

## Developing Inhibitors of the Epidermal Growth Factor Receptor for Cancer Treatment

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**Progress in identifying and understanding the molecular and cellular causes of cancer has led to the discovery of anomalies that characterize cancer cells and that represent targets for the development of cancer therapeutics. One such target is the epidermal growth factor receptor (EGFR), a transmembrane protein that is frequently dysregulated in cancer cells. Preclinical studies have demonstrated that pharmacologic interventions that abrogate EGFR dysfunction result in antitumor effects. On the basis of these findings, therapeutic strategies to inhibit EGFR and EGFR-related pathways, including the use of monoclonal antibodies against the extracellular ligand-binding domain of EGFR and small-molecule inhibitors of the tyrosine kinase activity of EGFR, have entered clinical testing where they have demonstrated favorable safety profiles and adequate clinical pharmacology. Further development of these agents has been fueled by evidence of their antitumor activities, both as single agents and in combination with chemotherapy and radiation therapy. Areas that require investigation are the definition of patient populations most likely to derive benefits from these drugs, the implementation of biologic correlative studies to aid the selection of pharmacodynamically relevant doses and schedules, the characterization of population pharmacokinetic parameters and pharmacogenomic variables, and the most appropriate clinical scenario for proceeding with the clinical development of these agents. [J Natl Cancer Inst 2003;95: 851-67]**

The past two decades have been a revolutionary period in biomedical research including cancer research. The substantial advances made in understanding the basic biologic principles underlying the genesis and progression of cancer have resulted in the identification of a number of molecular anomalies that are key for the development of cancer, many of which are sufficient to induce malignant transformation in normal cells. These discoveries have led to the development of pharmacologic interventions that target the molecular and cellular consequences of these alterations. Selected examples of aberrant targets that have been successfully exploited therapeutically include HER2, a transmembrane receptor that is involved in breast cancer pathogenesis, and c-kit, a receptor that is mutated in gastrointestinal stromal tumors. Other potentially relevant targets include, but are not limited to, the product of the Ras oncogene, components of the signal transduction network (e.g., mitogen-activated protein kinase [MAPK] and the mammalian target of rapamycin [mTOR]), cell cycle regulators, and factors involved in angiogenesis.

Membrane receptors of the tyrosine kinase family are some of the best characterized targets in cancer cells. The epidermal

growth factor (EGF) family of membrane receptors is one of the most relevant targets in this class. This family contains four members: epidermal growth factor receptor (EGFR; also known as ErbB1 or HER1), HER2 (also termed ErbB2 or Neu), HER3 (also termed ErbB3), and HER4 (also termed ErbB4). A series of seminal basic and preclinical studies conducted over the last two decades conclusively demonstrated that alterations in the functions of these membrane receptors, particularly EGFR, are associated with oncogenic transformation and that dysregulation of EGFR is associated with essentially all of the key features of cancer, such as autonomous cell growth, invasion, angiogenic potential, and development of distant metastases (1,2). Consequently, therapeutics that target this family were developed. For example, trastuzumab (Herceptin; Genentech, South San Francisco, CA), a monoclonal antibody (MAb) that targets HER2, demonstrated therapeutic benefit in breast cancer patients, thus providing clinical evidence that targeting the EGF family of membrane receptors is a valid therapeutic strategy (3). Over the last few years, an increasing number of compounds directed against EGFR have entered clinical development and are currently in clinical trials. Preliminary data from these studies, which have shown promising results in different disease settings, have fueled efforts by the pharmaceutical industry, governmental entities, and clinical and translational investigators to rapidly explore the overall therapeutic contributions of these agents. In this review, we summarize the current status of the clinical development of this class of agents. We also extrapolate from the basic operational functioning of the target and mechanisms of action of these agents, general concepts that may be useful for the future clinical development of this class of agents.

### EGFRs: STRUCTURE, FUNCTION, ACTIVATION, AND SIGNALING

The EGF family of receptors has a common molecular structure that consists of an amino-terminal extracellular domain, a single transmembrane-anchoring region, and a carboxyl-terminal intracellular domain that has tyrosine kinase (TK) activity (4,5). The extracellular domain contains 621 amino acid residues, and its key feature is the ligand-binding domain, which is formed by two domains called L1 and L2 (6,7). The role of the

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transmembrane domain, which contains 23 amino acid residues, in EGFR signaling remains uncertain, although recent studies suggest that it contributes to receptor stability (8). The intracellular domain of EGFR is composed of 542 amino acid residues and has TK activity, which functions as an activator of the cytoplasmic targets of the receptor (6).

Binding of activating ligands to the extracellular domain of EGFR activates the receptor and its signaling pathways, which results in the orchestrated activation or modulation of cellular processes such as proliferation, differentiation, migration, and survival (1,2,4,9). A large number of molecules, collectively known as the EGF-like growth factors, have been identified that can bind and activate the EGF family of receptors. Most of these molecules are cleaved and released from the plasma membrane by regulated mechanisms and can act in either an autocrine or a paracrine manner. These ligands have different affinities for the different receptor family members. For example, EGF, transforming growth factor- $\alpha$  (TGF- $\alpha$ ), and amphiregulin bind specifically to EGFR, whereas heparin-binding EGF, betacellulin, and epiregulin bind to both EGFR and HER4 (9,10). Another group of ligands, the neuregulins (NRGs; also known as Neu or heregulin), consists of alternatively spliced isoforms that bind to HER3 and HER4. NRG1 and NRG2 bind HER3 and HER4 (11,12), whereas the more recently identified isoforms NRG3 and NRG4 bind exclusively to HER4 (13,14). No specific ligand has thus far been identified for HER2, supporting the notion that HER2 potentiates receptor signaling by heterodimerizing with other members of the family (15–17). The binding of ligands to the receptor induces the formation of either homo- or heterodimers in a strictly hierarchical order (1,4,18,19). Although the mechanisms that regulate the formation of the different dimers are not totally elucidated, factors such as the nature of the ligand and the relative abundance of one member of the family versus others influence the dimerization pattern. Furthermore, the transforming potential and signaling pathways activated by the different dimers are also unique. For example, EGFR–HER2 heterodimers are associated with a more intense and sustained proliferative signal than EGFR–EGFR homodimers (20,21). This pleiotropic system greatly increases the signal diversity of the EGF family.

Dimerization induces conformational changes in EGFR that result in the activation of the intracellular tyrosine kinase moiety and receptor autophosphorylation. HER3 lacks intrinsic kinase activity and relies on dimerization with other receptor family members to become phosphorylated and capable of signaling (22,23). Phosphorylation of the receptor leads to the formation of intracellular docking sites for cytoplasmic amplifying molecules that contain Src homology 2 (SH2) domains or phosphotyrosine-binding sites (19). These molecules then activate a multitude of signal transduction molecules, including phospholipase C- $\gamma$ -1, MAPK, phosphatidylinositol 3,4,5 kinase (PI3K)/protein kinase B (PKB or Akt), the tyrosine kinase Src, stress-activated protein kinases, c-Jun kinase, and signal transducers and activators of transcription (4,21,24–26). The activation of downstream signaling pathways is increased by the ability of these molecules to form various docking sites and by the redundant expression of binding motifs (18).

