Review

A Novel Approach in the Treatment of Cancer: Targeting the Epidermal Growth Factor Receptor¹

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Abstract

The epidermal growth factor receptor (EGFR) autocrine pathway contributes to a number of processes important to cancer development and progression, including cell proliferation, apoptosis, angiogenesis, and metastatic spread. The critical role the EGFR plays in cancer has led to an extensive search for selective inhibitors of the EGFR signaling pathway. The results of a large body of preclinical studies and the early clinical trials thus far conducted suggest that targeting the EGFR could represent a significant contribution to cancer therapy. A variety of different approaches are currently being used to target the EGFR. The most promising strategies in clinical development include monoclonal antibodies to prevent ligand binding and small molecule inhibitors of the tyrosine kinase enzymatic activity to inhibit autophosphorylation and downstream intracellular signaling. At least five blocking monoclonal antibodies have been developed against the EGFR. Among these, IMC-225 is a chimeric human-mouse monoclonal IgG1 antibody that has been the first anti-EGFR targeted therapy to enter clinical evaluation in cancer patients in Phase II and III studies, alone or in combination with conventional therapies, such as radiotherapy and chemotherapy. A number of small molecule inhibitors of the EGFR tyrosine kinase enzymatic activity is also in development. OSI-774 and ZD1839 (Iressa) are currently in Phase II and III development, respectively. ZD1839, a p.o. active, selective quinazoline derivative has demonstrated promising in vitro and in vivo antitumor activity. Preliminary results from Phase I and II trials in patients with advanced disease demonstrate that ZD1839 and OSI-774 have an acceptable tolerability profile and promising clinical efficacy in patients with a variety of tumor types. This mini-review describes the EGFR inhibitors in clinical development.

Introduction

Role of the EGFR³-driven Autocrine Pathway in Cancer Development and Progression. Peptide growth factors modulate signaling pathways, which control cell proliferation and death in both normal and malignant cells. The EGF was one of the first growth factors to be discovered and is the prototype of a large family of closely related growth factors, which includes TGFα, amphiregulin, heparin binding-EGF, and beta-cellulin. Among these growth factors, TGFα has been identified as a key modulator in the process of cell proliferation in both normal and malignant epithelial cells. TGFα binds to its specific cell membrane receptor, the EGFR, with subsequent activation of the EGFR tyrosine kinase enzymatic activity that triggers the intracellular signaling pathway (1, 2).

The EGFR is part of a subfamily of four closely related receptors: EGF (or ErbB-1), HER-2/neu (ErbB-2), HER-3 (ErbB-3), and HER-4 (ErbB-4; Refs. 3 and 4). The receptors exist as inactive monomers, which dimerize after ligand activation. This causes homodimerization or heterodimerization between EGFR and another member of the erb receptor family. After ligand binding, the tyrosine kinase intracellular domain of the receptor is activated, with autophosphorylation of the intracellular domain, which initiates a cascade of intracellular events (4). The signaling pathway involves activation of ras and mitogen-activated protein kinase, which activates several nuclear proteins, including cyclin D1, a protein required for cell cycle progression from G1 to S phase (Ref. 5; Fig. 1). EGF signaling is not only critical for cell proliferation. Several studies have demonstrated that EGFR-mediated signals also contribute to other processes that are crucial to cancer progression, including angiogenesis, metastatic spread, and the inhibition of apoptosis (4–7).

Activation of the TGFα-EGFR autocrine growth pathway in cancer cells can be attributable to several mechanisms, such as overexpression of the EGFR, increased concentration of ligand(s), decreased phosphatase activity, decreased receptor turnover, and the presence of aberrant receptors, including EGFR gene alterations. In this context, the most common EGFR mutant found in human cancer is EGFRvIII (8). The rearranged EGFRvIII gene is often amplified, thus resulting in tumor cells overexpressing EGFRvIII (9). The EGFRvIII is a truncated EGFR that lacks domains I and II of the extracellular domain and is not capable of ligand binding (9). However, it has a constitutively activated tyrosine kinase domain which stimulates cell proliferation independently of ligand interaction. TGFα and/or EGFR are overexpressed in many different solid human

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³ The abbreviations used are: EGF, epidermal growth factor receptor; EGF, epidermal growth factor; TGFα, transforming growth factor α; TKI, tyrosine kinase inhibitor; MAb, monoclonal antibody; NSCLC, non-small cell lung cancer; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor.
cancers, including NSCL, breast, head and neck, gastric, prostate, bladder, ovarian, colorectal carcinomas, and glioblastomas, in which it is generally associated with advanced disease and poor prognosis (7, 10–12). Overexpression of EGFR has also been associated with resistance to hormonal therapy, cytotoxic agents, and radiotherapy (12–15).

