/2 activation [34], supporting the hypothesis that inhibition of the ERK1/2 MAP kinase pathway may be involved in the anti-proliferative effects of these agents. In addition, the effects of these EGFR-blocking agents on the ERK1/2 pathway may release cells from insensitivity to TGF-β-mediated growth inhibition, as evidence suggests that EGFR-mediated activation of the Ras–MEK–ERK pathway prevents the growth-inhibitory effects of TGF-β [35].

Inhibition of angiogenesis and metastasis

It has been postulated that the abrogation of angiogenesis contributes to the *in vivo* effects of EGFR-blocking agents, a theory that helps explain why many of these agents appear to be more effective against tumor xenografts than in cell culture models [36,37]. Several *in vitro* studies have demonstrated down-regulation of the pro-angiogenic factors VEGF, basic fibroblast growth factor, IL-8 or TGF-α following treatment of EGFR-expressing cells with cetuximab, gefitinib, and the mAbs ABX-EGF and ICR62 [37–40]. Similar observations have been made in xenograft tumor models, where down-regulation of angiogenesis factors by cetuximab, gefitinib and the tyrosine kinase inhibitor PKI-166 was accompanied by a reduction in the number of new blood vessels [37,38,41]. Consistent with its effects on angiogenesis, cetuximab has also been shown to reduce tumor metastases in human transitional cell carcinoma and pancreatic carcinoma xenograft models [36,37,42].

Induction of apoptosis

Although the effects of EGFR blockade are largely cytostatic, treatment of various cancer cell lines with cetuximab, EGFR antisense oligonucleotides and several EGFR tyrosine kinase inhibitors has been shown to increase apoptosis in subsets of tumor cells [31,43–48]. The mechanism of induction of apoptosis by anti-EGFR agents has not been fully elucidated, but it appears that pro-apoptotic molecules are potentiated, and anti-apoptotic molecules are depressed, by EGFR blockade. For example, cetuximab-induced apoptosis is accompanied by up-regulation of the pro-apoptotic molecule Bax and down-regulation of the anti-apoptotic molecule Bcl-2 in squamous cell carcinoma cell lines [48], and is accompanied by the sequential induction of pro-apoptotic caspases in DiFi colon adenocarcinoma cells [49]. In addition to promoting apoptosis in tumor cells, cetuximab has been shown to induce apoptosis in endothelial cells in xenograft models of prostate and pancreatic carcinoma [42,50], suggesting that EGFR-mediated apoptotic effects may contribute to the anti-angiogenic mechanism of EGFR blockade.

Increasing tumor sensitivity to cytotoxic therapy

A large body of evidence supports a role for EGFR blockade in potentiating the effects of conventional chemotherapy and radiation therapy [6,41,51–57]. The increase in efficacy from combining EGFR inhibition with cytotoxic therapy is at least additive and, in some cases, appears to be synergistic. Although the mechanism of

enhancement has not been fully elucidated, it may involve augmented activation of pro-apoptotic pathways, coupled with a decrease in proliferation and/or abrogation of angiogenesis [6,36]. In addition, there is evidence that EGFR blockade can inhibit tumor cell recovery and repair following cytotoxic therapy [58].

Specificity of EGFR blockade

Although the common goal of EGFR-targeted approaches is to inhibit receptor autophosphorylation and downstream signaling pathways, the various classes of anti-EGFR agents differ in their mechanism, specificity and selectivity of EGFR blockade, and thus may produce different effects in the clinic. This is illustrated by a comparison between the clinical experiences with the mAb cetuximab and the tyrosine kinase inhibitor gefitinib.

mAbs: cetuximab

Highly specific targeting of the extracellular ligand-binding domain of the EGFR with mAbs is a rational therapeutic approach for EGFR-expressing cancers. Cetuximab, a chimeric IgG1 mAb, was the first of the anti-EGFR mAbs to be developed and has demonstrated anti-tumor activity against a wide range of tumor cell lines and human tumor xenografts in numerous preclinical studies [6,59]. In addition, a number of studies have shown that enhanced anti-tumor activity is achieved by combining cetuximab with conventional cytotoxic therapies [36,42,49,60–65]. In contrast, VEGF inhibitors such as bevacizumab have not demonstrated enhanced activity in second-line combination with standard irinotecan and oxaliplatin-based regimens [66]. Consistent with the pleiotropic nature of EGFR-signaling pathways, the *in vivo* effects of cetuximab have been attributed to the inhibition of proliferation, induction of apoptosis, and abrogation of angiogenesis and metastasis. Cetuximab has recently been approved by the US Food and Drug Administration (FDA), either as monotherapy or in combination with irinotecan, for the treatment of advanced CRC with detectable EGFR expression. Extensive phase II and III clinical testing of cetuximab, either alone or in combination with chemotherapy or radiation, has continued in CRC [67–71], pancreatic carcinoma [72] and non–small cell lung cancer (NSCLC) [73,74], with particularly promising data in the treatment of SCCHN [43].

Like other anti-EGFR mAbs, cetuximab targets the extracellular domain of the EGFR and is exclusive to EGFR. Cetuximab was first developed as a murine mAb (mAb 225) that competitively inhibits EGFR ligand binding, but was chimerized subsequently with human IgG1 to minimize the potential for development of human anti-murine antibody (HAMA) responses. The chimerized version retains the properties of the original mAb, but was shown to bind to the EGFR with higher affinity (K_d = 0.1–0.2 nM, approximately 10 times greater than the natural ligands) and to produce greater growth inhibition of established A431 epidermoid carcinoma xenografts in nude mice compared with the murine version [75]. Published phase I studies have shown that as few as 0% of patients developed human anti-chimeric antibodies (HACAs) [76] and a review of 120 patients treated with cetuximab found HACAs in less than 4% of patients [77], demonstrating the success of the chimerized antibody approach. By comparison, 20% of patients receiving the rat anti-EGFR mAb ICR62 developed HAMA responses [78], thus increasing the potential for resistance to this antibody.

The binding of cetuximab prevents ligand-induced activation of the receptor tyrosine kinase and leads to receptor internalization to the endosomal compartment [79,80]. Once internalized, EGFR is no longer available for ligand binding at the cell surface. Studies with mAb 225 demonstrate that the antibody-induced receptor internalization and processing follow a time line similar to that induced by the natural ligands for EGFR, greatly reducing the half-life of cell-surface EGFR and effectively down-regulating the cell-surface expression of the receptor [81,82]. Once cetuximab-bound EGFR is internalized, EGFR is degraded [83]. Collectively, these results suggest that removal of EGFR from the cell surface may contribute to or potentiate the anti-tumor effects of cetuximab.

Therapeutic antibodies of the IgG1 isotype [84], such as cetuximab, potentially support immune system effector functions such as antibody-dependent cell-mediated cytotoxicity (ADCC) through the antibody Fc domain [85]. Although smaller bivalent $F(ab')_2$ forms of mAb 225 lacking the Fc region were able to inhibit the growth of human cancer xenografts [86]; in another set of experiments, both mAb 225 and cetuximab were shown to promote ADCC: mAb 225 through the activation of mouse splenocytes and cetuximab, through the activation of human peripheral blood mononuclear cells [87]. The mAbs ICR62 and EMD 72000 also have demonstrated strong anti-tumor ADCC *in vitro* [88]. These findings suggest that cetuximab (and other anti-EGFR mAbs) may enhance toxicity to EGFR-expressing tumor cells in human patients by initiating ADCC.

Phase I studies in patients with advanced EGFR-expressing solid tumors—including CRC, NSCLC, SCCHN, and breast, ovarian and prostate carcinoma—have demonstrated that cetuximab is well tolerated, either as a single agent or in combination with cisplatin or radiation therapy, with minimal overlapping toxicities to conventional therapy [76,89,90]. The most clinically relevant adverse events attributable to cetuximab were allergic reactions and skin toxicity (characterized as an acneiform rash) [91]. Allergic reactions were successfully managed with standard

medications, and skin toxicity was reversible and not dose limiting [92].

