Acta Pharmacol Sin 2007 Sep; 28 (9): 1476–1493

Invited review

Targeted cancer therapies based on antibodies directed against epidermal growth factor receptor: status and perspectives

Zhenping ZHU[1]

ImClone Systems Incorporated, New York, NY 10014, USA

Key words

wear particles; osteolysis; aseptic loosening; doxycycline

[1] Correspondence to Dr Zhenping ZHU.
Phn 1-646-638-5190.
Fax 1-212-645-2054.
E-mail zhenping.zhu@imclone.com

Received 2007-04-30
Accepted 2007-07-11

doi: 10.1111/j.1745-7254.2007.00681.x

Abstract

Compelling experimental and clinical evidence suggests that epidermal growth factor receptor (EGFR) plays an important role in the pathogenesis of a variety of human cancers; thus, providing a strong rationale for the development of receptor antagonists as effective and specific therapeutic strategies for the treatment of EGFR-expressing cancers. Monoclonal antibodies (mAb), owing to their high specificity towards a given target, represent a unique class of novel cancer therapeutics. A number of anti-EGFR mAb are currently being developed in our clinic, including two that have been approved by the United States Food and Drug Administration for the treatment of refractory metastatic colorectal cancer (mCRC) and squamous cell carcinomas of the head and neck (SCCHN). Cetuximab (Erbitux, IMC-C225), an IgG1 mAb, has demonstrated significant antitumor activity, both as a single agent and in combination with chemotherapeutics and radiation, in patients with refractory mCRC and SCCHN, respectively. Panitumumab (Vectibix), an IgG2 mAb, has been approved as a single agent for the treatment of patients with refractory mCRC. These mAb, via blocking ligand/receptor interactions, exert their biological activity via multiple mechanisms, including inhibition of cell cycle progression, potentiation of cell apoptosis, inhibition of DNA repair, inhibition of angiogenesis, tumor cell invasion and metastasis and, potentially, induction of immunological effector mechanisms. Anti-EGFR antibodies have demonstrated good safety profiles and potent anticancer activity in our clinic and may prove to be efficacious agents in the treatment of a variety of human malignancies.

Introduction

A large number of genes in eukaryotic cells encode for proteins that function as trans-membrane cell surface receptors, of which many are endowed with intrinsic protein tyrosine kinase activity. These receptor tyrosine kinases (RTK) play important roles in the control of some of the most fundamental cellular processes, including cell cycling, proliferation and differentiation[1,2]. Abnormal cell growth resulting from aberrant signal transduction has been implicated in the initiation and progression of a variety of human cancers[1,2], suggesting that various RTK pathways may represent excellent targets for effective cancer intervention[3,4]. In the past few years, many efforts have been made by both academic laboratories and pharmaceutical/biotechnology companies to identify and develop effective RTK antagonists for cancer therapy[3–6]. Our understanding of how each RTK signal transduction pathway participates in abnormal cell growth and the molecules responsible for these events has led to a variety of novel and increasingly mechanism-based approaches to the development of RTK antagonists for cancer therapy. As a result, a number of selective RTK antagonists, including monoclonal antibodies (mAb) and small molecular compounds that selectively inhibit growth factor receptor activation and signal transduction, have been successfully developed for the treatment of patients with a variety of cancers[5–9].

Epidermal growth factor receptor (EGFR) is a trans-membrane receptor encoded by the *c-erb*B1 proto-oncogene with a molecular weight of approximately 170 kDa[10–13]. EGFR belongs to the subclass I family of RTK and is the receptor to at least six distinct ligands, including EGF, transforming growth factor-α (TGF-α), heparin-binding EGF, amphiregulin, betacellulin and epiregulin[10–13]. The subclass I family of RTK consists of EGFR (also known as HER1), HER2/neu (*erbB-2*), HER3 (*erbB-3*) and HER-4 (*erbB-4*)[14–16]. Much evidence suggests that these receptors function in various homodi-meric and heterodimeric pairs, depending on their density on the cell surface, the concentrations of a particular ligand and intrinsic dimerization preference between the receptors[17]. EGFR is normally expressed in a wide variety of epithelial tissues as well as in the central nervous system. Binding of a ligand to the extracellular domain of EGFR leads to receptor dimerization, followed by activation of the intrinsic RTK activity and autophosphorylation of specific residues within the receptor's cytoplasmic domain. These phosphorylated residues serve as docking sites for other molecules involved in the regulation of intracellular signaling cascades. The major signaling cascades activated by EGFR include the Ras/MAP kinase, PLC-gamma, PI-3 kinase/Akt and STAT3 pathways. The integrated biological responses to EGFR signaling are pleiotropic and include enhanced cell mitogenesis, cell motility, protein secretion, cell adhesion, invasion, differentiation or dedifferentiation, and increased neovascularization[11–16].

A large body of experimental evidence supports a role for EGFR activation and signaling in the pathogenesis of human cancers. In 1984, analyses of the EGFR gene and protein revealed that its sequence was homologous to the v-erbB proto-oncogene[10,18]. Direct preclinical evidence for a role of EGFR in malignant transformation emerged from studies in which the transfection of EGFR or TGF-α cDNA was associated with cellular transformation[19]. EGFR is expressed in a variety of human solid tumors, including squamous cell cancer of the head and neck (SCCHN) and carcinomas of the colon and rectum, pancreas, lung, cervix, renal cell, prostate, bladder and breast, as well as melanoma, glioblastoma and meningioma[20,21]. Accumulating evidence suggests that the level of EGFR overexpression is an important factor that directly correlates with active proliferation of malignant cells and poor prognosis of patients[20,21]. In addition, several tumor types have been shown to co-express EGFR and its ligands, leading to an autocrine activation of the receptor and poor outcome in the clinic[20,21]. Finally, mutants of EGFR, due to gene rearrangements that result in in-frame deletion of portions of the extracellular domain of the receptor, have been found in a significant fraction of EGFR-expressing tumors. For example, the most common mutation, EGFR variant III (EGFRvIII), with a deletion of amino acids 6–273, which is frequently found in brain tumors such as glioblastoma, results in a protein with defective ligand binding capacity, but is constitutively activated and its tumorigenicity *in vivo* is enhanced[22,23]. Taken together, these data indicate that expression of EGFR in human cancers has a significant effect on their biological behavior; thus, providing the rationale for the development of EGFR antagonists as potentially useful therapeutic strategies for the treatment of EGFR-expressing cancers[3–8,24].

Monoclonal antibodies as cancer therapeutics

In recent years, mAb, owing to their high specificity towards a given target, have rapidly evolved from the ideal of a "magic bullet" to a new class of practical and efficacious cancer therapeutics. Traditional obstacles in antibody therapy, such as immunogenicity of rodent-derived antibodies and difficulty in producing antibodies in sufficient quantity and quality for commercial application, are being rapidly superseded by advancement in antibody engineering technologies, including antibody chimerization, humanization and direct generation of fully human antibodies from either phage display libraries or human transgenic mice[25–28], as well as the development of efficient manufacturing processes for high level production of mAb at costs that are more economical than ever[29]. Since 1994, the United States Food and Drug Administration (FDA) has approved 21 therapeutic mAb for clinical use, including 9 for oncology indications. In addition, there are several hundred mAb currently being tested in clinical trials worldwide for various indications.

The majority of antibodies currently being used for targeted therapy belong to the IgG class of immunoglobulins. The effectiveness of an antibody-based therapeutic depends on its ability to induce one of several biological mechanisms. These include:

1. Blocking growth factor/receptor interaction and/or downregulating expression of oncogenic proteins (or receptors) on the cell surface. By interfering with important growth factor/receptor signaling pathways, the mAb can influence the growth and survival of tumor cells, and may also potentiate the cytotoxic effects of chemotherapeutic drugs and radiation. Several antibodies are believed to exert their therapeutic efficacy mainly via this mechanism, including bevacizumab (Avastin, an IgG1 antibody to vascular endothelial growth factor [VEGF]), cetuximab (Erbitux, an IgG1 antibody to EGFR) and trastuzumab (Herceptin, an IgG1

antibody to HER2/neu)[30–32].

2. Recruiting effector mechanisms of the immune system, such as antibody-dependent cellular cytotoxicity (ADCC) and complement-mediated cytotoxicity (CMC). The ability to mediate both ADCC and CMC is dependent on the isotype of the mAb. For example, human IgG1 and IgG3 bind with significantly higher affinity to human Fc receptors and are much more effective mediators of ADCC than IgG2 and IgG4. There is evidence to suggest that effector mechanisms play an important role in the clinical antitumor efficacy of rituximab (Rituxan, an IgG1 antibody to CD20), trastuzumab and cetuximab[33–36]; all 3 mAb are of the human IgG1 isotype.

3. As a conjugate, the antibody acts as a carrier molecule to deliver an attached chemotherapeutic agent or toxin or radioisotope to cells displaying a specific antigen. A number of antibody conjugates have been approved by the FDA for oncology indications, including gemtuzumab ozogamicin (Mylotarg, an anti-CD30 IgG4 antibody-calicheamicin conjugate) for acute myeloid leukemia[37], ibritumomab tiuxetan (Zevalin, a ^{90}Y-labeled anti-CD20 antibody)[38] and tositumomab (Bexxar, an ^{131}I-labeled anti-CD20 antibody)[39] for non-Hodgkin's lymphoma.

4. Other mechanisms; for example, antibodies to stimulate the anti-idiotype network to generate antitumor anti-anti-idiotypic antibody response[40], and antibodies to enhance a patient's immune response to tumors by stimulating cytotoxic T lymphocytes via CD40[41] or by antagonizing endogenous immune inhibitory factors such as CTLA-4[42]. Recently, functional antibodies have been expressed intracellularly as "intrabodies". These intrabodies exert their biological effects through interfering with the function of the targeted molecules via a variety of mechanisms, including altering their intracellular trafficking, localization and/or surface expression, blocking their interaction with other molecules, or directly neutralizing their enzymatic activity (for kinase targets)[43,44].

Cetuximab (Erbitux, IMC-C225)

Cetuximab in preclinical studies Cetuximab is an IgG1 anti-EGFR antibody that is being developed for the treatment of EGFR-expressing human cancers (ImClone Systems Incorporated, New York, NY, USA). Cetuximab is a chimeric version of the murine anti-EGFR mAb M225[45,46]. It efficiently competes with EGF, and TGF-α for binding to EGFR, inhibits ligand-stimulated activation of the receptor and downstream signaling molecules, and inhibits tumor cell mitogenesis *in vitro*. Binding of cetuximab to EGFR also induces receptor internalization; hence, effectively stripping the receptor from the tumor cell surface. Cetuximab also induces apoptosis in some EGFR-overexpressing tumor cell lines. Furthermore, as a human IgG1 isotype, cetuximab mediates effective ADCC to a variety of human tumor cell lines in vitro, and the extent of cell lysis correlates directly with the expression level of EGFR by the tumor cells[47,48]. The tumor growth inhibitory activities of cetuximab were confirmed *in vivo* in a variety of human tumor xenograft models that include carcinomas of colon, epidermoid, lung, pancreas, bladder, breast, prostate and renal cells[49].

It has been shown that tumor cells often upregulate the expression of growth factors and their receptors; for example, EGF and EGFR, in response to cellular stress or cytotoxic insult in order to activate the survival mechanisms[50–53]. Several *in vivo* studies have demonstrated a direct correlation between increased EGFR expression and decreased response (ie the development of resistance) to chemotherapy and radiotherapy regimens for several different tumor types[54–56]. To this end, a number of preclinical studies have shown that cetuximab could augment the antitumor activity of various anticancer agents, including cisplatin, doxorubicin, fluorouracil, gemcitabine, paclitaxel and topotecan, both *in vitro* and *in vivo* in human tumor xenograft models in mice[57–62]. In these studies, combination treatment with both cetuximab and cytotoxic drugs resulted in markedly enhanced tumor inhibition over treatment with either agent alone, and in some models led to tumor regression and the eradication of established tumors. It is pertinent to note that the tumor cells used in some of these studies were poorly responsive to the cytotoxic agents alone, but were sensitized to these agents by concurrent cetuximab treatment[57,58]. For example, cetuximab has been shown to significantly potentiate, in an additive or synergistic manner, the antitumor activity of irinotecan (CPT-11) in a variety of preclinical animal models, in which irinotecan and/or cetuximab exhibit poor efficacy as monotherapies[63]. Similar enhancement of antitumor activity has been observed in studies in which cetuximab is used in combination with radiation[64–67]. These observations suggest that simultaneous inhibition of EGFR-mediated biological activities may improve (or sensitize) tumor response to conventional cytotoxic therapies.