Among the multiple signal transduction pathways activated by the EGF family, the MAPK pathway is one of the most relevant because it regulates cellular processes, such as gene transcription and proliferation, by activating a variety of sub-

strates located in the cytosol, nucleus, and plasma membrane (24–26). Another important signal transduction pathway activated by the EGF family of receptors is the PI3K/Akt signaling pathway, which mediates cell survival (27,28). The recruitment of Akt to the plasma membrane triggers a cascade of pro-survival signaling, which is mediated by increased expression of anti-apoptotic signals, decreased expression of pro-apoptotic signals, and the activation of mRNA translation (29–37). In addition to activating the receptor, the binding of ligands initiates receptor internalization for signal termination, usually within seconds of receptor activation. After the ligand–receptor complex is internalized, it is either degraded, leading to signal termination, or recycled to the cell surface for another round of signaling (1,38).

## EGFR EXPRESSION AND ITS IMPLICATIONS IN TUMORIGENESIS

The discovery that alterations in the EGFR signaling pathway result in malignant transformation was initially made in studies of oncogenic viruses that demonstrated that the EGFR is the cellular homolog of the avian erythroblastosis virus v-erbB oncogene, which encodes a carboxy-terminally truncated form of HER1 (2,5). Subsequently, it was discovered that activation of the EGFR signaling pathway mediates the malignant transformation of virus-infected cells (2,5). Additional supportive evidence of the critical role of EGFR in oncogenesis comes from studies in transgenic mice. For example, overexpression of TGF- $\alpha$  and/or neu, the mouse homolog of HER2, was associated with morphologic abnormalities in the mammary gland and with the development of spontaneous carcinomas, which was markedly enhanced by the simultaneous overexpression of both proteins (39–43). Furthermore, overexpression of members of the EGFR family in NIH 3T3 cells results in malignant transformation. Preclinical data suggest that dysregulation among the EGF family of receptors is associated with growth advantages for malignant cells. In addition, analysis of tumor tissues from patients with cancer indicates that aberrant expression and activation of EGFR is characteristic of many human cancers and is often associated with poor clinical outcome and chemoresistance (44–54).

Malignant transformation as a consequence of EGFR dysregulation can occur by different mechanisms, including receptor overexpression, activating mutations, alterations in the dimerization process, activation of autocrine growth factor loops, and deficiency of specific phosphatases. One of the most frequent mechanisms by which EGFR is implicated in cancer development and progression is gene overexpression without gene amplification, which is associated with activation by TGF- $\alpha$  in an autocrine loop (50,53,54). Variant forms of EGFR that contain mutations in the extracellular domain have recently been described, with EGFR variant III (EGFRvIII) being the predominant variant in most cancers (55–57). EGFRvIII is generated by rearrangement or alternative mRNA splicing, which leads to the loss of 801 base pairs (bp; bases 275–1075) in the extracellular ligand binding domain, resulting in a 145-kd glycoprotein with constitutive, ligand-independent activation of the receptor's tyrosine kinase activity (58,59). This deletion was initially discovered in malignant glioblastoma and has subsequently been detected in breast, non-small-cell lung, ovarian, and prostate cancers, but not in normal tissue (60–65). The exclusive presence of this mutation in malignant tissue makes this

mutated EGFR an attractive target for specific antitumor therapeutics.

## PHARMACOLOGIC STRATEGIES TO TARGET EGFR

Theoretically, a variety of approaches and strategies can be used to target EGFR, such as 1) using MABs that compete with the binding of activating ligands to the extracellular domain of the receptor, 2) using small-molecule inhibitors of the intracellular TK domain of the receptor, 3) using immunotoxin conjugates to deliver toxins that target EGFR to tumor cells, 4) reducing the level of EGFR through the use of antisense oligonucleotides, and 5) inhibiting downstream effectors of the EGFR signaling network. We discuss the first two of these strategies because they have been extensively explored in clinical trials.

### Anti-EGFR Antibodies

The use of MABs that inhibit EGFR was the first approach used in clinical studies to target the aberrant signaling of EGFR in malignant cells. The first murine anti-EGFR MABs developed, 528 and 225, showed preclinical activity in a variety of relevant *in vivo* models and acted synergistically with conventional cytotoxic treatments, such as chemotherapy or radiation therapy (66). One of the potential limitations in the development of anti-EGFR is the high incidence of human antimurine antibodies in patients, which could result in decreased efficacy. To avoid this complication, chimeric and humanized forms of the anti-EGFR mAbs were developed. IMC-C225 (cetuximab; ImClone Systems, New York, NY) is the first chimeric human-mouse antibody to be explored in clinical trials. Testing of IMC-C225 in phase III clinical studies among patients with a variety of solid tumors has been completed. In addition, a number of anti-EGFR antibodies have entered clinical development in the last few years, including the fully humanized MABs ABX-EGF (Abgenix, Fremont, CA), EMD 72000 (Merck KgaA, Darm-

stadt, Germany), and h-R3 (Table 1). The principal preclinical and clinical data for IMC-C225 and ABX-EGF are summarized below.

### IMC-C225

**Mechanism of action and preclinical studies.** IMC-C225 binds competitively to the extracellular domain of EGFR ( $K_d = 0.39$  nM), thereby inhibiting the binding of activating ligands to the receptor (66). Consequently, IMC-C225 binding inhibits autophosphorylation of EGFR and induces its internalization and degradation (67–69). Mechanistically, binding of IMC-C225 inhibits the progression of the cell cycle at the  $G_0/G_1$  boundary, increases expression of the cell cycle regulator p27<sup>KIP1</sup>, and induces apoptosis by either increasing expression of pro-apoptotic proteins [e.g., Bax and caspase-3, caspase-8, and caspase-9 (70,71)] or by inactivation of anti-apoptotic proteins (e.g., Bcl-2) by decreased expression or phosphorylation (72, 73). IMC-C225 has also been reported to inhibit the production of pro-angiogenic factors such as vascular endothelial growth factor, interleukin 8, and basic fibroblast growth factor; inhibition of those factors is associated with a decrease in new blood vessel formation and the development of distant metastases in orthotopic cancer models (74–76). Although the antitumor effects of IMC-C225 are also potentially related to its ability to induce an immunologic response, this is probably not a fundamental mechanism (77). In preclinical studies, IMC-C225 showed antitumoral activity against a variety of human tumor xenografts (67,78) and displayed synergistic effects when used with classical cytotoxic agents, such as doxorubicin, cisplatin, topotecan, paclitaxel, and radiation (79–84), suggesting that IMC-C225 may inhibit anti-apoptotic signaling, the accumulation of cells in radiosensitive phases of the cell cycle, DNA repair, autocrine EGFR activation upon DNA damage, or neo-angiogenesis.

**Clinical studies.** Tables 2 and 3 summarize the results of

**Table 1.** Phase I and II clinical trials with various anti-EGFR antibodies\*

Type of trial	Antibody	Tumor type	No. of patients	Dose	Regimen	Maximum tolerated dose, mg/m <sup>2</sup>	Toxicities	Activity	References	Comments
Phase I	EMD 72000	Head and neck Colorectal Esophageal Cervical Others	22	400–2000 mg/wk	Weekly for 5 weeks	1600 mg/wk	Headache, fever†	23% PR 17% SD	Tewes et al. 2002 (158)	EGFR-expressing tumors.
Phase I	h-R3	Head and neck	12	Part A: 50–400 mg/wk	Weekly for 6 weeks plus radiotherapy (60–66 Gy)	Not reached	Tremor, fever, chills, hypotension	67% CR	Crombert et al. 2002 (159)	Patients with advanced loco-regional disease.
			10	Part B: 200–400 mg/wk						EGFR-expressing tumors in part B.
Phase I	ABX-EGF	Renal Prostate Non-small-cell lung Pancreatic Esophageal Colorectal	33	0.01–2.5 mg/kg	Weekly for 4 weeks, then every other week	Not reached	Skin rash	3% MR 6% SD	Figlin et al. 2002 (101)	
Phase II	ABX-EGF	Renal	88	1.0–2.5 mg/kg	Weekly	Not reached	Rigors, vomiting, diarrhea, skin rash	6% PR/MR 50% SD	Schwartz et al. 2002 (102)	20 patients per dose level planned in part I of the study; 40 additional patients will be treated with the two highest dose levels.