Inhibition of EGFR Signaling in Cancer Therapy. A large body of experimental and clinical work supports the view that the EGFR is a relevant target for cancer therapy. Two therapeutic approaches have been shown most promising and are currently being used to inhibit the EGFR in clinical studies: (a) MAbs; and (b) small molecule inhibitors of the EGFR tyrosine kinase enzymatic activity. MAbs are generally directed at the external domain of the EGFR to block ligand binding and receptor activation. TKIs prevent the autophosphorylation of the intracellular tyrosine kinase domain of the EGFR. Other approaches currently in development for targeting the EGFR include the use of recombinant proteins containing TGFα or EGF fused to toxins, such as Pseudomonas aeruginosa toxin, EGF conjugated to genistein (a natural broad-spectrum TKI), EGFR-directed vaccine approaches, and the use of antisense oligonucleotides to the EGFR mRNA (Ref. 7; Fig. 1).

MAbs to the EGFR. The development of blocking MAbs to the EGFR as a cancer therapy was first proposed by J. Mendelsohn in the 1980s (16, 17). Mendelsohn’s group has generated two blocking anti-EGFR MAbs which inhibit the in vitro and in vivo growth of human cancer cell lines that express TGFα and EGFR. MAb 528 and MAb 225 are two mouse MAbs that have been characterized extensively for their biological and preclinical properties, and represent the first series of anti-EGFR blocking agents that have entered clinical evaluation in cancer patients (16, 17). MAb 528 and MAb 225 bind to the EGFR with affinity similar to EGF and TGFα, compete with these ligands for receptor binding, and block EGF- or TGFα-induced activation of EGFR tyrosine kinase. In addition, it has been shown that the combined treatment of nude mice bearing well-established human A431 epidermoid and MDA-MB-468 breast cancer xenografts with MAb 528 or with MAb 225 and with cytotoxic drugs, such as doxorubicin or cisplatin, significantly increases the antitumor activity of these drugs (Fig. 2; Refs. 18 and 19). To avoid human antimouse Ab production that can interfere with the therapeutic efficacy of repeated administrations of mouse MAbs in humans, a chimeric human-mouse MAb 225 (IMC-C225) that contains the human IgG1 constant region has recently been developed and purified for clinical use (16, 20).

IMC-C225: Preclinical Studies. IMC-C225 binds the EGFR with a greater affinity (Kd = 0.39 nM) than MAb 225 and is able to block EGF-induced autophosphorylation of the EGFR in cell lines in vitro (20, 21). In vitro studies demonstrated that IMC-C225 induces dimerization and internalization of the EGFR (21, 22). In this respect, removal of the EGFR from the cell surface may contribute to the inhibitory effects of this antibody. IMC-C225 also perturbs cell cycle progression by inducing a G1 arrest through an increase in the protein levels of the p27Kip1 inhibitor of cyclin-dependent kinases (23, 24). IMC-C225 significantly inhibits the growth of epidermoid, prostate, colon, and renal cell carcinoma xenografts in vivo, and this
effect is generally accompanied by a significant increase in survival of mice (20, 21, 25–27). Studies using human cancer xenografts growing either s.c. or orthotopically in nude mice have demonstrated that IMC-C225 inhibits tumor-induced angiogenesis (25, 28–31). This is probably attributable to reduced tumor expression of numerous angiogenic factors, including TGFβ, VEGF, interleukin-8, and bFGF (Table 1; Refs. 25 and 28–31). A dose-dependent additive increase in growth inhibition has been observed when cancer cells have been treated with IMC-C225 plus various cytotoxic agents, including doxorubicin, cisplatin, gemcitabine, and topotecan in vitro (16, 26, 29, 31). In addition, EGFR blockade with IMC-C225 in combination with gemcitabine, topotecan, or paclitaxel resulted in regression of human pancreatic, colon, or bladder carcinoma xenografts in nude mice, respectively (Refs. 26, 29, and 31; Fig. 3). In two orthotopic models of human pancreatic carcinoma and human bladder transitional carcinoma in nude mice, this was mediated in part by the inhibition of tumor-induced angiogenesis, leading to endothelial and cancer cell apoptosis and tumor regression (29, 31). These effects were potentiated when mice were treated with IMC-C225 in combination with gemcitabine or paclitaxel, respectively (29, 31). Several in vitro experiments and in vivo animal studies have also shown an enhancement of tumor response to ionizing radiations by IMC-C225 in human epidermoid, head and neck, and colon cancer xenografts (Fig. 4; Refs. 32–36). Among the potential mechanisms that contribute to the increased radiation sensitivity by treatment with IMC-C225, various studies have suggested an accumulation of cancer cells in the more radiosensitive cell cycle phases (G1, G2-M), a blockade of radiation-induced DNA repair mechanisms, and a reduction of VEGF production by cancer cells with inhibition of tumor angiogenesis (33, 34, 36).

