

Monoclonal Antibodies to Target Epidermal Growth Factor Receptor–Positive Tumors

A New Paradigm for Cancer Therapy

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BACKGROUND. Traditional cytotoxic approaches to tumor management are associated with efficacy and toxicity limitations. Blockade of the epidermal growth factor receptor (EGFR) and its ligands is a novel approach to the treatment of human tumors that offers a noncytotoxic alternative to cancer treatment.

METHODS. An English-language literature search was conducted to identify studies assessing the in vitro and in vivo effects of EGFR blockade with an emphasis on approaches that use monoclonal antibody therapy.

RESULTS. The EGF pathway regulates normal cellular processes and appears to be correlated with the development of malignancy. Approximately 30% of human tumors express EGFR, which has been reported to be correlated with poor prognosis and diminished disease-free and overall survival in selected tumor types. A number of anti-EGFR monoclonal antibodies have been developed, which currently are undergoing clinical trials in humans. Effective anti-EGFR monoclonal antibodies compete with endogenous ligands, primarily EGF and transforming growth factor- α , for receptor ligand-binding sites. Binding to EGFR blocks critical signaling pathways and interferes with the growth of tumors expressing EGFR. Anti-EGFR monoclonal antibodies that currently are under study include IMC-C225, EMD 55900, ICR 62, and ABX-EGF.

CONCLUSIONS. These antibodies have demonstrated promising results and appear to have been well tolerated. EGFR-targeted therapy addresses important, unmet needs in the treatment of human tumors, particularly EGFR-positive epithelial tumors including common malignancies of the head and neck, lung, and colon.

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Traditional cytotoxic therapies utilized in the management of solid tumors have been found to be associated with significant therapeutic and safety limitations. Because chemotherapy and radiation therapy generally do not discriminate between normal and malignant tissues, the successful administration of cytotoxic therapy can be limited by the nonspecific toxicity sustained by healthy tissues. Poor tolerance of chemotherapy and radiation therapy may result in subtherapeutic dosing or delays in therapy administration or necessitate discontinuation of therapy completely. Furthermore, toxicities to healthy tissues may prevent dose escalation. These limitations generally result in poor outcomes in terms of disease control and overall survival. In some cases, the administration of concomitant chemo-

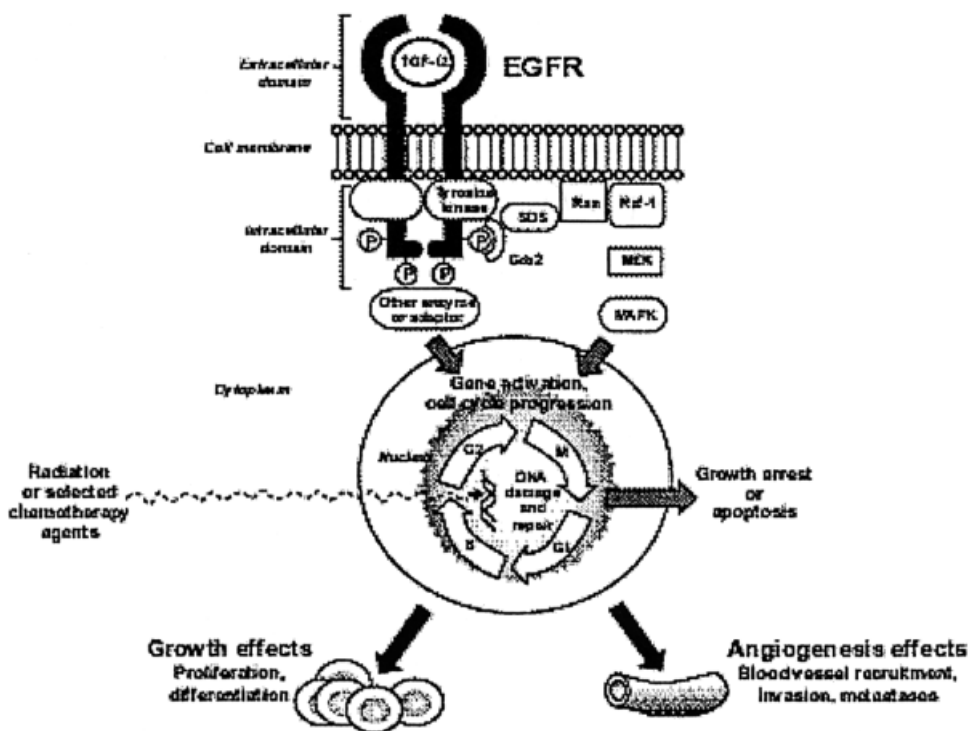


FIGURE 1. Epidermal growth factor receptor (EGFR) pathways. EGFR regulates cellular processes via multiple mechanisms (Reproduced with permission from Harari PM, Huang S-M. Modulation of molecular targets to enhance radiation. *Clin Cancer Res.* 2000;6:323–325.

therapy and radiation therapy is not tolerable owing to overlapping toxicities. In addition, tumors can become resistant to the effects of chemotherapy and/or radiation therapy, and in some cases cross-resistance develops to agents that have not yet been administered.

These limitations emphasize the need for treatment approaches that demonstrate efficacy in targeting tumor cells while limiting damage to healthy cells. Furthermore, an agent that could be administered in combination with chemotherapy and/or radiation therapy and that demonstrates synergistic activities with minimal if any increased toxicity would be ideal in overcoming barriers to efficacy. Monoclonal antibody therapy is one such approach that has undergone significant evolution and improvement in terms of production, safety, and efficacy over the past 20 years. Although monoclonal antibodies have been studied for many years, to our knowledge only recently has this therapy become a reality in terms of both safety and efficacy. Early clinical trials utilized only murine monoclonal antibodies, which unfortunately were associated with the development of a human antimouse antibody (HAMA) response against the murine proteins. The development of HAMA results in increased clearance of the monoclonal antibodies, thereby decreasing efficacy. Furthermore, the development of HAMA increases the risk of serious allergic reactions. Recent advances in genetic engi-

neering have made it possible to produce antibodies comprised of both murine and human sequences: chimeric antibodies. Chimeric antibodies are comprised of the variable regions of the murine antibody, that portion that is responsible for antigen binding and the constant region of the human Fc fragment.¹ Chimeric monoclonal antibodies have been reported to demonstrate specificity and a diminished incidence of HAMA reactions.^{2,3} In addition, humanized and fully human monoclonal antibodies have been developed in an attempt to overcome the immune responses that initially limited the widespread applicability of monoclonal antibody therapy. There are numerous applications for monoclonal antibody therapy in the treatment of human malignancies; the current article focuses on monoclonal antibodies that modulate the effects of growth factor receptors.

Treatment targeted to the growth factor receptors has shown promise in the management of solid tumors. Growth factor receptors are important in regulating cellular processes such as proliferation, differentiation, and survival (Fig. 1). There are four related growth factor receptors: HER-1 (epidermal growth factor receptor [EGFR] or *c-erb B-1*), HER-2 (*c-erb B-2*), HER-3 (*c-erb B-3*), and HER-4 (*c-erb B-4*). EGFR inhibitors block the HER-1 receptor. A number of anti-EGFR monoclonal antibodies have been tested in vitro and in vivo using animal models, and a few have been entered into clinical trials. Examples of those anti-

EGFR monoclonal antibodies that have undergone or currently are in clinical testing are IMC-C225, a chimeric monoclonal antibody; EMD 55900 (monoclonal antibody 425), a murine anti-EGFR monoclonal antibody;^{4–6} ICR 62, a rat monoclonal antibody;^{5,7} and ABX-EGF, a fully human anti-EGFR antibody.⁸ IMC-C225 is an anti-EGFR monoclonal antibody that currently is in Phase II and Phase III testing.