\*EGFR = epidermal growth factor receptor; PR = partial response; SD = stable disease; CR = complete response; MR = minor response.

†Dose limiting.

**Table 2.** Phase I and II clinical trials with IMC-C225\*

Type of trial	Tumor type	No. of patients	Loading/ maintenance dose, mg/m <sup>2</sup>	Regimen	Toxicities	Activity	References	Comments
Phase I	Head and neck Non-small-cell lung Colorectal Ovarian Prostate Pancreatic Breast Bladder	52	a) 0/5–100 b) 0/5–100 c) 0/5–400†	a) Single dose b) Weekly for 4 weeks c) Weekly for 4 weeks plus cisplatin‡	Diarrhea, skin rash, allergic reactions, fatigue, nausea, and vomiting	a) 54% SD§ b) 65% SD§ c) 50% SD§ 9% PR§	Baselga et al. 2000 (85)	Cisplatin starting dose was 100 mg/m <sup>2</sup> , which was decreased to 60 mg/m <sup>2</sup> after unexpected toxicity.
Phase I	Head and neck	12	100–500/ 100–250	Weekly for 6 weeks plus cisplatin	Fever, allergic reactions, skin rash	22% CR 44% PR	Mendelsohn et al. 1999 (86,160)	Patients with recurrent diseases.
Phase I	Head and neck	15	100–500/ 100–250	Weekly for 7 weeks plus radiotherapy¶	Mucositis, skin rash, allergic reactions	87% CR 13% PR	Bonner et al. 2000 (87) Ezekiel et al. 1999 (161)	Combined treatment in locally advanced head and neck carcinomas.
Phase II	Kidney	54	400/250	Weekly for 7 weeks	Skin rash	2% OR 25% SD	Gunnnett et al. 1999 (162)	
Phase II	Colorectal	57	400/250	Weekly	Skin toxicity, allergic reactions, asthenia	11% PR 23% SD	Saltz et al. 2002 (93)	EGFR-positive tumors.

\*SD = stable disease; PR = partial response; CR = complete response; OR = objective response; EGFR = epidermal growth factor receptor.

†Patients with non-small-cell lung and head and neck cancer only.

‡Cisplatin at 60 mg/m<sup>2</sup> every 4 weeks.

§Unconfirmed responses after 4 or 6 weeks of treatment.

||Cisplatin at 100 mg/m<sup>2</sup> every 3 weeks.

¶70 Gy (2.0 Gy given once daily) with IMC-C225 escalation and 76.8 Gy (1.2 Gy given twice daily) with highest IMC-C225 dose administered.

various clinical studies that have investigated the toxicity, clinical pharmacology, pharmacodynamics, and clinical efficacy of IMC-C225 in various tumor types, either alone or in combination with conventional chemotherapy and radiation therapy. Phase I studies of IMC-C225 given alone or in combination with cisplatin to subjects with EGFR-positive tumors explored doses of IMC-C225 that ranged from 5 to 400 mg/m<sup>2</sup> without reaching a maximum tolerated dose (85–87). However, continuous saturation of IMC-C225 clearance was reached in most patients treated with a loading dose of 400 mg/m<sup>2</sup> followed by a weekly maintenance dose of 250 mg/m<sup>2</sup>. Because IMC-C225 is cleared by binding to EGFR, it was assumed that saturation of clearance indicated complete saturation of the EGFR binding sites. Therefore, this dose regimen was selected for phase II and phase III studies in patients with different tumor types. In addition, inhibition of EGFR TK activity was demonstrated in sequential tumor biopsy samples obtained from some patients in the phase I studies, indicating sufficient target inhibition by IMC-C225 at this dose level (86). Treatment with IMC-C225 was generally well tolerated, with the predominant side effect being skin toxicity. Antitumor activity associated with IMC-C225 consisted of disease stabilization in the majority of patients, and encouraging objective responses were seen for patients who received IMC-C225 in combination with cisplatin or radiation therapy (Table 2). IMC-C225 treatment of patients with locally advanced head and neck cancers produced objective tumor responses in 15 of 15 patients; 13 of 15 patients had a complete response (median duration of response = 13.9 months) (87). These results generated high expectations that IMC-C225 could be used in combined-modality treatments for patients with head and neck carcinoma because the expected response rates for this subset of patients treated with radiation therapy alone is usually only 20%–60% (88–90). A large phase III study to confirm these

results is ongoing among patients with early-stage head and neck cancer.

The results reported thus far for phase II and phase III studies of IMC-C225 combined with other treatments are summarized in Table 3. These disease-oriented studies with IMC-C225 used a unique clinical trial design: Patients with documented progressive disease under a given chemotherapy regimen were treated with the same chemotherapy regimen plus IMC-C225. The results of two studies that used this strategy in patients with colorectal cancer and head and neck cancer have been reported: Addition of IMC-C225 to irinotecan or to cisplatin-based chemotherapy in patients with refractory colorectal and head and neck cancer, respectively, yielded response rates of 23% and 15%, respectively (91,92). However, this clinical trial design has important limitations, including the methodologic problems that are associated with the definition of resistance and, more importantly, the inability to determine whether the observed effect is a consequence of reversal of resistance to chemotherapy or of intrinsic antitumor activity of the antibody. Indeed, because the response rate of single-agent IMC-C225 in patients with colorectal cancer is only 11%–12% (93), the added benefit of continuing irinotecan treatment in patients with tumors resistant to this agent remains questionable. In these studies (all of the studies listed in Table 3), treatment with IMC-C225 was well tolerated, and the most common toxicities consisted of allergic reactions or acneiform skin rash. Interestingly, in the phase II study of IMC-C225 in patients with colorectal cancer (91), patients who developed skin toxicity appeared to have a better outcome than patients who did not (after adjustment for other confounding factors). If this relationship between skin toxicity and outcome is confirmed in subsequent studies, skin toxicity could be used as a marker to predict the outcome in individual patients.