**IMC-C225: Clinical Studies.** Three consecutive Phase I clinical trials have been carried out, in which IMC-C225 was administered as a single i.v. infusion, weekly infusions for 4 weeks, and weekly infusions in combination with cisplatin (37).
All were open-label, dose-escalation studies (5, 20, 50, and 100 mg/m²). IMC-C225 was additionally escalated to 200 and 400 mg/m² in the combination study with cisplatin (100 mg/m², later reduced to 60 mg/m²). Overall, 52 patients were included in these studies. All patients were immunohistochemically determined to have EGFR tumor overexpression (37). Antibodies against IMC-C225 were detected in only 1 of 52 patients. IMC-C225 toxicity was minimal and was not dose related or related to the number of cycles administered (37). The most frequent IMC-C225-related adverse events were skin toxicities (20.9%), fever and chills (13.5%), asthenia (13.5%), transient transaminase elevations (11.5%), and nausea (11.5%; Ref. 37). Skin toxicities were mainly flushing or acneiform rashes. Four grade 3–4 adverse events occurred when IMC-C225 was in combination with cisplatin 100 mg/m² [diarrhea (1 patient), epiglottis (1 patient), dyspnea (1 patient), and anaphylactoid reaction (1 patient)]. The maximum tolerated dose was not reached in any of these three Phase I studies (37). IMC-C225 has nonlinear pharmacokinetics, with antibody doses in the range of 200–400 mg/m² being associated with complete saturation of systemic clearance (37). Clearance did not change with repeated administration or with coadministration with cisplatin.

Fig. 3 Antitumor activity of topotecan and IMC-C225 on established GEO tumor xenografts (reproduced from Ref. 26). Mice were injected s.c. into the dorsal flank with 10⁷ GEO cells. After 7 days (average tumor size, 0.2 cm³), the mice were treated i.p. with topotecan alone (2 mg/kg/dose; twice weekly on days 1 and 2 of each week for 2 weeks), with IMC-C225 alone (0.25 mg/dose, twice weekly on days 3 and 6 of each week for 5 weeks), or with both drugs. Each group consisted of 10 mice. Results are shown as mean ±SD.

Fig. 4 Antitumor activity of IMC-C225 in combination with radiotherapy in squamous cell carcinoma xenografts (reproduced from Ref. 36). SCC-1 (10⁶) or SCC-6 (5 × 10⁵) cells were injected s.c. into the dorsal flank of athymic mice and treated with IMC-C225 (0.1 mg/dose) on days 10 and 13. Radiotherapy (XRT) was delivered with 12 Gy on day 15. Each group consisted of six mice. Values represent mean ±SE.
On the basis of the doses for complete saturation of antibody clearance, the recommended maintenance dose for Phase II studies has been 250 mg/m². Single-agent IMC-C225 treatment resulted in disease stabilization at 4 weeks for 7 of 12 (58%) and 11 of 16 (69%) patients, respectively (37). In combination with cisplatin, 9 of 13 (69%) patients treated with IMC-C225 doses ≥ 50 mg/m² completed 12 weeks of therapy. Two patients with head and neck cancer had a partial response (37). A Phase Ib clinical trial with IMC-C225 and cisplatin in 12 patients with advanced head and neck cancer has been reported recently (38). Tumor EGFR saturation after treatment with IMC-C225 was demonstrated by immunohistochemistry. Additionally, inhibition of EGFR tyrosine kinase activity after IMC-225 treatment within the tumor mass was demonstrated in some patients. The recommended loading dose was 400 mg/m² with a maintenance dose of 250 mg/m². Six of 9 evaluable patients achieved a major response, including two complete responses (38). In patients with EGFR-positive tumors refractory to, or in relapse from, previous therapeutic regimens, including surgery, radiotherapy, or chemotherapy, IMC-C225 (maintenance dose 100 or 250 mg/m²) in combination with cisplatin has shown positive antitumor activity (39). One patient with head and neck cancer and 1 patient with colorectal cancer had complete responses. In addition, 4 patients with head and neck cancer had partial responses. In a Phase II study of IMC-C225 monotherapy in 54 patients with metastatic renal cell carcinoma, there was one partial response and disease stabilization for ≥6 months in >25% of treated patients (40). An additional study evaluating IMC-C225 in combination with radiotherapy showed that of 15 patients with locally advanced head and neck cancer, 13 patients experienced a complete response, whereas an additional patient had a partial response when treated with IMC-C225 and radiotherapy, a response rate significantly higher than the expected 30–45% with radiotherapy alone (41). The median duration of response in this study was 17 months, with a range of 1–32 months. Preliminary results of a Phase II study of IMC-C225 in combination with gemcitabine in 41 advanced pancreatic cancer patients have been reported (42). Antitumor activity has been observed in 5 (12%) patients with a partial response and in 16 (39%) patients with a stable disease. The 1-year patient survival in this study was 32%. Results from two Phase II clinical trials of therapy with IMC-C225 in combination with CPT-11 or cisplatin in patients with diseases refractory to these cytotoxic drugs have also been reported recently. Saltz et al. (43) have shown that treatment with IMC-225 plus CPT-11 in advanced colorectal cancer patients that have failed a previous treatment with CPT-11 determines partial responses in 27 of 120 patients (22.5%) with a median duration of response of 186 days and disease stabilization for >12 weeks in 9 other patients (7%). In this study, IMC-C225 treatment was well tolerated and did not increase the toxicity attributable to CPT-11. Hong et al. (44) have treated with IMC-C225 plus cisplatin advanced head and neck cancer patients that had stable disease (41 patients) or progressive disease (27 patients) after two cycles of a cisplatin-based combination chemotherapy. In the first group of patients, the authors observed one complete response, nine partial responses, and 25 disease stabilizations, with a median duration of the response of 24 weeks. In the second group, 5 patients experienced a partial response, and 6 patients had a disease stabilization for ≥12 weeks. Phase II and III clinical trials of IMC-C225 in combination with a range of chemotherapeutic drugs, such as cisplatin or CPT-11, or with radiotherapy in patients with head and neck or colorectal cancers are currently in progress (45).