The HER-2/*neu* receptor is overexpressed in 25–35% of breast carcinomas.^{9,10} Those tumors have a significantly less favorable prognosis as evidenced by a shorter recurrence-free survival and overall survival.^{9,11,12} Trastuzumab, an anti-HER-2 humanized monoclonal antibody, is an example of a successful application of a monoclonal antibody targeted to HER-2. Binding of trastuzumab to the HER-2 receptor inhibits cellular proliferation of tumors that overexpress HER-2. Results have been encouraging among women with metastatic breast carcinoma whose tumors demonstrate increased HER-2 protein expression. Treatment with trastuzumab alone has demonstrated overall response rates of 11.6–15% in women with refractory breast carcinoma.^{13,14} Unfortunately, the majority of the other solid tumors express HER-2 at a rather low level, making its role as a therapeutic target marginal.

EGFR-targeted monoclonal antibodies offer a therapeutic option that addresses some of the limitations associated with chemotherapy and radiation therapy. With a mechanism of action that differs from traditional cytotoxic therapies and a high degree of specificity, anti-EGFR monoclonal antibodies offer a new treatment approach that can be combined safely and effectively with chemotherapy and radiation therapy. To our knowledge, these antibodies have not been associated with any major organ toxicity except for skin reactions, which are a mechanism-based direct result of the expression of EGFR on epithelial tissues. The toxicity profiles of anti-EGFR monoclonal antibodies generally are different from those associated with chemotherapy and radiation therapy; thus, overlapping toxicities are expected to be unlikely or minimal. Furthermore, data from clinical testing in humans suggest that anti-EGFR monoclonal antibody therapy demonstrates synergistic activity when combined with chemotherapy and radiation therapy.^{15,16}

Epidermal Growth Factor Receptor

There exist a group of growth factors and receptors that share similarities in structure and function. Some receptors in this group are associated with intrinsic tyrosine kinase activity. Related growth factor receptors are HER-1 (EGFR or *c-erb* B-1), HER-2 (*neu* or *c-erb* B-2), HER-3 (*c-erb* B-3), and HER-4 (*c-erb* B-4).

HER-1, HER-2 and HER-4 possess intrinsic tyrosine kinase activity whereas HER-3 does not. The EGFR is a 170-kilodalton (kD) transmembrane glycoprotein that is encoded by the *c-erb* B-1 protooncogene.^{17,18} HER-2 is a 185-kD transmembrane glycoprotein that is closely related to EGFR.¹⁹ EGFR and *c-erb* B-2 proteins demonstrate 82% homology in the tyrosine kinase domain,²⁰ and evidence suggests that cross-reactivity exists between EGFR and HER-2 receptors.^{21,22} The ligand-binding domain of HER-3 (or *c-erb* B-3) demonstrates approximately 40% homology to EGFR. Finally, HER-4 (or *c-erb* B-4) was the most recently discovered of the group and is a receptor for heregulin- α and - β .²³

A number of EGFR variants have been identified, with the most common being EGFRvIII. In contrast to EGFR, EGFRvIII is not expressed in normal tissue. Its presence has been identified in a variety of tumors including nonsmall cell lung, breast, and prostate carcinomas and 50% of all gliomas.^{4,24–26} EGFRvIII has a constitutively activated tyrosine kinase and, unlike EGFR, does not bind ligand or undergo receptor dimerization.²⁴ The identification of EGFRvIII suggests the possibility that agents such as anti-EGFRvIII monoclonal antibodies can be targeted directly to those tumors expressing the variant without affecting normal tissues. In addition, this type of variant suggests ways in which a cell might override an anti-EGFR antibody.

EGFR is expressed on healthy cells that originate from all three germ cell layers, particularly those of epithelial origin (e.g., the skin, liver, and gastrointestinal tract), as well as on malignant tissues.^{27–30} There are a number of endogenous ligands for the EGFR including epidermal growth factor (EGF), transforming growth factor- α (TGF- α), amphiregulin, heparin-binding EGF (HB-EGF), and betacellulin.²³ EGF and TGF- α are the most important stimulatory ligands for the EGFR. After ligands bind to the EGFR, the receptor undergoes dimerization, followed by internalization of the receptor/ligand complex and autophosphorylation.³¹ Finally, the tyrosine kinase signal transduction pathways that control cellular proliferation, differentiation, and survival are activated.³²

EGFR is important in the maintenance of normal cellular function and survival, and EGFR expression contributes to the growth and survival of tumor cells (Fig. 1). After ligand binding, EGFR elicits cellular responses through various divergent pathways. Ligand binding to EGFR activates the cytoplasmic catalytic function of tyrosine kinase by promoting receptor dimerization and self-phosphorylation on tyrosine residues. These residues serve as binding sites for the recruitment of signal transducers and activators of

transcription (STATS) and other downstream adaptor proteins (e.g., *ras*) or enzymes (e.g., phospholipase-C), which, in turn, stimulate an intracellular signal transduction cascade leading to a wide range of physiologic and pathogenic functions including cellular adhesion, differentiation, and apoptosis.³³

The EGFR signal transduction pathways have been correlated with various processes that contribute to the development of malignancy, such as effects on cell cycle progression, inhibition of apoptosis, angiogenesis, tumor cell motility, and metastasis. Given the role of the EGFR pathways in cell cycle progression and tumor proliferation, it has been proposed that combining anti-EGFR therapy with chemotherapy or radiation therapy may result in synergistic antitumor activities by inhibiting various processes that contribute to tumor growth.^{34–37}

The EGFR pathway is important in controlling cell cycle events that affect survival. The role of EGF in regulating the cell cycle was studied in a human prostate carcinoma cell line.³⁸ EGF induced cyclin D1, a protein that is important in cell cycle progression. Cyclin D1 activates the cyclin-dependent kinases, CDK4 and CDK6, which leads to phosphorylation of the retinoblastoma protein and progression through the G₁ phase of the cell cycle.³⁹ Additional data supporting the role of the EGFR pathways and cell survival come from the observation that tumors expressing EGFR are dependent on binding of the ligands EGF or TGF- α and activation of the tyrosine kinase pathways for downstream signaling activation. EGF also has been shown to inhibit apoptosis in cells expressing EGFR.⁴⁰

Angiogenesis is a process of new blood vessel formation that contributes to tumor growth and metastasis. There is *in vivo* and *in vitro* evidence that numerous growth factors, including EGF and TGF- α , possess angiogenic activity.⁴¹ In one study, both TGF- α and EGF were shown to be inducers of angiogenesis in the hamster cheek pouch assay. TGF- α has been shown to promote the expression of vascular endothelial growth factor (VEGF), which is important in the angiogenic process, specifically by promoting vascular cell permeability.^{42,43} Finally, previous studies have shown that coexpression of EGF and TGF- α is correlated with increased microvessel density in patients with invasive breast carcinoma.⁴⁴

The EGFR pathway has been shown to regulate tumor cell motility and metastasis in a variety of studies.^{45–48} The stimulatory effects of EGF on cellular proliferation, migration, and invasion were observed.⁴⁶ EGF is a potent stimulator of tumor growth and an effective stimulator of migration and cellular invasion in patients with primary gliomas. Modulation

TABLE 1
EGFR Overexpression in Tumors

Tumor type	Percentage of tumors overexpressing EGFR	Reference
Colon	25–77%	23, 53
Head and neck	80–100%	23, 54, 55
Pancreatic	30–50%	23, 56
Nonsmall cell lung carcinoma	40–80%	23, 57–59
Breast	14–91%	60–63
Renal carcinoma	50–90%	23, 64
Ovarian	35–70%	23, 65, 66
Glioma	40–63%	23, 67, 68
Bladder	31–48%	23, 69

EGFR: epidermal growth factor receptor.