**Table 3.** Phase II and III clinical trials with IMC-C225 in chemo-naïve and chemorefractory patients\*

Type of trial	Tumor type	No. of patients	Loading/maintenance dose, mg/m <sup>2</sup>	Regimen	Toxicities	Activity	References	Comments
Phase II	Colorectal	120	400/250	Weekly plus irinotecan†	Allergic reactions, skin rash	23% PR	Saltz et al. 2001 (91)	Tumors were EGFR-positive and resistant to both 5-FU and irinotecan.
Phase II	Non-small-cell lung	30	400/250	Weekly plus docetaxel‡	Skin rash, neutropenia, allergic reactions, fatigue	17% PR 10% PR§ 27% SD	Kim et al. 2002 (164)	EGFR-positive tumors. Patients progressing on platinum-based chemotherapy were treated with IMC-C225 and docetaxel.
Phase II	Head and neck	96	400/250	Weekly plus platinum-based chemotherapy	Skin rash	2% CR 13% PR 40% SD	Baselga et al. 2002 (92)	Progressing patients were treated with IMC-C225 using the same schedule as the previous therapy.
Phase II	Pancreatic	41	400/250	Weekly plus gemcitabine	Skin rash, fatigue	12% PR 39% SD	Abbruzzese et al. 2001 (165)	EGFR-positive tumors. Chemo-naïve patients.
Phase II	Colorectal	21	400/250	Weekly plus irinotecan plus high- and low-dose 5-FU/FA¶	Skin rash, diarrhea	5% CR 53% PR 37% SD	Schoeffski et al. 2002 (166)	EGFR-positive tumors. Responses were evaluated in 19 chemo-naïve patients.
Phase II	Colorectal	25	400/250	Weekly plus irinotecan plus 5-FU/FA#	Diarrhea, neutropenia, skin rash	44% PR 20% MR	Rosenberger et al. 2002 (167)	EGFR-positive tumors. Chemo-naïve patients.
Phase III	Head and neck	121	400/250	Weekly plus cisplatin vs. cisplatin plus placebo**	Allergic reactions, neutropenia, skin rash, treatment-related death	23% OR (IMC-C225) 9% OR (placebo)	Burtneess et al. 2002 (94)	Chemo-naïve patients with advanced or recurrent disease.

\*PR = partial response; EGFR = epidermal growth factor receptor; 5-FU = 5-fluorouracil; SD = stable disease; CR = complete response; FA = folinic acid; MR = minor response; OR = objective response.

†Irinotecan dose and schedule were maintained from the regimen used previously.

‡Docetaxel at 75 mg/m<sup>2</sup> every 3 weeks.

§Unconfirmed responses after 4 or 6 weeks.

||Gemcitabine was administered at 1000 mg/m<sup>2</sup> weekly for 7 weeks, followed by 1 week rest, then continued weekly for 3 weeks, and then once every 5 weeks.

¶Irinotecan was administered at 80 mg/m<sup>2</sup> along with 5-FU/FA at either 1500/500 mg/m<sup>2</sup> (low dose) or 2000/500 mg/m<sup>2</sup> (high dose) weekly for 6 weeks, and then once every 7 weeks.

#Irinotecan was administered at 125 mg/m<sup>2</sup> along with 5-FU/FA at 500/20 mg/m<sup>2</sup> weekly for 4 weeks, and then once every 6 weeks at the starting dose; irinotecan at 100 mg/m<sup>2</sup> along with 5-FU/FA at 400/20 mg/m<sup>2</sup> was the recommended dose.

\*\*Cisplatin was administered at 100 mg/m<sup>2</sup> every 4 weeks combined with either IMC-C225 or placebo.

The only phase III study of IMC-C225 with results reported to date has been conducted in chemo-naïve patients with advanced and recurrent carcinoma of the head and neck. In this study (94), cisplatin was administered in combination with either IMC-C225 or placebo. IMC-C225 and cisplatin achieved an objective response rate of 23%, which was substantially higher than that achieved with placebo and cisplatin (9%). This higher response rate, however, was not associated with longer progression-free or overall survival times. These results were unexpected on the basis of preclinical studies that had shown substantial synergy between IMC-C225 and cisplatin, and they call into question the benefits of combining IMC-C225 with chemotherapy (94). Ongoing clinical trials with IMC-C225 include phase III clinical studies in patients with head and neck cancer to investigate treatment with cisplatin or radiation therapy, either alone or in combination with IMC-C225.

## ABX-EGF

**Mechanism of action and preclinical studies.** ABX-EGF is a fully human MAb directed against EGFR that was generated using the XenoMouse model (95). ABX-EGF binds to the EGFR with high affinity, blocks the binding of EGF and TGF- $\alpha$  to

EGFR, and inhibits autophosphorylation of the TK domain of EGFR (96). In preclinical studies, ABX-EGF showed high antitumor activity against a broad panel of human xenograft tumors, and treatment efficacy appeared to be associated with EGFR expression levels (97). Furthermore, combined treatment with ABX-EGF and conventional chemotherapeutics showed additive effects in human epidermoid, prostate, and lung cancer xenograft tumors (98,99). On the basis of these results, a bio-mathematical model was developed to predict the antitumor efficacy of ABX-EGF in patients, and maximum antitumor activity was calculated for maintenance doses ranging from 1 to 3 mg/kg per week (100).

**Clinical studies.** ABX-EGF is currently undergoing development in clinical trials (Table 1). In the phase I trial, ABX-EGF was administered at doses ranging from 0.01 to 2.5 mg/kg weekly for 4 consecutive weeks and every other week thereafter (101). In that study, treatment with ABX-EGF was well tolerated and no dose-limiting toxicity was reported. The predominant toxicity in all patients was skin rash; no allergic reactions or human anti-human antibodies were observed. Three percent of patients treated in these studies achieved a minor response, and an additional 6% had disease stabilization after treatment. In a

disease-oriented phase II trial, the antitumor efficacy of ABX-EGF was evaluated among renal cancer patients who were unable to receive interferon-based therapy or whose cancers had progressed while receiving such therapy (102). Toxicities were generally mild to moderate, and no allergic reactions or human anti-human antibodies were observed. Responses were partial or minor in 6% of the patients, and disease stabilization was obtained in 50% of the patients.

### Small-Molecule Inhibitors of EGFR TK Activity

The second approach to inhibit the EGFR signaling network that has been widely investigated in clinical trials uses agents that block the activation of the EGFR TK domain. The basic mechanism of action of these agents is competitive inhibition of the binding of ATP to the TK domain of the receptor, resulting in inhibition of EGFR autophosphorylation. Although early studies used naturally occurring small molecules that inhibited the TK moiety of EGFR *in vitro*, the *in vivo* antitumor effects of those agents were limited. Subsequently, novel compounds with improved pharmacologic properties were discovered using a molecular modeling approach and were tested in preclinical studies. A growing number of these agents, the most representative of which are listed in Table 4, have entered clinical development. Although these agents share a common mechanism of action, they have different specificities for the various members of the EGF family and different potencies, and they differ in the reversibility of their interactions with the ATP-binding site. Below we describe the two agents that are in advanced stages of clinical development—OSI-774 (erlotinib [Tarceva]; OSI Pharmaceuticals, Uniondale, NY) and ZD1839 (gefitinib [Iressa]; AstraZeneca, Wilmington, DE). Other specific inhibitors of the EGF family, such as CI-1033, EKB-569, and PKI-166, have been developed and have entered clinical development. Table 5 summarizes the principal findings of clinical studies with these other agents.