Data from several phase II trials with cetuximab in combination with chemotherapy have been reported. In patients with recurrent and/or metastatic SCCHN who had previously progressed on a platinum-based regimen, the combination of cetuximab plus cisplatin or carboplatin produced a 14.6% overall response rate (two complete responses and 12 partial responses from 96 evaluable patients) and 39.6% of patients had stable disease or minor responses lasting at least 6 weeks [93]. The median survival time for responders was 269 days, compared with 178 days for all patients. The combination of cetuximab and platinum was well tolerated in this study. In another phase II trial of cetuximab plus docetaxel in patients with NSCLC who had failed one prior chemotherapy regimen, 13 of 47 evaluable patients (28%) achieved a partial response and eight patients (17%) had stable disease [94]. The regimen was well tolerated with minimal toxicities; the most common grade 3 toxicities were infection (21% of patients), fatigue (21%) and acneiform rash (19%).

Two studies have investigated the combination of cetuximab, irinotecan, fluorouracil and leucovorin in patients with previously untreated metastatic CRC. In the first trial, this combination produced a 44% partial response rate [95]; in the second, which used high-dose fluorouracil/leucovorin, 74% of patients achieved objective responses [96]. Dose reductions of cetuximab were not required, although most patients required irinotecan and/or fluorouracil dose modifications. The results of these trials demonstrate the feasibility and potential therapeutic benefit of incorporating cetuximab into first-line therapeutic regimens. Similarly, the combination of gemcitabine and cetuximab showed promising activity and was well tolerated in a phase II trial in chemotherapy-naive patients with advanced pancreatic cancer [72].

A phase II trial of cetuximab plus irinotecan in patients with CRC who had previously progressed on irinotecan alone produced encouraging results, with a 22.5% overall response rate (complete and partial responders) [97,98]. This response rate is notable given the treatment-refractory nature of the patient population under study. The median duration of response was 147 days and median time to progression was 186 days [97]. A second trial was conducted to determine the objective confirmed response rate associated with cetuximab plus irinotecan combination therapy or cetuximab monotherapy in patients with irinotecan-refractory metastatic CRC [70]. Results confirmed the findings from the study by Saltz *et al.*, showing a response rate of 22.9% (median time to progression 4.1 months) in patients receiving cetuximab plus irinotecan, and 10.8% (median time to progression, 1.5 months) in patients receiving cetuximab alone. In both studies, toxicities were consistent with the known safety profiles of irinotecan and cetuximab, and were manageable with standard therapies. Results from these studies in patients with CRC led to FDA approval of cetuximab, either as a single agent or in combination with irinotecan, for the treatment of EGFR-expressing, metastatic CRC in patients intolerant of or refractory to irinotecan-based chemotherapy, respectively [99].

In addition to the results achieved with cetuximab/irinotecan combination therapy, the aforementioned trial by Cunningham *et al.* also highlighted the clinical activity of cetuximab monotherapy in metastatic CRC [70]. A third phase II trial was conducted to investigate cetuximab monotherapy in patients with EGFR-expressing CRC refractory to both irinotecan and fluorouracil [71]. Cetuximab monotherapy produced a 9% partial response rate and 37% of patients had stable disease or minor responses. The median survival in these previously treated, chemotherapy-refractory CRC patients was 6.4 months [71].

Cetuximab has also recently demonstrated promising clinical activity in SCCHN. Cetuximab is the first and only anti-EGFR antibody in a phase III randomized, controlled clinical trial to demonstrate a statistically significant increase in survival and locoregional control [43]. In this study, the largest clinical trial to date of pharmacotherapy plus radiation therapy in the treatment of SCCHN, patients with locoregionally advanced disease who received cetuximab plus high-dose radiation showed a statistically significant 26-month improvement in overall survival compared to patients who received radiation alone.

Tyrosine kinase inhibitors: gefitinib

In contrast to the mAbs, EGFR tyrosine kinase inhibitors exert their activity intracellularly at the level of tyrosine kinase phosphorylation. These small-molecule, low-molecular-weight agents, usually quinazoline- or pyrazolo/pyrrolo/pyrido-pyrimidine derivatives, are thought to block tyrosine kinase activity by attaching to the adenosine triphosphate (ATP) binding site and preventing receptor autophosphorylation. The EGFR tyrosine kinase inhibitors differ among themselves in their potency, specificity for EGFR, reversibility of action and bioavailability. In addition to reversible (gefitinib, erlotinib) and irreversible (EKB-569, PKI-166) EGFR-selective tyrosine kinase inhibitors, several pan-ErbB family tyrosine kinase inhibitors have been developed. CI-1033, which inhibits EGRF, HER2 and HER4 tyrosine kinases (HER3 lacks an intrinsic tyrosine kinase domain), and GW-572016 (Lapatinib), which targets both EGFR and HER2, have both entered clinical trials. At present, there is insufficient data to determine whether any clinically significant differences exist between the different classes of tyrosine kinase inhibitors.

Gefitinib is a quinazoline-based reversible tyrosine kinase inhibitor that displays selectivity for the EGFR and is the best characterized of this class of agents. As is typical of the EGFR tyrosine kinase inhibitors, gefitinib selectively inhibits the EGFR tyrosine kinase with about 100-fold greater potency than other tyrosine or serine/threonine kinases [52]. Gefitinib has been shown to prevent autophosphorylation of EGFR and to block EGFR-mediated signal transduction pathways in a number of tumor cell lines in culture [52]. In preclinical studies, gefitinib produced reversible growth inhibition and growth delay in cultured tumor cells and human tumor xenografts [52]. As with cetuximab, anti-tumor activity was enhanced by combining gefitinib with a cytotoxic agent [44,57] In addition to NSCLC, gefitinib has entered phase II/III clinical testing in prostate, breast, bladder and renal cell carcinoma.

In a phase I trial conducted in patients with various EGFR-expressing tumors, including NSCLC and CRC as well as breast, ovarian and renal cell carcinoma, gefitinib was found to be well tolerated, with dose-limiting toxicity (grade 3 diarrhea) observed at doses at or above 700 mg/day [100]. Four of 16 NSCLC patients experienced a partial response to therapy.

Results from several phase II trials of gefitinib monotherapy have been reported. In a phase II study in patients with recurrent or metastatic SCCHN, gefitinib (500 mg/day) produced one complete response and four partial responses from 47 evaluable patients, with an overall response rate (complete and partial responders) of 10.6% and a disease control rate (complete response, partial response or stable disease) of 53% [101]. However, gefitinib monotherapy produced no objective responses in phase II trials in patients with advanced renal cell carcinoma [102], recurrent glioma [103] or CRC [104].

More encouragingly, in patients with advanced NSCLC who had failed one or two chemotherapy regimens, gefitinib achieved response rates of 18.4% (at 250 mg/day) and 19.0% (at 500 mg/day) [105]. In a second study conducted in patients with advanced NSCLC who had failed two or more previous chemotherapy regimens, the response rate (all partial responses) was 12% for patients receiving gefitinib 250 mg/day and 9% for patients receiving 500 mg/day [106]. Results from these phase II studies led to FDA approval of gefitinib as monotherapy for patients with locally advanced or metastatic NSCLC after failure of both platinum-based and docetaxel chemotherapies [107]. Why only a small subset of patients appears to respond to gefitinib is the subject of much debate. In a combined analysis of tumor samples from the above two trials, there were no consistent associations between levels of EGFR expression and radiographic or symptomatic improvement [108]. Other recent findings may help provide an answer [109,110]. These researchers independently identified somatic mutations in the tyrosine kinase domain of the EGFR gene in subsets of NSCLC patients; these somatic mutations confer susceptibility to gefitinib. Screening for these mutations may help identify patients with NSCLC who are more likely to respond to gefitinib or gefitinib-based chemotherapy regimens.