of matrix metalloproteinases (MMPs) may be important in regulating these effects; however, to our knowledge the precise role of MMP has not been elucidated fully.⁴⁹

EGFR Expression and Malignancy

EGFR and its ligands are known to be associated with the growth of malignant cells. EGFR expression in normal cells ranges from 40,000–100,000 receptors per cell.³¹ EGFR expression in a wide variety of malignant cells (including colon, head and neck, pancreatic, nonsmall cell lung, breast, kidney, ovarian, and bladder carcinomas and gliomas) has been documented extensively both *in vitro* and *in vivo*. In many cases, the number of EGFRs expressed in malignant cells is greater than that expressed in normal cells; up to 2 million EGFRs per cell were reported to be demonstrated in breast carcinoma.^{50–52} The percentage of tumors that express EGFR varies by tumor type; for example, in some malignancies, such as squamous cell carcinomas of the head and neck and lung, EGFR is expressed in the majority of tumors (Table 1). Increased expression of EGFR results from a variety of mechanisms including increased production of EGFR ligands, increased EGFR gene transcription, EGFR gene amplification, and mutations resulting in the constitutive activation of tyrosine kinase.⁵⁰

Given the role of EGFR in contributing to the development of malignancy and the presence of EGFR in numerous carcinoma cell types (in some cases at increased frequency), there is an opportunity to target and block the EGFR pathways to treat tumors that express EGFR. Because inhibition of EGFR targets tumor cells using a unique mechanism of action as well as blocks cell cycle progression, this is an important addition to the traditional forms of cytotoxic therapy, particularly when resistance to cytotoxic therapy de-

TABLE 2
Prognostic Value of EGFR Expression

Tumor type	Poor prognosis	Survival	Correlation with metastasis	Comment
Colon	Higher percentage of EGFR-expressing cells associated with poorer prognosis ⁷⁰			
Head and neck		Predictor of disease-free survival ⁷¹ ; EGFR correlated with overall survival ⁷²		EGFR expression occurs in premalignant and malignant lesions in the head and neck ⁵⁵
Pancreatic		Coexpression of EGFR and EGF/TGF- α associated with decreased survival ⁷³		Coexpression of EGFR and EGF/TGF- α associated with advanced stage and tumor size ⁷³
Nonsmall cell lung carcinoma	Correlates with poor prognosis ⁷⁴	Correlates with shorter overall survival ⁷⁵	Correlates with high metastatic rate ⁷⁶	
Breast		Correlates with worse overall survival and recurrence-free survival at 1- to 4-year follow-up (not significant at 10-year follow-up) ^{60,77}		
Ovarian	Associated with poor prognosis ⁶⁶	Associated with poor overall survival and disease-free survival ⁶⁵		Associated with resistance to chemotherapy ^{65,78}
Glioma				Correlates with degree of differentiation ⁶⁸
Bladder				Associated with increased risk of recurrence ^{69,79} and higher expression in invasive tumors ⁸⁰
Gastric carcinoma				Associated with increased risk of recurrence, ⁸¹ poor differentiation, and large tumor size ⁸²

EGFR: epidermal growth factor receptor; TGF- α : transforming growth factor- α .

velops or when the maximum tolerated (yet subtherapeutic) dose of cytotoxic therapy has been reached. Furthermore, the specificity of EGFR blockade by monoclonal antibodies results in a favorable safety profile that is different from that of chemotherapy and radiation therapy. Thus, additive toxicities are anticipated to be minimal when EGFR blockade is combined with chemotherapy or radiation therapy.

The prognostic significance of EGFR expression and its usefulness in predicting tumor-related outcomes has been studied widely. Several studies have demonstrated that EGFR expression correlates with reduced disease-free and overall survival, poor prognosis, increased risk of disease recurrence, advanced tumor stage, and increased risk of metastasis (Table 2). Other studies have reported conflicting results and found no such correlation between EGFR expression and less favorable outcomes.^{25,83} Difficulties associated with interpreting these data arise from the differences between studies with regard to the selection of the patient population, sample size, tumor type, methodology, measurement techniques, and selection

of endpoints. A meta-analysis of 5323 breast carcinoma patients studied in 40 different trials demonstrated a positive correlation between EGFR expression and lymph node status, tumor ploidy, and proliferative index.^{60,77} Further research is needed to clarify the implications of EGFR expression and determine its value in predicting response to therapy.

The determination of a tumor's EGFR status prior to treatment with an EGFR inhibitor may be important in identifying those patients who may benefit from treatment with an EGFR inhibitor. It is particularly meaningful to document EGFR expression in malignancies that exhibit variable EGFR expression; for example, the percentage of colon tumors expressing EGFR has been reported to range from 25–77% and the percentage of breast tumors expressing EGFR has been reported to range from 14–91% in different studies (Table 1). However, even when the receptor is not overexpressed, it is the entire autocrine loop including TGF- α and EGFR that might be impacted. Hence, even more tumors than originally believed may be dependent on this axis. A variety of techniques have been

used to assess EGFR expression including immunohistochemistry (IHC),^{54,55,84} fluorescent in situ hybridization (FISH),^{85,86} competitive radioligand-binding assay,^{57,84} solid matrix blotting techniques,^{51,87,88} reverse transcriptase-polymerase chain reaction (RT-PCR),⁸⁹ and enzyme-linked immunoadsorbent assay (ELISA).^{4,5} Advantages and disadvantages are associated with each test, and some have significant scientific and technical limitations that prohibit widespread clinical applicability. IHC, which detects protein expression, and FISH, which detects EGFR gene amplification, are the most commonly utilized methods. The majority of studies have used IHC because it is reliable, is relatively simple and quick to perform, and utilizes readily available reagents and equipment. FISH commonly has been utilized to detect amplification of EGFR and genes such as HER-2/*neu*. Elevated levels of HER-2 in carcinomas result from gene amplification or overexpression.⁹⁰ FISH testing has been applied successfully in the detection of HER-2/*neu* amplification that occurs in 25–30% of breast carcinomas to identify those patients who may benefit from trastuzumab therapy. In contrast, the majority of tumors that express EGFR demonstrate a low frequency of amplification at the gene level.^{91,92} Because the detection of EGFR utilizing FISH would generate false-negative results, it has limited applicability in assessing EGFR expression in human tumors. Research currently is underway to develop a standardized IHC test for the documentation of EGFR expression in tumor tissues. An EGFR screening kit for standardized IHC is being developed to identify patients who might benefit the most from treatment with the anti-EGFR monoclonal antibody IMC-C225.

Inhibition of EGFR Pathways

EGF has been shown to stimulate cell cycle progression and prevent apoptosis in a number of cell lines that express EGFR.^{40,93} Conversely, inhibition of the EGFR pathways in colorectal carcinoma, head and neck carcinoma, glioblastoma, and breast carcinoma cell lines via various mechanisms (such as anti-EGFR antibodies, EGFR tyrosine kinase inhibitors, and antisense EGFR oligonucleotides) was reported to block cell cycle progression and result in apoptosis.^{30,40,94–100} The anti-EGFR monoclonal antibody 225 has been shown to arrest DiFi human colorectal carcinoma cell lines in the G₁/G₀ peak and induce apoptosis.⁹⁵ Tyrosine kinase inhibitors have been reported to demonstrate inhibition of cell cycle progression and induction of apoptosis in both human tumor cell lines *in vitro* and in xenograft models.^{30,97,98}

Published data support the role of EGF, TGF- α , and EGFR in the process of angiogenesis.⁴¹ Blockade

of the EGFR with an anti-EGFR antibody, IMC-C225, resulted in inhibition of VEGF production both *in vitro* and *in vivo* in A431 carcinoma xenografts; *in vivo* inhibition of VEGF production was reported to be correlated with decreased angiogenesis.¹⁰¹ *In vitro* application of IMC-C225 to a metastatic human transitional carcinoma cell line resulted in inhibition of VEGF, interleukin-8, and basic fibroblast growth factor.¹⁰² Blockade of the EGFR pathway with the tyrosine kinase EGFR inhibitors genistein and PD166285 inhibited angiogenesis in murine mammary tumors, and genistein was reported to be associated with diminished microvessel formation in bladder tumors.^{103,104}

Stimulation of EGFR pathways also has been shown to promote tumor cell motility, adhesion, and metastasis.^{45–48,105} Finally, evidence suggests autocrine production of TGF- α in tumor cells,¹⁰⁶ which leads to activation of the EGFR.¹⁰⁷ Autocrine stimulation may help explain the recovery of tumor cells after cytotoxic therapy.