Other EGFR MAbs. ABX-EGF is a fully human IgG2 MAb specific for the EGFR, which inhibited the spontaneous production of angiogenic factors in vivo and caused significant growth inhibition of EGFR-positive tumors in vivo (46). A Phase I study of ABX-EGF has been initiated (46). Y10 is a MAb which recognizes both human and murine mutated EGFR-VIII. It inhibits cellular proliferation and induces cell-mediated cytotoxicity in vitro. Intratumoral injection of Y10 in mice bearing human brain tumor xenografts expressing the mutated EGFR-VIII increased median survival by ~3-fold with ~25% of mice achieving long-term survival (47).

Small Molecule Inhibitors of the EGFR Tyrosine Kinase. In the past 10 years, hundreds of TKIs have been synthesized and evaluated for potential preclinical activity (48–57). These molecules are generally reversible competitors with ATP for binding to the intracellular catalytic domain of the tyrosine kinase. Pharmacore modeling of the binding of compounds in the ATP pocket of tyrosine kinases has been used to successfully design potent and selective EGFR-TKIs (57). The most promising small molecule selective EGFR-TKIs are currently three series of compounds, which include 4-anilinoquinazolines, 4-[ar(alk)yl]amino) pyridopyrimidines, and 4-phe- nylnaphtho-pyrimidines (Fig. 5). A variety of these small molecule inhibitors has shown encouraging in vivo antitumor activity in preclinical models, including ZD1839 (Iressa), OSI-774, PD183805/C1-I033, PKI-166, CGP-59326A, GW2016, and PD153035 (Table 2). Of these EGFR-TKIs, ZD1839, OSI-774, CI-I033, and PKI-166 are currently in clinical development in cancer patients.

ZD1839: Preclinical Studies. ZD1839 [4-(3-chloro-4-fluoroanilino)-7-methoxy-6-(3-morpholinopropoxy) quinazoline] is a low molecular weight (447), synthetic anilinoquinazoline. ZD1839 is a p.o. active, selective reversible inhibitor of EGFR tyrosine kinase. ZD1839 inhibited autophosphorylation of EGFR isolated from A431 vulval carcinoma cells, with an IC₅₀
also demonstrated that ZD1839 inhibits EGFR signaling, e.g., cyclin-dependent kinase inhibitor (77). In vivo lines, via a dose- and time-dependent up-regulation of p27 Kip1 arrest in human head and neck squamous cell carcinoma cell. – 0.079 of 0.023 daily p.o. administration of single-agent ZD1839 (12.5 ker for EGFR mitogenic signaling activation (78). Moreover, nografts, there was a time- and dose-dependent decrease in c-fos after administration of ZD1839 to mice bearing A431 xe-(72). ZD1839, like IMC-225, has been shown to induce G 1 also be associated with a dose-related increase in apoptosis and NSCL (13, 72, 73 – 76). In addition, ZD1839 treatment can in vitro and in vivo and increased antitumor activity against other tyrosine kinases and several serine/threonine kinases. A generally cytostatic growth inhibiting activity of ZD1839 has been demonstrated in a wide range of human cancer cell lines that express functional EGFRs, including prostate, breast, ovarian, colon, epidermoid, small cell lung, and NSCL (13, 72, 73–76). In addition, ZD1839 treatment can also be associated with a dose-related increase in apoptosis in vitro (72). ZD1839, like IMC-225, has been shown to induce G1 arrest in human head and neck squamous cell carcinoma cell lines, via a dose- and time-dependent up-regulation of p27Kip1 cyclin-dependent kinase inhibitor (77). In vivo experiments have also demonstrated that ZD1839 inhibits EGFR signaling, e.g., after administration of ZD1839 to mice bearing A431 xenografts, there was a time- and dose-dependent decrease in c-fos mRNA, which can be considered a downstream nuclear biomarker for EGFR mitogenic signaling activation (78). Moreover, daily p.o. administration of single-agent ZD1839 (12.5–200 mg/kg) to athymic nude mice caused marked reductions in tumor growth in a variety of human cancer xenografts, including hormone-resistant prostate, ovarian, ductal carcinoma in situ of the breast, colon, vulval, small cell lung, and NSCL (72, 73, 76). Administration of ZD1839 (1.25–5 mg/mouse/day) to nude mice bearing human GEO colon cancer xenografts for 4 weeks produced a dose-dependent inhibition of tumor growth that was reversible, because tumors resumed a growth rate comparable with that observed in controls once treatment with ZD1839 ceased (Fig. 6; Ref. 72). ZD1839 demonstrates similar antitumor activity in xenograft models with differing levels of EGFR expression (76), suggesting that factors other than simply the number of EGFR per cell may influence cancer cell sensitivity to EGFR-targeted therapies. These include the levels of expression of the EGFR-specific ligands and of the other EGFR-related receptors that could form heterodimers with the EGFR. Enhancement of cell growth inhibition, induction of apoptosis, and increased antitumor activity in vitro and in vivo were observed when ZD1839 was combined with cisplatin, carboplatin, oxaliplatin, paclitaxel, docetaxel, doxorubicin, etoposide, topotecan, and raltitrexed (72, 76, 79). In some cases, ZD1839 in combination with cytotoxic agents produced tumor regression in nude mice bearing prostate, lung, and colon cancer xenografts (76, 80). After 4 weeks of treatment, ZD1839 combination therapy was associated with a significant increase in survival of nude mice with GEO tumor xenografts, especially with paclitaxel (Fig. 7; Refs. 72 and 80). Additionally, it has been shown recently that ZD1839 has a growth inhibitory effect and could restore the sensitivity to taxanes in MCF-7 ADR bcL-2 cells, a model of hormone-independent, multidrug-resistant human breast cancer cells (81). Preliminary data have been reported on the combination of ZD1839 and radiotherapy. ZD1839 treatment has an additive or frankly synergistic effect when combined with ionizing radiations in several human NSCL cancer of 0.023–0.079 μM (51). By contrast, ZD1839 exhibited minimal activity against other tyrosine kinases and several serine/threonine kinases. Clinical Cancer Research 2963