Trials of gefitinib in combination with chemotherapy in chemotherapy-naive patients with advanced stage III and IV NSCLC have been less successful. Two large phase III double-blind, placebo-controlled trials, known as the Iressa in NSCLC Trial Assessing Combination Therapy (INTACT) trials, were conducted in more than 2000 patients. In the first trial (INTACT 1), patients received gemcitabine and cisplatin with or without gefitinib [111]; and in the second (INTACT 2), patients received paclitaxel and carboplatin with or without gefitinib [112]. In both trials, the addition of gefitinib to chemotherapy in patients with NSCLC did not result in improved efficacy (survival benefit, time to progression or response rate) compared with chemotherapy alone [111,112]. The reasons for these disappointing results are unclear, but may reflect the fact that patients in these studies (as with most other studies of gefitinib) were not selected on the basis of tumor EGFR activation state or EGFR kinase domain mutation. Tissue from tumor biopsies from patients in the two studies is currently being examined in an attempt to identify the targets and mechanisms of response and resistance to therapy. Historically, three-drug chemotherapy regimens have not produced better survival outcomes than two-drug regimens for advanced NSCLC [113]. It is possible that gefitinib may be more effectively combined with chemotherapy when administered in a sequential or pulsatile fashion; such approaches are currently being investigated in preclinical studies [114,115].

Clinically relevant differences between anti-EGFR mAbs and tyrosine kinase inhibitors

In addition to their differences with respect to ADCC and receptor internalization, mAbs and tyrosine kinase inhibitors possess several different characteristics that may prove significant in a clinical setting. One major difference is in the route of administration of the two classes of agents. Whereas the mAbs are given by i.v. infusion, the tyrosine kinase inhibitors are typically administered orally. Thus, tyrosine kinase inhibitors come directly into contact with epithelial cells in the intestine, in contrast to mAbs, which are macromolecules and have limited access to the intestinal epithelium from the systemic circulation. Unlike most normal cells, the EGFR-expressing cells of the intestinal epithelium are in a constant state of renewal and may be susceptible to inhibition of EGFR function. This may explain why tyrosine kinase inhibitors, unlike mAbs, are associated with significant intestinal epithelial toxicity. As a

consequence, clinically significant diarrhea is commonly observed in patients receiving EGFR tyrosine kinase inhibitors [100,116–118]. In contrast to cetuximab clinical trials, gefitinib and erlotinib clinical trials are being conducted at or near their drug's maximum tolerated dose, with dose escalations limited by gastrointestinal and/or skin toxicities, and dose reductions are often required [100,116,119–121].

Unlike mAbs, which bind to the external ligand-binding site of EGFR and subsequently internalize the entire complex, tyrosine kinase inhibitors bind to the internal tyrosine kinase domain of the receptor, thus requiring that they diffuse across the cell membrane to reach their target. Given that these molecules are selective, but not completely specific, for EGFR, the tumor specificity of tyrosine kinase inhibitors may be reduced compared with mAbs. Many EGFR tyrosine kinase inhibitors inhibit other protein kinases at higher doses, increasing the potential for a lack of receptor selectivity and increased toxicity if dose escalation is attempted.

The small-molecule tyrosine kinase inhibitors, unlike mAbs, are eliminated from the body by cytochrome P450 enzymes. These enzymes are also involved in eliminating small-molecule chemotherapy drugs. Thus, although other small molecules may interfere with the elimination of EGFR tyrosine kinase inhibitors (or *vice versa*), they are highly unlikely to interfere with the elimination of EGFR mAbs. The different mechanisms of action and routes of elimination of mAbs and tyrosine kinase inhibitors may result in different dosing schedules in patients. Thus, mAbs such as cetuximab, which has a half-life of 4–7 days [76,89], may be administered once weekly, while the tyrosine kinase inhibitors require more frequent, usually daily, dosing.

Does EGFR expression level in tumors correlate with response to anti-EGFR therapy?

As the results of a growing number of preclinical studies and clinical trials become available, data are beginning to emerge about the relationship between the anti-tumor efficacy of EGFR inhibition and the level of EGFR expression on tumor cells. Using current immunohistochemical detection methods, a cell is deemed EGFR-positive if it expresses more than approximately 30 000 receptors per cell. By definition, agents targeting EGFR-positive tumors would not be expected to display efficacy toward tumors lacking any EGFR expression. This has been observed in tumor xenograft studies with ABX-EGF, where the anti-tumor effect of this mAb was only seen in tumors that expressed more than 17 000 EGFR receptors per cell [122]. Similarly, carcinomas with low EGFR protein levels (below 70 fmol/mg) were not susceptible to the mAb EMD 72000 in xenotransplants derived from cancer cell lines [123].

Consequently, clinical trials with agents other than gefitinib are being conducted in patients with EGFR-positive tumors, and cetuximab is indicated only for EGFR-positive colorectal tumors. Concurrent with the FDA approval of cetuximab, DakoCytomation also received FDA approval for the use of its EGFR pharmDx kit as an aid to identify CRC patients eligible for treatment with cetuximab [124]. Notably, however, preliminary analyses suggest that the clinical efficacy of anti-EGFR agents is not related to the relative levels of EGFR expression in these patients. In the phase II trial of cetuximab in combination with irinotecan in irinotecan-refractory CRC patients, response rates were not correlated with EGFR expression levels [98]. Similarly, in a phase II trial conducted in 56 patients with advanced NSCLC, tumor response to the tyrosine kinase inhibitor erlotinib was not associated with either a higher percentage of tumor cells positive for EGFR or more intensive EGFR staining [119].

Of note, these results contrast with the clinical response to the anti-HER2 mAb trastuzumab (Herceptin), which was found to correlate with a high level of HER2 expression resulting from gene amplification in patients with HER2-positive breast tumors [125].

Does skin rash predict response to EGFR blockade?

An important aspect of any cancer therapy, especially in patients with advanced cancers and shorter life expectancies, is the ability to predict response to therapy early in a therapeutic regimen. It is becoming increasingly apparent that the acne-like rash commonly observed in patients receiving EGFR inhibitory therapy [91,98,100, 116,126,127] may be predictive of a therapeutic response to EGFR blockade. The appearance of skin rash appears to be a class effect of EGFR inhibitors, relating to inhibition of EGFR-mediated signaling pathways in the skin. In support of this theory, up-regulation of $p27^{Kip1}$ has been observed in epidermal keratinocytes of cancer patients treated with cetuximab, gefitinib and erlotinib [128,130].

In the phase II trial of cetuximab and irinotecan in irinotecan-refractory CRC patients, 94 (78%) of the 120 patients who were classified as demonstrating progressive disease at the start of the trial developed at least 1 acne-like rash during the study [98]. These patients achieved a significantly higher rate of objective response compared to patients who did not develop a rash (28 versus 4%, $p < 0.021$). More recently, a phase II trial of cetuximab monotherapy in the treatment of metastatic CRC refractory to irinotecan, oxaliplatin and a fluoropyrimidine demonstrated statistically significant improvements in tumor response and overall survival in patients with acne-like rash grade 2 or higher [131]. Similarly, rash severity

was significantly associated with survival in patients with NSCLC receiving erlotinib [127]. As the majority of cutaneous side-effects are observed well below the maximum tolerated dose of anti-EGFR agents, the acneiform skin rash could prove to be a clinically useful efficacy marker for these agents.