Anti-EGFR-Targeted Approaches

Numerous EGFR blockers have been investigated to date, including anti-EGFR monoclonal antibodies, tyrosine kinase inhibitors, ligand conjugates, immunconjugates, and antisense oligonucleotides (Table 3). Anti-EGFR monoclonal antibodies target the extracellular receptor and thus are able to block the EGFR pathways effectively in a highly specific manner. Small molecules, such as the tyrosine kinase inhibitors, which target the intracellular tyrosine kinase signaling pathways, also inhibit the EGFR pathway. Early studies using naturally occurring kinase inhibitors (e.g., erbstatin) were limited by the poor correlation between cell-free systems (*in vitro*) and intact cells (*in vivo*).¹⁰⁸ However, recent attempts (1990s) at identifying new compounds via molecular modeling studies have led to the discovery of quinazoline inhibitors (e.g., ZD1839) and other tyrosine kinase inhibitors with promising clinical benefit (CI-1033, formerly PD183805; and OSI-774, formerly CP-358774). ZD1839, an orally active selective EGFR-tyrosine kinase inhibitor, has been reported to reduce human tumor growth from a variety of xenografts, including nonsmall cell lung, prostate, ovarian, breast, colon, and vulval.^{47,109–111} ZD1839 currently is in Phase III trials and is being evaluated for use in patients with nonsmall cell lung carcinoma.¹¹²

The effects of anti-EGFR monoclonal antibodies on EGFR-positive tumors have been studied extensively. They bind to the EGFR with a higher affinity compared with natural ligands; the chimeric anti-EGFR monoclonal antibody IMC-C225 has demonstrated a binding affinity that is approximately 1 log

TABLE 3
Inhibition of EGFR

EGFR inhibitor	Site of initial binding	Effect/comment
Anti-EGFR antibodies	Antibody binds to EGFR on cell surface	Ligand binding to receptor blocked Signal transduction cascade blocked Receptor-antibody complex internalized
Tyrosine kinase inhibitors	Inhibitors bind intracellularly to EGFR tyrosine kinase	Kinase activity inhibited Signal transduction cascade blocked
Antisense oligonucleotides	EGFR or TGF- α antisense oligonucleotides bind to DNA or RNA	May be delivered using liposome technology
Ligand conjugates	EGFR ligand (e.g., EGF or TGF- α) conjugated to toxin (ricin, <i>Pseudomonas</i> endotoxin)	
Immunoconjugates	Anti-EGFR antibody conjugated to ricin	

EGFR: epidermal growth factor receptor; TGF- α : transforming growth factor- α .

higher than that of natural ligands (0.1–0.2 nM vs. 1 nM).¹¹³ Competitive binding of the anti-EGFR monoclonal antibody to the EGFR prevents binding of the natural ligands, which in turn prevents phosphorylation of the EGFR and inhibits subsequent activation of tyrosine kinase-mediated signal transduction pathways.^{114–117} This was demonstrated in the human colorectal carcinoma cell line DiFi, which was cultured with an anti-EGFR monoclonal antibody (MoAb 225). The reduction in EGFR autophosphorylation likely was due to inhibition of EGF-induced tyrosine kinase activation, down-regulation of the receptors, and diminished receptor dimerization.⁹⁵ After anti-EGFR antibodies bind to the EGFR, the receptor is internalized and degraded and therefore no longer is available on the cell surface for binding to natural ligands.¹¹⁸

Anti-EGFR monoclonal antibodies have been shown to target malignant cells successfully,¹¹⁹ but not exclusively; they also will affect normal tissues. However, the effects to normal tissues appear to be minimal, because many healthy cells expressing EGFR are not turning over rapidly, resulting in down-regulation of the EGFR. The intestinal epithelium is a notable exception given that it is in a constant state of self-renewal. The newly formed epithelial cells that originate from the crypt epithelium express EGFR, which is important for maintaining normal intestinal epithelium. Although tyrosine kinase inhibitors, which are given orally, are associated with intestinal epithelial toxicity, anti-EGFR antibodies, which are given intravenously, are not associated with these effects. It has been speculated that the oral delivery directly exposes the intestinal epithelium to the toxic effects of the agent whereas the intravenous delivery poses difficulty in the agent directly accessing the epithelium from the systemic circulation. In vitro and in vivo studies have demonstrated that anti-EGFR antibodies successfully inhibit the growth of tumor cell lines that

express EGFR. Examples include colorectal carcinoma,⁹⁵ pancreatic carcinoma,^{35,120} breast carcinoma,³⁰ prostate carcinoma,^{118,121} renal cell carcinoma,¹²² and epidermoid carcinoma xenografts.^{113,123}

As a class, many EGFR inhibitors have demonstrated promising results in the treatment of solid tumors both in vitro and in vivo (Table 4). Given that anti-EGFR monoclonal antibodies are able to block the EGFR effectively by extracellular binding, anti-EGFR monoclonal antibodies may be more specific in blocking the EGFR pathway compared with the tyrosine kinase inhibitors. A number of agents have been entered into testing in human subjects (Table 5). As knowledge improves regarding the scientific and biologic implications of the EGFR pathway on the development and maintenance of malignancy, additional therapeutic agents and approaches will be identified.

It has been postulated that the combination of EGFR inhibitors and chemotherapy or radiation therapy may be synergistic with regard to antitumor activity. Synergistic activity with a variety of chemotherapeutic agents and radiation therapy has been demonstrated against various tumor cell lines in a number of preclinical trials (Table 6). Hypotheses regarding the mechanism of enhanced cytotoxic activity between EGFR inhibitors and cytotoxic therapy have been proposed. In the case of chemotherapy, enhanced activity may result from the mechanism of action of both agents. The administration of chemotherapy damages DNA, causing cells to arrest in the G₁ peak to undergo repair. Should this repair be unsuccessful, cells may undergo apoptosis. By preventing growth factor ligands from binding to the EGFR, the tyrosine kinase signal transduction pathways necessary for cellular proliferation are not activated. Thus, EGFR inhibitors and cytotoxic therapy may augment apoptosis in malignant cells.¹³³ Radiosensitization with EGFR inhibitors may occur by different mecha-

TABLE 4
EGFR Inhibitors

EGFR inhibitor	Chemical class	Description
IMC-C225	Monoclonal antibody	Chimeric anti-EGFR monoclonal antibody
ABX-EGF	Monoclonal antibody	Human anti-EGFR monoclonal antibody
ICR 62	Monoclonal antibody	Rat anti-EGFR monoclonal antibody
EMD 55900	Monoclonal antibody	Murine anti-EGFR monoclonal antibody
OSI-774 (formerly CP-358,774)	Quinazoline	Tyrosine kinase inhibitor
ZD-1839	Quinazoline	Tyrosine kinase inhibitor
PD153035	Quinazoline	Tyrosine kinase inhibitor
PKI-166	Pyridopyrimidine	Tyrosine kinase inhibitor
PD158780	Pyridopyrimidine	Tyrosine kinase inhibitor
EGF-ricin	Ligand conjugate	Protein synthesis inhibitor
EGF-genistein	Isoflavone conjugate	Ligand-tyrosine kinase inhibitor conjugate