### Table 2 Summary of small molecule inhibitors of the EGFR tyrosine kinase domain

<table>
<thead>
<tr>
<th>Drug</th>
<th>Inhibition of EGFR kinase (IC_{50})</th>
<th>Antitumor activity</th>
<th>Development stage</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>ZD1839</td>
<td>23 nM</td>
<td>+ (≥12 mg/kg)</td>
<td>Phase III</td>
<td>Woodburn et al., 1996 (51)</td>
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<tr>
<td>OSI-774</td>
<td>20 nM</td>
<td>+ (≥10 mg/kg)</td>
<td>Phase II</td>
<td>Kris et al., 2000 (57)</td>
</tr>
<tr>
<td>PKI-166</td>
<td>0.7 nM</td>
<td>+ (≥10 mg/kg)</td>
<td>Phase I</td>
<td>Moyer et al., 1997 (59)</td>
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<tr>
<td>OSI-774</td>
<td>100 nM</td>
<td>+</td>
<td>Phase I</td>
<td>Pollack et al., 1999 (60)</td>
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<tr>
<td>OSI-774</td>
<td>7.4 nM</td>
<td>+</td>
<td>Phase I</td>
<td>Karp et al., 2000 (61)</td>
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<tr>
<td>PKI-166</td>
<td>9.2 nM</td>
<td>+</td>
<td>Preclinical</td>
<td>Siu et al., 1999 (62)</td>
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<tr>
<td>OSI-774</td>
<td>27 nM</td>
<td>+ (≥1.5 mg/kg)</td>
<td>Preclinical</td>
<td>Traxler et al., 1999 (63)</td>
</tr>
<tr>
<td>OSI-774</td>
<td>9.2 nM</td>
<td>+</td>
<td>Preclinical</td>
<td>Bruns et al., 2000 (64)</td>
</tr>
<tr>
<td>PKI-166</td>
<td>20 nM</td>
<td>+</td>
<td>Preclinical</td>
<td>Sherwood et al., 1999 (65)</td>
</tr>
<tr>
<td>OSI-774</td>
<td>138 nM</td>
<td>+ (≥30 mg/kg)</td>
<td>Phase I</td>
<td>Driscoll et al., 1999 (66)</td>
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<tr>
<td>OSI-774</td>
<td>150 nM</td>
<td>+</td>
<td>Phase I</td>
<td>Lydon et al., 1998 (67)</td>
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<td>OSI-774</td>
<td>40 nM</td>
<td>+</td>
<td>Preclinical</td>
<td>Mullin et al., 2001 (68)</td>
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<tr>
<td>OSI-774</td>
<td>1.25 mg/kg</td>
<td>+</td>
<td>Preclinical</td>
<td>Modjtabahi et al., 1998 (69)</td>
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<tr>
<td>OSI-774</td>
<td>2.5 mg/kg</td>
<td>+</td>
<td>Phase I</td>
<td>Shin et al., 2001 (70)</td>
</tr>
<tr>
<td>OSI-774</td>
<td>5 mg/kg</td>
<td>+</td>
<td>Phase I</td>
<td>Garrison et al., 2001 (71)</td>
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* a, activity.

Fig 6 Dose-dependent effects of ZD1839 treatment on tumor growth in nude mice with established GEO tumor xenografts (reproduced from Ref. 72). Mice were injected s.c. into the dorsal flank with 10^7 GEO cells. After 7 days (average tumor size, 0.25 cm^3), the mice were treated i.p. on days 1–5 of each week for 4 weeks with ZD1839 at the indicated daily doses. Each group consisted of 10 mice. Results are shown as mean ±SD.
cell lines in vitro (82). Additionally, ZD1839 enhances the efficacy of radiotherapy in the LoVo human colon carcinoma xenograft model (83). A recent study has demonstrated that ZD1839 blocks tumor-induced angiogenesis (80). In fact, ZD1839 treatment determined a dose- and time-dependent growth inhibition accompanied by the decrease of VEGF, bFGF, and TGFα production in vitro and in vivo in several human cancer cell lines (Table 3). This effect was potentiated by the combination with paclitaxel (80). Finally, preliminary data from three independent groups have suggested that ZD1839 treatment prevents c-erbB-2 signaling in human breast cancer cell lines that overexpress c-erbB-2 and that express functional EGFRs, possibly by preventing EGFR/c-erbB-2 heterodimerization (84–86). Additionally, a cooperative or frankly synergistic antitumor activity in vitro and in vivo in these models has been demonstrated after treatment with ZD1839 in combination with trastuzumab (Herceptin; Refs. 84–86).