Mechanisms of action of cetuximab Cetuximab, once bound to EGFR, blocks ligands (EGF and TGF-α) from association with the receptor, resulting in inhibition of receptor activation and downstream signal transduction[49,68,69]. In addition, binding of cetuximab to cell surfaces induces internalization of the receptor, leading to surface receptor down-regulation[70]. As a result, the cellular process necessary for cell survival and proliferation does not ensue properly. Sev-

eral molecular mechanisms of action may play a role in the antitumor activity of cetuximab. These mechanisms include inhibiting cell cycle progression, inducing cell apoptosis, inhibiting angiogenesis, inhibiting invasion and metastasis, inhibiting DNA repair and recovery after chemotherapy and/or radiation, and inducing immunological effector responses (including both ADCC and CMC)[49,68,69]. In the case of combinational therapy, blockade of the EGFR pathway by cetuximab inhibits DNA synthesis and repairing processes following the cytotoxic insults, thereby enhancing the antitumor activity of chemotherapeutic agents and radiation[49,68,69].

Cetuximab in clinical studies in patients with metastatic colorectal cancer EGFR is expressed in a significant percentage (from 25% to 80%) of human colorectal tumors and its overexpression is usually associated with advanced diseases. Clinical trials have been conducted using both cetuximab alone and cetuximab in combination with conventional chemotherapeutic regimens in the treatment of metastatic colorectal cancer (mCRC) patients in various stages and settings. These trials aimed to demonstrate that treatment with cetuximab, either as a single agent or in combination with various chemotherapies, would not only be safe, but would also lead to significant antitumor activity, such as the activity observed in the preclinical studies.

EMR-007 (BOND), a randomized Phase II study, is designed to evaluate the activity of cetuximab alone or in combination with irinotecan in a prospective manner[71]. In this design, patients with EGFR-positive tumors who had recently failed irinotecan therapy were randomized in a 2:1 fashion to receive the combination of cetuximab and irinotecan or cetuximab alone. A total of 329 patients were randomized, 218 to receive the combination of irinotecan and cetuximab and 111 to receive cetuximab alone. Partial response (PR) (ie tumor shrinkage by more than 50% after treatment) was achieved in 22.9% of the patients who received both cetuximab and irinotecan, and in 10.8% of the patients treated with cetuximab alone (P=0.007). Time-to-progression (TTP) was also significantly different between the two groups in favor of the combination regimen; the median TTP for the combination arm is 4.1 months versus 1.5 months for the mono-therapy group (P<0.001). The most common toxicity associated with cetuximab treatment was an acne-like skin rash, which occurred in 80% of treated patients (grade 3/4 in 9.4% patients in the combination group and 5.2% patients in the monotherapy group). There seems to be a direct correlation between the patients' response to antibody therapy and the severity of the skin rash, but not to the EGFR staining intensity as determined by standard immunohistochemistry (IHC) methods (see Sections 4.1 and 4.2 for a detailed discussion). Severe anaphylactic reactions to cetuximab developed in 4 (1.2%) patients requiring the discontinuation of the treatment. Other grade 3 or 4 toxicities in the combination group included diarrhea (21.2%), asthenia (13.7%), neutropenia (9.4%), nausea and vomiting (7.1%) and dyspnea (1.4%), compared to rates of 1.7%, 10.4%, 0%, 4.3%, and 13%, respectively, in the antibody monotherapy group. The tumor response rate in this trial was consistent with that observed in an earlier trial in a similar patient population reported by Saltz *et al* (study CP02-9923). In this trial, a single group of 120 mCRC patients who had failed irinotecan was treated with a combination of cetuximab and irinotecan, and PR was observed in 22.5% of the treated patients[72]. Based on these observations, cetuximab was approved by the FDA in February 2004, both in combination with irinotecan for the treatment of EGFR-expressing mCRC who are refractory to irinotecan-based chemotherapy, and as a single agent for the treatment of EGFR-expressing mCRC patients who are intolerant to irinotecan-based chemotherapy.

Two non-randomized, single-arm studies provided additional data on the antitumor activity of cetuximab as a monotherapy. In a small single arm study (CP02-0141), 57 patients with documented progression on irinotecan or an irinotecan-based regimen were treated with cetuximab[73]. Five patients (9%) achieved a PR. Twenty-one additional patients had stable disease (SD) or minor responses. The median survival time was 6.4 months. Eighty-three percent of patients developed a skin rash (18% with grade 3), two patients (3.5%) had grade 3 allergic reactions, and 56% of patients experienced asthenia, fatigue, malaise or lethargy (9% grade 3). In another trial (IMCL-0144) reported by Lenz *et al*[74], cetuximab as a single agent yielded an 11.6% PR rate in 346 patients refractory to both irinotecan and oxaliplatin, with another 31.8% of patients experiencing SD for at least 6 weeks. Median overall survival was 6.7 months. The most common adverse events were very similar to those observed in the CP02-0141 trial, including an acne-like skin rash (90%, with 6% of grade 3/4) and fatigue/malaise (48%, grade 3/4 10%). Detailed analysis of the response rate among patient subgroups, who had received 2 to 9 regimens (median, 4) of prior chemotherapy (including 259 patients who had received oxaliplatin after irinotecan failure and 87 patients who had received irinotecan after or with oxaliplatin), revealed that cetuximab was equally active in all patient subgroups regardless of the numbers of prior therapy or the sequence of prior agents[75].

Most recently, a randomized, multi-center, Phase III trial

(NCIC CTG CO.17, also known as BMS-025) compared cetuximab plus best supportive care (BSC) to BSC alone in 572 patients with mCRC whose disease was refractory to all available chemotherapy, including irinotecan, oxaliplatin and fluoropyrimidines. The study met its primary end-point of overall survival:patients who received cetuximab lived an average of 6.1 months compared to 4.6 months for patients who received BSC alone, representing a 23% increase in overall survival (P=0.005). Cetuximab treatment also resulted in PR in 23 patients (8%) compared to 0% in patients who received BSC alone (P<0.0001), and a 32% reduction in the risk of disease progression (P<0.0001). Further, SD was seen in an additional 31.4% of patients receiving cetuximab, but only in 10.9% of patients on BSC. The antibody was generally well tolerated with a rash as the most common toxicity. These are the first data of an anticancer therapy to demonstrate overall survival in refractory mCRC patients.

Another Phase III randomized study, known as EPIC (Erbitux Plus Irinotecan in Colorectal Cancer), compared irinotecan to irinotecan plus cetuximab in second-line settings in patients whose disease was not responding to first-line oxaliplatin and fluoropyrimidine chemotherapy. A total of 1298 patients were randomized (1 to 1) into two groups receiving irinotecan plus cetuximab (Arm A) or irinotecan alone (Arm B) until disease progression, when the study treatment was stopped and further treatment was at the discretion of the physician. As a result, 47% of patients in Arm B received post-study cetuximab (87% of them in combination with irinotecan). The most common grade 3/4 adverse events included (Arm A vs Arm B): neutropenia (31.8% *vs* 25.4%), rash (8.2% *vs* 0.5%), infusion reaction (1.4% *vs* 0.8%) and hypomagnesemia (3.3% *vs* 0.4%), diarrhea (28.8% *vs*16.2%) and fatigue (9.2% *vs* 4.9%). Secondary efficacy end-points strongly favored the combination of cetuximab plus irinotecan over irinotecan alone; the response rate and progression-free survivaI (PFS) were 16.4% and 4.0 months in Arm A, respectively, compared to 4.2% (P<0.0001) and 2.6 months (P<0.0001) in Arm B, respectively. However, the primary end-point, overall survival was not significantly different between the two arms (10.7 months in Arm A *vs* 10 months in Arm B). Post-hoc analysis suggests this may result from the substantial post-study use of cetuximab, which potentially confounded the interpretation of this end-point. As observed in the two third-line studies (EMR-007 and NCIC CTG CO.17) discussed above, cetuximab, alone or in combination with irinotecan, significantly increased patients' response rates and/or overall survival after their failure with irinotecan therapy.

Cetuximab has also been evaluated for safety and efficacy in mCRC patients in first-line settings in combination with various chemotherapy regimens. In one Phase II study, cetuximab was given in combination with FOLFOX-4 (oxaliplatin/5-fluorouracil/leucovorin) to patients with non-resectable mCRC[76]. In a preliminary analysis of 42 patients, 10% had a complete response (CR), 71% had a PR and 17% had SD. Median PFS was 12.3 months. Nine patients (21%) subsequently underwent surgery of their metastases. The major grade 3/4 toxicities were acne-like rash (30%), neurotoxicity (30%), diarrhea (26%), neutropenia (21%) and stomatitis/mucositis (16%). Recently, ImClone Systems announced that the results of a Phase III study (CRYSTAL study) of cetuximab plus FOLFIRI (irinotecan/5-fluorouracil/leucovorin) met the primary end-point of increasing median duration of PFS over FOLFIRI alone in patients with previously untreated mCRC. This study enrolled more than 1000 patients around the world. Final results have been presented at the annual meeting of the American Society of Clinical Oncology (ASCO) in June 2007.

Currently, several additional Phase III randomized clinical trials with cetuximab in first-line settings in mCRC patients are being carried out. These trials include a trial comparing FOLFOX±cetuximab (OPUS trial, 300 patients, enrollment completed), a trial comparing continuous FOLFOX versus intermittent FOLFOX with or without cetuximab (COIN trial, 2400 patients, ongoing), a trial comparing XELOX (capecitabine plus oxaliplatin) plus bevacizumab with or without cetuximab (CAIRO II trial, 750 patients, ongoing), and an intergroup trial comparing chemotherapy (FOLFOX or FOLFIRI) plus bevacizumab versus chemotherapy plus cetuximab versus chemotherapy plus bevacizumab and cetuximab (CALGB 80405 trial, 2289 patients, ongoing). In mCRC adjuvant settings, cetuximab is being tested in two Phase III trials: NCCTG 147 intergroup trial (2300 patients, ongoing) and PETACC European trial (2000 patients, ongoing), both comparing FOLFOX±cetuximab in mCRC patients with a high likelihood of recurrence.

Cetuximab in squamous cell carcinoma of the head and neck The safety and efficacy of cetuximab was studied both as a single agent and in combination with radiation in patients with SCCHN. In a Phase III trial (IMCL-9815 study)[77], 424 patients with locally or regionally advanced SCCHN were randomized (1:1) to receive radiation alone or cetuximab plus radiation. The median duration of local-regional disease control, the primary end-point, was 24.2 months in the combination group compared with 14.9 months in patients who received only radiation (P=0.005). The medium overall survival in the combination group was

49 months compared with 29.3 months in the radiation alone group (P= 0.03). It is important to note that, except for an infusion reaction (3% in the combination group versus 0% in the radiation alone group) and skin rash, the use of cetuximab did not significantly increase the incidence of the major toxicities associated with radiation, particularly mucositis/stomatitis (56% in the combination group *vs* 52% in the radiation alone group). In another single-arm trial, cetuximab alone was given to 103 patients with recurrent or metastatic SCCHN with documented progression within 30 days after 2–6 cycles of a platinum-based chemotherapy. The PR rate was 13% and the median duration of response was 5.8 months. Based on these results, cetuximab was approved by the FDA in March 2006, in combination with radiation for the treatment of locally or regionally advanced SCCHN, and as a single agent for the treatment of patients with recurrent or metastatic SCCHN who had failed prior platinum-based therapy.