Summary

EGFR blockade represents a rational approach to tumor growth inhibition by exploiting the increased dependence of rapidly dividing, EGFR-expressing tumor cells on this signaling pathway relative to non-neoplastic cells. The high redundancy of growth factor signaling in most normal cell types further enhances the exquisite specificity of EGFR-targeted agents. In addition, the effects of anti-EGFR agents on normal tissues have proven to be manageable.

A variety of approaches have been taken to inhibit EGFR function, the relative merits of which remain to be determined in clinical trials. Three anti-EGFR agents, cetuximab, erlotinib and gefitinib, have been approved by the US FDA for the treatment of CRC (cetuximab) and NSCLC (erlotinib and gefitinib). Cetuximab has absolute specificity for the EGFR and thus exerts its effects only on EGFR-expressing cells. Importantly, the efficacy of cetuximab is not related to EGFR expression *per se*. Receptor internalization and ADCC, mechanisms attributed to mAbs, but not to tyrosine kinase inhibitors, may provide added clinical benefit with the use of therapeutic mAbs, possibly by providing a more continuous and constant blockade of receptor signaling.

Several important aspects of anti-EGFR therapy remain to be addressed. An increasing emphasis is being placed on the ability to predict response to cancer therapy. Studies are currently being conducted to determine whether pre-selecting tumors on the basis of EGFR, TGF-α, EGF and/or MAP kinase levels will predict which patients might respond to EGFR blockade. It is hoped that additional information will be provided by studies investigating the utility of acneiform skin rash as an early predictor of response to anti-EGFR therapy. The finding that mutations in the EGFR gene in subsets of patients with NSCLC confer susceptibility to gefitinib offers the potential to pre-select patients who are most likely to respond to tyrosine kinase inhibitors; however, it is unknown whether or not such mutations are relevant to responses to mAb therapy.

To date, most clinical trials have investigated the effects of EGFR blockade in patients with advanced disease. However, it may be that EGFR-blocking agents will prove valuable as part of first-line regimens in early-stage disease. Furthermore, the reduced toxicity profiles of EGFR-inhibiting agents suggest that these agents could be successfully used for maintenance therapy regimens following cytotoxic therapy.

Note added in proof

Recently, structural modeling and biochemical analysis of the tyrosine kinase domain was performed in a patient who developed clinical resistance to gefitinib. DNA sequence analysis of the EGFR gene in this patient's tumor revealed the presence of a second point mutation, resulting in a threonine to methionine mutation at position 790, conferring gefinitib resistance [132].

References

1 Beretta GD, Pessi MA, Poletti P, Mosconi S, Labianca R. New drugs and combinations in the palliative treatment of colon and rectal cancer. *Eur J Surg Oncol* 2001; **27**:595–600.
2 Kim ES, Kies M, Herbst RS. Novel therapeutics for head and neck cancer. *Curr Opin Oncol* 2002; **14**:334–342.
3 Kindler HL, Shulman KL. Metastatic colorectal cancer. *Curr Treat Options Oncol* 2001; **2**:459–471.
4 Lingen MW, Emami B, Clark JI. New therapeutic strategies for the treatment and prevention of head and neck cancer. *Expert Opin Invest Drugs* 2000; **9**:2855–2872.
5 Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000; **100**:57–70.
6 Mendelsohn J. The epidermal growth factor receptor as a target for cancer therapy. *Endocr Relat Cancer* 2001; **8**:3–9.
7 Barker FG, Simmons ML, Chang SM, Prados MD, Larson DA, Sneed PK, *et al.* EGFR overexpression and radiation response in glioblastoma multiforme. *Int J Radiat Oncol Biol Phys* 2001; **51**:410–418.
8 Fischer-Colbrie J, Witt A, Heinzl H, Speiser P, Czerwenka K, Sevelda P, *et al.* EGFR and steroid receptors in ovarian carcinoma: comparison with prognostic parameters and outcome of patients. *Anticancer Res* 1997; **17**:613–619.
9 Masuda M, Toh S, Koike K, Kuratomi Y, Suzui M, Deguchi A, *et al.* The roles of JNK1 and Stat3 in the response of head and neck cancer cell lines to combined treatment with all-*trans*-retinoic acid and 5-fluorouracil. *Jpn J Cancer Res* 2002; **93**:329–339.
10 Carpenter G. Receptors for epidermal growth factor and other polypeptide mitogens. *Annu Rev Biochem* 1987; **56**:881–914.
11 Wells A. EGF receptor. *Int J Biochem Cell Biol* 1999; **31**:637–643.
12 Yarden Y. The EGFR family and its ligands in human cancer: signalling mechanisms and therapeutic opportunities. *Eur J Cancer* 2001; **37(suppl 4)**:S3–S8.
13 Ennis BW, Lippman ME, Dickson RB. The EGF receptor system as a target for antitumor therapy. *Cancer Invest* 1991; **9**:553–562.
14 Moscatello DK, Holgado-Madruga M, Emlet DR, Montgomery RB, Wong AJ. Constitutive activation of phosphatidylinositol 3-kinase by a naturally occurring mutant epidermal growth factor receptor. *J Biol Chem* 1998; **273**:200–206.
15 Salomon DS, Brandt R, Ciardiello F, Normanno N. Epidermal growth factor-related peptides and their receptors in human malignancies. *Crit Rev Oncol Hematol* 1995; **19**:183–232.
16 Wikstrand CJ, Hale LP, Batra SK, Hill ML, Humphrey PA, Kurpad SN, *et al.* Monoclonal antibodies against EGFRvIII are tumor specific and react with breast and lung carcinomas and malignant gliomas. *Cancer Res* 1995; **55**:3140–3148.
17 Nicholson RI, Gee JM, Harper ME. EGFR and cancer prognosis. *Eur J Cancer* 2001; **37(suppl 4)**:S9–S15.
18 Verbeek BS, Adriaansen-Slot SS, Vroom TM, Beckers T, Rijksen G. Overexpression of EGFR and c-*erbB2* causes enhanced cell migration in human breast cancer cells and NIH3T3 fibroblasts. *FEBS Lett* 1998; **425**:145–150.
19 Wells A. Tumor invasion: role of growth factor-induced cell motility. *Adv Cancer Res* 2000; **78**:31–101.
20 Levitzki A. Signal-transduction therapy. A novel approach to disease management. *Eur J Biochem* 1994; **226**:1–13.
21 Powis G, Workman P. Signalling targets for the development of cancer drugs. *Anticancer Drug Des* 1994; **9**:555–568.
22 Prenzel N, Fischer OM, Streit S, Hart S, Ullrich A. The epidermal growth factor receptor family as a central element for cellular signal transduction and diversification. *Endocr Relat Cancer* 2001; **8**:11–31.

23 Ogiso H, Ishitani R, Nureki O, Fukai S, Yamanaka M, Kim JH, *et al.* Crystal structure of the complex of human epidermal growth factor and receptor extracellular domains. *Cell* 2002; **110**:775–787.

24 Rubin Grandis J, Drenning SD, Chakraborty A, Zhou MY, Zeng Q, Pitt AS, *et al.* Requirement of Stat3 but not Stat1 activation for epidermal growth factor receptor-mediated cell growth *in vitro*. *J Clin Invest* 1998; **102**:1385–1392.

25 Rubin Grandis J, Zeng Q, Drenning SD. Epidermal growth factor receptor-mediated stat3 signaling blocks apoptosis in head and neck cancer. *Laryngoscope* 2000; **110**:868–874.

26 Lu Z, Jiang G, Blume-Jensen P, Hunter T. Epidermal growth factor-induced tumor cell invasion and metastasis initiated by dephosphorylation and downregulation of focal adhesion kinase. *Mol Cell Biol* 2001; **21**: 4016–4031.

27 Niu G, Wright KL, Huang M, Song L, Haura E, Turkson J, *et al.* Constitutive Stat3 activity up-regulates VEGF expression and tumor angiogenesis. *Oncogene* 2002; **21**:2000–2008.