**ZD1839: Clinical Studies.** Five Phase I trials have been conducted to assess either intermittent ZD1839 (14 days treatment followed by 14 days observation; Refs. 57, 58, and 87) or continuous ZD1839 (28 consecutive days treatment) administration (58, 88, 89). ZD1839 was administered as a once-daily, p.o. dose (50–1000 mg/day). Nearly all of the 254 patients in these Phase I trials were heavily pretreated, with ~30% failing more than two prior chemotherapy regimens. Both intermittent and continuous dosing of ZD1839 were well tolerated. The most frequently reported National Cancer Institute-CTC grade 1–2 adverse events were diarrhea and an acneiform skin rash (57). Adverse events were reversible on discontinuation of treatment, and the skin rash resolved without scarring. Grade 3–4 adverse events were rare and usually related to disease progression (57). Intermittent ZD1839 treatment was also well tolerated in a Japanese study; grade 3 elevation of hepatic transaminases was observed in 3 patients, and grade 3 diarrhea was observed in 1 patient (58). Grade 3 diarrhea was dose limiting at the 700-mg dose level after intermittent dosing and 1000 mg after continuous dosing (87, 88). Pharmacokinetic assessments confirm that ZD1839 is suitable for once-daily dosing; the mean elimination half-life was 46 h (86). Steady-state plasma concentrations were achieved by day 7. Exposure to ZD1839 increased approximately linearly with dose, although exposure varied within each dose group (87). Antitumor activity has been observed in patients with colorectal, ovarian, NSCL, head and neck, renal, and hormone-resistant prostate cancers (57, 87, 88). In particular, encouraging results have been reported in patients with NSCLC. Of 99 patients with NSCLC, 8 patients have had a partial response lasting from 1 to 16 months, and 2 patients have had regression of nonmeasurable evaluable disease (57, 58). In addition, approximately one-third of patients have had long-lasting stable disease for ≥3 months, and preliminary quality of life assessment data suggest this corresponds with symptomatic relief (57). A pilot study has shown that ZD1839 in combination with carboplatin/paclitaxel appears feasible and well tolerated in previously untreated patients with advanced NSCLC (90). Preliminary pharmacokinetic data suggest that coadministration of ZD1839 does not affect the clearance of either carboplatin or paclitaxel (90). Preliminary antitumor activity data of this combination have been reported recently (91). Of 24 patients affected by advanced NSCLC and treated with carboplatin/paclitaxel plus ZD1839, 1 patient had a complete response (≥8 months), 5 patients experienced partial responses (range, 3.1–12.4 months), and 8 patients obtained a disease stabilization (91). Recently, preliminary results of a Phase I study of p.o. ZD1839 in combination with 5-fluorouracil and leucovorin in patients with advanced colorectal cancer have been reported (92). This study included 26 patients that were treated with ZD1839 doses from 250 to 500 mg/day. No significant increase in the frequency and the severity of diarrhea or skin toxicity beyond that seen with chemotherapy alone was observed in this trial (92). Two large multicenter Phase III studies of ZD1839 (250 or 500 mg daily) in combination with cytotoxic agents (carboplatin/paclitaxel or cisplatin/gemcitabine) have been started as first-line treatment in nonoperable stage III and stage IV NSCLC patients (Fig. 8). For both studies, patients accrual (~1030 patients in each trial) has been completed in March 2001.

**OSI-774: Preclinical Studies.** OSI-774 [6,7-bis(2-methoxy-ethoxy)-quinazolin-4-yl-(3-ethynylpheny)amine, formerly CP358,774] is a quinazoline derivative which reversibly inhibits the kinase activity of purified EGFR (IC50 = 2 nm) and autophosphorylation in intact cells (IC50 = 20 nm) in vitro. The proliferation of DiFi human colon tumor cell lines is inhibited by OSI-774 at submicromolar concentrations, and OSI-774 also blocks the cell cycle in G1, resulting in significant accumula-