Cetuximab has also been studied in combination with chemotherapeutic agents in patients with SCCHN. In a randomized Phase III trial, patients with recurrent or metastatic SCCHN were treated with cisplatin alone or in combination with cetuximab[78]. In 117 analyzable patients, response rates were 26% in the combination group and 10% in the cisplatin alone group (P=0.03). Median PFS and medium overall survival were 4.2 months and 9.2 months, respectively, in the combination group, and 2.7 months and 8.0 months, respectively, in patients who received cisplatin alone. Although there was a survival advantage for patients who received cetuximab, the difference was not significant mainly because of the fact that the trial was not sufficiently powered. In several other trials in SCCHN patients with advanced disease refractory to platinum-based regimen, cetuximab, either alone or in combination with the same dose and schedule of platinum (that patients had failed), yielded a response rate of 10% to 13%[79,80], which compares favorably to the expected response rate in a similar patient population treated with more toxic second-line chemotherapeutic agents. In April 2007, ImClone Systems announced that a first-line Phase III study of cetuximab combined with platinum-based chemotherapy met the primary end-point of increasing overall survival in patients with recurrent and/or metastatic SCCHN. The randomized, multi-center study, known as EXTREME, studied more than 400 patients treated with cetuximab in combination with 5-fluorouracil plus either cisplatin or carboplatin and compared the results to patients treated with 5-fluorouracil plus either cisplatin or carboplatin alone. Final results have been presented at the annual meeting of ASCO in June 2007.

Cetuximab in other cancers In addition to mCRC and SCCHN, cetuximab is also being tested in other cancer indications, including patients with pancreatic carcinoma[81], non-small cell lung carcinoma (NSCLC)[82, 83] and ovarian carcinoma[84; see 85,86 for reviews]. In April 2007, ImClone Systems announced the preliminary results of an open-label, randomized study comparing cetuximab plus gemcitabine to gemcitabine alone in more than 700 patients with pancreatic cancer in a first-line treatment setting. Conducted by the Southwest Oncology Group (SWOG) in centers throughout the United States and Canada, the study failed to meet its primary end-point of statistically improving overall survival. Detailed analysis of the data is currently being performed. Another large Phase III trial, the FLEX study, examining in first-line settings cisplatin/vinorelbine alone or in combination with cetuximab in patients with NSCLC, has been fully enrolled (1124 patients). The primary end-point for this study is overall survival and results are expected in late 2007.

Panitumumab (ABGX-EGF)

Panitumumab is a fully human anti-EGFR antibody generated using the transgenic XenoMouse technology being developed by Amgen (Thousand Oaks, CA, USA)[87,88]. The antibody binds to EGFR with a high affinity (~50 pmol/L) and is able to completely regress certain human xenografts in animal models as a single agent therapy[89]. As an IgG2 subclass antibody, panitumumab does not mediate a significant level of ADCC on EGFR-expressing tumor cells. In clinical studies panitumumab has been well tolerated at doses ranging up to 10 mg/kg, causing low infusion reaction (<1%), and has not elicited any human antibody response. In a dose-escalating trial, 88 patients with metastatic renal cell carcinoma were treated with panitumumab at weekly doses of 1.0, 1.5, 2.0, or 2.5 mg/kg with no loading dose. PR was achieved in three patients, and two patients had minor responses. Forty-four patients (50%) also had SD at their first 8-week assessment, and the median PFS was 100 days. The principal toxicity, skin rash, occurred in 68%, 95%, 87%, and 100% of patients who received at least three doses of ABGX-EGF at 1.0, 1.5, 2.0, and 2.5 mg/kg per week, respectively[90].

In a multi-center, open-label, single-arm study, a total of 148 CRC patients were grouped into two cohorts: cohort A with higher EGFR staining intensity (2+ or 3+ in >10% evaluated tumor cells [104 patients]) and cohort B with lower EGFR staining intensity (1+ or 2+ or 3+ in <10% evaluated tumor cells [44 patients]). The patients were first treated with panitumumab at 2.5 mg/kg every week for a total

of 8 doses, followed by radiographic evaluation of the tumor response and repeated 8-week treatment cycles until disease progression or unacceptable toxicity. The antibody was well tolerated, with the major toxicity, skin rash (including 3% grade 3), occurring in 95% of patients. Interim analysis revealed PR in 11% of cohort A patients and 9% of cohort B patients. Of the 15 patients with a response, 13 patients responded at the time of the first 8-week evaluation. The median overall survival time was 7.9 months and the median TTP was 2.0 months[91]. As seen in the cetuximab trials, there seems to be a direct correlation between tumor response and skin rash, but not between tumor response and EGFR staining intensity in the tumors, in panitumumab-treated patients. In a recently completed open-label Phase III trial, 463 patients with mCRC who had previously failed standard chemotherapy, including oxaliplatin and irinotecan, were randomized to receive panitumumab at 6 mg/kg every 2 weeks plus BSC (n=231) or BSC alone (n=232). The PR rate was 8% with panitumumab versus zero with BSC alone, and the median duration of response was 17 weeks. The SD rate was 28% with panitumumab versus 10% with BSC alone. Furthermore, patients who received panitumumab showed a 46% decrease in tumor progression rate versus those who received BSC alone. Approximately 75% of the BSC patients entered a crossover arm to receive panitumumab after their disease had progressed (n=174). Panitumumab treatment also showed a clinical benefit in patients who crossed over; in these patients, panitumumab treatment resulted in a 9% PR, 32% SD and one CR. Despite these findings, an interim analysis revealed that the overall survival between the two groups was similar. The investigators believed that rate (75%) and timing (median 7.0 weeks) of crossover from the BSC alone arm to receiving panitumumab, and the antitumor activity observed after crossover, are likely to have confounded the ability to demonstrate a treatment effect on overall survival[92]. Based on these observations, the FDA approved panitumumab in September 2006 for use as a single agent in patients with refractory mCRC.

In March 2007, Amgen announced that it had discontinued the Panitumumab Advanced Colorectal Cancer Evaluation (PACCE) trial, a Phase IIIb randomized, open-label clinical trial evaluating oxaliplatin-based and irinotecan-based chemotherapy and bevacizumab with and without panitumumab in the first-line treatment of patients with mCRC. The trial enrolled 1054 patients (824 patients were randomized to receive oxaliplatin-based chemotherapy and 230 patients were randomized to receive irinotecan-based chemotherapy) in the United States between the first quarter of 2005 and the third quarter of 2006. A pre-planned interim efficacy analysis scheduled after the first 231 events (death or disease progression) revealed a statistically significant difference in PFS in favor of the control arm (bevacizumab plus chemotherapy). An unplanned analysis of overall survival also demonstrated a statistically significant difference favoring the control arm. Furthermore, a review of the interim analysis showed an increased incidence of grade 3 severe events of diarrhea, dehydration and infections in the panitumumab-treated patients. In addition, increased incidence of pulmonary embolism was observed in patients who received panitumumab compared with those who did not (4% and 2%, respectively). One fatal event of pulmonary embolism occurred in a patient receiving panitumumab. Final results of this trial are due to be presented in late 2007.

Nimotuzumab (h-R3)

Nimotuzumab (YM Biosciences, Mississauga, ON, Canada, and Center of Molecular Immunology, Havana, Cuba) is a humanized IgG1 form of the murine IgG2a antibody R3 specific for EGFR[93,94]. Nimotuzumab binds to the EGFR extracellular domain with a moderate affinity (about 1 nmol/L), blocks EGF binding to its receptor and ligand-dependent receptor autophosphorylation, and inhibits cell growth in EGFR-expressing cells[94]. Studies have shown that the antitumor effect of nimotuzumab may result from its combined effects on tumor cell proliferation, survival and angiogenesis[95]. Multiple clinical trials are currently being conducted to examine the therapeutic efficacy of the antibody in a number of cancers, including a Phase III trial in pediatric pontine glioma (first-line in combination with radiation, data expected in 2007) and Phase II studies in patients with carcinomas of the pancreas, esophagus and cervix, and NSCLC and hormone-refractory prostate cancer. In a Phase II trial, pediatric patients (n=34) with relapsed or resistant high-grade gliomas received weekly nimotuzumab (150 mg/m^2) for 6 weeks, followed by a consolidation arm of four infusions in a 3-week interval if no disease progression were observed. One PR and 11 SD were observed and a median of 7.5 months of PFS was achieved in 8 patients in the consolidation phase of the trial. No severe side effects were observed. In a Phase I/II trial, 29 patients (16 glioblastoma, 12 anaplastic astrocytoma and 1 anaplastic oligodendroglioma) received 6-weekly infusions of nimotuzumab at a dose of 200 mg in combination with external beam radiotherapy. The antibody was very well tolerated without grade 3/4 adverse events. None of the patients developed acneiform rash or allergic reactions. One patient developed a positive anti-idiotypic response. The objective response

rate was 37.9% (17.2% CR, 20.7% PR) and SD occurred in 41.4% of the patients. With a median follow up time of 29 months, the median survival was 22.17 months for all subjects[96].

Nimotuzumab is also being tested in combination with radiotherapy in the treatment of locally advanced SCCHN patients. In one trial, 24 patients received 6-weekly infusions of nimotuzumab at 4 dose levels in combination with radiation. The combination therapy was well tolerated. Aside from infusion reactions, no skin or allergic toxicities were observed. Overall survival was significantly increased after the use of the higher antibody doses[97]. In another Phase II trial, nimotuzumab (100 mg, iv once weekly for 8 weeks) in combination with radiotherapy demonstrated greater efficacy against nasopharyngeal carcinoma than radiation alone. Of the 130 patients in the intent-to-treat analysis, 90.6% in the combination arm had a CR, compared with 51.5% in the radiation-alone group. Nimotuzumab in combination with radiation therapy has been approved for the treatment of locally advanced inoperable head and neck carcinomas in several countries, including Cuba, Argentina, Columbia, China and India.

To date about 600 patients have been treated with nimotuzumab. Early clinical experience with nimotuzumab suggests that it may lack some of the toxicities commonly associated with other EGFR-targeting agents, including cetuximab, panitumumab and small molecule tyrosine kinase inhibitors (TKI). In particular, grade 1/2 skin rash (no grade 3/4) has only been reported in about 6% patients, compared to the high frequency often seen with both cetuximab and panitumumab. Furthermore, no significant hypomagnesemia occurred in patients treated with nimotuzumab. Whether these observations have positive or negative clinical implications for nimotuzumab remains to be seen. A distinct toxicity profile may result from nimotuzumab binding to EGFR with lower affinity than other EGFR-specific antibodies. Alternatively, nimotuzumab may bind to a different epitope on the receptor, thereby inducing an alternative intracellular signal. Other toxicities of nimotuzumab observed to date include mild or moderate (grade 1/2) fever, hypotension/hypertension, vomiting, diarrhea and nausea, dry mouth, nail inflammation, and tremors; these were controlled with standard medications.

Matuzumab (EMD 72000)

Matuzumab is a humanized anti-EGFR antibody (Merck KGA, Germany) that has demonstrated antitumor activity in preclinical tumor models both as a single agent and in combination with chemotherapy and radiation[98]. To date, over 320 patients have been treated with the antibody and have tolerated it well, with the most commonly reported toxicities being skin rash, fever and headache. The maximum tolerated dose of matuzumab is 1600 mg, iv weekly, and the dose-limiting toxicities are grade 3 fever and headache. In a clinical trial (EMD 72000-018), 22 patients with mixed solid tumors were given the antibody at 400 mg, 800 mg, 1200 mg, 1600 mg or 2000 mg, iv per week. Grade 3 fever and headache were observed in patients at greater than 1600 mg dose levels. PR was achieved in 5 patients (2/5 of RCC patients, 3/5 SCCHN patients), with SD in an additional 6 patients[99]. In another single arm Phase II trial, 37 patients with recurrent, EGFR-positive ovarian or primary peritoneal cancers were treated with matuzumab. The antibody was well tolerated, and the most common toxicities included rash, acne, dry skin and paronychia, as well as headache, fatigue and diarrhea. Seven patients (21%) achieved SD and remained on therapy for more than 3 months[100].