28 Bancroft CC, Chen Z, Yeh J, Sunwoo JB, Yeh NT, Jackson S, *et al.* Effects of pharmacologic antagonists of epidermal growth factor receptor, PI3K and MEK signal kinases on NF-κB and AP-1 activation and IL-8 and VEGF expression in human head and neck squamous cell carcinoma lines. *Int J Cancer* 2002; **99**:538–548.

29 Di Gennaro E, Barbarino M, Bruzzese F, De Lorenzo S, Caraglia M, Abbruzzese A, *et al.* Critical role of both p27KIP1 and p21CIP1/WAF1 in the antiproliferative effect of ZD1839 ('Iressa'), an epidermal growth factor receptor tyrosine kinase inhibitor, in head and neck squamous carcinoma cells. *J Cell Physiol* 2003; **195**:139–150.

30 Fan Z, Shang BY, Lu Y, Chou JL, Mendelsohn J. Reciprocal changes in $p27^{Kip1}$ and $p21^{Cip1}$ in growth inhibition mediated by blockade or overstimulation of epidermal growth factor receptors. *Clin Cancer Res* 1997; **3**:1943–1948.

31 Moyer JD, Barbacci EG, Iwata KK, Arnold L, Boman B, Cunningham A, *et al.* Induction of apoptosis and cell cycle arrest by CP-358,774, an inhibitor of epidermal growth factor receptor tyrosine kinase. *Cancer Res* 1997; **57**:4838–4848.

32 Peng D, Fan Z, Lu Y, DeBlasio T, Scher H, Mendelsohn J. Anti-epidermal growth factor receptor monoclonal antibody 225 up-regulates $p27^{KIP1}$ and induces G_1 arrest in prostatic cancer cell line DU145. *Cancer Res* 1996; **56**:3666–3669.

33 Wu X, Rubin M, Fan Z, DeBlasio T, Soos T, Koff A, *et al.* Involvement of $p27^{KIP1}$ in G_1 arrest mediated by an anti-epidermal growth factor receptor monoclonal antibody. *Oncogene* 1996; **12**:1397–1403.

34 Albanell J, Codony-Servat J, Rojo F, Del Campo JM, Sauleda S, Anido J, *et al.* Activated extracellular signal-regulated kinases: association with epidermal growth factor receptor/transforming growth factor alpha expression in head and neck squamous carcinoma and inhibition by anti-epidermal growth factor receptor treatments. *Cancer Res* 2001; **61**: 6500–6510.

35 Lo RS, Wotton D, Massague J. Epidermal growth factor signaling via Ras controls the Smad transcriptional co-repressor TGIF. *EMBO J* 2001; **20**:128–136.

36 Inoue K, Slaton JW, Perrotte P, Davis DW, Bruns CJ, Hicklin DJ, *et al.* Paclitaxel enhances the effects of the anti-epidermal growth factor receptor monoclonal antibody ImClone C225 in mice with metastatic human bladder transitional cell carcinoma. *Clin Cancer Res* 2000; **6**:4874–4884.

37 Perrotte P, Matsumoto T, Inoue K, Kuniyasu H, Eve BY, Hicklin DJ, *et al.* Anti-epidermal growth factor receptor antibody C225 inhibits angiogenesis in human transitional cell carcinoma growing orthotopically in nude mice. *Clin Cancer Res* 1999; **5**:257–265.

38 Ciardiello F, Caputo R, Bianco R, Damiano V, Fontanini G, Cuccato S, *et al.* Inhibition of growth factor production and angiogenesis in human cancer cells by ZD1839 (Iressa), a selective epidermal growth factor receptor tyrosine kinase inhibitor. *Clin Cancer Res* 2001; **7**:1459–1465.

39 O-charoenrat P, Rhys-Evans P, Modjtahedi H, Eccles SA. Vascular endothelial growth factor family members are differentially regulated by c-*erbB* signaling in head and neck squamous carcinoma cells. *Clin Exp Metastasis* 2000; **18**:155–161.

40 Yang XD, Wang P, Fredlin P, Davis CG. ABX-EGF, a fully human anti-EGF receptor monoclonal antibody: inhibition of prostate cancer *in vitro* and *in vivo*. *Proc Am Soc Clin Oncol* 2002; abstr 2454.

41 Bruns CJ, Solorzano CC, Harbison MT, Ozawa S, Tsan R, Fan D, *et al.* Blockade of the epidermal growth factor receptor signaling by a novel tyrosine kinase inhibitor leads to apoptosis of endothelial cells and therapy of human pancreatic carcinoma. *Cancer Res* 2000; **60**:2926–2935.

42 Bruns CJ, Harbison MT, Davis DW, Portera CA, Tsan R, McConkey DJ, *et al.* Epidermal growth factor receptor blockade with C225 plus gemcitabine results in regression of human pancreatic carcinoma growing orthotopically in nude mice by antiangiogenic mechanisms. *Clin Cancer Res* 2000; **6**:1936–1948.

43 Bonner JA, Giralt J, Harari PM, Cohen R, Jones C, Sur RK, *et al.* Cetuximab prolongs survival in patients with locoregionally advanced squamous cell carinoma of head and neck: a phase III study of high dose radiation therapy with or without cetuximab. *Proc Am Soc Clin Oncol* 2004; abstr 5507.

44 Ciardiello F, Caputo R, Bianco R, Damiano V, Pomatico G, De Placido S, *et al.* Antitumor effect and potentiation of cytotoxic drugs activity in human cancer cells by ZD-1839 (Iressa), an epidermal growth factor receptor-selective tyrosine kinase inhibitor. *Clin Cancer Res* 2000; **6**:2053–2063.

45 Ciardiello F, Caputo R, Troiani T, Borriello G, Kandimalla ER, Agrawal S, *et al.* Antisense oligonucleotides targeting the epidermal growth factor receptor inhibit proliferation, induce apoptosis, and cooperate with cytotoxic drugs in human cancer cell lines. *Int J Cancer* 2001; **93**: 172–178.

46 Karnes WEJ, Weller SG, Adjei PN, Kottke TJ, Glenn KS, Gores GJ, *et al.* Inhibition of epidermal growth factor receptor kinase induces protease-dependent apoptosis in human colon cancer cells. *Gastroenterology* 1998; **114**:930–939.

47 Wu X, Fan Z, Masui H, Rosen N, Mendelsohn J. Apoptosis induced by an anti-epidermal growth factor receptor monoclonal antibody in a human colorectal carcinoma cell line and its delay by insulin. *J Clin Invest* 1995; **95**:1897–1905.

48 Huang SM, Bock JM, Harari PM. Epidermal growth factor receptor blockade with C225 modulates proliferation, apoptosis, and radiosensitivity in squamous cell carcinomas of the head and neck. *Cancer Res* 1999; **59**:1935–1940.

49 Liu B, Fang M, Schmidt M, Lu Y, Mendelsohn J, Fan Z. Induction of apoptosis and activation of the caspase cascade by anti-EGF receptor monoclonal antibodies in DiFi human colon cancer cells do not involve the c-jun N-terminal kinase activity. *Br J Cancer* 2000; **82**:1991–1999.

50 Karashima T, Sweeney P, Slaton JW, Kim SJ, Kedar D, Izawa JI, *et al.* Inhibition of angiogenesis by the antiepidermal growth factor receptor antibody ImClone C225 in androgen-independent prostate cancer growing orthotopically in nude mice. *Clin Cancer Res* 2002; **8**:1253–1264.

51 Aboud-Pirak E, Hurwitz E, Pirak ME, Bellot F, Schlessinger J, Sela M. Efficacy of antibodies to epidermal growth factor receptor against KB carcinoma *in vitro* and in nude mice. *J Natl Cancer Inst* 1988; **80**: 1605–1611.