EGFR: epidermal growth factor receptor.

nisms, including variations in the cell cycle, prevention of DNA repair after damage by irradiation, and inhibition of critical cellular signals during cell cycle arrest.¹³⁴

Development of IMC-C225: An EGFR Monoclonal Antibody

Early seminal studies evaluating the use of EGFR as an immunogen gave rise to different murine monoclonal antibodies, including M225.¹¹⁵ M225 was found to compete effectively with EGF for binding to the EGFR and inhibits EGF-induced tyrosine kinase-dependent phosphorylation and EGF. M225 also induces apoptosis in tumor cell lines that overexpress EGFR.¹³⁵ Despite these promising effects, all patients treated with M225 in early Phase I trials were reported to develop HAMAs.¹²⁰ Development of this HAMA reaction indicates that the potential benefits with repeated M225 treatment would likely be negated.¹²⁰ A human-mouse chimeric version (IMC-C225) was developed that can be used widely in humans. This was achieved via attaching the Fv regions of the mouse antibody with the human immunoglobulin G1 region. The resultant monoclonal antibody has been reported to demonstrate efficacy in tumor growth inhibition in various human xenograft models.¹¹³

Antitumor efficacy of IMC-C225 results from multiple mechanisms that include inhibition of cell cycle progression, promotion of apoptosis, antiangiogenesis, and potential enhancement of immunologic activity (antibody-dependent cellular cytotoxicity).¹³⁶ Furthermore, IMC-C225 may reduce metastasis for-

mation by down-regulating the expression of MMP.¹³⁷ IMC-C225 has demonstrated both in vitro and in vivo antitumor activity in tumor cell lines expressing EGFR.

In vitro and in vivo experience with IMC-C225

A number of both in vitro and in vivo studies have confirmed the antitumor activity of IMC-C225. Treatment of a human colorectal carcinoma cell line, DiFi, with IMC-C225 resulted in G₁ cell cycle arrest and induction of apoptosis as evidenced by a reduction in cell volume and DNA fragmentation.⁹⁴ IMC-C225 has been shown to inhibit cellular proliferation of a number of squamous cell carcinoma head and neck cell lines in vitro.³⁶ This study also demonstrated an accumulation of cells in the G₀/G₁ phase as well as a decrease in the number of cells in the S-phase. IMC-C225 resulted in enhanced radiosensitivity and radiation-induced apoptosis as well as significantly diminished cell survival. Recently, the effects of IMC-C225 were evaluated in human nonsmall cell lung carcinoma cells to determine its activity with radiation therapy and chemotherapy.¹³⁸ IMC-C225 was found to inhibit growth in highly EGFR-expressing nonsmall cell lung carcinoma cells (H226). Flow cytometric analysis showed the greatest cell cycle shifts (20%) in H226 cells, from the S-phase to the G₀/G₁ phase. Furthermore, synergistic/additive effects were observed with radiation therapy and chemotherapy in the high-moderate EGFR cells (i.e., H226, A549, and H157). Renal cell carcinoma cell lines treated with IMC-C225 demonstrated dose-dependent inhibition of DNA synthesis, resulting in inhibition of cellular proliferation.¹²¹ In this study, athymic mice treated with IMC-C225 demonstrated a significant increase in survival compared with control mice. Furthermore, athymic mice injected with SK-RC-29 cells and IMC-C225 demonstrated dose-dependent inhibition of tumor growth and reduced tumor volumes. When treated with IMC-C225, the human prostate carcinoma cell line DU145 demonstrated effects on cell-cycle progression.¹¹⁷ There was an increased G₁/G₀ peak compared with control cells and a decreased number of cells in the S-phase. IMC-C225 demonstrated significant inhibitory effects in the athymic nude mice that were inoculated with the human prostate carcinoma cell lines DU145 and PC-3 compared with control mice. Several mice achieved tumor remission and remained tumor-free for at least 90 days after cessation of IMC-C225 administration.

IMC-C225: An Anti-EGFR Monoclonal Antibody with Clinical Experience

Many data regarding the efficacy and safety of anti-EGFR monoclonal antibodies in solid tumors are de-

TABLE 5
EGFR Inhibitors in Clinical Trials

Agent	Tumor type	Trial phase	Manufacturer	Target
IMC-C225	Head and neck	II, III	ImClone	Ab EGFR1
	Colorectal	II		
	Pancreatic	II		
ZD-1839	Nonsmall cell lung carcinoma	II/III	Astra-Zeneca	TKI, EGFR1
OSI-774	Multiple advanced solid tumors	I	OSI Pharmaceuticals	TKI, EGFR1
ABX-EGF	Renal, prostate, pancreatic, nonsmall cell lung, and esophageal carcinomas	I/II	Abgenix	Ab EGFR1
PKI-166	Pancreatic/solid tumors	I	Novartis	TKI, EGFR1
CI-1033	Solid tumors	I	Pfizer	TKI, PAN erb B

EGFR: epidermal growth factor receptor; Ab EGFR1: antiepidermal growth factor receptor monoclonal antibody; TKI: tyrosine kinase inhibitor.

TABLE 6
Synergistic Effects Demonstrated for EGFR inhibitors in Combination with Cytotoxic Therapy

EGFR inhibitor	Cytotoxic agent	Tumor type	Reference
IMC-C225 (anti-EGFR MoAb)	Cisplatin	A431 squamous cell carcinoma xenografts	30, 96
IMC-C225	Doxorubicin	MDA-468 xenografts	123
IMC-C225	Topotecan	Colon carcinoma xenografts	124
IMC-C225	Paclitaxel	MDA-MB-468 breast carcinoma cells	125
IMC-C225	Irradiation	Head and neck carcinoma cell lines	36, 37, 129
IMC-C225	Irradiation	Glioma	130
IMC-C225	Irradiation	A431 tumor xenografts	131
EMD 55900 and ICR-62 (anti-EGFR antibodies)	Cisplatin	Head and neck carcinoma cell lines	4
OSI 776 (EGFR specific protein kinase inhibitor)	Doxorubicin, paclitaxel, or vinblastine	Breast carcinoma xenografts	126
ZD1839	Cisplatin, carboplatin, paclitaxel, or etoposide	GEO colon carcinoma cells	127
ZD1839	Cisplatin or paclitaxel	LoVo colorectal xenografts	128
EGFR tyrosine kinase inhibitor	Irradiation	Breast carcinoma cell line	132

EGFR: epidermal growth factor receptor; MoAb: monoclonal antibody.

rived from clinical experience with IMC-C225. The efficacy of IMC-C225 is optimal when serum levels completely saturate the EGF receptor. Dose-finding studies were based on the hypothesis that zero-order elimination of the antibody is associated with complete saturation of the EGF receptor.¹³⁹ Phase I dose-finding studies demonstrated that continuous saturation of IMC-C225 clearance (and hence the EGF receptor) is achieved in the majority of patients using an initial loading dose of 400 mg/m² followed by 250 mg/m² weekly.¹³⁹ The efficacy of IMC-C225 has been studied primarily in patients with squamous cell carcinomas of the head and neck and colon because a high percentage of these tumor lines express EGFR.