### OSI-774

**Mechanism of action and preclinical studies.** OSI-774 ([6,7-bis (2-methoxy-ethoxy)-quinazolin-4-yl]-[3-ethylphenyl]-amine) is a novel low-molecular-weight quinazolin derivative that acts as a potent and reversible inhibitor of EGFR TK activity. The drug inhibits the EGFR TK at concentrations of 2 nM in purified enzyme assays and 20 nM in whole-cells assays, whereas inhibition of unrelated TKs appeared at a 1000-fold higher concentration. In molecular analyses (103,104), the antitumor activity of OSI-774 was found to be associated with the increased expression of cell cycle regulatory proteins, such as

p27<sup>KIP1</sup>, and the induction of apoptosis. A recent report (105) indicates that submicromolar concentrations of OSI-774 can specifically inhibit the activation of EGFRvIII *in vitro*. In *in vivo* studies (106), administration of OSI-774 to mice bearing xenograft tumors derived from human head and neck cancer cells (HN5) inhibited the activation of the EGFR TK with an effective dose (ED)<sub>50</sub> of 9.2 or 9.9 mg/kg after either intraperitoneal or oral administration, respectively. In these studies, maximum inhibitory effects (90%) were obtained 1 hour after treatment, and 75%–85% inhibition was maintained for at least 12 hours; complete recovery to baseline occurred by 24 hours post-treatment (106–109). An important observation in the preclinical studies with OSI-774 was the direct relationship between target inhibition and antitumor effects in the animal model, suggesting that sufficient doses to inhibit the target in tumor tissues would need to be administered for this agent to be effective in the clinic.

**Clinical studies.** Table 6 summarizes the clinical trials conducted thus far with OSI-774. Phase I studies explored the administration of OSI-774, by either a continuous oral dosing schedule or a weekly oral administration schedule (110,111). The principal dose-limiting toxicities in these trials were reported to be acneiform skin rash and diarrhea (111). When administered on a continuous dosing regimen, the recommended dose of 150 mg/day of OSI-774 was well tolerated and exceeded plasma concentrations that were associated with activity in pre-clinical models. Antitumor activity was noted in patients with renal, non-small-cell lung, colon, and head and neck carcinomas. Parallel pharmacodynamic studies explored the biologic effects of OSI-774 in tumor and cutaneous tissues collected prior to and after 28 days of treatment. As expected, OSI-774 treatment was associated with the inhibition of EGFR activation, blockage of the EGFR signal transduction pathway, and increased expression of p27<sup>KIP1</sup> (112,113).

OSI-774 is currently being investigated in disease-oriented phase II and phase III clinical studies as either a single agent or in combination with conventional chemotherapeutics. Single-agent activity was observed among patients with non-small-cell lung cancer, head and neck carcinoma, and ovarian cancer who were previously treated with other chemotherapeutic agents (Table 6) (114–116). OSI-774 treatment of this heavily pretreated patient population produced objective responses in 6%–13% of the patients and disease stabilization in 29%–44% of the patients. Interestingly, EGFR expression level failed to predict clinical outcome among the patients with head and neck carcinoma in the phase II study (115). That study enrolled patients with various degrees of EGFR expression and retrospectively

**Table 4.** Small-molecule inhibitors of epidermal growth factor receptor (EGFR) currently in clinical development

Agent	EGFR IC <sub>50</sub> , nM*	Reversible inhibition	Development stage	References
ZD1839	23	Yes	Phase III	Ciardiello and Tortora 2001 (66)
OSI-774	20	Yes	Phase III	Hidalgo et al. 2001 (111), Moyer et al. 1997 (103)
CI-1033 (PD183805)	2	No	Phase I	Garrison et al. 2001 (168), Rinehart et al. 2002 (169)
PKI-166	0.7	Yes	Phase I	Hoekstra et al. 2001 (170), Murren et al. 2002 (171)
GW2016	9.2	Yes	Phase I	Mullin et al. 2001 (172)
EKB-569	38.5	No	Phase I	Hidalgo et al. 2002 (173)
PD168393	0.7	No	Preclinical	Fry et al. 1998 (174)
AG-1478	<3	No	Preclinical	Busse et al. 2000 (175)
CGP-59326A	27	Yes	Preclinical	Lydon et al. 1998 (176)

\*IC<sub>50</sub> = concentration inhibiting cell growth by 50%.

**Table 5.** Phase I clinical trials with CI-1033, PKI-166, and EKB-569\*

Agent	Tumor type	No. of patients	Dose, mg/day	Regimen	Maximum tolerated dose, mg/day	Toxicities	Activity	References	Comments
CI-1033	Several	68	2–220	Day 1–14, then every 3 weeks Day 1–21, then every 4 weeks Day 1–28, then every 5 weeks Continuous	Not determined	Stomatitis, skin rash	NA	Rinehart et al. 2002 (169)	
CI-1033	Non-small-cell lung Head and neck Skin Colon Breast Pancreatic	54	50–750	Daily for 7 days, then every 3 weeks	750	Diarrhea, nausea, hypersensitivity, thrombocytopenia	1.8% OR 24% SD	Zinner et al. 2001 (177)	
CI-1033	Several	34	100–560	Weekly for 3 weeks, then every 4 weeks	Not determined	Diarrhea, nausea, hypersensitivity	2.9% SD	Garrison et al. 2001 (168)	
PKI-166	Thyroid Renal A-CUP Others	15 16 25	50–100 50–400 50–900	Continuous M-W-F Day 1–14, then every 2 weeks	50	Transaminase elevations, diarrhea, skin rash, nausea, and emesis	7.1% SD	Hoekstra et al. 2001 (170), 2002 (178, 179)	Dose-limiting toxicity reached at 50 mg (countinuous administration), 400 mg (M-W-F) and 900 mg (2 weeks on and 2 weeks off).
PKI-166	Colorectal Head and neck Non-small-cell lung	32	50–600	Continuous for at least 4 weeks	NA	Transaminase elevations, diarrhea, skin rash, fatigue	3% PR 9% SD	Murren et al. 2002 (171)	
EKB-569	Colorectal Breast Head and neck Non-small-cell lung	30 11	25–125 25–50	Day 1–14, then every 4 weeks Continuous	75	Diarrhea, skin rash, nausea and vomiting, stomatitis	NA	Hidalgo et al. 2002 (173)	

\*NA = not available; OR = objective response; SD = stable disease; A-CUP = advanced cancer of unknown primary; M-W-F = Monday-Wednesday-Friday schedule; PR = partial response.

analyzed the relationship between parameters of outcome and levels of EGFR expression. These results underscore the complex nature of the EGFR signaling network in human cancers and suggest that EGFR expression may not be an appropriate predictor of antitumoral activity.

On the basis of promising results from combination therapy studies, exploratory phase I clinical trials of OSI-774 in combination with paclitaxel–carboplatin, gemcitabine–cisplatin, and docetaxel were initiated (117–119). The fact that toxicities of OSI-774 and conventional chemotherapeutic agents do not overlap permitted escalation of OSI-774 doses to the 100–150 mg/day range in combination with full doses of chemotherapeutics in both minimally and heavily pretreated patients. In these studies, dose-limiting toxicities consisted of neutropenia, acneiform skin rash, and diarrhea, requiring dose reductions in some protocols (118,119). Pharmacokinetic studies that were performed in parallel (118,119) did not reveal interactions between the tested chemotherapeutics and OSI-774. Combined treatment achieved promising objective responses in patients with non-small-cell lung cancer, head and neck cancer, ovarian cancer, and mesothelioma. Enrollment of subjects into pivotal phase III clinical trials is ongoing to explore the activity of OSI-774,

either alone or in combination with conventional chemotherapy, in non-small-cell lung and pancreatic cancer.