52 Baselga J, Averbuch SD. ZD1839 ('Iressa') as an anticancer agent. *Drugs* 2000; **60**(**suppl 1**):33–40.

53 Erlichman C, Boerner SA, Hallgren CG, Spieker R, Wang XY, James CD, *et al.* The HER tyrosine kinase inhibitor CI1033 enhances cytotoxicity of 7-ethyl-10-hydroxycamptothecin and topotecan by inhibiting breast cancer resistance protein-mediated drug efflux. *Cancer Res* 2001; **61**:739–748.

54 Hoffmann T, Hafner D, Ballo H, Haas I, Bier H. Antitumor activity of anti-epidermal growth factor receptor monoclonal antibodies and cisplatin in ten human head and neck squamous cell carcinoma lines. *Anticancer Res* 1997; **17**:4419–4425.

55 Pollack VA, Savage DM, Baker DA, Tsaparikos KE, Sloan DE, Moyer JD, *et al.* Inhibition of epidermal growth factor receptor-associated tyrosine phosphorylation in human carcinomas with CP-358,774: dynamics of receptor inhibition *in situ* and antitumor effects in athymic mice. *J Pharmacol Exp Ther* 1999; **291**:739–748.

56 Rao GS, Murray S, Ethier SP. Radiosensitization of human breast cancer cells by a novel ErbB family receptor tyrosine kinase inhibitor. *Int J Radiat Oncol Biol Phys* 2000; **48**:1519–1528.

57 Williams KJ, Telfer BA, Stratford IJ, Wedge SR. ZD1839 ('Iressa'), a specific oral epidermal growth factor receptor-tyrosine kinase inhibitor, potentiates radiotherapy in a human colorectal cancer xenograft model. *Br J Cancer* 2002; **86**:1157–1161.

58 Herbst RS, Shin DM. Monoclonal antibodies to target epidermal growth factor receptor-positive tumors: a new paradigm for cancer therapy. *Cancer* 2002; **94**:1593–1611.

59 Kim ES, Khuri FR, Herbst RS. Epidermal growth factor receptor biology (IMC-C225). *Curr Opin Oncol* 2001; **13**:506–513.

60 Bonner JA, Raisch KP, Trummell HQ, Robert F, Meredith RF, Spencer SA, *et al.* Enhanced apoptosis with combination C225/radiation treatment serves as the impetus for clinical investigation in head and neck cancers. *J Clin Oncol* 2000; **18**:47S–53S.

61 Baselga J, Norton L, Masui H, Pandiella A, Coplan K, Miller WHJ, *et al.* Antitumor effects of doxorubicin in combination with anti-epidermal growth factor receptor monoclonal antibodies. *J Natl Cancer Inst* 1993; **85**: 1327–1333.

62 Ciardiello F, Bianco R, Damiano V, De Lorenzo S, Pepe S, De Placido S, *et al.* Antitumor activity of sequential treatment with topotecan and anti-epidermal growth factor receptor monoclonal antibody C225. *Clin Cancer Res* 1999; **5**:909–916.

63 Fan Z, Baselga J, Masui H, Mendelsohn J. Antitumor effect of anti-epidermal growth factor receptor monoclonal antibodies plus *cis*-diamminedichloroplatinum on well established A431 cell xenografts. *Cancer Res* 1993; **53**:4637–4642.

64 Nasu S, Ang KK, Fan Z, Milas L. C225 antiepidermal growth factor receptor antibody enhances tumor radiocurability. *Int J Radiat Oncol Biol Phys* 2001; **51**:474–477.

65 Prewett MC, Hooper AT, Bassi R, Waksal HW, Hicklin DJ. Enhanced antitumor activity of anti-epidermal growth factor receptor monoclonal antibody ERBITUX™ (IMC-C225) in combination with irinotecan (CPT-11), 5-FU, and leucovorin against human colorectal carcinoma xenografts. *Proc Am Ass Cancer Res* 2002; abstr.

66 Chen HX, Mooney M, Boron M, Grochow L, Zwiebel J, Vena D, *et al.* Bevacizumab (BV) plus 5-FU/leucovorin (FU/LV) for advanced colorectal cancer (CRC) that progressed after standard chemotherapies: an NCI Treatment Referral Center trial (TRC-0301). *Proc Am Soc Clin Oncol* 2004; abstr 3515.

67 Badarinath S, Mitchell EP, Jennis A, Graham CD, Hansen VL, Henderson CA, *et al.* Cetuximab plus FOLFOX for colorectal cancer (EXPLORE): Preliminary safety analysis of a randomized phase III trial. *Proc Am Soc Clin Oncol* 2004; abstr 3531.

68 Rougier P, Raoul JL, Van Laethem JL, Peeters M, Husseini F, Brezault C, *et al.* Cetuximab + FOLFIRI as first-line treatment for metastatic colorectal CA. *Proc Am Soc Clin Oncol* 2004; abstr 3513.

69 Tabernero JM, Van Cutsem E, Sastre J, Cervantes A, Van Laethem JL, Humblet Y, *et al.* An international phase II study of cetuximab in combination with oxaliplatin/5-fluorouracil (5-FU)/folinic acid (FA) (FOLFOX-4) in the first-line treatment of patients with metastatic colorectal cancer (CRC) expressing epidermal growth factor receptor (EGFR). Preliminary results. *Proc Am Soc Clin Oncol* 2004; abstr 3512.

70 Cunningham D, Humblet Y, Siena S, Khayat D, Bleiberg H, Santoro A, *et al.* Cetuximab Monotherapy and Cetuximab plus Irinotecan in Irinotecan-Refractory Metastatic Colorectal Cancer. *N Engl J Med* 2004; **351**: 337–345.

71 Saltz LB, Meropol NJ, Loehrer Sr PJ, Needle MN, Kopit J, Mayer RJ. Phase II trial of cetuximab in patients with refractory colorectal cancer that expresses the epidermal growth factor receptor. *J Clin Oncol* 2004; **22**:1201–1208.

72 Xiong HQ, Rosenberg A, LoBuglio A, Schmidt W, Wolff RA, Deutsch J, *et al.* Cetuximab, a monoclonal antibody targeting the epidermal growth factor receptor, in combination with gemcitabine for advanced pancreatic cancer: a multicenter phase II trial. *J Clin Oncol* 2004; **22**:2610–2616.

73 Lynch TJ, Lilenbaum R, Bonomi P, Ansari R, Govindan R, Janne PA, *et al.* A phase II trial of cetuximab as therapy for recurrent non-small cell lung cancer (NSCLC). *Proc Am Soc Clin Oncol* 2004; abstr 7084.

74 Rosell R, Daniel C, Ramlau R, Szczesna A, Constenla M, Mennecier B, *et al.* Randomized phase II study of cetuximab in combination with cisplatin (C) and vinorelbine (V) vs. CV alone in the first-line treatment of patients with epidermal growth factor receptor (EGFR)-expressing advanced non-small-cell lung cancer (NSCLC). *Proc Am Soc Clin Oncol* 2004; abstr 7012.

75 Goldstein NI, Prewett M, Zuklys K, Rockwell P, Mendelsohn J. Biological efficacy of a chimeric antibody to the epidermal growth factor receptor in a human tumor xenograft model. *Clin Cancer Res* 1995; **1**:1311–1318.

76 Robert F, Ezekiel MP, Spencer SA, Meredith RF, Bonner JA, Khazaeli MB, *et al.* Phase I study of anti-epidermal growth factor receptor antibody cetuximab in combination with radiation therapy in patients with advanced head and neck cancer. *J Clin Oncol* 2001; **19**:3234–3243.