Clinical trials are also being carried out to investigate the antitumor activity of matuzumab in combination with chemotherapy. In a Phase I combination study (EMD 72000-020), matuzumab, at a weekly dose ranging from 100 to 800 mg, was given in combination with paclitaxel to advanced NSCLC patients. One patient at an antibody dose of 800 mg showed a grade 4 neutropenia. Grade 1/2 acneiform skin rash in 14 patients was the most frequent side effect. Grade 2 toxicities included pruritus (n=2), bronchospasm (n=1), fissures (n=1), abdominal pain (n=1) and hot flushes (n=1). Out of 18 patients evaluated, 1 CR (previously untreated), 3 PR (2 previously untreated) and 6 SD were achieved[101]. In another Phase I study, three groups of chemotherapy-naive advanced pancreatic adenocarcinoma patients (n=17) received escalating weekly doses of matuzumab (400 mg, 800 mg weekly, or 800 mg biweekly) and gemcitabine. Severe treatment-related toxicities were limited to grade 3 neutropenia (n=3), leucopenia (n=1) and decreased white blood cell count (n=1). Common study drug-related adverse events were skin toxicities (6 grade 2 and 7 grade 1) and fever (grade 1). Matuzumab inhibited EGFR phosphorylation and affected receptor-dependent signaling and transduction in tumor biopsies from all dose groups. PR or SD were achieved in 8 of 12 evaluated patients (66.7%), with 3 PR among 6 evaluated patients in the group receiving 800 mg weekly[102]. Matuzumab is currently also in Phase II trials in NSCLC, gastric and colorectal cancer patients.

Zalutumumab (HuMax-EGFr, SF8, 2F8)

Zalutuzumab (Genmab A/S, Copenhagen, Denmark) is a

human IgG1 anti-EGFR mAb generated using Medarex's (Princeton, NJ, USA) transgenic mouse technology. The antibody blocks the binding of growth factors to tumor cells, inhibits phosphorylation of EGFR and cell proliferation with an approximate EC50 value of 1 μg/mL, causes tumor cell killing by ADCC, and directly slows the rate of tumor growth[103]. In animal studies, complete eradication of tumors was observed between 9 and 14 days after three injections of the antibody. When administered 1 day after tumor inoculation, zalutumumab was capable of completely preventing tumor formation at a dose of 50 μg; the antibody was also capable of eradicating well-established tumors in mice at a total dose as low as 125 μg. A recent mechanistic study showed that the antibody locks the EGFR into an inactive configuration, preventing the growth factor from binding and the subsequent receptor dimerization and activation.

Results from a Phase I/II trial in patients with head and neck cancers were reported at the 2005 ASCO annual meeting. In this trial, 27 patients who had previously failed standard therapies were given a single dose of zalutumumab, ranging from 0.15, 0.5, 1.0, 2.0, 4.0 to 8.0 mg/kg, and were followed for 4 weeks before receiving 4 further doses at weekly intervals. Twenty patients received all five infusions. None of the patients receiving doses of up to 8 mg/kg experienced dose-limiting toxicity and preliminary pharmacokinetic data suggested that saturation of the EGFR was provided by doses close to 2 mg/kg. Assessed by 18-fluoro-2-deoxyglucose positron emission tomography (FDG-PET), 7 of 18 patients that could be evaluated achieved partial metabolic response and 4 had stable metabolic disease 1 week after their last treatment. These results were achieved in the 4 higher dose groups, including 9 out of 11 patients in the 2 highest dose groups. Computed tomography (CT) scans showed that 2 of 19 patients that could be evaluated achieved PR and 9 patients had SD according to Response Evaluation Criteria in Solid Tumors (RECIST) criteria. The PR was maintained at week 12 in 1 of the 2 patients. In the 2 highest dose groups, 7 out of 10 patients obtained PR or SD. The most frequent adverse event was acneiform rashes in 56% of the patients; this event was antibody dose dependent with 10 of 11 patients in the 4 and 8 mg/kg dose groups reporting the rash. Other adverse events included rigors, fatigue, pyrexia, nausea, flushing and increased sweating. One patient reported a serious adverse event, a grade 2 pyrexia, which developed during the first infusion. The patient recovered and completed the study.

In September 2006, Genmab started a randomized pivotal Phase III trial in 273 SCCHN patients who were refractory to or intolerant of platinum chemotherapy. In this study, patients have been randomized into 2 treatment groups: zalutumumab in combination with BSC or BSC alone. Patients in the antibody group will be given an initial dose of 8 mg/kg of zalutumumab, followed by weekly infusions of a maintenance dose until disease progression. The maintenance dose will be adjusted as necessary until the patient develops a dose-limiting skin rash, up to a maximum dose of 16 mg/kg. Disease status will be assessed every 8 weeks by CT scan or magnetic resonance imaging (MRI) according to RECIST criteria until disease progression, and patients will be followed for survival. In addition, a Phase I/II trial of zalutumumab in combination with chemoradiation in SCCHN patients was initiated in October 2006.

mAb 806

mAb 806 (Ludwig Institute for Cancer Research, New York, NY, USA) is an antibody raised against a truncated form of EGFR, delta2-7 EGFR (the variant III or EGFRvIII)[104]. The binding epitope(s) of mAb 806 is not exposed on inactive wild-type EGFR, but is exposed on a transitional form of the receptor[105,106]. This is supported by IHC staining demonstrating that the antibody binds to a broad range of epithelial cancers and to gliomas, but not to normal human tissues expressing wild-type receptor in the absence of gene amplification[104,107]. The antibody has significant anticancer activity against human tumor xenografts expressing amplified EGFR (such as A431 tumor model) or mutant EGFRvIII (such as U87MG delta2-7 model) as a single agent[108,109], and was synergistic with small molecular EGFR inhibitors[110]. Clinical trials using a chimeric version of mAb 806 (ch-806), which has similar binding affinity to the unique EGFR epitope as the parent antibody, were initiated in late 2003, and preliminary results were reported at the ASCO annual meeting in June 2006. The trials confirmed the excellent targeting of ch-806 to cancers including squamous cell carcinomas of the lung, head and neck, and skin, and colorectal cancer, mesothelioma and glioma. Importantly, there was no evidence of localization of ch-806 to normal tissue. No significant toxicities were observed[111].

IMC-11F8

IMC-11F8 is a fully human IgG1 antibody derived from a Fab fragment originally isolated from an antibody phage display library (ImClone Systems). The antibody binds to EGFR with high affinity, blocks EGF-stimulated receptor activation, signal transduction and cell proliferation, and mediates efficient ADCC on tumor cells expressing the receptor[112]. The antibody also showed significant tumor inhibitory activity

in vivo in a variety of xenograft models as a single agent[113–115] and demonstrated additive or synergistic antitumor effect when combined with conventional therapeutic agents. A Phase I clinical trial in patients of various advanced solid malignancies has been completed. The trial had 6 cohorts, testing weekly or biweekly doses of 100, 200, 400, 600, 800, or 1000 mg. The most frequent adverse events were grade 1/2 skin rashes, nausea, vomiting, fatigue and headache. No infusion reactions were observed. Interim analysis of the first 30 patients demonstrated 2 PR (1 with mCRC and the other with metastatic melanoma) and 9 SD of between 14 and 57 weeks[116]. Phase II/III trials are expected to begin in the latter half of 2007.

Perspectives

A number of anti-EGFR antibodies, exemplified by cetuximab, acting either as a single agent or in combination with other cytotoxic regimens including chemotherapy and radiation, have demonstrated good safety profiles[117,118] and significant antitumor activity both in preclinical studies and in the clinic in patients with various malignancies[85,86]. Cetuximab has been approved by the FDA for use, both as a single agent and in combination with chemotherapy or radiation, in patients with mCRC and SCCHN, and panitumumab has been approved as a single agent in patients with refractory mCRC. Despite the overall clinical success, there are a number of biological and clinical questions associated with anti-EGFR therapy that still need to be addressed. Answers to these questions will, undoubtedly, greatly facilitate further clinical development of these antibodies in a more rational and efficient manner.

EGFR expression and patient selection Unlike the case of trastuzumab in the treatment of HER2-expressing breast cancer, where overexpression of HER2 in tumors is positively associated with the patients' response, based on all the clinical trials carried out to date it seems apparent that the levels of EGFR expression in tumors, either as the percentage of EGFR-positive tumor cells or as the staining intensity determined by standard IHC, do not correlate with tumor response to anti-EGFR therapy. For example, in the EMR-007 study, patients with tumors that stained faint, weak, moderate, or strong for EGFR expression demonstrated response rates to cetuximab treatment of 20.8%, 24. 7%, and 22.7%, respectively (P=0.64). The response rates were also similar among patients with tumors that had either ≤10% EGFR-positive cells or >35% EGFR-positive cells[71]. It is even more intriguing that in a recent study in which 16 refractory EGFR-negative (by IHC) mCRC patients were treated with either cetuximab alone (2 patients) or in combination with irinotecan (14 patients), 4 PR (including 1 patient received cetuximab alone) and 2 minor responses were achieved[119]. Furthermore, it has been shown that expression of EGFR in primary tumors may not necessarily correlate with receptor status in metastatic sites. In a retrospective study, primary tumors and related metastatic sites from 99 mCRC patients were examined for EGFR expression using IHC. EGFR expression was seen in the primary tumors in 53 (53%) patients, but the corresponding metastatic sites from 19 (36%) of these patients were found to be EGFR negative. In contrast, 7 patients (15%) showed positive EGFR staining in the metastatic sites, but not in the primary tumors[120]. These results suggest that screening of EGFR expression using current IHC methods in primary CRC tumors may not be adequate for patient selection for anti-EGFR-based therapy.

As an alternative approach for assessing EGFR status in tumors, a recent study examined the gene copy numbers of the receptor using fluorescence in-situ hybridization (FISH) in 31 patients (10 responders and 21 non-responders) treated with cetuximab or panitumumab. Eight of 9 patients with an objective response had increased EGFR gene copy numbers compared to 1 of 20 non-responders, suggesting that gene amplification of EGFR may serve as a better criterion for patient selection and as an indicator of patient response to therapy[121]. Another recent report examining the correlation between EGFR gene amplification and protein expression by IHC revealed that only a small percentage of EGFR-positive (by IHC) tumors also harbor gene amplification (>5 copies/ nucleus) – in 158 primary or metastatic CRC tumors studied, positive IHC staining was detected in 85% of primary and 79% of metastatic tumors, whereas gene amplification was only seen in 12% of primary and 8% of metastatic tumors[122]. Taken together, all these findings suggest that large perspective clinical trials are clearly needed to further delineate the true relevance of the levels of EGFR expression (or gene amplification) in tumors and their response to anti-EGFR therapy.

Biomarkers for efficacy In addition to EGFR expression, significant efforts are aiming to identify other biomarkers, or surrogate markers, for patient stratification and for predicting patients' response to anti-EGFR therapies. One obvious phenomenon that is positively associated with patients' response to anti-EGFR therapy is the development of a skin rash or other types of skin reactions. As noted, patients who experienced skin rash were more likely to respond to antibody treatment than those who did not, and the severity of the skin rash seems to correlate well with

patients' response and overall survival[71–74]. For example, in the EMR-007 study, patients with any degree of skin reaction to cetuximab therapy yielded response rates of 25.8% (the combination group) and 13% (the antibody alone group) compared with 6.3% and 0% in patients without any skin reactions, respectively. Furthermore, patients who developed grade 3/4 skin reactions also showed higher response rates than those with grade 1/2 reactions; 55.2% versus 20.4% (in the combination group) and 33.3% versus 11.6% (in the antibody alone group), respectively. The median survival time among patients with skin reactions and those without skin reactions were 9.1 and 3.0 months, respectively, in the combination group, and 8.1 and 2.5 months, respectively, in the antibody alone group. These observations suggest that skin rash may be used as a pharmacodynamic marker; that is, an indication of EGFR inhibition, for biological activity of anti-EGFR antibody therapy. In contrast, one should keep in mind that activity seen in normal skin is not likely to be an accurate indication of tumor inhibition because the downstream consequences of EGFR blockade are clearly different in the skin and the tumors[123]. Nevertheless, clinical studies are currently being conducted where the patients are given escalating doses of cetuximab until the development of a skin rash occurs, in the hope of further enhancing the biological activity of the antibody by achieving maximum receptor saturation/blockade.