IMC-C225 in combination with radiation therapy

The efficacy and safety of IMC-C225 in combination with radiation therapy were studied in a Phase I trial of previously untreated patients with unresectable AJCC Stage III/IV squamous cell carcinoma of the head and neck.¹⁴⁰ Patients received 8 weekly infusions of IMC-

C225 with a loading dose ranging from 100–500 mg/m² and maintenance doses ranging from 100–250 mg/m². Conventional radiation therapy (70.0 grays [Gy] at a dose of 2.0 Gy daily) or hyperfractionated radiation therapy (76.8 Gy at a dose of 1.2 Gy twice daily) was initiated during the second week of the treatment course. Fifteen patients were evaluable for response of efficacy (one patient was not evaluable due to an anaphylactic reaction that occurred during the first infusion of IMC-C225). The combination of IMC-C225 and radiation therapy was associated with a 100% major (complete or partial) response rate; 87% of patients achieved a complete response and 13% demonstrated a partial response. This response rate was confirmed at follow-up 1 year later.¹⁶ The median duration of response to date is 13.9 months with 67% of patients reported to be progression-free. Because complete response rates to radiation therapy alone in this patient population were found to range from 31–46%,^{141–143} an 87% complete response rate is a possible appreciable improvement. With the exception of

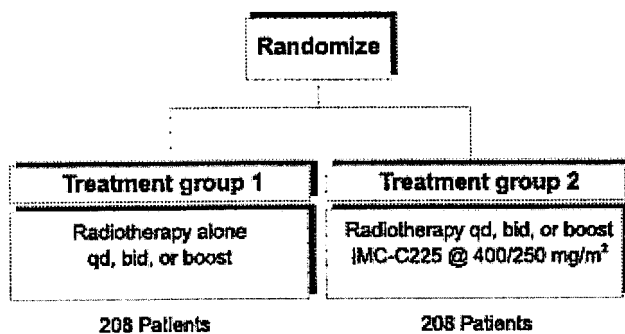


FIGURE 2. Phase II study of IMC-C225 and radiation therapy in patients with squamous cell carcinoma of the head and neck. qd: every day; bid: twice daily.

one patient who experienced an anaphylactic reaction during the first IMC-C225 infusion, treatment generally was well tolerated and many of the toxicities were those expected with radiation therapy alone (e.g., mucositis, xerostomia, and odynophagia). The administration of IMC-C225 was associated with acne-like rashes. The rashes were mostly localized to one body region, did not limit treatment, and resolved completely. A Phase III study currently is ongoing in early stage head and neck patients to confirm these promising findings (Fig. 2).

IMC-C225 in combination with chemotherapy

Initial studies with chemotherapy have been conducted in head and neck carcinoma cases because of their universal positivity for EGFR. A small Phase I dose escalation study of IMC-C225 in combination with cisplatin was conducted in patients with recurrent and/or metastatic EGFR-positive preselected head and neck squamous cell carcinoma that determined to be not curable with surgery and/or radiation therapy.¹⁵ Endpoints of this study included efficacy, safety, and EGFR saturation. Patients received weekly IMC-C225 at loading/maintenance doses of 100/100 mg/m², 400/250 mg/m², or 500/250 mg/m² in combination with cisplatin at a dose of 100 mg/m² every 3 weeks. Twelve patients were treated and evaluable for toxicity; 9 of these patients were evaluable for response. Overall, 67% of the patients achieved a major response (2 complete responses and 4 partial responses). Significantly, three responders had received prior cisplatin therapy; this is a group of patients who rarely responds to cisplatin alone. One patient each demonstrated a mixed response, stable disease, and progressive disease. Toxicities attributed to IMC-C225 included allergic reactions and an acne-like rash and were limited to Grades 2 and 3 in severity. The acne-like rashes resolved completely 4–6 weeks after the cessation of therapy. Other adverse events such as

fatigue, peripheral neuropathy, and orthostatic hypotension likely were related to cisplatin. Given these results, a Phase II study in patients with refractory disease and a Phase III front-line study in patients with untreated, locally advanced disease currently are in progress (Figs. 3 and 6).

In an ongoing Phase II study, patients with recurrent squamous cell carcinoma of the head and neck receive two cycles of a cisplatin-containing regimen, and patients with either progressive or stable disease go on to receive cisplatin plus IMC-C225 (Fig. 3). Preliminary analysis suggest that the combination of cisplatin with IMC-C225 appears to have clinical activity.¹⁴⁴ In another Phase II study, patients with advanced pancreatic carcinoma were treated with the combination of IMC-C225 plus gemcitabine (Fig. 4).¹⁴⁵ Encouraging efficacy and tolerability results will be confirmed as data are finalized.

Two ongoing Phase II trials are assessing the effects of IMC-C225 in patients with refractory disease. IMC-C225 currently is being studied in combination with irinotecan in patients with metastatic or recurrent colorectal carcinoma that previously was refractory to irinotecan (Fig. 5).¹⁴⁶ Patients are randomized to receive combination therapy or irinotecan alone. The basis for this trial was several anecdotal reports of activity when previously treated patients received IMC-C225 in combination with irinotecan. The primary endpoints of this study are the response rate and an evaluation of the safety profile of the combination therapy.¹⁴⁶ IMC-C225 also is currently being studied in patients with chemotherapy naive squamous cell carcinoma of the head and neck (Fig. 6). In this study, patients receive IMC-C225 in combination with cisplatin therapy. The primary objectives of this study are to determine the response rate to combination therapy, establish the safety profile of this combination, and determine the duration of response in these patients. Preliminary findings from these studies are encouraging, and data analyses are ongoing.

Safety Data

IMC-C225 in combination with chemotherapy or radiation therapy has appreciable activity in a number of tumor types that are advanced or have recurred, conditions that are associated with a poor prognosis. In addition to favorable response rates, to our knowledge IMC-C225 has not been associated with serious toxicity. A safety review demonstrated that adverse events generally were reported to be mild to moderate.¹⁴⁷ The percentage of adverse events of \geq Grade 3 was 12%. The major toxicities associated with IMC-C225 have been allergic and skin reactions. Of 189 patients treated, 2% experienced Grade 3 and 2% ex-

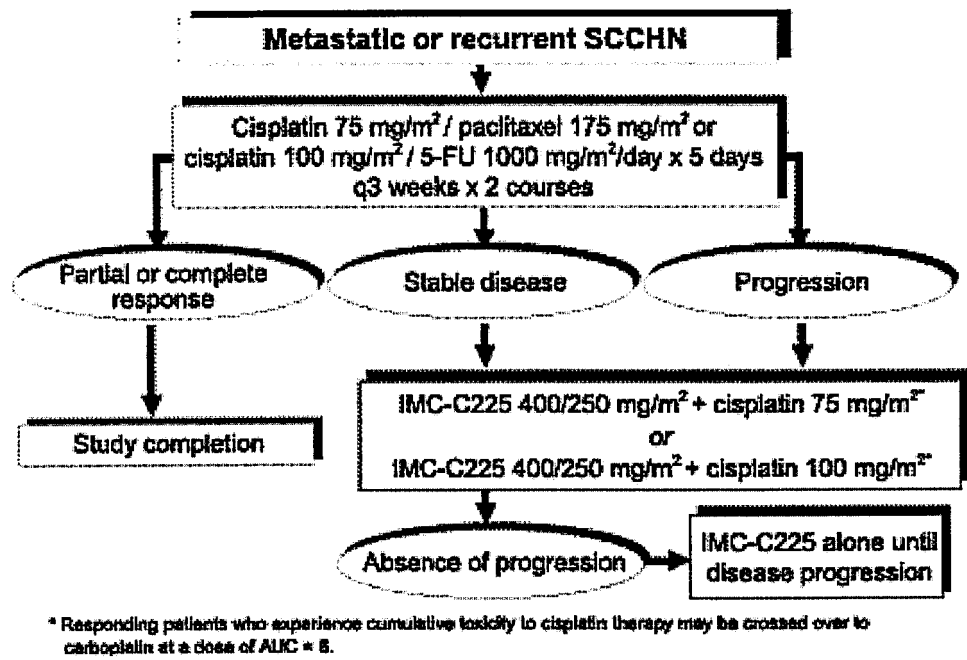


FIGURE 3. Phase II study of patients with squamous cell carcinoma of the head and neck treated with a cisplatin-containing regimen. q: every.