77 Khazaeli MB, LoBuglio AF, Falcey JW, Paulter VJ, Fetzer MK, Waksal HW. Low immunogenicity of a chimeric monoclonal antibody (MoAb), IMC-C225, used to treat epidermal growth factor receptor-positive tumors. *Proc Am Soc Clin Oncol* 2000; 207a (abstr 808).

78 Modjtahedi H, Hickish T, Nicolson M, Moore J, Styles J, Eccles S, *et al.* Phase I trial and tumour localisation of the anti-EGFR monoclonal antibody ICR62 in head and neck or lung cancer. *Br J Cancer* 1996; **73**:228–235.

79 Prewett M, Rockwell P, Rockwell RF, Giorgio NA, Mendelsohn J, Scher HI, *et al.* The biologic effects of C225, a chimeric monoclonal antibody to the EGFR, on human prostate carcinoma. *J Immunother Emphasis Tumor Immunol* 1996; **19**:419–427.

80 Prewett M, Rothman M, Waksal H, Feldman M, Bander NH, Hicklin DJ. Mouse-human chimeric anti-epidermal growth factor receptor antibody C225 inhibits the growth of human renal cell carcinoma xenografts in nude mice. *Clin Cancer Res* 1998; **4**:2957–2966.

81 Sunada H, Magun BE, Mendelsohn J, MacLeod CL. Monoclonal antibody against epidermal growth factor receptor is internalized without stimulating receptor phosphorylation. *Proc Natl Acad Sci USA* 1986; **83**:3825–3829.

82 Sunada H, Yu P, Peacock JS, Mendelsohn J. Modulation of tyrosine, serine, and threonine phosphorylation and intracellular processing of the epidermal growth factor receptor by antireceptor monoclonal antibody. *J Cell Physiol* 1990; **142**:284–292.

83 Hadari YR, Doody JF, Wang Y, Patel SN, Apblett RL, Loizos N, *et al.* The IgG1 monoclonal antibody cetuximab induces degradation of the epidermal growth factor receptor. *Proc Am Soc Clin Oncol* 2004; abstr 234.

84 Carter P. Improving the efficacy of antibody-based cancer therapies. *Nat Rev Cancer* 2001; **1**:118–129.

85 Burton DR, Woof JM. Human antibody effector function. *Adv Immunol* 1992; **51**:1–84.

86 Fan Z, Masui H, Altas I, Mendelsohn J. Blockade of epidermal growth factor receptor function by bivalent and monovalent fragments of 225 anti-epidermal growth factor receptor monoclonal antibodies. *Cancer Res* 1993; **53**:4322–4328.

87 Naramura M, Gillies SD, Mendelsohn J, Reisfeld RA, Mueller BM. Therapeutic potential of chimeric and murine anti-(epidermal growth factor receptor) antibodies in a metastasis model for human melanoma. *Cancer Immunol Immunother* 1993; **37**:343–349.

88 Bier H, Hoffmann T, Haas I, van Lierop A. Anti-(epidermal growth factor) receptor monoclonal antibodies for the induction of antibody-dependent cell-mediated cytotoxicity against squamous cell carcinoma lines of the head and neck. *Cancer Immunol Immunother* 1998; **46**:167–173.

89 Baselga J, Pfister D, Cooper MR, Cohen R, Burtness B, Bos M, *et al.* Phase I studies of anti-epidermal growth factor receptor chimeric antibody C225 alone and in combination with cisplatin. *J Clin Oncol* 2000; **18**: 904–914.

90 Shin DM, Donato NJ, Perez-Soler R, Shin HJ, Wu JY, Zhang P, *et al.* Epidermal growth factor receptor-targeted therapy with C225 and cisplatin in patients with head and neck cancer. *Clin Cancer Res* 2001; **7**: 1204–1213.

91 Cohen RB, Falcey JW, Paulter VJ, Fetzer KM, Waksal HW. Safety profile of the monoclonal antibody (MoAb) IMC-C225, an Anti-epidermal growth factor receptor (EGFr) used in the treatment of EGFr positive tumors. *Proc Am Soc Clin Oncol* 2000; 474a (abstr 1862).

92 Needle MN. Safety experience with IMC-C225, an anti-epidermal growth factor receptor antibody. *Semin Oncol* 2002; **29**:55–60.

93 Baselga J, Trigo JM, Bourhis J, Tortochaux J, Cortes-Funes H, Hitt R, *et al.* Cetuximab (C225) plus cisplatin/carboplatin is active in patients (pts) with recurrent/metastatic squamous cell carcinoma of the head and neck (SCCHN) progressing on a same dose and schedule platinum-based regimen. *Proc Am Soc Clin Oncol* 2002; 226a (abstr 900).

94 Kim ES, Mauer AM, Tran HT, Liu D, Gladish G, Dicke K, *et al.* A phase II study of cetuximab, an epidermal growth factor receptor (EGFR) blocking antibody, in combination with docetaxel in chemotherapy refractory/resistant patients with advanced non-small cell lung cancer: final report. *Proc Am Soc Clin Oncol* 2003; 642 (abstr 2581).

95 Rosenberg AH, Loehrer PJ, Needle MN, Waksal H, Hollywood E, Ramos L, *et al.* Erbitux (IMC-C225) plus weekly irinotecan (CPT-11), fluorouracil (5FU) and leucovorin (LV) in colorectal cancer (CRC) that expresses the epidermal growth factor receptor (EGFR). *Proc Am Soc Clin Oncol* 2002; 135a (abstr 536).

96 Folprecht G, Lutz M, Schoeffski P, Seufferlein T, Haag C, Beutel G, *et al.* Cetuximab/irinotecan/high-dose-5-fluorouracil/leucovorin (HD-5-FU/LV) in the first-line therapy of metastatic colorectal cancer (CRC). *Presented at Am Soc Clin Oncol Gastrointestinal Cancers Symp* 2004; abstr 283.

97 Saltz L, Rubin M, Hochster H, Tchekmeydian NS, Waksal H, Needle M, *et al.* Cetuximab (IMC-C225) plus irinotecan (CPT-11) is active in CPT-11-refractory colorectal cancer (CRC) that expresses epidermal growth factor receptor (EGFR). *Proc Am Soc Clin Oncol* 2001; 3a (abstr 7).

98 Saltz L, Rubin MS, Hochster HS, Tchekmeydian NS, Waksal HW, Needle MN, *et al.* Acne-like rash predicts response in patients treated with cetuximab (IMC-C225) plus irinotecan (CPT-11) in CPT-11-refractory colorectal cancer (CRC) that expresses epidermal growth factor receptor (EGFR). *Presented at AACR–NCI–EORTC Int Conf* 2001; abstr 559.

99 ImClone Systems. Erbitux™ (Cetuximab). *US Prescribing Information*. Princeton, NJ: ImClone Systems; 2004.

100 Ranson M, Hammond LA, Ferry D, Kris M, Tullo A, Murray PI, *et al.* ZD1839, a selective oral epidermal growth factor receptor-tyrosine kinase inhibitor,

is well tolerated and active in patients with solid, malignant tumors: results of a phase I trial. *J Clin Oncol* 2002; **20**:2240–2250.

101 Cohen EE, Rosen F, Stadler WM, Recant W, Stenson K, Huo D, *et al.* Phase II trial of ZD1839 in recurrent or metastatic squamous cell carcinoma of the head and neck. *J Clin Oncol* 2003; **21**:1980–1987.

102 Drucker B, Bacik J, Ginsberg M, Marion S, Russo P, Mazumdar M, *et al.* Phase II trial of ZD1839 (IRESSA) in patients with advanced renal cell carcinoma. *Invest New Drugs* 2003; **21**:341–345.

103 Rich JN, Reardon DA, Peery T, Dowell JM, Quinn JA, Penne KL, *et al.* Phase II trial of gefitinib in recurrent glioblastoma. *J Clin Oncol* 2004; **22**:133–142.