On a molecular level, several preclinical and clinical studies have been carried out in an attempt to identify molecular markers as predictive indicators for the outcome of anti-EGFR therapy. One preclinical study examined the proteome profile of two CRC cell lines with high expression of EGFR, but a different response to cetuximab. Using two-dimensional electrophoresis and subsequent mass spectrometry, 14 proteins were identified that expressed differentially among the two cell lines, the responder Caco-2 and the non-responder HRT-18. While all the proteins identified are involved in the metabolic pathways and malignant growth, expression of certain proteins, such as fatty acid binding protein and heat shock protein 27, were implicated in the anti-apoptotic activity responsible for the non-responsiveness to cetuximab treatment by the HRT-18 cells[124]. In a retrospective clinical study, tumor specimens from 39 patients enrolled in the IMCL-0144 trial were examined for intratumoral mRNA levels of EGFR, VEGF, cyclin D1, cyclooxygenase 2 and interleukin 8, using real-time RT-PCR following laser-capture microdissection. High levels of VEGF were associated with resistance to cetuximab, whereas the combination of low levels of EGFR, cyclooxygenase 2 and interleukin 8 were significantly associated with longer overall survival[125]. Both findings were independent of skin rash, which in itself is correlated to survival. In several early clinical studies using EMD72000 or small molecule EGFR TKI it was noted that while there was complete inhibition of tumor phosphorylated EGFR and MAP kinases in treated patients, phosphorylated Akt and Ki67 (a cell proliferation marker) were only inhibited in patients who responded to the therapy[79,126,127]. Recently, K-ras mutation was found to be associated with rapid disease progression and significantly decreased TTP in patients treated with cetuximab. K-ras mutation was detected in 22 out of 59 chemotherapy refractory patients treated with cetuximab and, remarkably, none was detected in the 12 patients who had a clinical response to the antibody, suggesting that K-ras mutation may represent a highly predictive factor to cetuximab efficacy[125]. Taken together, these observations suggest that in addition to the target EGFR itself, careful and detailed analysis of downstream signaling pathways may yield molecular biomarkers that can be used as useful indicators for the prediction of the efficacy of anti-EGFR-based therapies.

EGFR mutation and efficacy of anti-EGFR therapy It has been shown that in a number of clinical trials in NSCLC patients treated with two small molecular TKI to EGFR, gefitinib and erlotinib, a subset of patients, including those with bronchioalveolar carcinoma, women, never-smokers and Asian patients, had higher response rates and better clinical outcomes. In addition, it was later discovered that NSCLC patients with somatic mutations in the EGFR kinase domain had a much better response to gefitinib and erlotinib[129–134,for review see 135]. Subsequent sequencing studies of a large panel of lung cancer specimens revealed that the overall rate of EGFR kinase mutations was approximately 19.6% (149 mutations out of 759 tumors studies), and the mutations were more frequent in tumors from women (37.5%) than men (13%), never-smokers (50.8%) than smokers (9%), adenocarcinomas (31.3%) than tumors of other histology (2.3%), and from patients of Asian origin (29.1%) than those of non-Asian origin (7.9%)[126–128,133]. Overall, the incidence of EGFR kinase mutations in patients from the United States is 9.5%. This observation is rather intriguing in that the subgroups of patients harboring higher EGFR kinase mutation rates represent the same population of patients who had a better response to gefitinib and erlotinib. Detailed analysis of the mutation sites (192 in total identified to date) demonstrated that the majority of the mutations occur in two hot spots – one (55.8%) is an in-frame deletion of 4 highly conserved amino acids (LREA) encoded by exon 19 and the other (44.2%) is a point mutation in exon 21 that lead to an amino acid substitution at position 858 (L858R)[129–131,136,137].

Amid all these findings, perspective clinical trials to correlate these mutations with actual patient responses to either gefitinib or erlotinib are, however, yet to be conducted. In contrast, it is very encouraging to note that two recent preclinical studies showed that cetuximab was very effective in inhibiting both *in vitro* and *in vivo* growth of tumor cells expressing either wild-type EGFR or mutant EGFR (HCC-827 cell line with delE746-A750 mutation, and NCI-1940 cell line with L858R and T790M mutations)[138,139]. Furthermore, cetuximab also demonstrated good inhibitory activity to tumor cells that expressed the EGFRvIII variant[140]. Recently, Wong *et al* reported that transgenic mice with inducible expression of either L858R or LREA deletion mutant in type II pneumocytes developed lung adenocarcinoma after sustained EGFR mutant expression, which is also essential for tumor maintenance. Treatment with small molecular TKI (erlotinib) or cetuximab led to dramatic tumor regression in these mice[141]. Taken together, these observations strongly suggest that cetuximab may represent an effective therapeutic agent against both wild-type and/or mutant EGFR-expressing tumors.

In contrast to NSCLC patients, earlier studies failed to positively identify any EGFR TK domain mutations in other tumors[136,142,143]. Two recent reports have, however, revealed that EGFR kinase mutations may also be present in other human malignancies, including CRC and SCCHN. In a study by Nagahara *et al*[144], although none of the 11 CRC cell lines examined exhibited somatic mutations, 4 of 33 clinical tumors (12%) exhibited mutations in the EGFR kinase domain. Similarly, Lee *et al*[145] observed 3 mutations (7.3%), all the same in-frame deletion mutation in exon 19 (del746-750), in 41 SCCHN patients analyzed. In another recent report, EGFR kinase domain in tumor specimens from 38 NSCLC and 39 mCRC patients participating in two separate cetuximab monotherapy studies were sequenced. Three mutations were identified in the 38 NSCLC patients – two del746-750 mutations in 13 patients experiencing SD and one L861Q mutation in 21 patients with progressive disease. No mutations were found in the one patient who achieved a PR and 3 patients whose response data were unavailable. In the 39 mCRC patients, including 20 experiencing PR and one CR, no mutations were identified[146]. Further sequencing analysis of 160 biopsy samples of previously untreated CRC tumors from patients outside of cetuximab trials did not reveal any mutations in exons 18, 19 and 21 in the EGFR kinase domain. Taken together, these results suggest that, in contrast to the case of small molecular EGFR TKI, the presence of EGFR kinase mutations may not represent a major predictive and prognostic factor for the efficacy of cetuximab therapy in CRC patients.

Cetuximab in combination with other targeted agents

One of the hallmarks of effective cancer treatment is the use of combinational therapeutic regimens comprising several cytotoxic or cytostatic agents that target cancer cells via different mechanisms. To this end, cetuximab has demonstrated significant enhanced antitumor activity in combination with either chemotherapeutics or radiation in the clinic; for example, with irinotecan in mCRC and with radiation in SCCHN. The side-effects of these combination therapies are usually associated with the cytotoxic components in the regimens. Based on these observations, it is plausible that a combination of anti-EGFR antibodies with other targeted therapeutic agents, including small molecular TKI and mAb directed against different tumor-associated targets, may yield enhanced therapeutic activity without adding severe unwanted systemic toxicities. A number of preclinical studies have shown that combination of cetuximab with a small molecular TKI, gefitinib or erlotinib, resulted in enhanced tumor growth inhibition both *in vitro* and *in vivo* of a number of different tumor cell lines[147,148]. There was, however, at least one study that showed that the combination of cetuximab and gefitinib was rather antagonistic[149]. Taken together, these observations suggest that the concept of combining an anti-EGFR antibody with a small molecular TKI for double-hitting the same target in tumor cells, while encouraging, needs more preclinical validation and should proceed with caution in clinical trials. Similarly, additive or synergistic antitumor activity has also been observed when cetuximab was used in combination with antisense oligonucleotides targeting other molecules, such as protein A kinase and VEGF[150,151].

Cetuximab has also demonstrated additive or synergistic antitumor activities in various xenograft models when used in combination with mAb targeting other growth factor receptors, including those directed against HER2[152], VEGF receptor 2[153, 154] and insulin-like growth factor receptor[115]. In a recent Phase II clinical trial (NCI-6444), patients with mCRC refractory to irinotecan were given both cetuximab and bevacizumab, with or without irinotecan. Of 41 patients who received cetuximab/bevacizumab plus irinotecan, 37% had a PR and the median TTP was 7.9 months. Of 40 patients who received cetuximab/beva-cizumab alone, 20% had a PR and the median TTP was 5.6 months. The most commonly reported adverse events in the cetuximab/bevacizumab/irinotecan arm were skin rash (grade 2, 60%; grade 3, 17%), diarrhea (grade 2, 29%; grade 3/4, 24%), fatigue (grade 2, 32%; grade 3, 10%) and neutropenia (grade 3/4, 22%), and the most commonly reported adverse event in the cetuximab/

bevacizumab alone arm was skin rash (grade 2, 65%; grade 3, 20%)[155]. As a historical control, in the EMR-007 trial, patients who received cetuximab alone or cetuximab plus irinotecan had PR rates of 10.8% and 22.9%, respectively[71]. Furthermore, it is also very intriguing to note that bevacizumab has failed to demonstrate significant clinical benefits in refractory mCRC patients who have exhausted standard chemotherapeutic options, including both irinotecan-based and oxaliplatin-based regimens. In a single-arm, multi-center trial, 350 refractory patients were treated with bevacizumab plus bolus or infusional fluorouracil (FU) and leucovorin (LV). Of the first 100 patients evaluated, PR was confirmed in only one patient and medium PFS was 3.5 months[156]. Based on these results, a randomized Phase III trial is being conducted by CALGB/NCI in which chemotherapy naïve patients (about 2300 patients) are randomized into three groups to receive cetuximab plus chemotherapy (FOLFOX or FOLFIRI depending on the choice of the individual investigators), bevacizumab plus chemotherapy or cetuximab and bevacizumab plus chemotherapy in first-line settings.