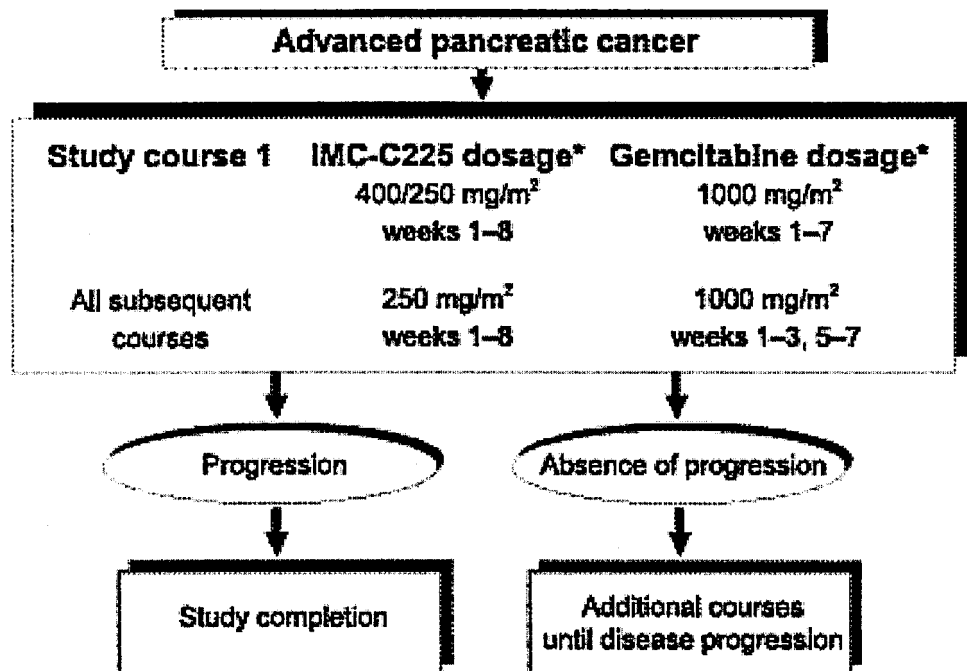


FIGURE 4. Phase II pancreatic carcinoma study with gemcitabine therapy.

perienced Grade 4 allergic reactions. Patients with low-grade allergic reactions were continued successfully on therapy by the administration of prophylactic antihistamine therapy and by slowing the infusion rate. At current Phase II dosing, nearly all patients developed some form of a dose-related, acne-like rash. These sterile, suppurative rashes, characterized as multiple pustular lesions that generally occur on the face, neck, and upper trunk, tend to manifest during

the first 2 weeks of therapy (Fig. 7). Despite their frequent occurrence, the acne-like rashes were not found to be dose-limiting and resolved completely, without scarring, on cessation of therapy in all cases. Given that EGFRs are expressed in epithelial tissues, skin reactions are a toxicity shared by the class of EGFR inhibitors. Additional Phase II and III trials currently are ongoing to determine the efficacy of IMC-C225 in combination with chemotherapy or radiation

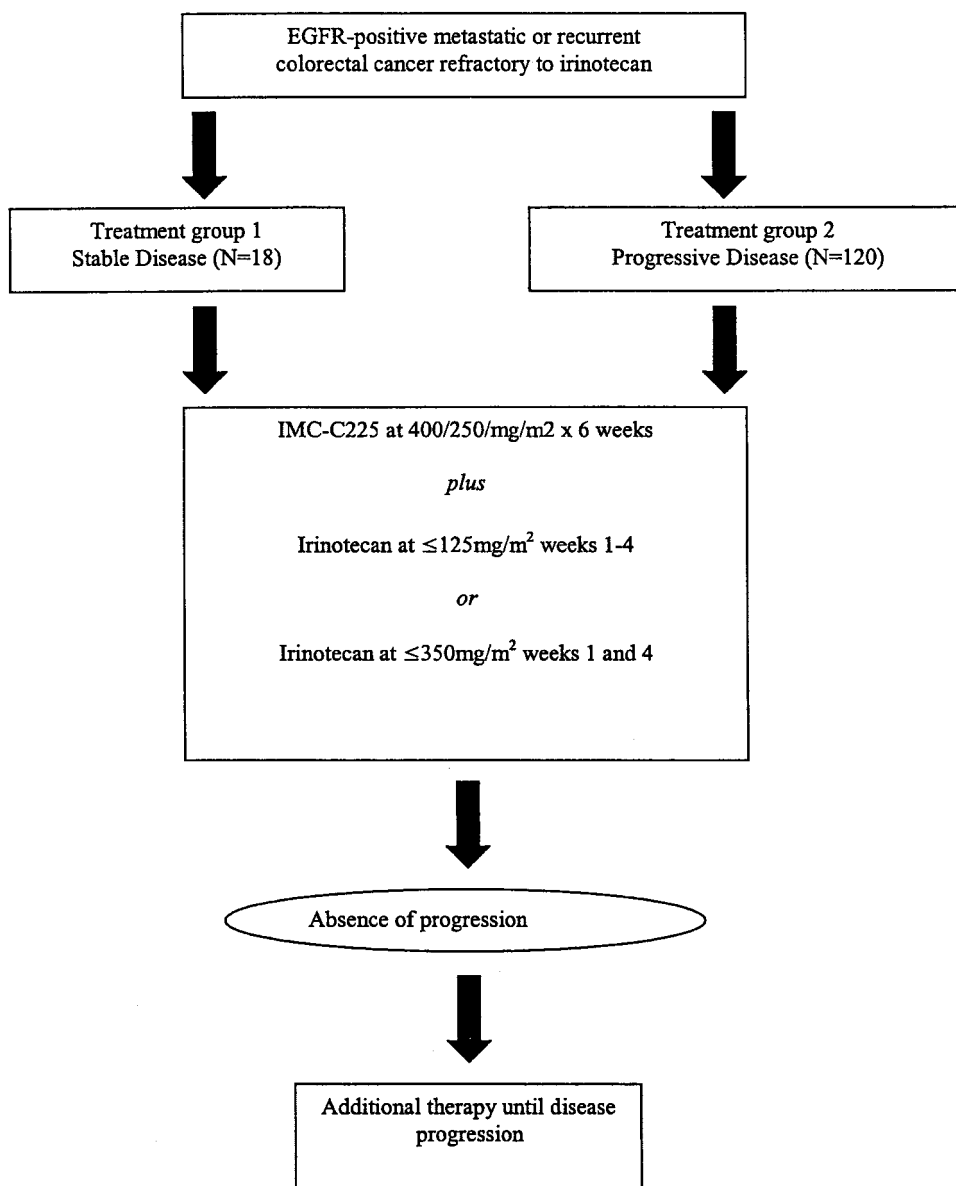


FIGURE 5. Phase II colorectal cancer study with irinotecan. EGFR: epidermal growth factor receptor.

therapy in various tumor types. These trials are evaluating patients with locally advanced, metastatic, or refractory disease.

Anti-EGFR Monoclonal Antibodies in Early Testing

A number of other anti-EGFR monoclonal antibodies are in the early phases of preclinical or clinical testing. ABX-EGF, a fully human monoclonal antibody, was found to inhibit the growth of human epidermoid carcinoma A431 xenografts in murine models and eradicate established tumors.¹⁴⁸ ABX-EGF currently is undergoing Phase I testing in a variety of tumors, including renal, prostate, pancreatic, nonsmall cell lung, and esophageal carcinomas.