104 Goss GD, Stewart DJ, Hirte H, Miller W, Major P, Batist G, *et al.* Initial results of part 2 of a phase I/II pharmacokinetics (PK), pharmacodynamic (PD) and biological activity study of ZD1839 (Iressa): NCIC CTG IND.122. *Proc Am Soc Clin Oncol* 2002; 16a (abstr 59).

105 Fukuoka M, Yano S, Giaccone G, Tamura T, Nakagawa K, Douillard JY, *et al.* Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer. *J Clin Oncol* 2003; **12**:2237–2246.

106 Kris MG, Natale RB, Herbst RS, Lynch Jr TJ, Prager D, Belani CP, *et al.* Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: a randomized trial. *J Am Med Ass* 2003; **290**:2149–2158.

107 AstraZeneca Pharmaceuticals. *Iressa™ (Gefitinib Tablets). US Prescribing Information*. Wilmington, DE: AstraZeneca Pharmaceuticals; 2003.

108 Bailey LR, Kris M, Wolf M, *et al.* Tumor EGFR membrane staining is not clinically relevant for predicting response in patients receiving gefitinib ('Iressa', ZD1839) monotherapy for pre-treated advanced non-small-cell lung cancer: IDEAL 1 and 2. *Proc Am Ass Cancer Res* 2003; 1362 (abstr LB-170).

109 Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, *et al.* Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2004; **21**:2129–2139.

110 Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, *et al.* EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 2004; **304**:1497–1500.

111 Giaccone G, Herbst RS, Manegold C, Scagliotti G, Rosell R, Miller V, *et al.* Gefitinib in combination with gemcitabine and cisplatin in advanced non-small-cell lung cancer: a phase III trial—INTACT 1. *J Clin Oncol* 2004; **22**:777–784.

112 Herbst RS, Giaccone G, Schiller JH, Natale RB, Miller V, Manegold C, *et al.* Gefitinib in combination with paclitaxel and carboplatin in advanced non-small-cell lung cancer: a phase III trial—INTACT 2. *J Clin Oncol* 2004; **22**:785–794.

113 Bunn PA Jr. Treatment of advanced non-small-cell lung cancer with two-drug combinations. *J Clin Oncol* 2002; **20**:3565–3567.

114 Solit DB, She Y, Moasser M, Hudis C, Kris M, Scher H, *et al.* Pulsatile administration of the EGF receptor inhibitor (Iressa, ZD1839) is significantly more effective than continuous dosing for sensitizing tumors to Taxol. *Presented at AACR–NCI–EORTC Int Conf on Molecular Targets and Cancer Therapeutics* 2003; abstr A83.

115 Xu JM, Azzariti A, Colucci G, Paradiso A. The effect of gefitinib (Iressa, ZD1839) in combination with oxaliplatin is schedule-dependent in colon cancer cell lines. *Cancer Chemother Pharmacol* 2003; **52**:442–448.

116 Hidalgo M, Siu LL, Nemunaitis J, Rizzo J, Hammond LA, Takimoto C, *et al.* Phase I and pharmacologic study of OSI-774, an epidermal growth factor receptor tyrosine kinase inhibitor, in patients with advanced solid malignancies. *J Clin Oncol* 2001; **19**:3267–3279.

117 Hidalgo M, Erlichman C, Rowinsky EK, Keopp-Norris J, Jensen K, Boni J, *et al.* Phase I trial of EKB-569, an irreversible inhibitor of the epidermal growth factor receptor (EGFR), in patients with advanced solid tumors. *Proc Am Soc Clin Oncol* 2002; 17a (abstr 65).

118 Murren JR, Papadimitrakopoulou VA, Sizer KC, Vaidyanathan S, Ravera C, Abbruzzese JL. A phase I dose-escalating study to evaluate the biological activity and pharmacokinetics of PKI166, a novel tyrosine kinase inhibitor, in patients with advanced cancers. *Proc Am Soc Clin Oncol* 2002; 95a (abstr 377).

119 Perez-Soler R, Chachoua A, Huberman M, Karp D, Rigas J, Hammond L, *et al.* A phase II trial of the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor OSI-774, following platinum-based chemotherapy, in patients (pts) with advanced, EGFR-expressing, non-small cell lung cancer (NSCLC). *Proc Am Soc Clin Oncol* 2001; 310a (abstr 1235).

120 Rowinsky EK, Hammond L, Siu P, Jerabek J, Rizzo J. Dose-schedule-finding, pharmacokinetic (PK) biologic, and functional imaging studies of OSI-774, a selective epidermal growth factor receptor (EGFR) tyrosine kinase (TK) inhibitor. *Proc Am Soc Clin Oncol* 2001; 2a (abstr 5).

121 Soulieres D, Senzer NN, Vokes EE, Hidalgo M, Agarwala SS, Siu LL. Multicenter phase II study of erlotinib, an oral epidermal growth factor receptor tyrosine kinase inhibitor, in patients with recurrent or metastatic squamous cell cancer of the head and neck. *J Clin Oncol* 2004; **22**: 77–85.

122 Yang XD, Jia XC, Corvalan JR, Wang P, Davis CG. Development of ABX-EGF, a fully human anti-EGF receptor monoclonal antibody, for cancer therapy. *Crit Rev Oncol Hematol* 2001; **38**:17–23.

123 Hambek M, Solbach C, Schnuerch HG, Roller M, Stegmueller M, Sterner-Kock A, *et al.* Tumor necrosis factor alpha sensitizes low epidermal growth factor receptor (EGFR)-expressing carcinomas for anti-EGFR therapy. *Cancer Res* 2001; **61**:1045–1049.

124 Dako Cytomation. *EGFR pharmDx™ Package Insert*. Carpinteria, CA: Dako; 2004.

125 Vogel CL, Cobleigh MA, Tripathy D, Gutheil JC, Harris LN, Fehrenbacher L, *et al.* Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. *J Clin Oncol* 2002; **20**:719–726.

126 Lee MW, Seo CW, Kim SW, Yang HJ, Lee HW, Choi JH, *et al.* Cutaneous side effects in non-small cell lung cancer patients treated with Iressa (ZD1839), an inhibitor of epidermal growth factor. *Acta Derm Venereol* 2004; **84**:23–26.

127 Perez-Soler R. Can rash associated with HER1/EGFR inhibition be used as a marker of treatment outcome? *Oncology (Huntingt)* 2003; **17**:23–28.

128 Albanell J, Rojo F, Baselga J. Pharmacodynamic studies with the epidermal growth factor receptor tyrosine kinase inhibitor ZD1839. *Semin Oncol* 2001; **28**:56–66.

129 Busam KJ, Capodieci P, Motzer R, Kiehn T, Phelan D, Halpern AC. Cutaneous side-effects in cancer patients treated with the antiepidermal growth factor receptor antibody C225. *Br J Dermatol* 2001; **144**: 1169–1176.

130 Malik SN, Siu LL, Rowinsky EK, deGraffenried L, Hammond LA, Rizzo J, *et al.* Pharmacodynamic evaluation of the epidermal growth factor receptor inhibitor OSI-774 in human epidermis of cancer patients. *Clin Cancer Res* 2003; **9**:2478–2486.

131 Van Cutsem E, Mayer RJ, Gold P, Stella PJ, Cohn A, Pippas AW, *et al.* Correlation of acne rash and tumor response with cetuximab monotherapy in patients with colorectal cancer refractory to both irinotecan and oxaliplatin. *Presented at EORTC–NCI–AACR Symp on Molecular Targets and Cancer Therapeutics* 2004; abstr 279.

132 Kobayashi S, Boggon TJ, Dayaram T, Janne PA, Kocher O, Meyerson M. *et al.* EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2005; **352**:786–792.