### ZD1839

**Mechanism of action and preclinical studies.** ZD1839 [4-(3-chloro-4-fluoroanilino)-7-methoxy-6-(3-morpholinopropoxy)quinazoline] is a low-molecular-weight (447 kd) quinazolin derivative that specifically inhibits the activation of EGFR TK through competitive binding of the ATP-binding domain of the receptor. ZD1839 inhibits EGFR TK activity with IC<sub>50</sub> values that range from 0.023  $\mu$ M to 0.079  $\mu$ M and is approximately 100-fold less effective in inhibiting HER2 TK activity (IC<sub>50</sub>>3.7  $\mu$ M) (120–125). Interestingly, ZD1839 has been reported to inhibit autophosphorylation of HER2 in breast cancer cells in which HER2 is preferentially activated by heterodimerization with EGFR at an IC<sub>50</sub> of 1  $\mu$ M (124,126). In preclinical studies, exposure to ZD1839 inhibited the growth of a wide range of cancer cell lines that overexpress EGFR (123,127,128). Mechanistically, ZD1839 treatment was associated with cell cycle arrest at the G<sub>0</sub>/G<sub>1</sub> boundary in a dose- and time-dependent manner that involved the increased expression of the cyclin-dependent kinase inhibitor p27<sup>KIP1</sup> (129,130). Subsequent *in vivo*

**Table 6.** Clinical trials with OSI-774\*

Type of trial	Tumor type	No. of patients	Dose, mg/day	Regimen	Maximum tolerated dose, mg/day	Toxicities	Activity	References	Comments
Phase I	Colorectal Breast Renal Non-small-cell lung Prostate	40	25–200	3 days/wk for 3 weeks, then every 4 weeks Weekly for 3 consecutive weeks, then every 4 weeks Continuous	150	Diarrhea,‡ skin rash‡	5% OR 15% SD	Hidalgo et al. 2001 (111)	
Phase I	Head and neck Others Lung Prostate Colorectal Head and neck Breast Renal Liposarcoma	28	100–1600	Continuous Days 1, 8, and 15, then every 4 weeks	Not reached	Diarrhea	NA	Karp et al. 2000 (110)	
Phase II	Non-small-cell lung	57	150	Continuous	NA	Skin rash	2% CR 14% PR 26% SD	Perez-Soler et al. 2001 (114)	
Phase II	Head and neck	124	150	Continuous	NA	Diarrhea, skin rash, nausea, vomiting, headache, fatigue	6% PR 40% SD	Senzer et al. 2001 (115)	
Phase II	Ovarian	34	150	Continuous	NA	Diarrhea, skin rash, nausea, vomiting, headache, fatigue	6% PR 50% SD	Finkler et al. 2001 (116)	Two unconfirmed PR.
Phase I	Head and neck Non-small-cell lung Others	9	100–150	Continuous plus paclitaxel plus carboplatin§	NA	Neutropenia,‡ diarrhea,‡ rash‡	11% PR 22% MR 22% SD	Forero et al. 2002 (117)	
Phase I	Mesothelioma Non-small-cell lung Others	7	100	Continuous plus gemcitabine plus cisplatin	NA	Neutropenia,‡ renal toxicity, increased prothrombin time	30% MR 30% SD	Ratain et al. 2002 (118)	Six patients assessable for response.
Phase I	Nasopharyngeal Non-small-cell lung Bladder Ovarian Gastric Others	22	100–150	Continuous plus docetaxel¶	NA	Febrile neutropenia‡	5% CR 5% MR 23% SD	Forouzesh et al. 2002 (119)	

\*OR = objective response; SD = stable disease; NA = not available; CR = complete response; PR = partial response; MR = minor response.

†Recommended dose.

‡Dose-limiting toxicity (DLT).

§Paclitaxel administered at 225 mg/m<sup>2</sup> with carboplatin area under the concentration versus time curve (AUC) at 6 mg · h/L by six intravenous injections every 3 weeks.

||Gemcitabine administered at 1000 mg/m<sup>2</sup> on days 1, 8, and 15 along with cisplatin at 100 mg/m<sup>2</sup> on day 1, then every 28 days; regimen was reduced to gemcitabine at 1000 mg/m<sup>2</sup> on days 1 and 8 and cisplatin at 60 mg/m<sup>2</sup> on day 1, then every 21 days.

¶Docetaxel administered at 75 mg/m<sup>2</sup> was reduced to 60 mg/m<sup>2</sup> after reaching the DLT.

studies demonstrated that ZD1839 treatment is associated with profound antitumor effects in human xenograft tumors, including those derived from hormone-resistant prostate cancer, ovarian cancer, ductal carcinoma *in situ* of the breast, and colon, vulval, and small-cell and non-small-cell lung cancers (122,123,131,132). Although ZD1839 treatment was associated with the rapid regression of established tumors, the tumors re-grew when the drug was discontinued, suggesting that long-term drug administration will be required to maintain a tumor response in

patients. ZD1839 treatment was associated with similar antitumor activity among xenograft models with variable EGFR expression, suggesting that other factors besides EGFR expression may predict tumor response to ZD1839. These factors could include expression levels of activating ligands, dimerization partners, the presence of mutated receptors, and the activation pattern of downstream signaling pathways by EGFR. As discussed below, these findings have important clinical and developmental implications regarding which patients would derive



the most benefit from treatment with ZD1839. As predicted on the basis of the mechanism of action of ZD1839, combinations of this agent with conventional chemotherapy and radiation therapy were associated with improved therapeutic outcome (80,123,128,132). Furthermore, recent preclinical studies have indicated that simultaneously blocking the TK activities of EGFR and HER2 with the combination of ZD1839 and trastuzumab was associated with additive or synergistic effects in *in vitro* and *in vivo* models (124,133).

**Clinical studies.** The results of published clinical trials with ZD1839 are displayed in Tables 7 and 8. Four phase I trials investigated intermittent and continuous administration of ZD1839 as a single agent among a total of 250 patients using doses that ranged from 50 to 800 mg/day. Treatment with ZD1839 was well tolerated; reversible, noncumulative, grade 1

and grade 2 cutaneous and gastrointestinal toxicities predominated. Dose-limiting toxicity (diarrhea) was observed at doses greater than 700 mg/day. The principal pharmacokinetic parameters derived from these studies include the time to peak plasma levels (3–7 hours after oral dosing) and the elimination half-life (27–85 hours). Plasma concentrations of ZD1839 above the levels expected to be associated with activity in preclinical studies were achieved at doses well below the doses associated with toxicity, indicating that the drug has a high therapeutic index (134–139). In addition, correlative pharmacodynamic studies in serial skin biopsy samples indicate that doses of ZD1839 greater than 100 mg/day are associated with inhibition of the activation and signaling of EGFR (140). Importantly, and probably unexpectedly for an agent that was thought to be predominantly cytostatic, treatment with ZD1839 was associated with objective