References

1 Ullrich A, Schlessinger J. Signal transduction by receptors with tyrosine kinase activity. Cell 1990; 61: 203–12.
2 Schlessinger J. Cell signaling by receptor tyrosine kinases. Cell 2000; 103: 211–25.
3 Noonberg SB, Benz CC. Tyrosine kinase inhibitors targeted to the epidermal growth factor receptor subfamily: role as anticancer agents. Drugs 2000; 59: 753–67.
4 Krause DS, Van Etten RA. Tyrosine kinases as targets for cancer therapy. N Engl J Med 2005; 353: 172–87.
5 Bianco R, Daniele G, Ciardiello F, Tortora G. Monoclonal antibodies targeting the epidermal growth factor receptor. Curr Drug Targets 2005; 6: 275–87.
6 Tibes R, Trent J, Kurzrock R. Tyrosine kinase inhibitors and the dawn of molecular cancer therapeutics. Annu Rev Pharmacol Toxicol 2005; 45; 357–84.
7 Starling N, Cunningham D. Monoclonal antibodies against vascular endothelial growth factor and epidermal growth factor receptor in advanced colorectal cancers: present and future directions. Curr Opin Oncol 2004; 16: 385–90.
8 Albanell J, Gascon P. Small molecules with EGFR-TK inhibitor activity. Curr Drug Targets 2005; 6: 259–74.
9 Paz K, Zhu Z. Development of angiogenesis inhibitors to vascular endothelial growth factor receptor 2. Current status and future perspective. Front Biosci 2005; 10: 1415–39.
10 Ullrich A, Coussens L, Hayflick JS, Dull TJ, Gray A, Tam AW, *et al*. Human epidermal growth factor receptor cDNA sequence and aberrant expression of the amplified gene in A431 epidermoid carcinoma cells. Nature 1984; 309: 418–25.
11 Wells A. EGF receptor. Int J Biochem Cell Biol 1999; 31: 637–43.
12 Carpenter G. The EGF receptor: A nexus for trafficking and signaling. Bioessays 2000; 22: 697–707.
13 Yarden Y. The EGFR family and its ligands in human cancer signalling mechanisms and therapeutic opportunities. Eur J Cancer 2001; 37: S3–S8.
14 Normanno N, Bianco C, Strizzi L, Mancino M, Maiello MR, De Luca A, *et al*. The ErbB receptors and their ligands in cancer: an overview. Curr Drug Targets 2005; 6: 243–57.
15 Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. Nat Rev Mol Cell Biol 2001; 2: 127–37.
16 Grant S, Qiao L, Dent P. Roles of ERBB family receptor tyrosine kinases, and downstream signaling pathways, in the control of cell growth and survival. Front Biosci 2002; 7: d376–89.
17 Gullick WJ. Type I growth factor receptors: current status and future work. Biochem Soc Symp 1998; 63: 193–8.
18 Downward J, Yarden Y, Mayes E, Scrace G, Totty N, Stockwell P, *et al*. Close similarity of epidermal growth factor receptor and v-erb-B oncogene protein sequences. Nature 1984; 307: 521–7.
19 Di Fiore PP, Pierce JH, Fleming TP, Hazan R, Ullrich A, King CR, *et al*. Overexpression of the human EGF receptor confers an EGF-dependent transformed phenotype to NIH 3T3 cells. Cell 1987; 51: 1063–70.
20 Salomon DS, Brandt R, Ciardiello F, Normanno N. Epidermal growth factor-related peptides and their receptors in human malignancies. Crit Rev Oncol Hematol 1995; 19: 183–232.
21 Nicholson RI, Gee JM, Harper ME. EGFR and cancer prognosis. Eur J Cancer 2001; 37: S9–15.
22 Nishikawa R, Ji XD, Harmon RC, Lazar CS, Gill GN, Cavenee WK, *et al*. A mutant epidermal growth factor receptor common in human glioma confers enhanced tumorigenicity. Proc Natl Acad Sci USA 1994; 91: 7727–31.
23 Pedersen MW, Meltorn M, Damstrup L, Poulsen HS. The type III epidermal growth factor receptor mutation. Biological significance and potential target for anti-cancer therapy. Ann Oncol 2001; 12: 745–60.
24 Arteaga C. Targeting HER1/EGFR: a molecular approach to cancer therapy. Semin Oncol Suppl 2003; 7: 3–14.
25 Morrison SL, Johnson MJ, Herzenberg LA, Oi VT. Chimeric human antibody molecules: mouse antigen-binding domains with human constant region domains. Proc Natl Acad Sci USA 1984; 81: 6851–5.
26 Jones PT, Dear PH, Foote J, Neuberger MS, Winter G. Replacing the complementarity-determining regions in a human antibody with those from a mouse. Nature 1986; 321: 522–55.
27 Winter G, Griffiths, AD, Hawkins RE, Hoogenboom HR. Making antibodies by phage display technology. Annu Rev Immunol 1994; 12: 433–55.
28 Lonberg N, Huszar D. Human antibodies from transgenic mice. Int Rev Immunol 1995; 13: 65–93.
29 Chadd HE, Chamow SM. Therapeutic antibody expression technology. Curr Opin Biotechnol 2001; 12: 188–94.
30 Ferrara N Hillan KJ, Gerber HP, Novotny W. Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer. Nat Rev Drug Discov 2004; 3: 391–400.
31 Li S, Schmitz KR Jeffrey PD, Wiltzius JJ, Kussie P, Ferguson KM. Structural basis for inhibition of the epidermal growth factor receptor by cetuximab. Cancer Cell 2005; 7: 301–11.
32 Slamon DJ, Leyland-Jones B, Shak S. Use of chemotherapy plus

a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 2001; 344: 783–92.
33 Lewis GD, Figari I, Fendly B. Differential responses of human tumor cell lines to anti-p185HER2 monoclonal antibodies. Cancer Immunol Immunother 1993; 37: 255–63.
34 Reff ME, Carner K, Chambers KS. Depletion of B cells *in vivo* by a chimeric mouse human monoclonal antibody to CD20. Blood 1994; 83: 435–45.
35 Clynes RA, Towers TL, Presta LG. Inhibitory Fc receptors modulate *in vivo* cytoxicity against tumor targets. Nat Med 2000; 6: 443–6.
36 Zhang W, Gordon M, Schultheis AM. Two immunoglobulin G fragment C receptor polymorphisms associated with clinical outcome of EGFR-expressing metastatic colorectal cancer patients treated with single agent cetuximab. Proceedings of the 42nd Annual Meeting of the American Society for Clinical Oncology, Atlanta, USA, 2006; abstract 3028.
37 Sievers EL, Linenberger M. Mylotarg: antibody-targeted chemotherapy comes of age. Curr Opin Oncol 2001; 13: 522–7.
38 Krasner C, Joyce RM. Zevalin: 90yttrium labeled anti-CD20 (ibritumomab tiuxetan), a new treatment for non-Hodgkin's lymphoma. Curr Pharm Biotechnol 2001; 2: 341–9.
39 Cheson B. Bexxar (Corixa/GlaxoSmithKline). Curr Opin Invest Drugs 2002; 3: 165–70.
40 Defreitas E, Suzuki H, Herlyn D. Human antibody induction to the idiotypic and anti-idiotypic determinants of a monoclonal antibody against a gastrointestinal carcinoma antigen. Curr Top Microbiol Immunol 1985; 119: 75–89.
41 French RR, Chan HT, Tutt AL, Glennie MJ. CD40 antibody evokes a cytotoxic T-cell response that eradicates lymphoma and bypasses T-cell help. Nat Med 1999; 5: 548–53.
42 Hurwitz AA, Foster BA, Kwon ED. Combination immunotherapy of primary prostate cancer in a transgenic mouse model using CTLA-4 blockade. Cancer Res 2000; 60: 2444–8.
43 Williams B, Zhu Z. Intrabody-based approaches to cancer therapy: status and prospects. Curr Med Chem 2006; 13: 1473–80.
44 Paz K, Brennan LA, Iacolina M, Doody J, Hadari YR, Zhu Z. Human single-domain neutralizing intrabodies directed against Etk kinase: a novel approach to impair cellular transformation. Mol Cancer Ther 2005; 4: 1801–9.
45 Sato JD, Kawamoto T, Le AD, Mendelsohn J, Polikoff J, Sato GH. Biological effects *in vitro* of monoclonal antibodies to human epidermal growth factor receptors. Mol Biol Med 1983; 1: 511–29.
46 Goldstein NI, Prewett M, Zuklys K, Rockwell P, Mendelsohn J. Biological efficacy of a chimeric antibody to the epidermal growth factor receptor in a human tumor xenograft model. Clin Cancer Res 1995; 1: 1311–8.
47 Kurai J, Chikumi H, Hashimoto K, Yamaguchi K, Yamasaki A, Sako T, *et al*. Antibody-dependent cellular cytotoxicity mediated by cetuximab against lung cancer cell lines. Clin Cancer Res 2007; 13: 1552–61.
48 Kawaguchi Y, Kono K, Mimura K, Sugai H, Akaike H, Fujii H. Cetuximab induce antibody-dependent cellular cytotoxicity against EGFR-expressing esophageal squamous cell carcinoma. Int J Cancer 2007; 120: 781–7.
49 Waskal HW. Role of an anti-epidermal growth factor receptor in treating cancer. Cancer Metastasis Rev 1999; 18: 427–36.
50 Dent P, Reardon DB, Park JS, Bowers G, Logsdon C, Valerie K, *et al*. Radiation-induced release of transforming growth factor α activates the epidermal growth factor receptor and mitogen-activated protein kinase pathway in carcinoma cells, leading to increased proliferation and protection from radiation-induced cell death. Mol Biol Cell 1999; 10: 2493–506.
51 Dent P, Yacoub A, Contessa J, Caron R, Amorino G, Valerie K, *et al*. Stress and radiation-induced activation of multiple intracellular signaling pathways. Radiat Res 2003; 159: 283–300.
52 Kari C, Chan TO, Rocha de Quadros M, Rodeck, U. Targeting the epidermal growth factor receptor in cancer: apoptosis takes center stage. Cancer Res 2003; 63: 1–5.
53 Talapatra S, Thompson CB. Growth factor signaling in cell survival: implications for cancer treatment. J Pharmacol Exp Ther 2001; 298: 873–8.
54 Nicholson S, Sainsbury JR, Halcrow P, Chambers P, Farndon JR, Harris AL. Expression of epidermal growth factor receptors associated with lack of response to endocrine therapy in recurrent breast cancer. Lancet 1989; 1: 182–5.
55 Volm M, Efferth T, Mattern J. Oncoprotein (c-myc, c-erbB1, c-erbB2, c-fos) and suppressor gene product (p53) expression in squamous cell carcinomas of the lung. Clinical and biological correlations. Anticancer Res 1992; 12: 11–20.
56 Sheridan MT, O'Dwyer T, Seymour CB, Mothersill CE. Potential indicators of radiosensitivity in squamous cell carcinoma of the head and neck. Radiat Oncol Invest 1997; 5: 180–6.
57 Baselga J, Norton L, Masui H, Pandiella A, Coplan K, Miller WH Jr, *et al*. Antitumor effects of doxorubicin in combination with anti-epidermal growth factor receptor monoclonal antibodies. J Natl Cancer Inst 1993; 85: 1327–33.
58 Fan Z, Baselga J, Masui H, Mendelsohn, J. Antitumor effect of anti-epidermal growth factor receptor monoclonal antibodies plus *cis*-diamminedichloroplatinum on well established A431 cell xenografts. Cancer Res 1993; 53: 4637–42.
59 Aboud-Pirak E, Hurwitz E, Pirak ME, Bellot F, Schlessinger J, Sela M. Efficacy of antibodies to epidermal growth factor receptor against KB carcinoma *in vitro* and in nude mice. J Natl Cancer Inst 1988; 80: 1605–11.
60 Prewett M, Rockwell P, Rose C, Goldstein N. Antitumor and cell cycle responses in KB cells treated with a chimeric anti-EGFR monoclonal antibody in combination with cisplatin. Int J Oncol 1996; 9: 217–24.
61 Prewett M, Rockwell P, Rose C, Zuklys K, Goldstein N. Altered cell cycle distribution and cyclin-CDK protein expression in A431 epidermoid carcinoma cells treated with doxorubicin and a chimeric monoclonal antibody to the epidermal growth factor receptor. Mol Cell Differ 1996; 4: 167–86.
62 Ciardiello F, Bianco R, Damiano V, De Lorenzo S, Pepe S, De Placido S, *et al*. Antitumor activity of sequential treatment with topotecan and anti-epidermal growth factor receptor monoclonal antibody C225. Clin Cancer Res 1999; 5: 909–16.
63 Prewett MC, Hooper AT, Bassi R, Ellis LM, Waksal HW, Hicklin J. Enhanced antitumor activity of anti-epidermal growth factor receptor monoclonal antibody IMC-C225 in combination with irinotecan (CPT-11) against human colorectal tumor xenografts. Clin Cancer Res 2002; 8: 994–1003.