### Table 3 Immunohistochemical analysis of GEO colon cancer xenografts after treatment with ZD1839

| Dose of ZD1839 (mg/day) | Tumor volume (cm³ ± SD) | % positive cells (±SD) | Factor VIII-related antigen
d | Ki67 | TGFα | bFGF | VEGF |
<table>
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<td>0</td>
<td>0.92 ± 0.15</td>
<td>75 ± 10</td>
<td>55 ± 8</td>
<td>45 ± 10</td>
<td>50 ± 4</td>
<td>19 ± 3</td>
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<tr>
<td>1.25</td>
<td>0.68 ± 0.08</td>
<td>50 ± 7</td>
<td>40 ± 6</td>
<td>25 ± 3</td>
<td>35 ± 2</td>
<td>8 ± 2</td>
<td></td>
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<tr>
<td>2.5</td>
<td>0.44 ± 0.05</td>
<td>25 ± 2</td>
<td>10 ± 2</td>
<td>15 ± 4</td>
<td>5 ± 1</td>
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<tr>
<td>3.75</td>
<td>0.31 ± 0.07</td>
<td>20 ± 2</td>
<td>5 ± 3</td>
<td>5 ± 1</td>
<td>4 ± 2</td>
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<tr>
<td>5</td>
<td>0.16 ± 0.04</td>
<td>25 ± 2</td>
<td>5 ± 1</td>
<td>3 ± 2</td>
<td>4 ± 1</td>
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*Reproduced with modifications from Ref. 80. Mice bearing GEO tumor xenografts were treated as follows. Briefly, 10⁶ cells were injected, after being suspended in 200 µl Matrigel, into the dorsal flank of 4–5-weeks-old nude mice on day 0. Treatment was started on day 7 after tumor cell injection, when tumor volume was ~0.25 cm³. Mice were treated i.p. daily from days 1 to 5 with the indicated doses of ZD1839 for 2 weeks. Analysis was performed on day 21 after tumor cell injection. Each group consisted of six mice. The percentage (±SD) of specifically stained GEO cancer cells for Ki67, TGFα, bFGF, and VEGF was recorded. The number of microvessels for field (±SD) was measured using a monoclonal antibody raised against the human factor VIII-related antigen and was scored by averaging five field counts of three individual tumors for each group.

*Figures for factor VIII-related antigen are the numbers of positively staining microvessels.
PKI-166. PKI-166 is a reversible pyrrolo-pyrimidine inhibitor of the EGFR tyrosine kinase in vitro (IC$_{50}$ = 1 nM; Ref. 63). PKI-166 inhibited EGFR autophosphorylation, c-fos mRNA expression, and cell proliferation in the submicromolar concentrations of cell cycle inhibitor p27$^{kip1}$ (59). OSI-774 induces apoptosis in vitro and has activity against various human tumor xenografts in vivo (59, 60, 93, 94). OSI-774 in combination with cisplatin produced substantial growth inhibition of human cancer xenografts with no detectable effects on body weight or lethal toxicity in mice (61).

OSI-774: Clinical Studies. OSI-774 has been evaluated in two Phase I dose-escalation pharmacokinetic trials in patients with advanced solid tumors (61, 62, 95). p.o. OSI-774 (100–1600 mg) was administered once weekly every 3 of 4 weeks (n = 28; Ref. 61), on 3 consecutive days for 3 weeks, or every day for 3 weeks (n = 27; Ref. 62). All these treatment schedules were followed by a week of rest. All of the patients had prior treatment for advanced solid tumors. The maximum tolerated dose was not reached in the once-weekly schedule, and 1600 mg weekly was a well-tolerated dose (61). Like ZD1839, dose-limiting toxicity was diarrhea (at the 200-mg dose level) in the continuous once-daily dose schedule (62). Half of the patients in the continuous dose schedule have reported grade 1–2 acneiform skin rashes (62). Preliminary pharmacokinetic analysis showed large intra and interpatient variability but dose-proportional increases in exposure (61, 62). Of 28 patients receiving OSI-774 weekly, 12 patients remained alive (9–22 months), including 5 of 11 with lung cancer and 3 of 5 with head and neck cancer (61). OSI-774 is currently in Phase II development in advanced head and neck, NSCL, and ovarian cancer. Preliminary results of a Phase II study of OSI-774 in patients with pretreated, advanced head and neck cancer have been presented recently (96). A group of 124 patients (98 patients with EGFR overexpressing cancer as detected by immunohistochemistry) received OSI-774, 150 mg daily. Major toxicities were skin rash in 74.2% patients (11.3%, grade 3) and diarrhea (3.2%, grade 3). Partial responses were observed in 7 patients (5.6%), and stable disease occurred in 39 patients (33.9%). The median survival was 5.8 months, with a 1-year survival of 24% patients. A Phase II study in ovarian cancer patients has also been reported recently (97). Patients (34) with advanced, heavily pretreated, ovarian cancer received p.o. OSI-774, 150 mg daily. Major toxicities were skin rash (88% patients, 9%, grade 3) and diarrhea (35% patients, 6%, grade 3). Two patients had partial responses, and 16 experienced disease stabilization. The median survival for the patients in this study was 242 days (97). Finally, evidence of antitumor activity of OSI-774 in patients with advanced NSCLC that had failed a platinum-based therapy was reported recently (98). A complete response occurred in 1 of 57 patients, and partial responses were recorded in 6 other patients. An additional 17 patients had stable disease.