**Table 7.** Phase I clinical trials with ZD1839\*

Tumor type	No. of patients	Dose, mg/day	Regimen	Maximum tolerated dose, mg/day	Toxicities	Activity	References	Comments
Non-small-cell lung Colorectal Head and neck Ovarian Breast Prostate Others	64	50–700	Days 1–14, then every 4 weeks	700	Diarrhea, skin rash†	3% PR 3% MR 3% SD	Ranson et al. 2002 (154)	Responses were evaluated in non-small-cell lung cancer patients.
Non-small-cell lung Colorectal Head and neck Breast	31	50–700	Days 1–14, then every 4 weeks	700	Diarrhea, transaminase elevation†	9% OR 4% SD	Negoro et al. 2001 (139)	
Non-small-cell lung Colorectal Head and neck Ovarian Prostate Others	127	150–800	Continuous	NA	Diarrhea, skin rash	2% PR 2% MR	Baselga et al. 2000 (136)	800-mg dose under evaluation.
Non-small-cell lung Colon Endometrial Others	28	150–800	Continuous	NA	Diarrhea	3% PR 7% MR	Goss et al. 2001 (135)	No further dose escalation based on results from other phase I studies.
Non-small-cell lung Esophageal A-CUP Others	18	250 or 500	Continuous ZD1839 from day 2 on plus gemcitabine–cisplatin‡	NA	Diarrhea, skin rash, asthenia, transaminase elevation, vomiting	53% PR 41% SD	Gonzalez-Larriba et al. 2002 (143)	17 chemo-naïve patients assessable for response.
Non-small-cell lung	25	250 or 500	ZD1839 on days 1–14 or 22–36 in combination with carboplatin–paclitaxel§	NA	Skin rash	28% PR 40% SD	Miller et al. 2001 (142)	
Colorectal	26	250–500	ZD1839 on days 1–14 or 22–35 in combination with 5-FU/LV	500	Diarrhea,† skin rash,† neutropenia	11% PR 8% PR¶	Hammond et al. 2001 (141)	

\*PR = partial response; MR = minor response; SD = stable disease; OR = objective response; A-CUP = advanced cancer of unknown primary; 5-FU = 5-fluorouracil; LV = leucovorin.

†Dose-limiting toxicity (DLT).

‡Gemcitabine administered at 1250 mg/m<sup>2</sup> for 30 minutes by infusion on days 1 and 8 and then every 21 days. Cisplatin administered at 80 mg/m<sup>2</sup> for 2 hours by infusion after administration of gemcitabine.

§Carboplatin area under the concentration versus time curve (AUC) at 6 mg · h/L and paclitaxel administered at 200 mg/m<sup>2</sup> on days 8 and 36 (with ZD1839 on days 1–14) or on days 1 and 29 (with ZD1839 on days 22–36).

||5-FU administered at 370 mg/m<sup>2</sup> along with leucovorin at 20 mg/m<sup>2</sup> by intravenous push on days 8–12 and 36–40 (with ZD1839 on days 1–14) or on days 1–5 and 29–33 (with ZD1839 on days 22–35).

¶Unconfirmed.

**Table 8.** Phase I and II clinical trials with ZD1839\*

Type of trial	Tumor type	No. of patients	Dose, mg/day	Regimen	Toxicities	Activity	References	Comments
Phase I/II	Colorectal	27	750	Continuous	Skin rash, diarrhea, fatigue, pruritus	17% SD	Goss et al. 2002 (180)	Dose reduction in one-third of the patients required. 24 patients assessable for response.
Phase II	Non-small-cell lung	210	250 or 500	Continuous	Diarrhea, skin rash, pruritus	19% OR 53% DCR	Fukuoka et al. 2002 (144)	IDEAL 1: patients who failed 1 or 2 chemotherapy regimens were eligible (Europe, Japan).
Phase II	Non-small-cell lung	216	250 or 500	Continuous	Skin rash, diarrhea	11% OR 29% SD	Kris et al. 2002 (145)	IDEAL 2: patients who failed 2 or more prior chemotherapy regimens were eligible (USA).
Phase II	Head and neck	52	500	Continuous	Skin rash, diarrhea, neuropathy, fatigue, hypercalcemia, infection	8% CR 12% PR 35% SD	Cohen et al. 2002 (181)	Recurrent or metastatic squamous cell carcinoma included. 40 patients assessable for response.
Phase II	Renal	18	500	Days 1–28	Skin rash	No response	Drucker et al. 2002 (182)	16 patients assessable for response.

\*SD = stable disease; OR = objective response; DCR = disease control rate (SD + OR); IDEAL = Iressa Dose Evaluation in Advanced Lung Cancer; CR = complete response; PR = partial response.

responses in the phase I setting, particularly among patients with non-small-cell lung cancer. Eight of a total of 99 patients with non-small-cell lung cancer treated in phase I studies with ZD1839 had disease stabilization. In addition, eight patients had an objective response of 1–16 months duration. Phase I clinical trials of ZD1839 in combination with 5-fluorouracil, paclitaxel–carboplatin, and cisplatin–gemcitabine have been completed; the results of those trials are summarized in Table 7. Those studies generally explored doses of ZD1839 that were thought to be effective for single-agent ZD1839 (250 or 500 mg) with a full dose of the standard chemotherapy regimen (141–143). In general, the regimens were well tolerated, and no increases in toxicities were observed compared with regimens of ZD1839 or chemotherapy alone or with pharmacologic interactions for the combined treatments.

The observation of antitumor activity in non-small-cell lung cancer in phase I clinical trials led to the decision to further explore the use of ZD1839 for treatment of this disease. Four clinical trials (144,145,183,184) that have evaluated the activity of ZD1839, either alone or in combination with chemotherapy, have been completed. Two of those trials (144,145) investigated the activity of single-agent ZD1839 in previously treated patients with non-small-cell lung cancer. The Iressa Dose Evaluation in Advanced Lung Cancer (IDEAL) 1 and IDEAL 2 studies were conducted in Europe and Japan and in the United States, respectively, among patients with non-small-cell lung cancer that was resistant to platinum-based therapy (IDEAL 1, one or two previous regimens) or platinum and docetaxel chemotherapy (IDEAL 2, two or more regimens) (Table 8). Among the 208 patients who were assessable for response in the IDEAL 1 study, there were no differences between the high- and low-dose treatment arms with regard to response rates (18.4% and 19.0%, respectively) or disease control rates (54.4% and 51.4%, respectively). In addition, response rates were not associated with the number of prior chemotherapy regimens a patient had undergone. However, patients treated with 500 mg/day ZD1839 experienced a higher incidence of adverse events than patients

treated with 250 mg/day (144). Similarly, objective response and stable disease rates were similar for both treatment arms among the 216 patients who were enrolled in IDEAL 2. On the basis of these studies, the U.S. Food and Drug Administration approved ZD1839 in 2003 for third-line treatment of patients with non-small-cell lung cancer.

In parallel to the IDEAL trials, two randomized clinical trials that compared the activity of two doses of ZD1839 versus placebo in combination with conventional chemotherapy in patients with advanced non-small-cell lung cancer were completed (Iressa Non-small-cell lung cancer Trial Assessing Combination Treatment [INTACT] trials 1 and 2). These randomized placebo-controlled phase III trials in patients with stage IIIB or stage IV non-small-cell lung cancer had overall survival as the primary endpoint. Each trial had a three-arm design, in which patients were randomly assigned to receive conventional treatment with either gemcitabine–cisplatin (INTACT 1) or paclitaxel–carboplatin (INTACT 2) plus either ZD1839 (250 or 500 mg) or placebo. The first results of these studies (183,184) were recently presented and are summarized in Table 9. In these two studies, the addition of ZD1839 to the conventional chemotherapy regimens did not improve any parameter of outcome. In addition to these trials, a large number of phase I, II, and III clinical trials, some of which are summarized in Table 8, are currently ongoing with ZD1839 as a single agent or in combination with other agents and therapeutic modalities.