64 Huang SM, Bock JM, Harari PM. Epidermal growth factor receptor blockade with C225 modulates proliferation, apoptosis, and radiosensitivity in squamous cell carcinomas of the head and neck. Cancer Res 1999; 59: 1935–40.
65 Saleh MN, Raisch KP, Stackhouse MA, Grizzle WE, Bonner JA, Mayo MS, *et al*. Combined modality therapy of A431 human epidermoid cancer using anti-EGFr antibody C225 and radiation. Cancer Biother Radiopharm 1999; 14: 451–63.
66 Milas L, Mason K, Hunter N, Petersen S, Yamakawa M, Ang K, *et al*. *In vivo* enhancement of tumor radioresponse by C225 antiepidermal growth factor receptor antibody. Clin Cancer Res 2000; 6: 701–8.
67 Raben D, Helfrich B, Chan DC, Ciardiello F, Zhao L, Franklin W, *et al*. The effects of cetuximab alone and in combination with radiation and/or chemotherapy in lung cancer. Clin Cancer Res 2005; 11: 795–805.
68 Mendelsohn J. Epidermal growth factor receptor inhibition by a monoclonal antibody as anticancer therapy. Clin Cancer Res 1997; 3: 2703–7.
69 Mendelsohn J. Jeremiah Metzger Lecture. Targeted cancer therapy. Trans Am Clin Climatol Assoc 2000; 111: 95–110.
70 Sunada H, Magun BE, Mendelsohn J, MacLeod CL. Monoclonal antibody against epidermal growth factor receptor is internalized without stimulating receptor phosphorylation. Proc Natl Acad Sci USA 1986; 83: 3825–9.
71 Cunningham D, Humblet Y, Siena S, Khayat D, Bleiberg H, Santoro A, *et al*. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med 2004; 351: 337–45.
72 Saltz L, Rubin M, Hochster H, Tchekmeydian NS, Waskal H, Needle M, *et al*. Cetuximab (IMC-C225) plus irinotecan (CPT-11) is active in CPT-11 refractory colorectal cancer (CRC) that expresses epidermal growth factor receptor (EGFR). Proc Am Soc Clin Oncol 2001; 20, abstract 7.
73 Saltz LB, Meropol NJ, Loehrer PJ Sr, Needle MN, Kopit J, Mayer RJ. Phase II trial of cetuximab in patients with refractory colorectal cancer that expresses the epidermal growth factor receptor. J Clin Oncol 2004; 22: 1201–8.
74 Lenz HJ, Mayer RJ, Gold PJ, Mirtsching B, Stella PJ, Cohn AL, *et al*. Activity of cetuximab in patients with colorectal cancer refractory to both irinotecan and oxaliplatin. Proc Am Soc Clin Oncol 2004; 23, abstract 3510.
75 Lenz HJ, Mayer RJ, Mirtsching B, Cohn AL, Pippas A, Windt P, *et al*. Consistent response to treatment with cetuximab monotherapy in patients with metastatic colorectal cancer. Proc Am Soc Clin Oncol 2005; 24, abstract 3536.
76 Rubio ED, Tabernero J, van Cutsem E, Cervantes A, André T, Humblet Y, *et al*. Cetuximab in combination with oxaliplatin/5-fluorouracil (5-FU)/folinic acid (FA) (FOLFOX-4) in the first-line treatment of patients with epidermal growth factor receptor (EGFR)-expressing metastatic colorectal cancer: An international phase II study. Proc Am Soc Clin Oncol 2005; 24, abstract 3535.
77 Bonner JA, Harari PM, Giralt J, Azarnia N, Shin DM, Cohen RB, *et al*. Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. N Engl J Med 2006; 354: 567–78.
78 Burtness B, Goldwasser MA, Flood W, Mattar B, Forastiere AA, Eastern Cooperative Oncology Group. Phase III randomized trial of cisplatin plus placebo compared with cisplatin plus cetuximab in metastatic/recurrent head and neck cancer: an Eastern Cooperative Oncology Group study. J Clin Oncol 2005; 23: 8646–54.
79 Baselga J, Trigo JM, Bourhis J, Tortochaux J, Cortes-Funes H, Hitt R, *et al*. Phase II multicenter study of the antiepidermal growth factor receptor monoclonal antibody cetuximab in combination with platinum-based chemotherapy in patients with platinum-refractory metastatic and/or recurrent squamous cell carcinoma of the head and neck. J Clin Oncol 2005; 23: 5568–77.
80 Herbst RS, Arquette M, Shin DM, Dicke K, Vokes EE, Azarnia N, *et al*. Epidermal growth factor receptor antibody cetuximab and cisplatin for recurrent and refractory squamous cell carcinoma of the head and neck: a phase II, multicenter study. J Clin Oncol 2005; 23: 5578–87.
81 Mochlinski K, Cunningham D, Sharma B, Uzzell M. Metastatic pancreatic adenocarcinoma in complete remission after three and a half years of combination therapy with cetuximab. Proc Am Soc Clin Oncol 2005; 24, abstract 4278.
82 Rosell R, Daniel C, Ramlau R, Szczesna A, Constenla M, Mennecier B, *et al*. Randomized phase II study of cetuximab in combination with cisplatin (C) and vinorelbine (V) *vs* CV alone in the first-line treatment of patients (pts) with epidermal growth factor receptor (EGFR)-expressing advanced non-small cell lung cancer (NSCLC). Proc Am Soc Clin Oncol 2004; 23, abstract 7012.
83 Lilenbaum R, Bonomi P, Ansari R, Lynch T, Govindan R, Janne P, *et al*. A phase II trial of cetuximab as therapy for recurrent non-small cell lung cancer (NSCLC): final results. Proc Am Soc Clin Oncol 2005; 24, abstract 7036.
84 Aghajanian C, Sabbatini P, Derosa F, Gerst S, Spriggs DR, Dupont J, *et al*. A phase II study of cetuximab/paclitaxel/carboplatin for the initial treatment of advanced stage ovarian, primary peritoneal, and Fallopian tube cancer. Proc Am Soc Clin Oncol 2005; 24, abstract 5047.
85 Kim ES, Vokes EE, Kies MS. Cetuximab in cancers of the lung and head and neck. Semin Oncol Suppl 2004; 1: 61–7.
86 Ng M, Cunningham D. Cetuximab (Erbitux) — an emerging targeted therapy for epidermal growth factor receptor-expressing tumours. Int J Clin Pract 2004; 58: 970–6.
87 Chua YJ, Cunningham D. Panitumumab. Drugs Today (Barc) 2006; 42: 711–9.
88 Yang XD, Jia XC, Corvalan JR, Wang P, Davis CG. Development of ABX-EGF, a fully human anti-EGF receptor monoclonal antibody, for cancer therapy. Crit Rev Oncol Hematol 2001; 38: 17–23.
89 Yang XD, Jia XC, Corvalan JR, Wang P, Davis CG, Jakobovits A. Eradication of established tumors by a fully human monoclonal antibody to the epidermal growth factor receptor without concomitant chemotherapy. Cancer Res 1999; 59: 1236–43.
90 Rowinsky EK, Schwartz GH, Gollob JA, Thompson JA, Vogelzang NJ, Figlin R, *et al*. Safety, pharmacokinetics, and activity of ABX-EGF, a fully human anti-epidermal growth factor receptor monoclonal antibody in patients with metastatic renal cell cancer. J Clin Oncol 2004; 22: 3003–15.
91 Tyagi P, Jain V, Chu E, Jain V. Recent results and ongoing trials

with panitumumab (ABX-EGF), a fully human anti-epidermal growth factor receptor antibody, in metastatic colorectal cancer. Clin Colorectal Cancer 2005; 5: 21–3.

92 Gibson TB, Ranganathan A, Grothey A. Randomized phase III trial results of panitumumab, a fully human anti-epidermal growth factor receptor monoclonal antibody, in metastatic colorectal cancer. Clin Colorectal Cancer 2006; 6: 29–31.

93 Fernandez A, Spitzer E, Perez R, Boehmer FD, Eckert K, Zschiesche W, *et al*. A new monoclonal antibody for detection of EGF-receptors in Western blots and paraffin-embedded tissue sections. J Cell Biochem 1992; 49: 157–65.

94 Mateo C, Moreno E, Amour K, Lombardero J, Harris W, Perez R. Humanization of a mouse monoclonal antibody that blocks the epidermal growth factor receptor: recovery of antagonistic activity. Immunotechnology 1997; 3: 71–81.

95 Crombet-Ramos T, Rak J, Perez R, Viloria-Petit A. Antiproliferative, antiangiogenic and proapoptotic activity of h-R3: A humanized anti-EGFR antibody. Int J Cancer 2002;101: 567–75.

96 Ramos TC, Figueredo J, Catala M, Gonzalez S, Selva JC, Cruz TM, *et al*. Treatment of high-grade glioma patients with the humanized anti-epidermal growth factor receptor (EGFR) antibody h-R3: report from a phase I/II trial. Cancer Biol Ther 2006; 5: 375–9.

97 Crombet T, Osorio M, Cruz T, Roca C, del Castillo R, Mon R, *et al*. Use of the humanized anti-epidermal growth factor receptor monoclonal antibody h-R3 in combination with radiotherapy in the treatment of locally advanced head and neck cancer patients. J Clin Oncol 2004; 22: 1646–54.

98 Kim T. Technology evaluation: Matuzumab, Merck KGaA. Curr Opin Mol Ther 2004; 6: 96–103.

99 Vanhoefer U, Tewes M, Rojo F, Dirsch O, Schleucher N, Rosen O, *et al*. Phase I study of the humanized antiepidermal growth factor receptor monoclonal antibody EMD72000 in patients with advanced solid tumors that express the epidermal growth factor receptor. J Clin Oncol 2004; 22**:** 175–84.

100 Seiden MV, Burris HA, Matulonis U, Hall JB, Armstrong DK, Speyer J, *et al*. A phase II trial of EMD72000 (matuzumab), a humanized anti-EGFR monoclonal antibody, in patients with platinum-resistant ovarian and primary peritoneal malignancies. Gynecol Oncol 2007; 104: 727–31.

101 Kollmannsberger C, Schittenhelm M, Honecker F, Tillner J, Weber D, Oechsle K, *et al*. A phase I study of the humanized monoclonal anti-epidermal growth factor receptor (EGFR) antibody EMD 72000 (matuzumab) in combination with paclitaxel in patients with EGFR-positive advanced non-small-cell lung cancer (NSCLC). Ann Oncol 2006; 17: 1007–13.

102 Graeven U, Kremer B, Sudhoff T, Killing B, Rojo F, Weber D, *et al*. Phase I study of the humanised anti-EGFR monoclonal antibody matuzumab (EMD 72000) combined with gemcitabine in advanced pancreatic cancer. Br J Cancer 2006; 94: 1293–9.

103 Bleeker WK, Lammerts van Bueren JJ, van Ojik HH, Gerritsen AF, Pluyter M, Houtkamp M, *et al*. Dual mode of action of a human anti-epidermal growth factor receptor monoclonal antibody for cancer therapy. J Immunol 2004; 173: 4699–707.

104 Johns TG, Stockert E, Ritter G, Jungbluth AA, Huang HJ, Cavenee WK, *et al*. Novel monoclonal antibody specific for the de2-7 epidermal growth factor receptor (EGFR) that also recognizes the EGFR expressed in cells containing amplification of the EGFR gene. Int J Cancer 2002; 98: 398–408.

105 Johns TG, Adams TE, Cochran JR, Hall NE, Hoyne PA, Olsen MJ, *et al*. Identification of the epitope for the epidermal growth factor receptor-specific monoclonal antibody 806 reveals that it preferentially recognizes an untethered form of the receptor. J Biol Chem 2004; 279: 30375–84.

106 Sivasubramanian A, Chao G, Pressler HM, Wittrup KD, Gray JJ. Structural model of the mAb 806-EGFR complex using computational docking followed by computational and experimental mutagenesis. Structure 2006; 14: 401–14.

107 Jungbluth AA, Stockert E, Huang HJ, Collins VP, Coplan K, Iversen K, *et al*. A monoclonal antibody recognizing human cancers with amplification/overexpression of the human epidermal growth factor receptor. Proc Natl Acad Sci USA 2003; 100: 639–44.

108 Mishima K, Johns TG, Luwor RB, Scott AM, Stockert E, Jungbluth AA, *et al*. Growth suppression of intracranial xenografted glioblastomas overexpressing mutant epidermal growth factor receptors by systemic administration of monoclonal antibody (mAb) 806, a novel monoclonal antibody directed to the receptor. Cancer Res 2001; 61: 5349–54.

109 Luwor RB, Johns TG, Murone C, Huang HJ, Cavenee WK, Ritter G, *et al*. Monoclonal antibody 806 inhibits the growth of tumor xenografts expressing either the de2-7 or amplified epidermal growth factor receptor (EGFR) but not wild-type EGFR. Cancer Res 2001; 61: 5355–61.

110 Johns TG, Luwor RB, Murone C, Walker F, Weinstock J, Vitali AA, *et al*. Antitumor efficacy of cytotoxic drugs and the monoclonal antibody 806 is enhanced by the EGF receptor inhibitor AG1478. Proc Natl Acad Sci USA 2003; 100: 15871–6.