EMD 55900, a murine monoclonal antibody,

demonstrated antiproliferative effects in vitro in seven head and neck squamous cell carcinoma lines.⁵ When it was combined with cisplatin, there was additive growth inhibition in these cell lines. EMD 55900 was administered safely in a Phase I trial to patients with advanced head and neck squamous cell carcinoma.⁶ Safety and efficacy were studied further in a Phase I/II trial of 16 patients with recurrent malignant gliomas.¹⁴⁹ In contrast to normal brain tissue, which lacks significant EGFR expression, EGFR is expressed in up to 80% of malignant gliomas. Thus, this tumor type was hypothesized to represent a viable target for EGFR blockade. EMD 55900 generally was well tolerated and surprisingly, only one patient developed a HAMA response. This

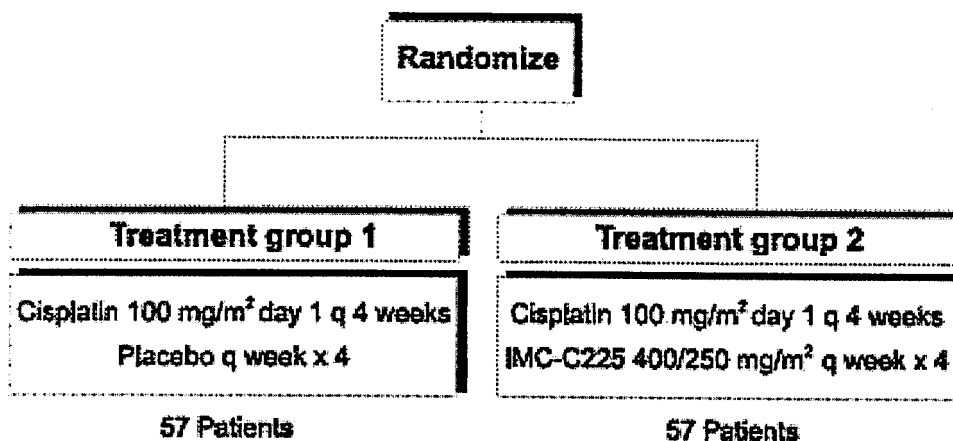


FIGURE 6. Phase II study of IMC-C225 with cisplatin or placebo in squamous cell carcinoma of the head and neck.

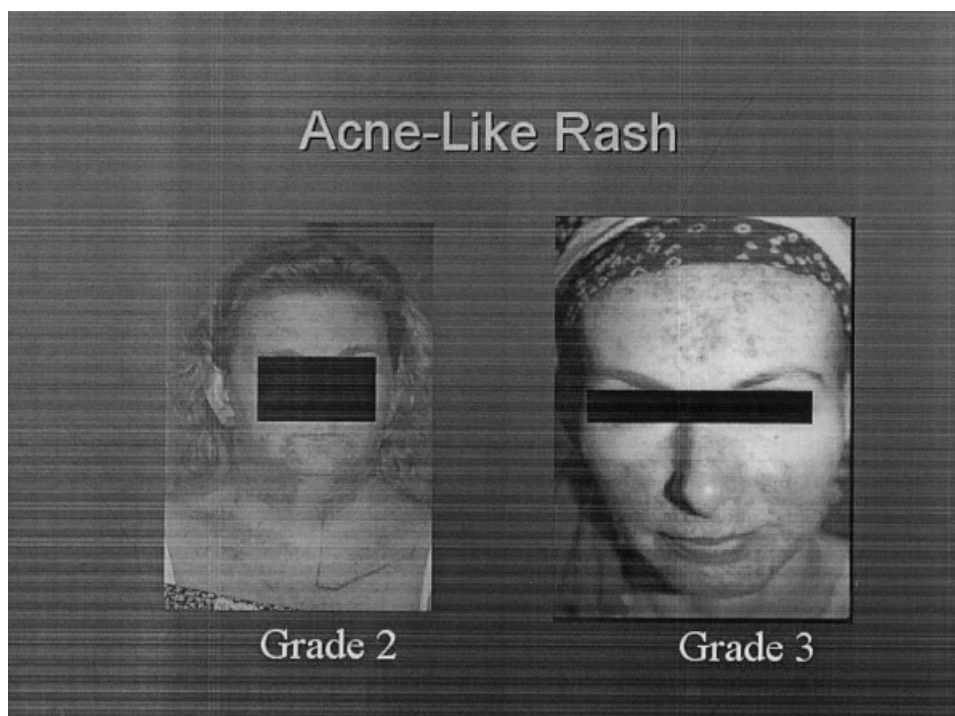


FIGURE 7. Transient skin reactions in patients.

patient had elevated HAMA values prior to treatment with EMD 55900. Thirteen patients were evaluable for response. There were no complete or partial responses reported; 54% of the patients were found to have stable disease and 46% demonstrated progressive disease after 1 treatment cycle. All patients developed disease progression by 3 months. EMD 55900 monotherapy did not demonstrate favorable antitumor activity in this patient population.

ICR 62, a rat monoclonal antibody, exerted anti-proliferative effects on seven head and neck squamous cell carcinoma cell lines in vitro.⁵ Similar to EMD 55900, when cisplatin was combined with ICR 62, there were additive effects on tumor growth inhibi-

tion. In a Phase I trial, ICR 62 was evaluated in 11 patients with squamous cell carcinoma of the head and neck and in 9 patients with squamous cell carcinoma of the lung.⁷ Four patients developed a human antirat antibody (HARA) response. Otherwise, ICR 62 was well tolerated. Biopsies of selected metastatic lesions revealed that ICR 62 localized to tumor cells. As with the other monoclonal antibodies, data are not yet fully complete.

Conclusions

The EGFR pathway plays an important role in cellular proliferation, angiogenesis, and survival. Given its effects on cell cycle progression, apoptosis, angiogene-

sis, tumor cell motility, and metastasis, it clearly is associated with the growth and progression of tumors. EGFR is expressed in approximately 33% of human tumors and this expression can affect important functions, particularly those relative to overall prognosis and survival. Expression of EGFR in tumors, particularly those that demonstrate a high percentage of expression such as head and neck squamous cell carcinoma and colorectal carcinoma, makes it an attractive target for anticancer therapy. Various mechanisms of EGFR inhibition have been proposed and studied including anti-EGFR monoclonal antibodies, tyrosine kinase inhibitors, EGFR-ligand conjugates, immunconjugates, and antisense oligonucleotides. Many of these agents have been shown to inhibit the growth of tumors expressing EGFR.

Monoclonal antibodies directed at the EGFR have been studied extensively. The clinical utility of monoclonal antibodies has been enhanced greatly over the past 20 years, particularly with regard to efficacy and safety. Improvements in technology, primarily the introduction of hybridoma technology, have allowed for the increased production of monoclonal antibodies for clinical use. The introduction of humanized or chimerized monoclonal antibodies resulted in monoclonal antibodies that are less immunogenic than murine monoclonal antibodies. Thus, the incidence of HAMA reactions that previously were problematic in terms of both efficacy and safety has decreased greatly.

The efficacy of EGFR blockade has been demonstrated in vitro and in vivo in both animal and human models for a variety of tumor types. EGFR blockade has resulted in inhibition of tumor growth, diminished tumor volume, and improved survival. Anti-EGFR monoclonal antibodies in particular have demonstrated synergistic antitumor activity when administered concomitantly with chemotherapy and radiation therapy. EGFR inhibition has been shown to sensitize cells to the effects of chemotherapy or radiation therapy. This has potentially important implications in treating patients with resistant disease. In addition to the synergistic activities, the nonoverlapping toxicity profile of EGFR blockade allows concomitant administration with other forms of cytotoxic therapy. EGFR inhibition thus addresses an important limitation associated with chemotherapy and radiation therapy.

Results of ongoing trials with EGFR inhibitors are anticipated to provide valuable information regarding documentation of EGFR expression, the effect of EGFR expression on prognosis, and the efficacy and safety of these agents alone and in combination with chemotherapy and radiation therapy.

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