## CHALLENGES IN THE DEVELOPMENT OF EGFR-TARGETED THERAPIES

The clinical development of EGFR inhibitors illustrates the potential difficulties and challenges associated with the development of novel targeted agents. Phase II and III clinical testing, using developmental strategies similar to those used for classical anticancer agents, has already been completed for many of these inhibitors. The results of these studies have demonstrated that

**Table 9.** Phase III clinical trials with ZD1839\*

Tumor type (Trial)	No. of patients	Regimen	Response rate, %	Median time to tumor progression, mo	Median survival, mo	References
Non-small-cell lung (INTACT 1)	1093	Gemcitabine-cisplatin† plus placebo	44.8	5.98	11.07	Giaccone et al. 2002 (183)
		250 mg ZD1839	50.2	5.85	9.86	
		500 mg ZD1839	49.7	5.55	9.92	
Non-small-cell lung (INTACT 2)	1037	Carboplatin-paclitaxel‡ plus placebo	33.5	5.06	9.92	Johnson et al. 2002 (184)
		250 mg ZD1839	35.0	5.32	9.82	
		500 mg ZD1839	32.1	4.67	8.74	

\*INTACT = Iressa Non-small-cell lung cancer Trial Assessing Combination Treatment.

†Gemcitabine administered at 1250 mg/m<sup>2</sup> for 30 minutes by infusion on days 1 and 8 along with cisplatin at 80 mg/m<sup>2</sup> for 2 hours by infusion after gemcitabine on the first day of every 21-day cycle.

‡Carboplatin area under the concentration versus time curve (AUC) at 6 · h/L and paclitaxel at 200 mg/m<sup>2</sup> on the first day of every 21-day cycle.

some of the initial assumptions, such as the lack of dose-related toxicities and inability to induce an objective tumor response, were incorrect and that inhibitors of EGFR resemble conventional chemotherapy drugs in many respects. There are, however, still some unique features of this class of drugs that could be better exploited by a more specific clinical development plan (146–148). In this section of the review, we summarize some of the most relevant issues that are pertinent to the EGFR inhibitors, including selection of patients most likely to benefit from this class of agents, selection of the most appropriate dose and schedule of administration, incorporation of pharmacodynamic markers into clinical studies, and identification of surrogates of patient outcomes.

Selection of the appropriate population of patients for participation in clinical studies with these compounds has generated the most debate. The original hypothesis—that only patients who overexpressed EGFR would benefit from treatment with these agents—was based on preclinical data that suggested that there was a relationship between the expression level of the receptor and its susceptibility to the inhibitors (124,149–151). However, the results of several studies conducted thus far suggest that EGFR expression alone is not sufficient to predict the response of cell lines to these agents *in vitro* (150,151). Other factors, such as the activation of downstream signaling pathways, the dependence of cell growth and proliferation on EGFR-regulated pathways, the presence of activating growth factors, and the relative expression of EGFR versus other members of this family of receptors, have been shown to influence the cellular response to these inhibitors in preclinical models (124,125,149,150). These data suggest that a useful pharmacodiagnostic marker for EGFR inhibitors should incorporate various elements of downstream signaling pathways. In this regard, the development and validation of immunohistochemical methods to measure the activation of signaling pathways with phosphorylation-specific antibodies could be important (152,153). In addition, tumors that express EGFRvIII could be classified as EGFR-negative, depending on the method used to measure the expression of the receptor, when indeed, cells with vIII mutations are inhibited by some of these compounds (105). The lack of properly validated assays to measure the expression and activation of EGFR, as well as the semiquantitative and subjective nature of the most commonly used immunohistochemical methods of measuring EGFR expression/activation, also further complicate the issue of patient selection. Most clinical trials currently conducted with EGFR inhibitors have not selected patients on the basis of a specific molecular feature. This decision appears ap-

propriate considering the lack of data to support the selection of a specific subset of patients and the availability of adequate assays to measure EGFR dependence.

The second relevant aspect in the development of EGFR-directed therapies is the selection of appropriate dose and schedule of administration for phase II and III studies. In studies with EGFR inhibitors, the selection of phase II dose has been based on toxicity as well as pharmacokinetic parameters, such as plasma concentrations above a biologically relevant level or saturation of clearance, which suggests complete occupancy of drug-binding sites (85,111,154). Subsequent disease-oriented studies have been conducted that use an optimal dose that was determined in phase I evaluations or have explored, in randomized phase II studies, different dose levels. This strategy was used in the IDEAL 1 and IDEAL 2 studies, in which two doses of ZD1839 below the recommended phase II dose (which was based on results of phase I clinical studies) were given to patients with non-small-cell lung cancer (144,145). It should be noted, however, that there was substantial interpatient variability in the plasma concentrations of ZD1839 achieved after administration of either dose, suggesting a probable overlap in the exposure obtained with the two dose levels that would limit a dose-response effect.

Two general observations about ZD1839 and EGFR are relevant to the appropriate selection of dose and schedule of this class of drugs. First, there is a linear relationship between target inhibition and antitumor activity, and second, only tumors in which inhibition of the receptor results in inhibition of downstream signaling pathways are growth arrested (106,150,151). These observations suggest that the optimal dose of an EGFR inhibitor is the dose at which the target (i.e., EGFR) is inhibited. The definition of the pharmacologically active dose is, however, more difficult to establish. The approach that has been used to determine the biologically relevant dose is to collect tumor biopsy samples before and after treatment to measure pharmacodynamic effects. The limitation of this approach, however, is that it is difficult to collect tumor tissues for biologic studies from a sufficient number of patients to draw meaningful conclusions (112,155). To overcome this barrier, investigators have used normal tissues and, when testing EGFR inhibitors, normal skin to develop pharmacodynamic surrogates of EGFR inhibition. Results of these studies have demonstrated that treatment of patients with EGFR TK inhibitors is associated with the inhibition of EGFR activation and signaling in normal epidermis (112,140,153). The principal limitation of such studies is that there is not necessarily a direct relationship between EGFR inhibition in

normal skin and in tumor tissues. In addition, it is possible that the downstream effects of inhibiting EGFR in skin tissues and tumor tissues are different because tumors tend to have mutations and alterations of downstream signaling elements that could result in constitutive activation of these pathways.

Finally, it is important to establish how the activity of an EGFR inhibitor should be defined in phase II studies. On the basis of preclinical data, it was expected that the principal effect of these agents would be to induce tumor stabilization rather than tumor response. However, results of phase II studies demonstrated that these agents indeed are capable of inducing tumor response, although the meaningful response rate for this class of agents is not known. Methods that could be used in phase II studies to determine the activity of an EGFR inhibitor include functional imaging techniques such as positron emission tomography to follow tumor size and metabolic activity. In addition, the application of novel clinical trial designs, such as the randomized discontinuation design and the multinomial method, may facilitate the evaluation of drugs that primarily induce stable disease (141,156,157). In addition, the pharmacodynamic markers of biologic activity mentioned in the previous paragraph should be explored as surrogates of activity in phase II disease-oriented studies.

In conclusion, EGFR is a valid target for therapeutic intervention in patients with cancer. Work conducted over the past two decades has culminated in the clinical development of a substantial number of specific inhibitors of this target. The results from the clinical studies completed thus far are promising, and one of these drugs (ZD1839) has already been approved for cancer treatment. Important questions, such as the selection of patients most likely to benefit from these types of agents, the definition of the optimal biologic dose and schedule of administration, and the appropriate endpoints and assessment of outcome in disease-oriented clinical trials remain to be addressed and will constitute the focus of future research.

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## NOTE

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