111 Scott AM, Lee FT, Tebbutt N, Herbertson R, Gill SS, Liu Z, *et al*. A phase I clinical trial with monoclonal antibody ch806 targeting transitional state and mutant epidermal growth factor receptors. Proc Natl Acad Sci USA 2007; 104: 4071–6.

112 Liu M, Zhang H, Jimenez X, Ludwig D, Witte L, Bohlen P, *et al*. Identification and characterization of a fully human antibody directedagainst epidermal growth factor receptor. Proc Am Assoc Cancer Res 2004; 45, abstract 706.

113 Prewett M, Tonra J, Bassi R, Hooper A, Makhoul G, Finnerty B, *et al*. Antitumor activity of a novel, human anti-epidermal growth factor receptor (EGFR) monoclonal antibody (IMC-11F8) in human tumor xenograft models. Proc Am Assoc Cancer Res 2004; 45, abstract 5353.

114 Lu D, Zhang H, Ludwig D, Persaud A, Jimenez X, Burtrum D, *et al*. Simultaneous blockade of both the epidermal growth factor receptor and the insulin-like growth factor receptor signaling pathways in cancer cells with a fully human recombinant bispecific antibody. J Biol Chem 2004; 279: 2856–65.

115 Lu D, Zhang H, Koo H, Tonra J, Balderes P, Prewett M, *et al*. A fully human recombinant IgG-like bispecific antibody to both the epidermal growth factor receptor and the insulin-like growth factor receptor for enhanced antitumor activity. J Biol Chem 2005; 280: 19665–72.

116 Kuenen B. A phase I study of IMC-11F8, a fully human anti-epidermal growth factor receptor (EGFR) IgG1 monoclonal antibody in patients with solid tumors. Interim results. Proc Am Soc Clin Oncol 2006; 25, abstract 3024.

117 Needle MN. Safety experience with IMC-C225, an anti-epider-

mal growth factor receptor antibody. Semin Oncol Suppl 2002; 14: 55–60.

118 Thomas M. Cetuximab: adverse event profile and recommendations for toxicity management. Clin J Oncol Nurs 2005; 9: 332–8.

119 Chung KY, Shia J, Kemeny NE, Shah M, Schwartz GK, Tse A, *et al*. Cetuximab shows activity in colorectal cancer patients with tumors that do not express the epidermal growth factor receptor by immunohistochemistry. J Clin Oncol 2005; 23: 1803–10.

120 Scartozzi M, Bearzi I, Berardi R, Mandolesi A, Fabris G, Cascinu S. Epidermal growth factor receptor (EGFR) status in primary colorectal tumors does not correlate with EGFR expression in related metastatic sites: implications for treatment with EGFR-targeted monoclonal antibodies. J Clin Oncol 2004; 22: 4772–8.

121 Moroni M, Veronese S, Benvenuti S, Marrapese G, Sartore-Bianchi A, Di Nicolantonio F, *et al*. Gene copy number for epidermal growth factor receptor (EGFR) and clinical response to anti-EGFR treatment in colorectal cancer: a cohort study. Lancet Oncol 2005; 6: 279–86.

122 Shia J, Klimstra DS, Li AR, Qin J, Saltz L, Teruya-Feldstein J, *et al*. Epidermal growth factor receptor expression and gene amplification in colorectal carcinoma: an immunohistochemical and chromogenic *in situ* hybridization study. Mod Pathol 2005; 18: 1350–6.

123 Perez-Soler R. Can rash associated with HER1/EGFR inhibition be used as a marker of treatment outcome? Oncology Suppl 2003; 12: 23–8.

124 Skvortsov S, Sarg B, Loeffler-Ragg J, Skvortsova I, Lindner H, Werner-Ott H, *et al*. Different proteome pattern of epidermal growth factor receptor-positive colorectal cancer cell lines that are responsive and nonresponsive to C225 antibody treatment. Mol Cancer Ther 2004; 3: 1551–8.

125 Vallbohmer D, Zhang W, Gordon M, Yang DY, Yun J, Press OA, *et al*. Molecular determinants of cetuximab efficacy. J Clin Oncol 2005; 23: 3536–44.

126 Cappuzzo F, Magrini E, Ceresoli GL, Bartolini S, Rossi E, Ludovini V, *et al*. Akt phosphorylation and gefitinib efficacy in patients with advanced non-small-cell lung cancer. J Natl Cancer Inst 2004; 96: 1133–41.

127 Pao W, Miller VA, Venkatraman E, Kris MG. Predicting sensitivity of non-small-cell lung cancer to gefitinib: is there a role for P-Akt? J Natl Cancer Inst 2004; 96: 1117–9.

128 Di Fiore F, Blanchard F, Charbonnier F, Le Pessot F, Lamy A, Galais MP, *et al*. Clinical relevance of KRAS mutation detection in metastatic colorectal cancer treated by cetuximab plus chemotherapy. Br J Cancer 2007; 96: 1166–9.

129 Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, *et al*. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 2004; 350: 2129–39.

130 Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, *et al*. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 2004; 304: 1497–500.

131 Pao W, Miller V, Zakowski M, Doherty J, Politi K, Sarkaria I, *et al*. EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. Proc Natl Acad Sci USA 2004; 101: 13306–11.

132 Han SW, Kim TY, Hwang PG, Jeong S, Kim J, Choi IS, *et al*. Predictive and prognostic impact of epidermal growth factor receptor mutation in non-small-cell lung cancer patients treated with gefitinib. J Clin Oncol 2005; 23: 2493–501.

133 Taron W, Ichinose Y, Rosell R, Mok T, Massuti B, Zamora L, *et al*. Activating mutations in the tyrosine kinase domain of the epidermal growth factor receptor are associated with improved survival in gefitinib-treated chemorefractory lung adenocarcinomas. Clin Cancer Res 2005; 11: 5878–85.

134 Mitsudomi T, Kosaka T, Endoh H, Horio Y, Hida T, Mori S, *et al*. Mutations of the epidermal growth factor receptor gene predict prolonged survival after gefitinib treatment in patients with non-small-cell lung cancer with postoperative recurrence. J Clin Oncol 2005; 23: 2513–20.

135 Pao W, Miller V. Epidermal growth factor receptor mutations, small-molecule kinase inhibitors, and non-small-cell lung cancer: current knowledge and future directions. J Clin Oncol 2005; 23: 2556–68.

136 Shigematsu H, Lin L, Takahashi T, Nomura M, Suzuki M, Wistuba II, *et al*. Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. J Natl Cancer Inst 2005; 97: 339–46.

137 Sordella R, Bell DW, Haber DA, Settleman J. Gefitinib-sensitizing EGFR mutations in lung cancer activate anti-apoptotic pathways. Science 2004; 305: 1163–7.

138 Perez-Torres M, Guix M, Gonzalez A, Arteaga CL. Epidermal growth factor receptor (EGFR) antibody down-regulates mutant receptors and inhibits tumors expressing EGFR mutations. J Biol Chem 2006; 281: 40183–92.

139 Steiner P, Joynes C, Bassi R, Wang S, Tonra JR, Hadari YR, *et al*. Tumor growth inhibition with cetuximab and chemotherapy in non-small cell lung cancer xenografts expressing wild-type and mutated epidermal growth factor receptor. Clin Cancer Res 2007; 13: 1540–51.

140 Patel D, Patel, S, Franklin, M, Kang X. Activity and binding mechanism of cetuximab (Erbitux®) to the type III EGF deletion-mutant receptor. Proc 97th Am Assoc Cancer Res 2006; 47, abstract 1238.

141 Ji H, Li D, Chen L, Shimamura T, Kobayashi S, McNamara K, *et al*. The impact of human EGFR kinase domain mutations on lung tumorigenesis and *in vivo* sensitivity to EGFR-targeted therapies. Cancer Cell 2006; 9: 485–95.

142 Barber TD, Vogelstein B, Kinzler KW, Velculescu VE. Somatic mutations of EGFR in colorectal cancers and glioblastomas. N Engl J Med 2004; 351: 2883.

143 Lee JW, Soung YH, Kim SY, Park WS, Nam SW, Lee JY, *et al*. Absence of EGFR mutation in the kinase domain in common human cancers besides non-small cell lung cancer. Int J Cancer 2005; 113:510–1.

144 Nagahara H, Mimori K, Ohta M, Utsunomiya T, Inoue H, Barnard GF, *et al*. Somatic mutations of epidermal growth factor receptor in colorectal carcinoma. Clin Cancer Res 2005; 11: 1368–71.

145 Lee JW, Soung YH, Kim SY, Nam HK, Park WS, Nam SW, *et al*. Somatic mutations of EGFR gene in squamous cell carcinoma of

the head and neck. Clin Cancer Res 2005; 11: 2879–82.
146 Tsuchihashi Z, Khambata-Ford S, Hanna N, Janne PA. Responsiveness to cetuximab without mutations in EGFR. N Engl J Med 2005; 353: 208–9.
147 Matar P, Rojo F, Cassia R, Moreno-Bueno G, Di Cosimo S, Tabernero J, *et al*. Combined epidermal growth factor receptor targeting with the tyrosine kinase inhibitor gefitinib (ZD1839) and the monoclonal antibody cetuximab (IMC-C225): superiority over single-agent receptor targeting. Clin Cancer Res 2004; 10: 6487–501.
148 Huang S, Armstrong EA, Benavente S, Chinnaiyan P, Harari PM. Dual-agent molecular targeting of the epidermal growth factor receptor (EGFR): combining anti-EGFR antibody with tyrosine kinase inhibitor. Cancer Res 2004; 64: 5355–62.
149 Fischel JL, Formento P, Milano G. Epidermal growth factor receptor double targeting by a tyrosine kinase inhibitor (Iressa) and a monoclonal antibody (Cetuximab). Impact on cell growth and molecular factors. Br J Cancer 2005; 92: 1063–8.
150 Ciardiello F, Caputo R, Bianco R, Damiano V, Pomatico G, Pepe S, *et al*. Cooperative inhibition of renal cancer growth by anti-epidermal growth factor receptor antibody and protein kinase A antisense oligonucleotide. J Natl Cancer Inst 1998; 90: 1087–94.
151 Ciardiello F, Bianco R, Damiano V, Fontanini G, Caputo R, Pomatico G, *et al*. Antiangiogenic and antitumor activity of anti-epidermal growth factor receptor C225 monoclonal antibody in combination with vascular endothelial growth factor antisense oligonucleotide in human GEO colon cancer cells. Clin Cancer Res 2000; 6: 3739–47.
152 Ye D, Mendelsohn J, Fan Z. Augmentation of a humanized anti-HER2 mAb 4D5 induced growth inhibition by a human-mouse chimeric anti-EGF receptor mAb C225. Oncogene 1999; 18: 731–8.
153 Jung YD, Mansfield PF, Akagi M, Takeda A, Liu W, Bucana CD, *et al*. Effects of combination anti-vascular endothelial growth factor receptor and anti-epidermal growth factor receptor therapies on the growth of gastric cancer in a nude mouse model. Eur J Cancer 2002; 38: 1133–40.
154 Tonra JR, Deevi DS, Corcoran E, Li H, Wang S, Carrick FE, *et al*. Synergistic antitumor effects of combined epidermal growth factor receptor and vascular endothelial growth factor receptor-2 targeted therapy. Clin Cancer Res 2006; 12: 2197–207.
155 Saltz, LB, Lenz H, Hochster H, Wadler S, Hoff P, Kemeny, N, *et al*. Randomized phase II trial of cetuximab/bevacizumab/irinotecan (CBI) versus cetuximab/bevacizumab (CB) in irinotecan-refractory colorectal cancer. Proc Am Soc Clin Oncol 2005; 24, abstract 3508.
156 Chen HX, Mooney M, Boron M, Vena D, Mosby K, Grochow L, *et al*. Phase II multicenter trial of bevacizumab plus fluorouracil and leucovorin in patients with advanced refractory colorectal cancer: an NCI Treatment Referral Center Trial TRC-0301. J Clin Oncol 2006; 24: 3354–60.