**PD183805/CI-1033.** PD183805 [4-(-3-(chloro-4-fluoro-phenylamino)-7-(3-morpholin-4-yl-propoxy)-quinazolin-6-yl)-acrylamide dihydrochloride] is an irreversible inhibitor of the EGFR (IC$_{50}$ = 7.4 nM against EGF-stimulated A431 cells) and of the other EGFR family members (65). PD183805 has demonstrated antitumor activity against A341 and H125 tumors in vivo (66). PD183805 is currently in Phase I development in patients with head and neck, breast, and NSCL cancer. The water-soluble analogue of PD183805, CI-1033, inhibited EGFR tyrosine kinase in vitro and p.o. CI-1033 suppressed human epidermoid A431 xenografts in nude mice. A Phase I trial in advanced cancer patients has been reported recently (70). Patients (50) were treated with escalating doses of CI-1033 (50–650 mg p.o., daily for 7 days, every 3 weeks). Toxicities included skin rash and diarrhea, whereas the dose-limiting toxicities were grade 3 hypersensitivity at 560-mg dose level and grade 4 thrombocytopenia at 560-mg dose level. A similar Phase I study was reported (71). Also in this trial, hypersensitivity and thrombocytopenia were the major toxic side effects. A disease stabilization for 12 weeks was observed in 6 of 53 patients treated in this study (71).
Targeting the Epidermal Growth Factor Receptor cell lines efficacy in relevant preclinical models, such as human cancer treatment. All of the EGFR inhibitors described above have shown most successful areas of translational research in cancer treatment of selective and potent inhibitors of EGFR activation that pathway is a rational target for cancer therapy. The identification of the EGFR-driven autocrine range (63). PKI-166 demonstrated antitumor activity in four EGFR-expressing tumor xenograft models in nude mice (63). In addition, PKI-166 decreased the growth and metastasis of a human pancreatic cancer growing orthotopically in nude mice, increased survival via reduced production of proangiogenic molecules, and increased apoptosis (64). PKI-166 is planned to enter Phase I trials in patients.

**GW2016.** GW2016 is a 6-thiazolyquinazoline derivative that selectively inhibits with equimolar potency both the EGFR and the ErbB-2 tyrosine kinases (IC50 = ~10 nM for both kinases in vitro; Ref. 99). Recently, data have been presented on the antitumor activity of GW2061 in nude mice bearing human HNS squamous carcinoma xenografts (68). GW2016 is planned to enter Phase I trials in cancer patients.

**Conclusions.** Considerable experimental evidence over the last decade has shown that the EGFR-driven autocrine pathway is a rational target for cancer therapy. The identification of selective and potent inhibitors of EGFR activation that could be developed as anticancer agents has been one of the most successful areas of translational research in cancer treatment. All of the EGFR inhibitors described above have shown efficacy in relevant preclinical models, such as human cancer cell lines in vitro and human tumors xenografted to immuno-deficient mice in vivo.

Three different inhibitors of EGFR signaling, IMC-C225, ZD1839, and OSI-774, have shown in vitro and in vivo antitumor activity, as well as encouraging results in clinical trials. Although IMC-C225 and the small molecule EGFR-TKI have different mechanisms of action, they all ultimately lead to G1 arrest via accumulations of p27kip1. IMC-C225 administered i.v. and ZD1839 or OSI-774 administered p.o. displayed different pharmacokinetic profiles. IMC-C225 demonstrated nonlinear saturable pharmacokinetics, whereas ZD1839 and OSI-774 showed linear pharmacokinetics.

The hope for new agents targeting the EGFR is that specific inhibition of the EGFR will have therapeutic efficacy without the toxicities associated with virtually all currently available anticancer cytotoxic drugs. Toxicities associated with IMC-C225, ZD1839, and OSI-774 were similar and very mild. Grade 1–2 rash-like, skin toxicity was common with IMC-C225, ZD1839, and OSI-774. In this respect, skin toxicity is often viewed as an indirect marker of clinically relevant EGFR targeting in vivo. Although the IMC-C225, ZD1839, and OSI-774 dose-escalation Phase I trials were not designed to specifically analyze clinical response, they have all shown encouraging clinical results. Moreover, preliminary results from Phase II studies with these agents confirm antitumor activity of anti-EGFR-directed therapies in advanced human cancer. In fact, treatment with IMC-C225, ZD1839, or OSI-774 as single agents resulted in disease stabilization, as well as in a number of major responses in a variety of cancers.

Preclinical results with IMC-C225, ZD1839, and OSI-774 and preliminary clinical results with IMC-C225 suggest that the potential of these agents will increase when combined with standard cytotoxics and/or radiotherapy. Taken together, these studies support the hypothesis that cellular damage induced by chemotherapy or by ionizing radiations can convert EGFR ligands from growth factors into survival factors for cancer cells that express functional EGFR (100, 101). In this situation, the blockade of EGFR signaling in combination with cytotoxic drugs or with radiotherapy could cause irreparable cancer cell damage leading to increased programmed cell death. The enhancement of anticancer activity of conventional cytotoxic treatments by interfering with EGFR activation may have relevant clinical implications. In this respect, treatment with conventional doses of cytotoxic drugs or of radiotherapy in combination with signal transduction inhibitors, such as the anti-EGFR selective agents, could be an effective novel anticancer strategy, which is less toxic and more tolerable than other clinical approaches for increasing the activity of cytotoxic drugs, such as high-dose chemotherapy (100, 101).

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